

UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF NEW YORK

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ASSOCIATION FOR MOLECULAR)
PATHOLOGY; AMERICAN COLLEGE OF)
MEDICAL GENETICS; AMERICAN)
SOCIETY FOR CLINICAL PATHOLOGY;)
COLLEGE OF AMERICAN)
PATHOLOGISTS; HAIG KAZAZIAN, MD;)
ARUPA GANGULY, PhD; WENDY)
CHUNG, MD, PhD; HARRY OSTRER, MD;)
DAVID LEDBETTER, PhD; STEPHEN)
WARREN, PhD; ELLEN MATLOFF, M.S.;)
ELSA REICH, M.S.; BREAST CANCER)
ACTION; BOSTON WOMEN’S HEALTH)
BOOK COLLECTIVE; LISBETH CERIANI;)
RUNI LIMARY; GENAE GIRARD;)
PATRICE FORTUNE; VICKY)
THOMASON; KATHLEEN RAKER,)
))
Plaintiff,)
))
-against-)
))
UNITED STATES PATENT AND)
TRADEMARK OFFICE; MYRIAD)
GENETICS; LORRIS BETZ, ROGER)
BOYER, JACK BRITTAIN, ARNOLD B.)
COMBE, RAYMOND GESTELAND,)
JAMES U. JENSEN, JOHN KENDALL)
MORRIS, THOMAS PARKS, DAVID W.)
PERSHING, and MICHAEL K. YOUNG, in)
their official capacity as Directors of the)
University of Utah Research Foundation,)
))
Defendant.)

No. Civil Action No. 09-4515 (RWS)

ECF Case

DECLARATION OF JOHN J. DOLL

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I, John J. Doll, hereby declare that:

1. I reside in Maryland.

2. I served as the Acting Under Secretary of Commerce for Intellectual Property and Acting Director of the United States Patent and Trademark Office during the change of administration (January 2009 to August 2009). As the Acting Under Secretary, I advised the President, the Secretary of Commerce, and the Administration about all intellectual property matters. As Acting Director, I administered the laws of granting Patents and Trademarks, and the day-to-day management of the \$2.1 billion Agency for over 9,600 employees. I developed and articulated Administration positions on all Patent, Trademark and Copyright issues, both domestic and foreign while promoting strong Intellectual Property policy globally, including strategies to thwart the theft of U.S. Intellectual Property around the world. *See* Curriculum Vitae (Exhibit 1)

3. I was the Deputy Under Secretary of Commerce for Intellectual Property and Deputy Director of the United States Patent and Trademark Office (November 2008 to September 2009). In this capacity, I assisted the Under Secretary in developing and promoting Administration positions on all Patent, Trademark and Copyright issues domestically and internationally. I also served as the Chief Operating Officer in planning, measuring and improving the mission performance and achievement of the Agency. I was responsible for maintaining and growing domestic and international leadership roles in Intellectual Property Rights policy by strengthening Intellectual Property protection. I was also responsible for providing customers with the highest levels of quality and service in all aspects of Agency operations.

4. In 2005 I was appointed as the Commissioner for Patents by the Secretary of Commerce. In this capacity from August 2005 to October 2009, I served as the Chief Operating Officer for all aspects of patent related operations, planning and policy with the mission of properly applying the patent laws and regulations of the United States in the examination of patent applications. I oversaw a budget of \$1.3 billion and a staff of over 7000 employees. I was responsible for Patent's strategic planning and execution, budget formulation and execution, information technology systems, staffing, employee development, labor management relations, customer outreach, congressional relations, public advisory committee relations, and patent policy formulation.

5. Prior to my appointment as Commissioner for Patents, I served as the Acting Commissioner for Patents from April 2005 to August 2005. I was appointed by the Under Secretary of Commerce for Intellectual Property and the Director of the United States Patent and Trademark Office to perform the duties of the Commissioner for Patents until the Secretary of Commerce appointed a new Commissioner for Patents. Prior to and during this appointment, I served as the Deputy Commissioner for Patent Resources and Planning from January 2005 to August 2005. I was responsible for the formulation and execution of the \$1.3 budget as well as strategic planning for the Patent Office.

6. Before my appointment as Acting Commissioner, I served as the Director or Deputy Director of three different Patent Examination Technology Centers from August 1992 to August 2005. I was responsible for all patent examination issues in the chemistry, pharmaceutical, biotechnology and design art areas. During my tenure, from March 2004 to January 2005, I served as the Special Assistant to the Under Secretary and Director of the United

States Patent and Trademark Office from March 2004 to January 2005. In this capacity, I provided technical, examination and legal advice on all Patent related issues.

7. From September 1974 to August 1992, I was a Patent Examiner, Supervisory Patent Examiner, and Quality Assurance Examiner. I examined and supervised the examination of patent applications encompassing pharmaceuticals, herbicides, pesticides, dyestuffs, inorganic chemistry, hydrometallurgy, zeolite catalysts, buckminsterfullerenes, proteins, and peptides.

8. I have been honored on multiple occasions for my service at the United States Patent and Trademark Office. Among my honors, I received the Department of Commerce Bronze Medal for examination and supervisory accomplishments; a second Bronze Medal for the implementation of Patent Application Location and Monitoring (PALM) system; a Department of Commerce Silver Medal for the development of automated examiner office action tools; the Vice Presidential Hammer Award for establishing the Biotechnology Customer Partnership; a Department of Commerce Gold Medal for implementation of the Image File Wrapper system.

9. I received my Master of Science in Physical Chemistry in 1974 from The Pennsylvania State University. I received my Bachelor of Science in Chemistry and Physics in 1971 from Bowling Green State University.

10. In preparing this Declaration, I reviewed and considered the Plaintiffs' Statement of Material Facts ("SMF"); Plaintiffs' Memorandum of Law in Support of Motion for Summary Judgment ("Memo"); Dr. John Sulston's and Dr. Myles Jackson's Declarations; the Utility Examination Guidelines (66 Fed. Reg. 1092 (January 5, 2001)); United States Patent Nos. 5,747,282 ("the '282 patent"); 5,837,492 ("the '492 patent"); 5,693,473 ("the '474 patent"); 5,710,001 ("the '001 patent"); 5,753,441 ("the '441 patent"); 6,033,857 ("the '857 patent"); 5,709,999 ("the '999 patent"), (collectively "Myriad patents").

THE USPTO ADMINISTERS THE LAWS ENACTED BY CONGRESS

11. The U.S. Patent and Trademark Office (“USPTO” or the “Office”) is an administrative agency charged with examining applications for patent. The USPTO issues a patent if the application meets the statutory requirements of Title 35 United States Code, Title 37 Code of Federal Regulations and the procedures of the Manual of Patent Examining Procedure (“MPEP”). The USPTO must apply the laws Congress has enacted and the Federal Circuit and the Supreme Court have interpreted.

12. On October 17, 1994, Commissioner Bruce Lehman conducted a public hearing in San Diego on the patenting of biological inventions before the USPTO. Public Hearing on the Patenting of Biotechnological Inventions before the United States Patent and Trademark Office (Oct. 17, 1994), located at <http://www.uspto.gov/web/offices/com/hearings/biotech/biotrans.html> (Exhibit 2). The purpose of the public hearing was to obtain input from the industry in connection with the Office’s review of its examining procedures to ensure that examination was of sufficiently high quality, and that the USPTO was responsive to the industry’s needs.

13. The recurrent theme of the testimony received from industry representatives at that hearing, sometimes referred to as “the San Diego Massacre,” was that the USPTO applied a more stringent and uneven standard for biotechnology inventions, and that we were “killing the industry” by not issuing patents for biotechnology inventions. Mostly, the complaints related to uneven examination standards, peer review rather than legal review of patent applications, and that a special higher standard for patentability (in particular, utility) was being applied to biotechnology inventions.

14. In response, the USPTO issued utility examination Guidelines to address the concerns aired at the public hearing. Utility Examination Guidelines, 60 Fed. Reg. 36263 (1995) (reproduced in MPEP § 706.03(a)(1) (July 1998) (hereafter “1995 Utility Guidelines”)) (Exhibit

3). The Guidelines are not law or substantive rules. Rather, the Guidelines define the procedures to be followed by Office personnel in their review of patent applications for compliance with 35 USC § 101. The legal analysis supporting the Guidelines articulated the basis for the procedures that were established.

15. In February of 1995, I was asked to be the Director of the Patent Examination Technology Center 1800, which examined patent applications claiming biotechnological inventions. Shortly after taking the position, I was told of a backlog of unexamined applications in Group 1800 relating to “ESTs” (“expressed sequences tags”). An EST is a short fragment of a cDNA that represents a portion of an expressed gene. Patenting “anonymous” EST fragments was controversial. There was a concern that patents on ESTs without knowledge of the function of the gene, could potentially dominate or preclude a patent to those who later deciphered the structure of the full length gene and the function of the gene. The Technology Center was holding the EST applications and not examining them since they were unsure how to properly apply the patent laws and regulations to EST applications claiming, sometimes, thousands of ESTs in a single application. Thus, there was a moratorium on examination of these applications.¹

16. After I was briefed on the EST applications, it was apparent that the examination of EST applications presented some unique examination issues. I began discussions with the Office of the General Counsel and the Solicitor’s Office to determine the appropriate examination procedures for EST applications and to get the examination of these applications started.

¹ Plaintiffs’ expert, Jackson, is incorrect when he states that ESTs were easy to patent in the mid-1990s (Jackson Declaration, p. 11, n.13). Patents on ESTs were not issued during this time period, and ultimately, patentability of ESTs without knowledge of function of the corresponding gene product was denied by the Federal Circuit (In re Fisher, 421 F.3d 1365 (1995)).

17. A team from the Office of the General Counsel, including the Solicitor's Office, and the Technology Center convened and studied the law on patent eligibility of DNA molecules and the criteria that would be required to address the concerns about ESTs. The results showed that utility was the main issue that needed to be addressed. The USPTO also consulted with various groups, including Pharmaceutical Research and Manufacturers of America ("PhRMA") and Biotechnology Industry Organization ("BIO"), and agencies, including the National Institutes of Health ("NIH"), the Department of Commerce ("DOC"), and the Office of Management and Budget ("OMB"), to develop a framework for examining DNA EST patent applications.

18. Through this process, the USPTO refined its procedures for examining all inventions including biotechnology inventions to require a specific, credible and substantial utility for DNA molecules claimed. This was done to ensure that patents did not issue on DNA molecules without a proper disclosure of utility satisfying 35 USC § 101. These criteria were published in the revised Guidelines. Revised Interim Utility Examination Guidelines, 64 Fed. Reg. 71440 (December 21, 1999) (hereinafter "1999 Interim Guidelines") (Exhibit 4). The Utility Examination Guidelines that were released in 2001 (Utility Examination Guidelines, 66 Fed. Reg. 1092 (January 5, 2001) (hereinafter "2001 Utility Guidelines") (Exhibit 5)) were based on the 1999 Interim Guidelines. The USPTO incorporated these Guidelines into the MPEP. U.S. Pat. & Trademark Off., MPEP § 2107 (8th ed. 2001) (Exhibit 6).

19. In order to explain our position regarding the examination of patent applications relating to DNA molecules to the public, I worked with the Office of Public Affairs, the Office of the General Counsel including the Solicitor's Office, and the Technology Center, and wrote an

article published in *Science*. Doll, J.J., 1998, "The Patenting of DNA" *Science* 280: 689-690 (Exhibit 7).

20. During the evolution of the Examination Guidelines, several of the plaintiffs and plaintiffs' experts in this action participated in the commentary and expressed their concern with the possible impact of patents granted for DNA-related inventions, particularly with respect to ESTs. [Association for Molecular Pathology, Debra Leonard, Wayne W. Grody, Mark E. Sobol, The American College of Medical Genetics, David H. Ledbetter]. A number of the participants commended the USPTO's approach since it addressed their major concern in that the Guidelines would prevent the patenting of DNA molecules where the function of the gene product was unknown. *See* Letter from R. Rodney Howell, President, American College of Molecular Genetics to Commissioner, USPTO (March 20, 2000), *available at* <http://www.uspto.gov/web/offices/com/sol/comments/utilguide/acmg.pdf> (Exhibit 8); Letter from Debra G.B. Leonard, President, Association for Molecular Pathology to Commissioner, USPTO (March 17, 2000), (hereinafter "AMP Letter"), *available at* <http://www.uspto.gov/web/offices/com/sol/comments/utilguide/amp.pdf> (Exhibit 9).

21. The only remaining concern expressed was whether patents in this area were in the public interest (AMP Letter ¶ 3) – an issue mirrored in the plaintiffs' complaint. *See* Plaintiffs' Complaint, ¶¶ 2, 81-101. This is a policy issue that can only be addressed by Congress, not the courts and not the USPTO.

22. I will now explain what criteria were used to assess patentability of biotechnological inventions, in particular for DNA molecules, and how the USPTO applied them.

ISOLATED DNA MOLECULES ARE PATENT ELIGIBLE SUBJECT MATTER

23. New areas of technology do not create the need for a whole new specialized area of patent law. The patent statutes and regulations are the same for all inventions regardless of whether they are genes, semiconductors, computers, pesticides or doorbells. Neither the Congress nor the courts have indicated that patent law should be changed with the introduction of genetic technology or any other specific technologies. Had Congress wanted the USPTO to apply different standards to different technologies, I believe Congress would have expressly done so.

24. The same patentability analysis has always been applied by USPTO regardless of the technology. The laws have consistently remained the same through every new class of inventive effort because they established a patent regime that has worked well.

25. As I pointed out in my Science article, in many ways, Plaintiffs' arguments resemble those voiced 30 to 40 years ago when polymer chemistry was an emerging technology. At that time, it was argued that patents on the building blocks of basic polymers would devastate the industry. In fact, no such disaster occurred. For example, the issuance in 1965 of a basic patent broadly claiming a vulcanizable copolymer of aliphatic mono-olefins and unsaturated bridged-ring hydrocarbons (*i.e.*, ethylene-propylene-diene monomer ("EPDM") rubber) did not preclude the later issuing of patents to different inventors for several copolymers of this type (Exhibit 7 ¶ 3-4).

26. Isolated or purified DNA molecules are chemical compositions that are eligible for patent protection under 35 USC § 101 as compositions of matter. They are not naturally occurring substances and they are not just information.

27. Isolated or purified DNA molecules are chemical compositions that possess physical, chemical and structural properties that differ from their naturally occurring counterpart

genes. In their natural state, genes are chemically bonded into long strands of nucleotides that make up chromosomes. Genes do not float around in cells as separate entities; one cannot simply reach into a cell and pull a gene out of the body. It requires research and discovery to identify, isolate or synthesize and sequence a gene from the vast run of nucleotides on a chromosome. There is no roadmap as to where the genes are located on a chromosome or what function the gene products have.

28. Isolating or purifying a gene is not simply copying its information into another format. The isolated DNA is chemically, physically and structurally different from the gene in the body. Just like any other chemical composition, DNA contains various groupings of atoms. DNA specifically contains carbon, hydrogen, nitrogen, oxygen and phosphorous atoms that are bonded in a manner that determines its properties, just as the properties of any chemical composition are determined by the atoms that comprise it and the manner in which they are bonded. Even if the information in an isolated DNA molecule is the same as its genomic counterpart, it would not preclude the isolated DNA from being patent eligible subject matter.

29. A critical aspect of any chemical compound is its inherent chemical, physical and structural properties, such as chemical reactivity, catalytic properties, and biological interactions. This is also true of DNA. An isolated DNA has a different number of atoms of various elements that are bonded in a manner that is distinct from the gene sequence found in the body. As a result, an isolated DNA has properties, functions and utilities that the gene in the body does not have. For example, the isolated DNA, but not the naturally occurring gene, can be used as a diagnostic probe, molecular marker, or source primer.

30. The Plaintiffs contend that genetic sequences are information and should not be patentable. SMF ¶¶ 48-49, 59, 65, 96, 114. The Plaintiffs fail to see that the inherent

information contained in a composition of matter is not what is patented in a composition of matter claim. What is patented is the composition of matter itself and the patent excludes others from making using selling or importing the patented invention which includes all of chemical, physical, structural and informational properties for that specific composition of matter.

31. A DNA molecule is a chemical composition that is composed of structural units called nucleotides. A nucleotide consists of an phosphate, a deoxyribose sugar, and one of four bases: Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). The letters are just assigned to the nucleotides so that a DNA molecule may be more easily depicted. This system is analogous to the chemical formulas used for describing other chemical compounds. For example, the chemical formula for water is H₂O, where “H” represents a hydrogen atom, “2” represents the number of hydrogen atoms present in the compound, and “O” represents an oxygen atom. The recitation of a chemical formula in a claim does not mean that the inventor is patenting the chemical formula – it is the chemical composition of matter is being patented. So analogously, when a nucleotide sequence is recited in a claim, the inventor is not claiming the sequence, but the DNA molecule as a chemical composition.

32. As the Plaintiffs acknowledge, cDNAs are not natural forms of DNA that are found in the human body. They are synthesized by reverse transcribing mRNA in a laboratory. SMF ¶ 62. They are chemical compositions of matter that are chemically, physically and structurally different from naturally occurring genes.

33. For example, cDNAs are not the same as the gene sequence in the body – they lack the introns that are present in the naturally occurring gene. Even if we assume for argument’s sake that a sequence of a cDNA was identical to a DNA sequence in the body, the cDNA would be a very small part of a chromosome’s huge string of nucleotides present in the

body, and thus would have totally different properties than any naturally occurring composition of matter. Thus, Plaintiffs are wrong when they claim that “[t]he sequence of a cDNA ... is identical to that in the body.” SMF ¶¶ 62, 63.

34. Plaintiffs incorrectly conclude that genes and their mutations are unpatentable because they are manifestations of laws of nature. SMF ¶¶ 73, 81. Every composition of matter ever patented can be said to embody the inherent laws of nature dictated by the chemical, physical and structural properties of that particular chemical combination of atoms – this does not render those compositions unpatentable. Determining specifically which nucleotides on a chromosome relate to a gene of a specific function and which variations of that gene indicate a predisposition to a specific disease is certainly a discovery which is patent eligible under § 101. Claims covering isolated DNA molecules with a specific, substantial and credible utility, and methods using them to screen, analyze, or diagnose are patent eligible.

35. The USPTO has examined countless applications using the standards I discussed. I searched the USPTO database and found that over the past 29 years, the USPTO has issued some 2,645 patents claiming “isolated DNA.” <http://patft.uspto.gov/netahtml/PTO/search-adv.htm> (search query ACLM/”isolated DNA”) (Exhibit 10). Seven of these patents are the Myriad Patents challenged by the plaintiffs.

36. Moreover, I am aware of several patents that are related to genes implicated in different diseases and that have been issued to some of the plaintiffs in this case - Dr. Haig Kazazian 5,407,796, (“Cystic Fibrosis Mutation Cluster”) (Exhibit 11); Dr. Stephen T. Warren (Patents 6,107,025 and 6,180,337 (both entitled “Diagnosis of the Fragile X Syndrome”) (Exhibit 12 and 13); and Dr. David H. Ledbetter (Patent 6,143,504 (“Methods and Compositions for the Diagnosis of Fragile X Syndrome”) (Exhibit 14).

MYRIAD'S BRCA GENE PATENT CLAIMS MEET THE STANDARDS OF § 101

37. I believe that all of the Myriad patent claims in question here meet the requirements for patent eligibility reflected in 35 USC § 101, the Regulations, Guidelines, and the USPTO's practice and procedures set forth in the Manual of Patent Examining Procedure (MPEP) for composition of matter and method claims.

38. Challenged claim 1 of the '473 patent, claims 1 and 2 of the '282 patent, and claims 1,6 and 7 of the '492 patent recite isolated BRCA1/2 DNA molecules. Chemical compositions of matter were patented in these patents, not functionality or information.

39. The utility of the BRCA1/2 composition of matter claims are specific, credible and substantial, thus meeting the elevated utility standard set forth in the 2001 Utility Guidelines. The Myriad patents describe the function of the gene products and discovered the significance of the mutations; these patents are different from the EST patents I discussed earlier.

40. The language of Claim 1 of patents '999, '001, and '441 and Claims 1 and 2 of patent '857 is typical for method claims found throughout thousands of patents for detecting or screening a sample against a normal sample and meet all of the statutory requirements for patentability.

41. The language of Claim 2 of patents '857, patent appears to be the same as thousands of other patented diagnostic claims and meet all of the statutory requirements for patentability.

42. The language of Claim 20 of '282 patent is common for method claims found in thousands of patents for screening drugs using cell culture and meet all of the statutory requirements for patentability.

43. Plaintiffs allege that “[s]ome of Myriad’s patents cover mutations that were found by other scientists.” SMF ¶ 98. If this is true and if Myriad is not the first true inventor of patented subject matter, there are established procedures to address this concern.² However, this is irrelevant to the issue of patent eligibility of the subject matter claimed.

PATENTS PROMOTE RESEARCH AND COMMERCIALIZATION

44. The Plaintiffs express concern that the right to exclude others from using the patented invention will limit the number of other laboratories that can provide the same services as Myriad. SMF ¶¶ 10-12, 87, 139. What the Plaintiffs fail to acknowledge, however, is that the patentee’s right to exclude is limited in time, after which the patented invention is donated to the public to freely use. The Constitution expressly permits this quid pro quo contract with the government which the patent system ensures.³

45. Patents encourage the sharing of inventions and discoveries by discouraging trade secrets. Without patents and the protections they grant, the world would have less information and many fewer life saving drugs and therapies. More people would have died and there would be less information to use as the basis for future research.

46. Without patents which allow researchers to profit from their discoveries, many discoveries would not have been made. Public funding alone simply could not fund all the research and commercialization that the combination of public and private funding currently accomplishes. Many of the life saving inventions that the world now enjoys would never have been discovered or commercialized if it were not for the potential profits afforded by the temporary exclusive rights granted by the patent system. Undoubtedly, many researchers would

² Which party first invented the commonly claimed invention can be determined through an interference proceeding under 35 USC § 135(a).

³ U.S. Constitution, Article 1, Section 8, Clause 8 clearly states “The Congress shall have the Power to promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries.”

not have embarked on their research in the first place because they could not afford it or did not have a place to conduct the desired research. Also, the information disclosed in patents provides other researchers with the knowledge and a new starting point to begin research based on the information disclosed in the patent. The discovery and characterization of a gene permits a multitude of other patent possibilities, e.g., diagnostic probes, methods of activating or deactivating, gene splicing, gene replacement therapies, and discovering genes within genes.

47. Contrary to the clinicians and physicians who complain that gene patents interfere with an individual's ability to make informed health decisions (SMF ¶¶ 7-9, 13, 14, 17, 82, 83), gene patents give people options that may not have been available if not for the exclusive rights granted by patents allowing researchers and companies the opportunity to recover the research and development ("R&D") cost and to possibly return a profit for corporations, shareholders and venture capitalists. Many of the life saving inventions that the world now enjoys would never have been discovered or commercialized if it were not for the exclusive rights granted by the patent system.

48. Myriad might not have spent the monies on the R&D that provided the BRCA1 and BRCA2 information to the world if it were not for the exclusive rights granted by the patent system and the possibility of recovering their R&D costs and to return a profit for their shareholders and venture capitalists. There is nothing of record to suggest that, without Myriad's R&D, the world would have the benefit of the life saving advancements claimed and disclosed in their patents.

49. The Plaintiffs also argue that DNA molecules should not be patentable because DNA patents "can serve as a disincentive to innovation in molecular testing and medicine because they deny access to a vital baseline of genomic information that cannot be 'invented

around.” SMF ¶¶ 1, 3, 50, 114, 175-192. The Supreme Court captured the overbreadth of this criticism in *Diamond v. Chakrabarty*, 447 U.S. 303, 317 (1980), “[t]he grant or denial of patents ... is not likely to put an end to genetic research ... patentability will not deter the scientific mind from probing into the unknown ... Whether respondent’s claims are patentable may determine whether research efforts are accelerated by the hope of reward or slowed by want of incentives, that is all.” Although the new discoveries resulting from the R&D of “inventing around” are a benefit of the patent system, the ability to invent around an invention is not a statutory patentability requirement. That an invention is fundamental to human nature and scientific inquiry does not preclude it from patentability. This issue is not part of the calculus of the patent statutes established by Congress and repeatedly interpreted by the Courts or the guidelines set forth by the USPTO.

50. Many women and men have benefited from the Myriad patented compounds and methods. These benefits probably would not have come about if it were not for Myriad’s R&D which is highly likely to have been supported by the profits from their patents. Myriad is using the patent system for exactly what it was intended - to exclude others from making, using, selling and importing their patented invention(s) for a limited time, after which the invention becomes available to the public to freely use.

51. Pursuant to 28 U.S.C. § 1746, I declare under penalty of perjury that the foregoing is true and correct.

Executed on December 22, 2009


John Doll

APPENDIX 1

LIST OF EXHIBITS

Exhibit No.	Title
Exhibit 1	<i>Curriculum Vitae</i>
Exhibit 2	Public Hearing on the Patenting of Biotechnological Inventions Before the United States Department of Commerce and Patent and Trademark Office (Oct. 17, 1994), located at http://www.uspto.gov/web/offices/com/hearings/biotech/biotrans.html
Exhibit 3	<i>Utility Examination Guidelines</i> , 60 Fed. Reg. 36263 (1995) (reproduced in MPEP § 706.03(a)(1) (July 1998))
Exhibit 4	Revised Interim Utility Examination Guidelines, 64 Fed. Reg. 71440 (December 21, 1999)
Exhibit 5	Utility Examination Guidelines, 66 Fed. Reg. 1092 (January 5, 2001)
Exhibit 6	U.S. Pat. & Trademark Off., MPEP § 2107 (8th ed. 2001)
Exhibit 7	Doll, J.J., 1998, "The Patenting of DNA" <i>Science</i> 280: 689-690
Exhibit 8	Letter from R. Rodney Howell, President, American College of Molecular Genetics, USPTO (March 20, 2000), <i>available at</i> http://www.uspto.gov/web/offices/com/sol/comments/utilguide/acmg.pdf
Exhibit 9	Letter from Debra G.B. Leonard, President, Association for Molecular Pathology to Commissioner, USPTO (March 17, 2000), <i>available at</i> http://www.uspto.gov/web/offices/com/sol/comments/utilguide/amp.pdf
Exhibit 10	http://patft.uspto.gov/netahtml/PTO/search-adv.htm (search query ACLM/"isolated DNA")
Exhibit 11	U.S. Patent No. 5,407,796 (filed Jan. 4, 1991)
Exhibit 12	U.S. Patent No. 6,107,025 (filed May 24, 1991)
Exhibit 13	U.S. Patent No. 6,180,337 (filed Aug. 29, 1991)
Exhibit 14	U.S. Patent No. 6,143,504 (filed Oct. 27, 1999)

CERTIFICATE OF SERVICE

This is to certify that on December 23, 2009, a true and correct copy of the foregoing document has been served on all counsel of record via the court's ECF system.

/s/ Brian M. Poissant

Brian M. Poissant

EXHIBIT 1

John Doll
3780 Waterfield Court
Huntingtown, MD 20639
Email: johnjdoll@comcast.net
Phone: 410 535 3508
Mobile: 410 610 1252

**Acting Under Secretary of Commerce for Intellectual Property and
Acting Director of the United States Patent and Trademark Office**
January 2009 to August 2009

Served as the Acting Under Secretary and Acting Director of the United States Patent and Trademark Office during the change of Administration. As the Acting Under Secretary, advised the President, the Secretary of Commerce, and the Administration about all intellectual property matters. As Acting Director, administered the laws of granting Patents and Trademarks, and the day-to-day management of the \$2.1 billion Agency for over 9,600 employees. Developed and articulated Administration positions on all Patent, Trademark and Copyright issues, both domestic and foreign while promoting strong Intellectual Property policy globally, including strategies to thwart the theft of U.S. Intellectual Property around the world.

**Deputy Under Secretary of Commerce for Intellectual Property and
Deputy Director of the United States Patent and Trademark Office**
November 2008 to September 2009

Assisted the Under Secretary in developing and promoting Administration positions on all Patent, Trademark and Copyright issues domestically and internationally. Served as the Chief Operating Officer in planning, measuring and improving the mission performance and achievement of the Agency. Responsible for maintaining and growing domestic and international leadership roles in Intellectual Property Rights policy by strengthening Intellectual Property protection. Also responsible for providing customers with the highest levels of quality and service in all aspects of Agency operations.

Commissioner for Patents
August 2005 to October 2009

Appointed by the Secretary of Commerce and served as the Chief Operating Officer for all aspects of patent related operations, planning and policy with the mission of properly applying the patent laws and regulations of the United States in the examination of patent applications. Oversaw a budget of \$1.3 billion and a staff of over 7000 employees. Responsible for Patent's strategic planning and execution, budget formulation and execution, information technology systems, staffing, employee development, labor management relations, customer outreach, congressional relations, public advisory committee relations, and patent policy formulation.

Deputy Commissioner for Patent Resources and Planning

January 2005 to August 2005

Responsible for the formulation and execution of the \$900K budget as well as strategic planning for the Patent Office

Special Assistant to the Director of the United States Patent and Trademark Office

March 2004 to January 2005

Provided technical, examination and legal advice on all Patent related issues

Director of Patent Examination Technology Center

August 1992 to August 2005

Responsible for all patent examination issues in the chemistry, pharmaceutical, biotechnology and design art areas

Patent Examiner, Supervisory Patent Examiner, Quality Assurance Examiner

September 1974 to August 1992

Examined and supervised the examination of patent applications encompassing pharmaceuticals, herbicides, pesticides, dyestuffs inorganic chemistry, hydrometallurgy, zeolite catalysts, buckminsterfullerenes, proteins, and peptides

Awards

Department of Commerce Bronze Medal for examination and supervisory accomplishments; a second Bronze Medal for the implementation of Patent Application Location and Monitoring (PALM) system; a Department of Commerce Silver Medal for the development of automated examiner office action tools; the Vice Presidential Hammer Award for establishing the Biotechnology Customer Partnership; a Department of Commerce Gold Medal for implementation of the Image File Wrapper system.

Education:

MS Physical Chemistry The Pennsylvania State University 1974

BS Chemistry / Physics Bowling Green State University 1971

EXHIBIT 2

Transcripts of the Public Hearings on Biotechnology

PUBLIC HEARING ON THE

PATENTING OF BIOTECHNOLOGICAL INVENTIONS

BEFORE THE UNITED STATES DEPARTMENT OF COMMERCE

PATENT AND TRADEMARK OFFICE

The Copper Room

San Diego Concourse

202 C Street

San Diego, California

Monday, October 17, 1994, 9:00 a.m.

Panel Members From the United States Patent and Trademark Office:

THE HONORABLE BRUCE LEHMAN, CHAIRMAN

Assistant Secretary of Commerce and Commissioner of Patents and
Trademarks

CHARLES VAN HORN, ESQ.

Deputy Assistant Commissioner for Patent Policy

NANCY LINCK, ESQ.

Solicitor

JEFF KUSHAN, ESQ.

Attorney Advisor

Office of Legislative and International Affairs

BARRY RICHMAN

Director, Group 1800

CHARLES WARREN, ESQ.

Deputy Director, Group 1800

WITNESSES

Congressman Dana Rohrabacher, 45th District, California

Ken Widder, Molecular Biosystems, Inc.

William Rastetter, Idec Pharmaceuticals

Congressman Bob Filner, San Diego, California

Jerry D. Caulder, Mycogen Corporation

Dave Gollaher, California Health Care Institute

Bernie Rhinerson, Biomedical Industry Council of California

Bill Otterson, National Cancer Patients Association

Eugene Schonfeld, National Kidney Cancer Association

Martin Simpson, Office of Technology Transfer University of California

William Beers, The Scripps Research Institute

Kathy Behrens, Robertson, Coleman and Stephens

Douglas Obenshain, Ernst and Young

William N. Epstein, Hoffman-La Roche, Inc.

Laura Handley, Weil, Gotshal and Manges

Timothy Gens, Fenwick and West

William Kennedy, Morrison and Foerster

Elizabeth Enayati, Weil, Gotshal and Manges

William J. Scanlon, Foley and Lardner, Biotechnology Industry
Organization

Stanley Crooke, Isis Pharmaceuticals

Vincent Gioia, Christie, Parker and Hale

Mark G. Toohey, Spencer, Frank and Schneider

Allen E. Dow, Klarquist, Sparkman, Campbell, Leigh and Whinston

Ned A. Israelsen, Knobbe, Martens, Olson and Bear and Vical, Inc.,
Alliance Pharmaceutical

Barbara Rae-Venter, Weil, Gotshal and Manges

Timothy Gens, Fenwick and West

Elizabeth Lassen, Calgene, Inc.

Daniel N. Chambers, Viagene, Inc.

Thomas G. Wiseman, Cushman, Darby and Cushman

George Johnston, Law Department, Hoffman-La Roche

Richard C. Peet, Foley and Lardner

John Sanders, Mycogen Corporation

Micheal B. Farber, Merchant and Gould

Michael J. Roth, Pioneer Hi-Bred International

Margaret Connor, Office of Technology Transfer, Agricultural Research Service, USDA

Steven Brostoff, The Immune Response Corporation

Robert Schaffer, Darby and Darby

Bertram I. Rowland, Flehr, Hohbach, Test, Albritton and Herbert

John W. Schlicher, Crosby, Heafey, Roach and May

Alain Schreiber, Vical Corporation

Eric C. Woglom, The Association of the Bar of the City of New York

Ronald Tuttle, Houghton Pharmaceuticals, Inc.

Jeffrey Miller, IXSYS Corporation

Gail M. Kempler, Regeneron Pharmaceuticals, Inc.

Andrew D. Fortney, Oblon, Spivak, McClelland, Maier and Nestadt

Robert Benson, Genelabs, Incorporated

Lynne Parshall, Isis Pharmaceuticals

Susan Perkins, Cambell and Flores

Robert Sobol, San Diego Regional Cancer Center

David A. Lowin, Syntex, Inc.

Jeffrey G. Sheldon, Sheldon and Mak

Ted Green, Amylin Pharmaceuticals

William J. Scanlon, Foley and Lardner, Wisconsin Biotechnology Association

P R O C E E D I N G S (9:00 a.m.) COMMISSIONER LEHMAN: I'm going to start the hearing right now. We're expecting Congressman Filner, San Diego, to welcome us and introduce us, and when he comes we will interrupt for him so that he can do that officially. But I'd like to begin with my opening remarks so that we can get the hearing underway and try to keep on our schedule as much as possible; otherwise, we'll be here until midnight tonight.

First of all I'd like to introduce who we are. I'm Bruce Lehman the Commissioner of Patents and Trademarks and with me here are Chuck Warren, Deputy Group Director of Group 1800, our biotechnology examining group; Barry Richman, Group Director, Group 1800; Charles Van Horn, Deputy Assistant Commissioner for Patent Policy; Nancy Linck, the solicitor, our head lawyer in the Patent and Trademark Office; and Jeff Kushan of the Office of Legislative and International Affairs, and Jeff organized this hearing and has been dealing with quite a few of you. Now, Mike O'Neil is here someplace. He's there at the door. If you have any administrative problems or questions or anything like that, please, ask Mike O'Neil about it, and Jeff Kushan also would be sort of a second backup on substantive matters.

Also here we have Esther Kepplinger and Dave Lacey of Group 1800 who are in the audience.

Since I was appointed the Commissioner of Patents and Trademarks by President Clinton, we've worked hard to make this office responsive to our customers, and our customers are the users of the patent system and this hearing is an example. In fact, this is our fourth round of public hearings on patent issues and our second round on patent issues in California. I think it's appropriate that we come out here to California because, really, more than any other state, the center of creativity and probably where we get most of our business and the largest single concentration of our customers for the Patent and Trademark Office are in this particular state.

Last January we were in Silicon Valley and at that time we held hearings about problems in the -- patent problems in the software industry. Last July we held hearings in Washington, D. C. on the obviousness standard, and just about a year ago we held hearings on patent law harmonization and those were really our first hearings in this area.

We've used these public hearings to identify the needs and concerns of our customers -- the patent applicants -- as well as their goals for the patent system. For example, we use the public record from our patent law harmonization hearings when we decided to suspend negotiations on the patent law harmonization treaty. We relied on comments made during our harmonization and software hearings, both of them, to shape the legislation to implement the Uruguay round agreement which is now pending before Congress.

By the way, I note that nearly every witness in our harmonization and in our software hearings that addressed the question of patent terms supported a patent term of 20 years from filing.

We also listened carefully to what the software industry said to us during our public hearings in Silicon Valley. Their comments were very important, if not the deciding factor, in our decision to forward legislation to Congress to reform re-examination procedures and then our decision to support prior user rights legislation. That's why these hearings are very important because they can help us identify problems, provide us with useful solutions and create an important public record of where our customers stand on the important issues in the patent system. This is particularly true for the biotechnology industry which is particularly dependent on effective and meaningful patent protection.

The availability of patent protection has made it possible for biotechnology companies to attract investments, undertake risky product development and ultimately put products on the market. And because patents play such an important role in this industry, we have a special obligation to ensure that the patent system is serving its needs.

We cannot grant patents that can be taken to the bank -- literally -- or impose requirements that are so strict the patents cannot be obtained. We can't do that, we will undercut a primary purpose of the patent system which is not only to promote innovation but also commercialization, and this is very important. That's really what the patent system does, it promotes the commercialization of the remarkable advances in biotechnology that we are witnessing today.

Before we began to hear from our witnesses, I'd like to give you some feeling for where the office stands with respect to its biotechnology examining operation.

During the mid-1980s, we began to feel the strain of an explosion of activity in the biotechnology industry. Applications were being filed at a rate that exceeded our capacity to examine. This created a backlog of cases waiting resolution and led to increasingly long patent pendency; a pendency on a per application basis that reached nearly 27 months in 1987. This prompted us to make a serious commitment to improving the

examining operation in this particular area and we started by creating a special biotechnology examining group, which is now Group 1800, in 1988. It then recruited highly-skilled examiners, invested in advanced computer systems, to support searching DNA and protein sequences, and increased its collections of non-patent literature and expanded its access to on-line information resources.

The office also reached out to industry, academia, the bar, and government organizations by establishing the Biotechnology Institute to assist in scientific and legal training of examiners. These efforts have all paid off and pendency has decreased from nearly 27 months, in this particular area in 1987, to a current average of 20.8 months, despite the fact that filings have nearly doubled during that period. And we have cut the time that we take to issue a first office action from 14 months, in 1988, to less than eight months today. I think this shows that we can keep patent pendency down, if we simply keep on top of the system.

I'm particularly proud of our success in recruiting extremely qualified examiners in Group 1800. Currently there are 165 examiners in this group, up from 67 in 1988. Seventy percent of these individuals hold advanced degrees in biotech-related disciplines. In fact, over half of our biotechnology examiners have Ph.D. degrees.

With regard to our efforts to automate, the biotech group has spent over \$2 million so far on advance computing equipment related to sequence searching. This is addition to our larger capital project to automate the entire search system which is pretty much complete now in the Patent and Trademark Office.

We have also increased our holdings of specific non-patent literature such that we now have over 1,700 periodicals in our library holdings and through the National Library of Medicine, the National Agricultural Library, and the Library of Congress we have ready access to over 8,000 periodicals.

We currently use 12 sequence information databases and we have access to over 30 more through commercial on-line services, and we're studying more solutions to getting usable information on prior art.

All of these steps represent a serious commitment by the Patent and Trademark Office to provide an effective, efficient examining operation for biotechnology applications.

The Clinton administration has also supported the biotechnology industry in other ways. When we heard that the biotechnology industry had concerns with the Uruguay round implementing legislation -- and I should note that the Uruguay round itself will provide protection for

biotechnology inventions in many, many markets and countries in the world where there currently are no such protections, such as Brazil, for example, which provide no protection whatsoever for most pharmaceutical inventions. And when you think that the pharmaceutical industry alone in the United States is a \$100 billion a year industry and that we're receiving less than \$1 billion in revenues from a country of 120 million people, you can imagine what the impact of the Uruguay round is going to mean; it's going to mean billions more dollars flowing into the United States economy because we recognize protection for intellectual property in this area. But we also recognize that it's very important to fine tune the implementing legislation and so when we talked to the industry and knew that they had concerns we listened very carefully. And I would note that Senator Dianne Feinstein was very instrumental in bringing these concerns to our attention and worked closely with us to amend the legislation before it was introduced so that these concerns could be addressed.

We have also supported legislation that would change how determinations are made with regard to obviousness of biotechnology process inventions. For example, we testified in support of legislation in both the House and the Senate that would have addressed problems that were identified with the biotechnology industry.

Our hearings today represent another example of our commitment to serving customer needs. We will take your input today and we will use it to refine our examining operations and to make policy decisions that will ensure that our biotechnology group not only produces a high quality patent but is also responsible to the biotechnology industry's needs.

Now, before we begin the hearings, I'd like to briefly review the ground rules for the witnesses. We have 57 people today and so these turned out to be the most popular, probably, set of hearings that we've had. We keep getting more and more people and we should have arranged for two days but we didn't expect this large number and so we'll just stay here until the end of the evening, as long as necessary, to make certain everybody does get to speak. It does limit us a little bit in that it makes it a little harder for us to have a dialogue and answer and ask, you know, questions with witnesses, which is what we ideally like to do.

Each witness will have approximately nine minutes to present their remarks, and to the extent that you can do it in less than that, we would really appreciate it, and I think Jeff has talked to some of you about special needs that individual people have and so we have that taken into consideration. But, again, to the extent that we wish to have anybody in the panel -- any dialogue, if you can keep your remarks limited it would be very helpful. Also, to the extent that the points have already been made by other people, especially as we go through the day with 57 witnesses, it may not be necessary to repeat all those points. You can

just say, "I agreed with Mary Smith or Joe Small," and we'll write that down and make certain that we have that -- that we can consult their testimony.

Now, to help you out keeping on time, we're going to have a monitor. Apparently we've have had some technical problems for the first time during these hearings. We're going to have a monitor but for right now we'll have to do it manually, and Jeff will be responsible for timing people and he'll signal you when you're two minutes away from your nine minutes being up. In other words, at the seventh minute. And, hopefully, a little later we'll have a computer monitor here that will help you on that, and when we have that set up the screen will turn red when your time is up and it will turn yellow when you have two minutes to go.

We have, as I mentioned, Jeff Kushan has consulted with just -- or Mike O'Neil, at least one of the two of them, with all of the witnesses and we know that some of them have asked for additional time to address particular issues where they represented more than one group or had more than one set of issues and we have tried to accommodate those requests. And, as we go through the hearing, if you feel there are problems, that you need additional time, you can try to contact Jeff or get a message into Mike O'Neil who is back here and we'll try to see if we can provide the time that everyone needs, but keeping in mind that we have 57 people.

Has Congressman Filner arrived yet? If he has not, then we will recess for him, but I do see that our distinguished representative, Mr. Rohrabacher, has come all the way down. Got up early in the morning, I presume, and drove down here from Orange County, a neighboring county to San Diego, and so we've had lots of dialogue with Congressman Rohrabacher over the last few months. We have a few disagreements but I know that we share a common goal and that is a dynamite intellectual property system that's second to none in the world. So I'd like to start out -- our protocol in Washington is that we let the members of Congress go first so I'd like to start out with Congressman Rohrabacher and invite him to be our first witness.

CONGRESSMAN DANA ROHRABACHER-45TH DISTRICT CALIFORNIA

CONGRESSMAN ROHRABACHER: Thank you very much, and I appreciate the fact that we do have disagreements and that I am given this forum to express my opinions on the things that are happening in terms of biotech but also in terms as it relates to the overall patent situation. I will try to keep my remarks to ten minutes, and I appreciate that, and I will refer to you as Mr. Chairman whereas you are the chairman today of this hearing.

Mr. Chairman, America is in the forefront of the biotech revolution and that we can all be proud of. Biotech is an American creation financed by private American capital and brought to market by Americans. The German government tried to develop the biotech industry but it failed, turning instead to American technology. Given the German experience and the similar experiences in the rest of Europe and in Japan, government subsidization of industry start-ups has certainly had a dubious success and I would say it's cast a pall on that concept. But our foreign competitors in the biotech arena are not giving up, they are watching the American biotech industry and are waiting for the technology and markets to fully develop and then the copying will begin.

Our patent system acts as a strong shield protecting America's innovators from this theft, thus maintaining the incentive for the investment of future venture capital in R&D. So it should be no surprise to any of those here today that our competitors are working at this moment to weaken our patent system. The evidence of a wide-ranging and heretofore low-key assault on patent rights -- on the patent rights -- enjoyed by Americans is now beginning to surface and that is why I am here today.

The Clinton administration, the Japanese and multinational interests are the aggressors and they are justifying their aggression by waving a banner of patent harmonization. If this is successful, U.S. patent holders or technology creators will be robbed of billions of dollars in royalties by those who will use their technology; people such as huge foreign corporations who will be let off the hook for the licensing revenue they would otherwise owe to Americans under current law. This, Mr. Chairman, is what I consider to be a crime in progress. That is why I am so upset and that's why I have been so vocal on this issue. If the perpetrators are not stopped, American technology will be stolen and used against us just as the incentives for future investment in technology creation evaporates.

The growth in our biotech industry, in particular, would be severely stunted. This insidious reduction of patent rights is being, as we have discussed before, Mr. Chairman, done under the cover of GATT, the General Agreement on Tariffs and Trade. The questionable intent of this administration is apparent when one considers that the most egregious threat to the patent term -- and there's the change that is being proposed by this administration -- is not, and I repeat -- is not -- mandated by the Uruguay round agreements.

According to Article 33 of GATT, a patent must have a minimum -- the operative phrase "minimum" -- of 20- years protection from the filing date. Therefore, we would be in full compliance with GATT by changing the patent term to 17 years from grant or 20 years from filing, whichever is longer. Our historic patent term of 17 years from grant has

guaranteed U.S. investors and inventors a fixed period of protection regardless of delays in the issuing of the patent.

Conversely, starting the patent clock running at filing leaves little incentive for the Patent Office to be diligent in issuing the patents and being diligent in the reforms that you have outlined today, seeing that they are implemented. Any delays will detract from the patent life and, therefore, its economic value and, therefore, lessen the incentive. For example, a biotechnology patent on average takes 10 years to issue. Under the present system the inventor still receives 17 years worth of protection; however, under the proposed change, with the term measured from filing date, the inventor would only get 10 years worth of protection.

The U.S. patent commissioner, yourself Mr. Lehman, suggests that 20 years from filing that date term will actually give inventors a longer period of protection. That's something you've said over and over again, and I'm here today to testify that I believe that is a falsehood and every inventor's group in this country recognizes it as a falsehood. If what is being said is true, the administration should have no problem whatsoever, and should have had no problem, with the compromise that we proposed. Those of us who were vocal in Congress, we proposed a 20 year from filing or 17 years from grant, whichever is longer. That compromise would have guaranteed a minimum of 17 years of patent protection and be fully compliant with GATT. This administration rejected that language because I believe the intent of this administration is to cut the time of patent protection, not to improve the system.

I believed, Mr. Lehman, that the -- that it is a misleading statement also to say that the patent pendency average is 19 months, which is something else that you've stated. The 19 month figure treats totally inconsequential matters, like the stripe on the bottom of a toothpaste tube, to be on par with world-changing inventions like the ones we are seeing in biotech and other such inventions. This figure also treats incremental and revolutionary patents on an equal footing. The claim that a patent term of 20 years from filing would produce longer patent terms is, I believe, disingenuous at best. I think the American people deserve to be leveled with.

Under this change that is being proposed, valuable patents whose revolutionary products keep America competitive will receive shorter terms and less protection. It will mean billions of dollars in royalties that should be going to American inventors will now be in the bank accounts of huge Japanese companies and multinational corporations. It has been stated that the U.S. must change to the filing date term because of mischievous and avaricious inventors who distort the system with submarine patents. Well, that, reducing patent protection for 99.5 percent of all inventors who do not practice submarine patents in order

get at that one-half of one percent is ridiculous, but also we actually proposed a change -- those of us in Congress proposed a change that would have dealt with the submarine issue but, again -- and that was basically if the inventor delays the time of grant then it will be published with the -- the patent would be published immediately. But this was also rejected what again demonstrates that the purpose of this administration is to reduce the time of patent protection for American inventors, not make the system better.

And if anyone has any doubts as to who officials are looking out for, there is an agreement that was signed with a Japanese patent office on August 16th which basically will require a change in U. S. patent law so that all of our patent applications will be published after 18 months. Yes, you've got it, this is premature publication mandated by an agreement by our own government. This blanket publication provision will open U.S. patents to scrutiny to foreign competitors in most instances even before the patent is issued and our technology protected; it will invite foreign competition to steal American technology and to use it against us when our own corporations have exhausted the revenue in the development of that technology.

In Japan, which has both a 20 year from filing term and 18-month publication, the competition can use the published patent application to practice patent flooding, and that is basically surrounding the original invention with patents of their own with unfair cross-licensing. Basically, premature publication would also allow the competition to challenge the issuance of American patents. Patent applicants should only be published -- it should only be published after -- after -- a patent is issued. The U.S. patent system has served our country well. This is no time to delude its protections.

Let me say in summary, American biotech companies are at this moment in a very dangerous position. They need large capital infusions which will not and cannot come from government. So far the private sector has financed tens of billions of dollars worth of research and development. Tens of billions of dollars more will be needed to protect and perfect this technology, but the private sector will not fund this great scientific advance if their work can be copied and if royalties are diminished by a weakened patent system. Trying to muffle the cries of pain from industry, the administration has offered a small, small compromise of relief, and I would say that is a scrap of relief based on a horrible proposal. This scrap of relief will provide a windfall to lawyers but will not protect this industry from its foreign competition, and it's sad to see that the industry has knuckled under to this intimidation. And my recommendation, in closing, to the biotech industry is that they should fight this because it's not good for your industry and it's not good for America. We've got to stay in the technological forefront and this change that's being done in a very disingenuous way

will reduce the patent protection and hurt all Americans and American competitiveness in the future. Thank you very much.

COMMISSIONER LEHMAN: Mr. Rohrabacher, could I ask you just one question?

CONGRESSMAN ROHRABACHER: Go right ahead.

COMMISSIONER LEHMAN: You know, you referred to the Japanese agreement which was basically designed to open up the Japanese patent system so it would be more effective for U.S. applicants and obviously you disagree with some of the modifications that are proposed in U.S. laws as part of that agreement. What would be your recommendation as to how to get effective patent protection in Japan?

CONGRESSMAN ROHRABACHER: Well, it certainly wouldn't be to try to harmonize our patent system with what the Japanese do, which seems to be the Japanese have a very -- I know, you know, they are very notorious for their ability to negotiate but the last thing we should be doing is trying to harmonize our patent system by basically making our system more like theirs. Our system is based on individual creativity; it's based on our culture. The Japanese don't have that same cultural appreciation for the individual. And, in terms of publication, the specific question that you're asking, I don't think that should have been a compromise at all. We should never have reached that agreement with the Japanese.

COMMISSIONER LEHMAN: So your answer is that you have no recommendation for how you would open up --

CONGRESSMAN ROHRABACHER: We have a market -- We have a market in the United States that the Japanese depend on in order to have any type of a thriving economy. The economic well being of their people is totally tied to our market. Now, if we can't use this incredible leverage that we have in order to prevent the Japanese from forcing publication of our inventors' inventions so they can steal them, well, then, we -- whoever is handling the negotiations on our side is incompetent, because it's not just a matter of an issue of patent negotiations, this is a matter of relationships between the United States and Japan. And, in that relationship, we have tremendous leverage which should have been brought to bear so we wouldn't have had to give up this sacred right which I consider the property rights of inventors to be on the same par as the property rights of homeowners, of farmers, and the rights of freedom of speech and every other right that we hold dear in the United States.

COMMISSIONER LEHMAN: Thank you very much.

I think our monitor is set up now that I described so we'll -- you're bringing it up now? -- get it set up and then Mr. Widder can come

forward. If Ken Widder could come forward, please. And I think what we'll do is we'll do you manually and then we'll have the monitor for everybody else.

KEN WIDDER, MOLECULAR BIOSYSTEMS, Inc.

MR. WIDDER: I'm Ken Widder, Chairman and Chief Executive Officer, Molecular Biosystems, a biomedical company here in San Diego. I appreciate the opportunity speaking with you and also I wish to thank you for coming to San Diego. We're honored to have you here.

Aside from being chairman and CEO of MBI, I'm also chairman of the San Diego Technology Council, a member of the Governor's Advisory Council on Biotechnology here in California, and president of the San Diego Biomedical Industry Council, a consortium of CEOs -- local CEOs -- of 65 of the local biotech companies. I'm also representing BIO here for the purposes of this hearing.

For your information the biotech industry here in California represents over 50-percent of the companies total in the country and here in San Diego we have about 120 biotech companies spanning pharmaceutical development, device development, and agriculture. San Diego itself is really the fourth largest conglomeration, if you will of biotech companies in the country.

From our standpoint in the biotech are, the work of the Patent and Trademark Office is absolutely critical to the biotechnology industry. Without strong intellectual property laws we, as an industry, would cease to exist. I think it's safe to say that patents represent almost a cornerstone of our industry.

Mark Twain called inventors "the creators of the world after God." That may have been stretching it a little bit but the bottom line is that the technology laws that govern this country are critical to us as an industry to raise money. Without it, we would not be able to raise one dime.

A little bit about my company just to give you a sense of where I'm coming from. Our company started in 1980. We originally were involved with the development Antisense and DNA probes. Both of those patents, incidentally, took about 10 years to issue and still are in some form of prosecution. So we have had some excellent experience in the Patent Office with patents issuing very early or much sooner, but some key, very generic patents that we were involved did take a very long time to get through the system.

We are now are involved, though, since about 1986, in developing contrast imaging agents for ultrasound, MR, and CT. Our first product

has just been approved by the FDA which has been heavily patented, without which we would never have been able to raise the money it would have taken to get through the FDA process.

Throughout your history we've raise a little over \$150 million. That's since 1980, and that's really been based on two factors:

One, the quality of our technology and, secondly, the strength of our patent positions. And virtually no money would have been raised if the latter hadn't been in place because the first question anybody asks is: "What patents do you have and what protections do you have?"

Biotechnology companies, in general, are constantly raising money. That's an activity as a CEO that you are constantly involved in. You never rest because there's never enough money. One could look at biotech companies virtually as money machines, we just constantly are consuming it. Very few companies are profitable. Only a handful are really making significant revenues. In fact, I think someone was asked what's the definition of a -- "What is a biotech company?" and the answer was that a biotech company is very similar to a pharmaceutical company but unincumbered by revenues. And I think that's a fair analogy of what the situation is even today. It's a very competitive field. It burns a lot of money to get product through the pipeline, roughly \$100 million to \$150 million depending on the type of product you have, and it's a long time coming through.

Not only do we have to wait for patents to issue in the length of time as the Congressman described, but, additionally, the extra burden of FDA approval can take anywhere from five to eight years so I think there will be a number of comments about GATT and the 20-year proposal. I won't get into that but I just ask you to consider the fact that it's not just the patent review time that's of question, it's also the development time for a product and the FDA approval time that a company could easily burn up a significant amount at 20 years just getting the product out and maybe have two or three years of patent exclusivity at the back end.

COMMISSIONER LEHMAN: Can I ask a question?

MR. WIDDER: Yes.

COMMISSIONER LEHMAN: Do you favor a revision of the patent term extension legislation that was enacted I think in 1984?

MR. WIDDER: I think that's very helpful. I know it's a very complicated formula in terms of -- getting an additional patent extension is based on a variety of factors, and I'm sure there will be some discussion of that later and I think that's very --

COMMISSIONER LEHMAN: If we just did away with the formula and said however many years of FDA delay there is you get --

MR. WIDDER: You would add on?

COMMISSIONER LEHMAN: -- an extension of patent term?

MR. WIDDER: Well, we're just in the process of extending one patent and I think we have -- you know, we have gone back and utilized that formula to try to figure out which patent would be the best to extend, so I'm not familiar with if the laws have changed on that.

COMMISSIONER LEHMAN: Well, the laws haven't changed but what I'm saying is if that's a problem, if what you're saying is that FDA approval is a serious problem so that in any event you end up losing patent term, then, you know, shouldn't we change that law?

MR. WIDDER: I guess that's one reasonable suggestion, yes, because -- I think some of the other CEOs will discuss this, but it is a long, long process and we are constantly looking at -- I mean, you're penalized basically for having a patent issue early. If you start from the creative process, go through the product development cycle into clinical trials and then --

COMMISSIONER LEHMAN: I understand that, and the difficulty is that, generally speaking, in most technologies you want the patent to issue early because most technologies, if you're in the electronic business, for example, which is a very rapidly moving business you -- as a matter of public policy I think you want to encourage the innovator to get in there, make the innovation quickly and get into the marketplace quickly, compete, because you've got global competition, and you're in an unusual circumstance so --

MR. WIDDER: That's correct. We are in a different --

COMMISSIONER LEHMAN: So probably the best way to deal with that, since you have the problems of regulatory delay, is to -- and which Congress has already recognized -- is to go back and look at the solution that they have devised for that and make that work better.

MR. WIDDER: I would endorse that. I think extending -- instead of some formula over a percentage of the time that the product is being put through the FDA, I think giving full credit for that would certainly be a benefit. I think -- How am I doing on time here? Yellow. One minute, okay.

I'd just like to say two more things: One, I think the retention of patent examiners is a very important item, from our perspective, having people there that have some perspective on the other types of patents

that are going through that have a decreasing turnover I think is critical from our point of view. And one other item: We found it very useful and I would certainly endorse to continue the policy of the interview process. I think it's been very helpful when very tricky patents or ones that are tough to describe in writing, to be able to go in and interview has been a very positive experience for us and would hope that that process would continue within certain bounds so the examiners aren't overburdened, but it really has been a very good type of activity for us. And we're committed as an industry to work with the Patent Office and try to make recommendations that hopefully will streamline the process. With that, I think I'll stop and turn it over to Bill Rastetter.

COMMISSIONER LEHMAN: Thank you very much. I'd just like to say that retention of patent examiners is very important. It's extremely important that we have the resources that we need to do our job in the Patent Office that has been provided for us in legislation and that we are fully fee-funded agency. However, Congressman Rohrabacher and other critics of the system have voted consistently to take fee money from the Patent and Trademark Office and divert it and use it as tax revenues. This year some \$30 million is being diverted, so you might want to bring that to their attention and others.

MR. WIDDER: I appreciate that, yeah. I think we'd clearly like to keep the money in the Patent Office where it can do the most good.

COMMISSIONER LEHMAN: Thank you.

Mr. Rastetter?

WILLIAM RASTETTER, IDEC PHARMACEUTICALS

MR. RASTETTER: Mr. Lehman and colleagues, thank you for coming to San Diego this morning. My name is Bill Rastetter. I'm the president and chief executive officer of IDEC Pharmaceuticals. I also serve on the board of directors of two trade groups in town: BIC, the Biomedical Industrial Council, and BIOCOM, the Biocommerce Association. I also serve on the Governor's Council on Biotechnology. I should also mention that IDEC is a member of BIO and the opinions I'll express today are mine and those of IDEC Pharmaceuticals.

We are one of about 150 biotech or biomed companies here in San Diego which collectively employ about 14,000 individuals. The biomedical community here in San Diego is certainly a very active and cohesive one and we work very closely with the city. The city, in fact, has embraced us as part of its economic future and I think, in no small part, the issues which we will discuss today will certainly impact our companies' ability to timely secure and build patent portfolios which will

ultimately drive the growth of our companies. And that growth, I think it's important to point out, will entail a transition from our status as R&D organizations which, as Doctor Widder pointed out, consume copious amounts of capital to companies that are driven by cash flow from product sales. And certainly that transition will ultimately truly drive economic growth in this region, so patents are a critical part of our future.

I'd like to place the comments I'll make this morning in the larger context of strangely enough health care reform. It has been said recently by many that health care reform is dead, and I'd like to point out that nothing could be further from the truth. In fact, health care reform is occurring and is occurring rapidly through private sector market forces without legislation and without direct government intervention. And certainly those forces are changing the way that every company or every practitioner in health care does business.

And to continue to place my comments in context, the largest pharmaceutical companies seem to be gaining ground on the mid-sized companies. I would not include the biotech companies in the mid-size -- we're certainly much smaller than that -- companies like Merck and SmithKline who have moved aggressively to acquire pharmacy benefit managers as their paradigm for doing business changes. The analogy might be that the hardware makers (read the drug developers) will now also be in the business of software (read pharmacy benefits management and outcomes research.) In doing so, they will optimize the way their drugs are used and they will capture further economic benefit.

But I think they're doing this because the pipelines of the large pharmaceutical companies are not as full as they might be and revenue growth is eroding with blockbuster drugs coming off patent, and with generic substitution, and the broad pricing pressures that we're seeing from the private-sector-driven health care reform. Well, to finish that context, all of this is actually good news for the biotech company if we can continue to innovate, and provided that we can timely secure and build intellectual property positions.

Now, the revenue erosion and pressure on the bottom line for large pharma is leading to internal R&D budget cutbacks at large pharma, if you will. So they will be looking increasingly to small companies, such as IDEC or Molecular BioSystems for late-stage, development products where the risks, both technical or clinical risks and the intellectual property risk, has been largely removed from the product development process. So, small may be beautiful, speaking of companies, provided we can continue to discover and efficiently develop protectable, proprietary products.

The small company in the era of health care reform may well emerge as the predominant engine of innovation in partnership with large companies.

The large companies will consolidate their power as the worldwide marketing partners. Clearly, patent protection is paramount to the health of such partnerships.

We have all heard that the era of health care reform has damaged the ability to raise capital from the public markets for biotech. It is, indeed, impossible today for small companies to rely routinely on the public equity markets as a source of capital. We look increasingly then to a large pharmaceutical company to fund research and development and to sustain us as viable R&D organizations until our products are launched and royalties and/or profit sharing arrangements begin to pay the bills. And that may take 10 to 12 years after a company hires its first scientist to reach that position of cash flow sufficiency.

Let me make two points from the perspective of a small company:

First, the trend at the Patent Office towards requiring human clinical data to demonstrate utility hurts the small company. Generally, we need to find corporate partners in order to fund clinical development because clinical development is extraordinarily expensive. But without clear patent position it can be hard to get a partner. Catch 22.

The second point, the "first to invent" system in the U.S. certainly helps the small company. I am certainly glad we have not changed this element of our patent system. We, as small companies, just cannot file patent applications as efficiently or as early as the large companies. We just don't have the resources. We need to spend as much as we can on discovery and on development of product leads. A "first-to-file" system would divert dollars away from R&D, curtail innovation, and weaken the small company. You know as well as I do that a "first-to-file" system, rather than the "first-to-invent" would also burden the Patent Office with premature applications on incompletely conceived inventions.

We had the pleasure of hosting a group of patent examiners at IDEC to talk about our technology. We talked about our products which are antibodies to treat cancer and autoimmune disease. We talked about our innovative, we hope, proprietary system for making antibodies in Macaque monkeys for treatment of chronic diseases. We talked about our host/vectors' system. It was a very productive interaction with the examiners.

We also would urge that the system of interviews with the Patent Office be continued. We think it's mutually beneficial, and especially for very complex systems tends to expedite the process.

I see that my time has expired.

COMMISSIONER LEHMAN: I would like to ask a lot of questions. I'll try

to be brief. You know, we're setting up in Sunnyvale a -- it's really inexpensive to do this when you think of the benefits. We're setting up a video conferencing facility so that people can go there, you know, up in Silicon Valley, and interview. You know, conduct face-to-face interviews with the examiners. Would something like that be helpful down here in San Diego?

MR. RASTETTER: Yes. Absolutely. We at the company have used that quite extensively. We had two locations: One in the Bay area and one down here and were connected by 24-hour -- just walk into a conference room and your colleagues 500 miles away were there. Very, very useful system.

COMMISSIONER LEHMAN: Also, we have included in the legislation that is now pending in Congress, it's part of the 20-year fast-track legislation, but it's a provision for provisional applications so that -- which you can file for \$75; it's very inexpensive, and then have a full year for the full application. I assume that will help you when you say that, you know, it's hard for you to get into the system. At least that will put your marker down and help you out a lot.

MR. RASTETTER: Certainly. The first I've heard of this but it sounds --

COMMISSIONER LEHMAN: Well, we have done that. I just also would like to say that changing our system from a "first to invent" to a "first to file" system, the Bush administration had proposed to do exactly that, along with all of the other horrors that Congressman Rohrabacher outlined. So I would just point out this is not exactly a Clinton administration plot. In fact, we pulled back from those efforts because we're not going to -- we're trying to develop a world patent system that will favor U.S. inventors. That is our primary purpose, and that means your company, too. We really appreciate your input here and thank you very much.

MR. RASTETTER: Thank you for coming today.

COMMISSIONER LEHMAN: Next I'd like to ask Jerry Caulder to come forward from Mycogen Corporation.

Oh, he is here. I'm sorry -- Mr. Filner, we have 57 witnesses so we wanted to get started right away but we'd be happy to have you welcome us right now to San Diego.

CONGRESSMAN ROBERT FILNER - 50TH DISTRICT CALIFORNIA

CONGRESSMAN FILNER: Thank you very much. I apologize for being late. My name is Bob Filner and I represent the 50th Congressional District in

the United States Congress, and we certainly do welcome you here in San Diego today. It's important. It's timely. Certainly the issues around the intellectual property protection apported to biotechnology inventions is one that's critical to San Diego. Not only San Diego, to California and the nation and we are very concerned, obviously about the local development of our biotech industry. It's a growing source of investment and employment, and with its solid base of academic institutions, non-profit research institutions, and innovative companies San Diego is surely on the cutting edge of this whole movement.

You all know the policy issues involved. I would like to take a few minutes, if it's appropriate, to talk about some of the intellectual property issues, the way I see it as a member of Congress. I'll try to do it very, very briefly. It's certainly well known but I want to make sure that everybody knows that the Congress also knows it; that valid and enforceable patent rights are a prerequisite for success in this industry.

I think it's essential that the Patent and Trademark Office be provided the funding and resources it needs to properly examine patent applications and I support full appropriation of fees collected by the PTO and oppose measures that would diminish the PTOs ability to handle the volume of application that it presently receives. In particular, I opposed the version of withholding appropriations of fees collected from patent applicants for any purpose other than PTO operations.

I think you'll hear from the industry its serious objections to the practice of PTO examiners and others regarding standards set for determination of the utility of an invention. These objections undermine the competitiveness of the biotech industry and it will make it much more difficult to become profitable. The patent protection afforded in the United States is in many cases less than that afforded to the same inventions abroad and we cannot put ourselves at a competitive disadvantage in this highly competitive international marketplace.

The issue is not about the patent laws. What the industry needs is a non-discriminatory application of those laws to biotech inventions. And I urge the PTO to review those issues carefully and forcefully instruct its staff to apply a standard for biotech that is consistent with the standard applied to other industries.

I think you're well aware of the Biotechnology Protection Act that the industry has championed, HR-760 in the House. Those bills did not go through the present session. I hope that you will continue your support of those. They are absolutely necessary for competitiveness.

With regard to international trade. I have concerns, like many in this room, with the 20-year patent term requirement in the GATT-implementing

legislation. I urge the PTO to look very carefully at this issue to ensure that the 20-year term does not apply to any biotechnology patent applications pending at the PTO and that biotech firms do not lose patent term with the shift of the 20-year term. And I think implementation of the PTO of the recommendations on the utility and other issues that you will hear about will go a long way to ensure that the patent term is not in fact lost.

Those are the issues that I have -- at least want to highlight for you. Obviously, the PTO can play a major role in providing the protection in which this industry is entitled to under current patent laws. We really welcome your visit here to San Diego. We thank you for your openness to the initiatives that will be talked about today. Thank you so much for getting me on.

COMMISSIONER LEHMAN: Thank you very much, Congressman Filner, and I really appreciate your looking into these issues and we will work closely with you to make certain that the interests of the biotechnology industry are taken care of in the Patent and Trademark Office in all the policy decision. And anyone that represents a constituency where there are 14,000 jobs in biotech certainly has a strong incentive to work with us and I'm really looking forward to having a friend in Congress next year.

CONGRESSMAN FILNER: Thank you so much.

COMMISSIONER LEHMAN: Thank you.

Next, Jerry Caulder.

JERRY CAULDER, MYCOGEN CORPORATION

MR. CAULDER: I assume since I've been introduced I get twice as much time; right? Wrong.

Good morning. I'm Jerry Caulder, Chairman and President and CEO, of Mycogen Corporation. We certainly appreciate this opportunity, as Bill and Ken, to have you come to California and let us talk to you about what we're trying to do in this industry to turn our products into jobs.

By way of background, I grew up on a cotton farm in Missouri and I've been involved in agriculture all my life, and we're the only agriculture biotechnology firm here in California. I also served as chairman of bio trade organization, Industrial Biotechnology, for three years, so I worked early on in getting the Patent Office and the trade association working together about eight or nine years ago in the problems that we were having. So this is certainly not new to me and I certainly appreciate the opportunity to see that we're continuing to do this. I know I worked with Charles Van Horn quite a bit when Don Peterson was there.

I've been CEO of Mycogen since 1984. That's almost as long as some of our patents pending, so I do know that we have some and this 19-month thing is kind of interesting to me. We're a diversified agriculture company. We're basically in the business of developing alternatives to chemical pesticides using recombinant DNA and using biotechnology.

We're building our business around these genetically engineer microbes so that we can have substitutes for chemical pesticides, and we're commercializing these things through two basic delivery systems that we consider breakthroughs: One is Mycogen's proprietary cell cap system which is a delivery system that allows us to efficiently produce these insecticidal proteins that we then put in microbes and then we put these on plants to keep the plants from being harmed by particular insects that are present there.

We are the first company to receive EPA registration for products that are genetically engineered as alternatives to chemical pesticides. And I would say that the question that you asked Doctor Widder about the length -- it is not just the FDA, we also have delays in EPA, and USDA. So, I would suggest to you and submit to you that anything that -- and we don't want to delay the patent situation, we want to speed up the regulatory situation, so don't confuse those two. We would certainly like to get our patents as quickly as possible because it does allow us to establish our property rights. And I would suggest that the patent starts when you get regulations, if you're delayed in any of the regulatory arenas. Because this does put us behind the eight ball when you get a patent and then you spend 10 more years getting it through the EPA, so you've used up most of your patent life.

I think the spirit is you have 17 years of monopoly for marketing your product so I think the regulatory delay was totally unanticipated.

COMMISSIONER LEHMAN: I should point out that's one of the weakness of the existing patent term extension legislation. It's called "the drug price competition," a patent term extension legislation and it doesn't deal with EPA delay, and this might be something that we would want to look into to see that we deal with that problem as well.

MR. CAULDER: Well, agriculture, as you know, is the Rodney Dangerfield of biotech. Drugs get all of the play and we are not unincumbered by revenues. We are making money. We are one of the profitable companies so we feel that agriculture should be specifically addressed because our needs are different.

COMMISSIONER LEHMAN: Having grown up in Wisconsin, I'm very sensitive to agriculture.

MR. CAULDER: Good. Good.

The second thing we're doing is transforming plants, particularly cotton and corn. We're putting genes in these plants that protect them against insects and we're using these as alternatives. Two weeks ago we filed for an experimental use permit that would allow us to test our first genetically engineered corn plant that's resistant to European corn bore, and we hope to receive that registration pretty sure so we can get into field tests. We do not have our patents covering that yet.

We spent over 12 years and tens of millions of dollars developing our technology and we recognize that the Patent Office is faced with an unprecedented number of broad, fundamental biotechnology patent applications representing inventions that we think have truly opened up many new fields in agriculture, pharmaceuticals, medical devices, and other industries. These inventions and their related patent applications represent pioneering, next generation technology. They are not incremental improvements to old technology but rather whole new fields.

Many of these patent applications have been under examination for five, ten, even more years. We ask they question, why is it taking so long? I think there are fundamentally two reasons: One is a reluctance now to grant broad applications in this area. This is influenced primarily by the fact that the fundamental nature of many of the biotechnology inventions are being evaluated in hindsight. You mentioned you have a lot of new patent examiners with advanced degrees. Many of them were still in high school when we applied for some of these patents and we are very biased by this area in which we live. So the things that we are doing today may look obvious but 10, 12 years ago they were not quite as obvious. Some of the things it took a post-doc to do 10 years ago high school kids can do today.

Second, the Patent Office is not consistent in applying the fundamental standard of patentability. Patent law requires that to have a patentable invention you need to satisfy two things:

One, the claimed discovery must be unobvious to a person of ordinary skills, and I emphasize "ordinary skills," in the art. In other words, the discovery can't be easy and it can't be obvious to figure it out.

The second is the description of the invention in the patent must be sufficiently detailed to teach a person of ordinary skills the claims of the invention. This is where we think a problem comes in. On the front end of the invention, of determining whether the invention was easy or obvious to figure out by a person of ordinary skills, that mythical person of ordinary skills is presumed to be damn near a genius. On the back end, however, once we have convinced you after four or five years that there is truly an invention, this person is viewed as nearly

incompetent. So, in order to get a biotechnology breakthrough recognized as an invention, you have to have a real stroke of genius, argue the point for five or ten years, after you have convinced the examiner that you have an invention the scope of your claimed invention is then narrowed because some mythical person of ordinary skill is not viewed as being competent to apply it, this breakthrough, across the board.

The benefits of the true pioneering nature of these biotechnology inventions are being lost by the companies, and these are the small companies, not the large ones, I think, and I'm biased toward that, who are responsible for these inventions. The patent process is biased against us. Consistency by the patent office in applying this standard of ordinary skill I think is the single most important element to patenting biotechnology innovations and inventions. Consistency on this issue would help the ability of our industry to attract the financial and people resources that you've heard both Ken and Bill talk about earlier.

Thank you very much.

COMMISSIONER LEHMAN: I take it that the problem here, from your point of view, is that you get some -- when you say "consistency" is really the issue, you get some examiners who have a unrealistic view of what is ordinary skill in the art, and so it's a little bit Russian roulette, is that the problem?

MR. CAULDER: It comes from this hindsight thing. If you have someone who has recently gotten a Ph.D or a postdoc in biotech, or a master's degree, they view biotech -- and they're 22, 23 years old, maybe 25, they view it very differently than the pioneering people who made the invention in 1980.

COMMISSIONER LEHMAN: I see.

MR. CAULDER: So to get them to look at the context of the invention as it existed in 1980, rather than 1994, is a big problem.

COMMISSIONER LEHMAN: I understand that. And you find that with the younger examiners who come right out of --

MR. CAULDER: Well, I don't want to categorize it. My personal opinion is all of us have that problem, that when you are totally immersed in something you tend to judge it the way it is today rather than the way it was submitted to you 12 years ago.

COMMISSIONER LEHMAN: Okay. Thank you very much.

MR. CAULDER: Thank you.

COMMISSIONER LEHMAN: Our next witness is Dave Gollaher from California Health Care Institute.

DAVE GOLLAHER, CALIFORNIA HEALTH CARE INSTITUTE

MR. GOLLAHER: Thank you, Commissioner Lehman, for presenting us with this opportunity to give you the perspective of our industry. What I'd like to do this morning is open up the lens and talk about the perspective of California as a whole when it comes to biotechnology and what we see as the broader collection of health care technology industries that are becoming so important to the state's economy.

I'm David Gollaher. I'm the director of the California Health Care Institute which is an organization of some 80 research universities, private research institutes, like Scripps, and La Jolla Cancer, biotechnology companies like many of the ones that will testify today, as well as pharmaceutical and medical device firms who have a significant stake in the State of California.

The State of California is interesting economically in the sense that if California were a separate nation it would be the sixth largest economy in the world. It would be the seventh largest trade export economy in the world, so its magnitude makes it virtually a nation state, and yet the interest of the emerging biotechnology and health care technology companies in California have, it seems to many of us, received less attention than their economic scale merits.

What I would like to do is briefly review a couple of statistics from a report that we're in the process of preparing to be released next month on the current state of the industry with respect to jobs, employment growth and it's overall scope and scale in the California economy.

To begin with, in 1993 alone California companies in the broader area of biotechnology invested more than \$2 billion seeking solutions to diseases like AIDS, cancer, heart disease, genetic disorders like cystic fibrosis, and multiple sclerosis, Alzheimer's, et cetera.

By and large, biotech -- let me just skip ahead because I realize in summarizing this report that I'm not going to be able to make my time. I'd like to start with our estimate of current jobs in California this year. We have estimated in our report that approximately 111,000 Californians are directly employed by organizations developing diagnostics and therapeutics using recombinant DNA technology as well as other technologies that are largely labeled biotech.

In addition, there are 33,000 Californians who work in health care research in the life sciences at major universities and federal facilities within the state. In fact, the University of California alone

accounts for nearly 22,000 jobs in biomedical research and an additional 8,500 people are employed at the California Institute of Technology, Stanford, and the University of Southern California.

If you look at direct employment in the health care technology industry in the state, you see that already in 1994 health care technology jobs -- 111,000 jobs -- place the industry ahead of aerospace, ahead of computers, and not far behind electronic components. In other words, this industry which is growing quickly will soon overtake aircraft with its 150,000 jobs as a major backbone of the California economy.

What makes the industry all the more attractive is its annual average salaries. Currently, pharmaceutical manufacturing and biotechnology salaries in the State of California average over \$43,000 a year which puts them far in advance of average manufacturing salaries at \$33,500 a year in the State of California. Because it's based on information and knowledge, biotechnology and health care technology is exactly the kind of industry that the administration has pointed to as an industry of the future; high tech, high growth, information-based jobs.

Manufacturing employment for pharmaceuticals and medical devices is up 45-percent since 1984, up 45-percent, in other words, during the past decade with direct employment in those sectors at 70,000 plus jobs. In fact, by itself the manufacturing segment of the health care technology industry will likely employ more than 100,000 Californians by the turn of the century.

If we look not only at the direct jobs, though, but look in the spinoff jobs, the spinoff technology that sort of ripples around the center in areas like construction, we can see that the health care technology industry accounted for \$2.4 billion in new construction in the period from 1987 through 1992, and over the next four years that amount is expected to increase to \$3.7 billion. Again, this is in marked contrast to California's overall construction picture which has reflected the downturn in the aerospace and defense-based industries that sustained California after World War II.

In terms of revenue growth, revenues for the 142 largest California health care technology companies increased by nearly \$2 billion to \$13 billion for 1993 despite a general decline for many high technology industries. Much of the gain can be attributed to new products in the biopharmaceutical sector. In fact, 47 biopharmaceutical companies reported revenues of \$3.57 billion in 1993. This is up almost \$800 million, even though we've read many reports that the biotech sector is having trouble attracting new capital and has been having trouble meeting its cash flow needs. Nonetheless, even in the overall context of a down economy, it has managed not only to hold its own but to grow in California during the past year.

Finally, health care technology companies spend more of their revenues on research and development than virtually any other industry. So the industry is a very good investment with respect to its spinoff revenue. In fact, biotechnology spends three times more than any other high tech industry and five times as much as the U. S. average on R&D as a percentage of its revenues.

How am I doing on time? I have two minutes.

I guess I would like to close in saying that we had a board meeting a couple of days ago, a roundup, a biotech meeting that happens once a year in Laguna Niguel, and it was the strong consensus of our board of directors and most of the chief executive officers I talked to there to support the GATT treaty in its current form and to -- in fact, we wrote a letter to all members of the California delegation urging them to support it in the lame duck session which is coming up. Our attitude was that the treaty is far from perfect but that we should not let the perfect become the enemy of the good, and that the potential for biopharmaceutical companies to realize gains with the lowering of tariff barriers represented by GATT far outweighed some of the intellectual property and patent term concerns that many companies had. As Doctor Widder said, most biotech companies are not embarrassed by revenues but they all hope to be soon.

Thank you.

COMMISSIONER LEHMAN: Thank you very much. That was really helpful testimony and I'd really like to have a copy of those statistics that you gave us.

MR. GOLLAHER: We'll provide a copy of the report. It will be published within the next 30 days.

COMMISSIONER LEHMAN: Great. Because that really -- you know, in going around the country, Secretary of Commerce Ron Brown and myself and others have been making exactly those points that you've been making; that this is where the jobs are; this is where the growth in the American economy is and we really have to fine tune that economy to support everything that you're -- that industries like the ones you've described are doing. That's why we're here in San Diego, to listen and see what we can do to adjust public policy to give you all the support that you need. So thanks very much.

MR. GOLLAHER: Just a follow-up comment. We found in talking with members of the congressional delegation that over time there's been a kind of automatic response on the part of every member of the delegation to classical California industries -- defense, movies -- so that when

issues about those industries come up they're automatically interested. That hasn't been true up to now with biotechnology but it should be, and we're working hard to make that the case.

COMMISSIONER LEHMAN: Thank you very much.

Next I like to call on Bernie Rhinerson of the Biomedical Industry Council of San Diego.

BERNIE RHINERSON, BIOMEDICAL INDUSTRY COUNCIL

MR. RHINERSON: Thank you very much. I'm also going to give you a little bit of background about the industry here in San Diego. Much of what I was going to say has already been mentioned by previous speakers, so I can be brief.

I want to thank you for coming to San Diego on behalf of the Biomedical Industry Council. We're a local trade organization that's associated with BIO at the national level. We work closely with Mr. Gollaher and the California Health Care Institute, and other trade associations. We represent about 60 local San Diego biotechnology and medical device companies. We range in size from very small companies urgently in need of patent protection with employees of less than 20, somewhere 10 to 20 employees, very small start-up firms, to companies that have manufacturing operations and sales with 500 to 1,000 employees.

Biotechnology is very important to this local area, to San Diego. You've heard that mentioned. We have over 100 companies here and we estimate, as you heard earlier, about 14,000 employees, and we expect that that could grow to 30,000 to 40,000 employees here locally by the turn of the century.

Nationally, according to the Ernst and Young report, Biotech '94, San Diego ranks fourth in the nation as a concentration of publicly held biotechnology companies. In 1993 alone approximately \$223 million was spent in our local economy on research and development, and that was just by the publicly held companies alone. To that number you have to add tens of millions of dollars that were spent by companies that have not yet gone public that are working with venture capital, and moneys spent by our public and private research institutions.

San Diego is a hotbed for biotechnology research, specifically because we are graced with excellent research and university institutions in this area: The University of California at San Diego; UCSD; the Salk Institute; the Scripps Research Foundation; La Jolla Cancer Research, just to name a few. These institutions are the principal sources of the ideas that come out into commercial biotechnology ventures and become start-up companies here in San Diego and elsewhere in the nation.

Protecting those innovations as they move from those initial stages into the commercial ventures is very critical, and that's of vital interest to us here in San Diego. We're working very hard here at the local level to continue to help these companies grow and to take these ideas into research and into manufacturing. You've heard one of the concerns here about the waits that these companies experience with FDA approvals and that has been a concern that they're not penalized for that wait for patent protection. The Patent Term Registration Act is a partial remedy to that problem.

We're also working hard with local companies to help them to be in manufacturing here locally so that they don't have to move that manufacturing process offshore and into other companies; they have to compete in a global marketplace and it's very important that patent protections be applied to manufacturing processes also.

Here locally, over the last three years, many companies have begun to move into that manufacturing process. Several companies have built major manufacturing facilities here in San Diego: IDEC who you heard from earlier, Telios, Tenomi Research have all built facilities that represent millions of dollars of investment in research and manufacturing.

Basically I'd like to just end with those remarks and thank you for being here, and we appreciate your interest.

COMMISSIONER LEHMAN: Thank you very much, Mr. Rhinerson.

Next I'd like to call on Bill Otterson from the National Cancer Patients Association.

BILL OTTERSON, NATIONAL CANCER PATIENTS ASSOCIATION

MR. OTTERSON: Good morning and thank you very much. I am Bill Otterson and I'm a spokesperson for the National Association of Cancer Patients. We were asked by BIO to speak to you today from the standpoint of cancer patients, not from the companies but from the ultimate beneficiary of patents. I'm certainly not an expert in the arcane rules of the Patent Office but you might be surprised to learn that patients support patents. Perhaps we have a broader view of patents as benefitting us. Without patent protection, we cannot expect biotech companies and medical research institutions to put in the enormous amounts of money that they must do after the research is done at a SCSD or UCSF or whatever, so we do understand the need for patents.

I take alpha interferon and, as you all know, the alpha interferon -- I take it three times a week. I am a cancer patient myself. I've been taking it for three years. This is an outgrowth of the recombinant DNA

patent initially issued I believe to UCSF and to Stanford which then became the basis for Genetech's work in recombinant interferon that was then passed on to Hoffman-La Roche. My bottle was produced in Switzerland at Hoffman-La Roche.

What I would like to say is that these companies are permitted extended time to enjoy the benefits of their research, an extension for regulatory delays through the FDA, which I understand you are looking at, and perhaps extending to 20 years the time that the companies will have protection. You might say but that's going to cost you more as a cancer patient. I think we all understand that the free market -- you may give 20-years protection but just at this time other people are working very hard to develop alternative interferons and the free market will surely chase Hoffman-La Roche to produce better and faster interferons. Thank you very much.

COMMISSIONER LEHMAN: Thank you very much, Mr. Otterson. You're sort of speaking to the converted on this point about the importance of patent protection, but I think one of the things we will consider very seriously as a result of this hearing, assuming some of our other legislative reforms passes, is a revisitation of the patent term extension legislation. But I can tell you right now that there are going to be people on the other side that are going to say, no, you know, we need more generic drugs, and they're going to be representing themselves as being from consumers, just like you. So I think people just like you are a very important linchpin of this, and there undoubtedly will be a big fight over that. But already I think we're starting to hear that the existing drug price competition and patent term registration legislation is not adequate to deal with the problem so we'll be wanting to work with you and others as we move on studying that issue. Thanks.

MR. OTTERSON: I am available to testify whereas and if you would like me. Thank you.

COMMISSIONER LEHMAN: Thank you.

Next I like to ask Eugene P. Schonfeld from the National Kidney Cancer Association to come forward.

EUGENE P. SCHONFELD, NATIONAL KIDNEY CANCER ASSOCIATION

MR. SCHONFELD: By way of introduction, I am a cancer patient. I've also founded five high tech companies, although not in the biotech or health care area.

Patent policy in biotechnology is extremely relevant to the well-being of patients with cancer, Alzheimers, AIDS, and other life-threatening illnesses. Patent policy can speed scientific progress or retard it,

accelerate products to patients or delay cures. Therefore, as health care consumers, patients cannot be indifferent to the work of the Patent and Trademark Office. If these folks are your customers and users, I'm your shareholders; you work for me.

Sometimes we find people in government get confused about that, as to who they are supposed to be working for, so I just want to take a moment to remind you. When patent policy is either too restrictive or too lax, corporate managers and outside investors would be less willing to commit capital to research and to research intensive ventures. At the same time, if it's -- well, too restrictive or too lax, either way it's no good.

What we're looking for is a patent policy that maximizes the value of invention and stimulates the process of innovation.

The second thing I would like to recommend to you, from a patient's point of view, is that you should make all patents for life -- or patent applications for life saving inventions special and treat them as a special class of patents. The public has a unique and special interest in such inventions as opposed to inventions which are purely, shall we say, commercial. There is a significant precedent for doing this. During the Carter administration, at the time of the energy crisis, certain patents for energy conservation inventions were treated as special patent applications, and I think the public deserves no less today when it comes to life saving innovations. We must remember that moving these things to the public faster will help reduce our health care costs. President Clinton tapped into the latent discontent of many Americans when he proposed health care reform and he's expressed his concern about health care costs. I believe the Patent and Trademark Office can help address this issue by moving life saving inventions through the system faster.

The second thing I'd like to comment on is the whole question of practical utility and clinical trial data. It's very nice for a patent examiner to have before him extensive clinical trial data. Unfortunately, he usually doesn't have it. In fact, there is the whole question of whether clinical trial data should be the basis for approving a biotechnology patent.

In recent times, the FDA itself has moved away from using clinical trial information as the gold standard for FDA approval of drugs. In fact, the FDA in the case of AIDS has moved to surrogate markers, surrogate end points and other ways of looking at application for marketing drugs.

I believe the patent office itself needs to also think along similar lines as to what end points and markers might be an adequate substitute for clinical trial information. The reason it should do this is that clinical trials, as we know them today, are, frankly, becoming obsolete.

The National Kidney Cancer Association started a research project three years ago to try to develop technology which would allow physicians to better match specific therapy to a specific patient, rather than simply saying the average response rate for a drug is, you know, 20-percent. What you really care about is whether you are one of the 20 or one of the 80, if you're a patient.

What we need to develop is patient specific therapies and techniques and technologies which will allow us to move away from the time-worn clinical trial paradigm which only deals with average response rates. In doing these things, the Patent Office should accelerate the approval of patent applications for life saving inventions.

Let me sum up by saying that we really think of a number of different goals for the Patent and Trademark Office. First, the development of a patent policy which maximizes the value of patented inventions, a policy which is neither too restrictive nor too lax.

The second is to adopt a policy of making special all patent applications which involve life saving inventions and, in so doing, accelerate the patent process for these inventions.

Operationally, the Patent Office needs to beef up the corps of biotechnology examiners, particularly retain them. As I understand it, there's a significant employee retention problem within PTO in this area.

Also it needs to develop, as I say, a system of standards moving away from clinical trials, making clinical trial data less important in the approval process. It needs to develop surrogates perhaps to do this, and perhaps it needs to even reach outside and build an advisory board which would advise the Patent and Trademark Office on the use of surrogate end points and surrogate markers. There is I believe expertise in the outside world related to this and it may be beneficial if the examiners had an external group to go to in developing a comprehensive system of surrogate end points and markers. Thank you.

COMMISSIONER LEHMAN: Thank you very much, Mr. Schonfeld. I would like to just point out that we do actually at the present time have a system whereby biotech applicants can make their cases a special case. If they're a small business, which is still a pretty big sized business, it's up to I think \$5 million in revenue and 500 employees, just for a \$65 fee you can expedite your case. And we're finding that only one and a half of one percent of the applicants are doing this, even for big companies. It's only --

MR. SCHONFELD: Well, I should tell you something. As a manager, you know, I believe that the specialness of something should not be related

to the size of the company.

COMMISSIONER LEHMAN: I said for a big company it's \$135.

MR. SCHONFELD: I see.

COMMISSIONER LEHMAN: So, in other words, everybody, every patent applicant can make their case special. It's just we have a two-tiered fee system. You know, we have a -- for all the fees. It's half for a small company. In effect, the big companies help to subsidize the small companies, but for \$135, even if you're Hoffman-La Roche, you can get your application special.

MR. SCHONFELD: Can you tell us what the time length is for those patents to move through the system?

COMMISSIONER LEHMAN: Well, there --

MR. SCHONFELD: As I say, we judge by results, not effort.

COMMISSIONER LEHMAN: As I indicated, you know, the average is 20.7 months for all biotech patents, so presumably, if you've made yours special, it's even less. But, you know, I didn't want to have an argument about it. I just wanted to point out that we've already done that. But I would point out that you're absolutely on target in that we have to have the resources necessary to do this job, and that's why I mentioned -- everybody wants to criticize us and so on and so forth but there are things that we can do and will do as a result of this hearing.

Internally, we will make changes in our internal policies but there are some things that we cannot do without help or without permission from our board of directors, and, in effect, our board of directors is Congress. And you've already heard it said that \$30 million a year are being siphoned off of our fee revenues -- \$30 million a year that could go to providing better biotech patent examiners in this area.

In addition to that, we have had imposed upon us a reduction in the number of employees. We're one of the few growing businesses in America probably that's fully supported by revenues. Every time you file a patent applicant a check comes along -- a patent application -- a check comes right along with the patent application to sustain doing the business and yet we find that we have some arbitrary limitations being put on us in the number of examiners that we can hire simply because of government bureaucracy. So I think organizations like yourself need to work with us so that we can go to these outsiders that have some impact on us and -- to enable us to do our job better. So hopefully we can work with you on not only making these changes, which will be made, we're going to make some changes as a result of these hearings. We're going to

propose legislation probably as a result of these hearings, but there are things that we are going to need help from the outside and what we'll need are customers like --

MR. SCHONFELD: I've twisted a few arms on Capital Hill. I'll be happy to twist a few for you.

COMMISSIONER LEHMAN: Great. Thank you very much.

Next I'd like to call on Martin Simpson from the Office of Technology Transfer, University of California.

MARTIN SIMPSON, UNIVERSITY OF CALIFORNIA

MR. SIMPSON: Thank you for coming to listen. Universities license their patents in order to get them commercialized so a strong patent system is essential to having technology transfer work properly from a university. In addition, when we're talking about resources, over 80-percent of our licenses are to small businesses so resources are a problem for our licensees as well.

When you're talking about the first topic of your Notice about practical utility of biotechnology inventions, you have Brenner v Manson stating that any substantial or practical utility is enough. And that traditional chemical standard we would urge would be something that is predictable and it will permit people to make investment decisions on pending cases.

University inventions are frequently at an early stage. When you're talking about utility, you can have utility in the research market; you can have utility in a diagnostic market; in a vaccine market or in a therapeutic market. But when you're talking about university inventions that are early stage, you're talking about things such as assays that may be detectors; you're talking about things that may be screening tools; you're talking about things that may be probes with fluorescent markers on them. If they're being used as a tool and actually showing that something is happening, that's not simply research. When you're talking about early commercialization, one of those characteristics is no or minimal FDA approval; one of them is much less investment for a return and you see, from a university point of view, our commercialization is through finding licensees, that's why this is important; therefore, it's a lower barrier to finding licensees.

If you have early commercialization, you have an early utility supporting work on a later utility, thus the adverse effect on university licensing by demanding what one could call the highest utility is you decrease the number of licensees we can find because of lack of available patent protection and you also mean that our programs will have fewer big

hits, which help pay for the programs.

One of the things that happens is that if -- Licensing of patents is a very empirical process so you get them out there, you license them and some of them work and some of them don't when people start trying to develop the technology from the early stage, typically at a university.

When you talk about the topic of "Proof of Operability for Human Therapeutic Inventions," I come back to the theme you'll see running through it about the chemical art as being your guide, but the key is predictability. And if you have in vitro data that is predictive, why isn't that sufficient for a minimal standard? If you have animal data that is predictive, why isn't that sufficient? If you have human data short of an FDA efficacy showing, why isn't that predictive? It is in the chemical arts. And when you're talking about FDA showings of efficacy you have to remember those are very highly stylized: they have a certain dosage given certain lengths of time and they can't vary it in the middle of the proceedings. It may be also that they show growing hair on bald men in the middle of the process that may be another utility. So, again, you just have these adverse effects if you decrease the available patent protection by how the system is operated.

With respect to the next topic of "Nonobviousness and Enablement," I have to agree with the chairman/CEO of Mycogen that we're seeing that instead of treating these as straight chemical art, you're seeing that there's increasingly narrow room between enablement and utility. And if you get it so narrow that you have virtually no protection available then that means that you either get a narrow patent through the system or no patent through the system and, again, that doesn't call forth the venture capital needed to fund a new invention through development.

With respect to pending legislative patent reform, the 20-year term is a disproportionate disadvantage in biotechnology. There is frequently lengthy prosecution and, as in the Mycogen case, we are seeing very lengthy prosecutions, far beyond the 20 months you're talking about. We see frequently interferences. We've been in multiple-party interferences. We see also -- frequently on appeal in this area of developing law -- and you also run into a disincentive in pioneering cases if the patent examiners are, in essence, nervous about issuing broad claims.

With respect to prior users rights. They are really the antithesis of the patent system. You've got somebody who decided to keep something secret instead of doing what the Supreme Court termed in *Brenner v Manson*, that it's the incentive to disclose, not the other way around. That's, in essence, a royalty free compulsory license.

With respect to restriction practice. One of the things that's

happening there is that by saying you get a 12-way restriction and when you try to talk to the examiner about, well, can't you group these together and can't you group these together, and they say, no, what you're doing for either a university, such as we are, or for -- with our over 80-percent licensees who are small businesses, you're telling them that you've going to have to spend a lot more money for that patent application to get that coverage that you tried to file for. And if you go to a 20-year term, you're also saying you have to spend it simultaneously instead of stretching it out if you really want that protection.

With respect to broadening patent term extension. We would suggest that with respect to these things like lengthy prosecution, interferences, appeals, and so forth, so that when somebody runs into a longer process than is normal that there is some provision for patent term extension. Also, the patent is the right to exclude others. If you effectively don't have the right to exclude then the question becomes: What right do you have? So some thought, we would suggest, would be given to some form of patent trial court or something that would be speedy and inexpensive, moreso than the current process. Also, I want to say thank you for keeping "first to invent."

With respect to the experimental use defense. Basically the comment is, please keep 271(e) narrow.

With respect to plant patent issues. Recently plant variety protection was amended to include the harvest products, such as fruit as well as parts of the plant down to cell lines. We would ask that that be done for plant patents asexually reproduced as well. Also add import and export as they are for plant variety protection and for asexually reproduced plants.

The University of California is into about every kind of technology you can think of so whether it's agriculturally related or it's straight biotechnology into pharmaceuticals for humans, we're into all of it, so we're interested in a very strong patent system, and we very much appreciate your coming to listen. Thank you.

COMMISSIONER LEHMAN: Thank you very much. First, I'd note that the University of California has a very effective Washington operation and, you know, you might want to work with them a little bit and work with the California delegation on perfecting some of these ideas that require statutory approval. You know, we can do that, the administration can submit legislation but obviously you are another venue there, I just point out, to work with.

I had a question about -- getting back to this utility issue, really, which I could have asked other witnesses too, but assume hypothetically

that we adopted a presumption that the utility standard had been met with some very low threshold.

One of the problems that I feel that we have is that we could, in effect, almost do away with it, I mean, internally in terms of our examining procedures. But then the problem is we issue the patents; they get out to the court of appeals for the Federal Circuit and the court of appeals for the Federal Circuit overturns one of our patents that we issued because we haven't met the utility standard. That's where we're really getting this from, we're getting it from the courts. That tends to run a little counter to the notion that what you really need is a patent that you can take into the marketplace, that you can take to the bank. So I'm wondering what kind of thoughts you have in dealing with that. If you could ask us to do anything -- I mean, would you be satisfied if we simply -- if we administratively in the Patent and Trademark Office established a presumption of utility and then we I guess would fight it out in the courts.

MR. SIMPSON: I think we would because right now what's happening is that we're spending a lot of time on the subject and that takes away from the resources up front where you have nothing on the market with your licensee, and what you're doing is spending a lot of time worrying about the topic when you would rather have the time spent later on where there are a lot of resources if it's successful.

COMMISSIONER LEHMAN: Thank you very much.

MR. SIMPSON: Thank you.

COMMISSIONER LEHMAN: Next I'd like to ask William Beers from Scripps Research Institute to come forward, please.

WILLIAM BEERS, SCRIPPS RESEARCH INSTITUTE

MR. BEERS: Mr. Lehman, members of the panel, I'd like to thank you for this opportunity to come here this morning.

I'm the senior vice president and chief operating officer at the Scripps Research Institute. The Research Institute is a non-profit biomedical research operation. We have over 200 individuals that if we were a university you would consider faculty. We have in total about 2,000 employees and of those more than 700 are at the Ph.D. or M.D. level.

We get a lot of government support for our research and most of that comes from the National Institute of Health. What that means is that our technology transfer, which we very aggressively pursue, is governed by the Bayh-Dole Act which was passed in 1980. And, under Bayh-Dole, an institution such as ours, which is in receipt of federal funds, may elect

to retain title to discoveries made with those federal dollars. If a decision is made to retain title, the institution must be able to demonstrate that it is duly attempting to convert the discovery into a product. And at the Scripps Research Institute that conversion is accomplished by licensing discoveries to for profit entities for further development.

Now, in order to be licensable, the discovery must be protected by a patent. Without that protection no company will try to develop it. Now, with that as a background, I would just like to comment on a couple of issues that are being raised here this morning. The first has to do with what should a patentable invention be in biotechnology. My research institute is in the business of basic research, we are not in the business of developing technology. And, as I indicated, a strong patent system is what makes our technology transfer system work. That is, if we are able to license our technology it must be covered by a well defined patent. As I said, the ability to license provides us with our means to comply with Bayh-Dole.

We believe that this means that patents should not be issued for ideas or for discoveries before use is known. If ideas become patentable a restrictive and counterproductive research environment will result. Consequently, we strongly endorse and support a historically consistent view that the utility requirement should remain for biotechnological inventions.

The second issue I'd like to touch on is that of proof of operability for human therapeutic inventions. In an academic research environment such as ours, timely publication of results is terribly important. Consequently, it is also important if technology transfer is going to occur that patents be issued relatively early on, that is once the plausibility of operability has been demonstrated in vitro or in vivo but non-human models' systems. In essence, that is to say when it's fairly clear that there is good predictive data available.

If proof of operability would have to wait for complete or advanced human trials, the pressure the academy puts on rapid publishing would likely put the information in the public domain and thereby deny exclusivity which is an important engine driving technology transfer. Indeed, it's next to impossible to license technology that is not protected by a patent. We have been in that position a few times and it's terribly, terribly hard. It simply isn't worth the risk to industry. Consequently, this situation would terminate the development of promising but not necessarily fully proven therapeutics when the original findings emerge from an academic research environment.

Thank you very much.

COMMISSIONER LEHMAN: Thank you very much for those statements, Mr. Beers. I'm glad you brought out the connection between NIH funding, Bayh-Dole Act, an institution like yours and the commercialization of the technology. I think it does point out that we are capable from time to time of making adjustments in the system that works, and that's one that has worked pretty well. And actually I was involved in that myself back when it was enacted in I think it was 1978. I think roughly 1978.

MR. BEERS: That sounds about right.

COMMISSIONER LEHMAN: That's when I was counsel on Capital Hill. Thank you very much.

Next I'd like to ask Douglas Obenshain of Ernst and Young to come forward, please.

I really want to thank our witnesses for being cooperative and moving right along because we're on schedule. We're actually a little bit ahead of schedule so it will help us get through our 57 appearances that we have today.

I guess Mr. Obenshain isn't here. How about Kathy Behrens?

KATHY BEHRENS, ROBERTSON, COLEMAN and STEPHENS

MS. BEHRENS: Thank you. You've heard a lot of numbers, you're going to hear a few more, unfortunately.

I'm Kathy Behrens. I'm a managing director with Robertson and Stephens and Company. Robertson and Stephens is an investment banking firm focused on providing investment banking, brokerage, money management services and venture capital to emerging growth companies and investors. The company manages approximately \$1.2 billion in public and private equity funds. And I would add that my comments reflect both the views of the venture group at Robertson and Stephens as well as the investment banking group.

By way of introduction, as everybody in this room knows, patents play a fundamental and a critical role in the availability of capital and willingness of institutions and individuals to invest in biotechnology. They are as important as any factor in the decision to invest in any idea, entrepreneur or company in this field. The reason for such dependency upon patents is that they provide the favorable economics required to justify substantial capital investment for successful product development.

Historically, biotechnology companies with successful proprietary products have generated significant products' sales levels during periods

of market exclusivity yielding considerable profit margins and high returns on investment. These returns have been necessary given the significant level of risk associated with technology development, regulatory hurdles, clinical testing demands, limited availability of capital and prolonged product development cycles.

While most of the historical information accumulated for this industry has been generated by human pharmaceutical and diagnostic products sold both by biotechnology and pharmaceutical companies, there remains a tremendous commercial opportunity in other areas such as animal and plant of schedule so it will help us get tagriculture. The substantial level of private and public investment in biotechnology made over the last 15 years has accelerated technology development and enhanced U.S. competitiveness.

Over the last two years, considerable new pressures have been exerted upon young emerging biotechnology companies. Enormous uncertainties associated with health care reform and unrealistic performance goals related to quickly moving products to market have contributed to disappointments with this industry by the private and public markets. As a result, capital investment is down from prior years. Additional uncertainty is now being generated by the proposed patent changes included in the general agreement on tariffs and trade. Any alteration of patent practice that might shorten the current period of exclusivity or increase the magnitude of capital required to develop or protect products will have a negative impact on the availability of capital for biotechnology. The cumulative effect of such proposed changes and preexisting market uncertainty could be devastating.

Let me get to a couple of numbers now. Patents have made a significant contribution to the emergence of multiple successful, vertically integrated biotechnology companies. The most mature of these include Amgen, Chiron, Genetech, Byogen, Genzyme, and Immunex which generated combined 1993 product-associated revenues of \$3.1 billion.

Sales of the biotechnology industry exceeds \$7 billion a year, with 12 biotechnology products contributing estimated 1994 worldwide sales booked by biotechnology and pharmaceutical companies in excess of \$4.5 billion.

Since 1980, over \$4 billion in private equity has been raised for more than a thousand privately held companies. However, in the first nine months of 1994 the investment rate has been down considerable compared with 1993, and the year will probably close with the lowest amount of private capital invested in biotechnology since 1985.

I have included in the text of my comments a summary of every year since 1980: the individual year, the amount of dollars invested by the private industry, and the number of companies that have been financed in that

time period. And what I, unfortunately, do not have a slide for and that I was making reference to is that through the 5th of October, 1994, the invested dollars by the private sector has been about \$168 million compared with close to \$494 million for the full year 1993. While there is a considerable lag in generating those numbers in the private industry, it's pretty clear we're going to be at half and perhaps lower than half of the prior year.

Now, getting the public market sector for a minute. Nearly \$10 billion in capital has been invested since 1980 by the public equity markets into more than 300 biotechnology companies. The public market investment rate in biotechnology is also down in 1994, as is the number of companies financed and the average offering size. The 1994 public investment rate will probably be the lowest in the last four years. And, similar to my last comments, I've got text that includes a chart showing all of the dollars raised since 1980 in yearly aggregate and the number of companies and the average offering size for those companies.

To give you just 1994 compared with '93, through September of 1994 the public market equity invested in biotechnology is approximately \$826 million compared with the full year of 1993 of \$1.5 billion. The number of companies is 37 compared with 53 in 1993, and the average offering size in 1994 is \$22 million versus \$29 million in 1993.

As I mentioned earlier, several factors have contributed to the lower 1994 public and private investment activity in biotechnology companies. In somewhat greater detail, these factors include investor uncertainty due to health care reform and the associated negative impact upon pharmaceutical products and companies; product development disappointments related to aggressive forecasting and the urgency to speed biotechnology products to market; and the large number of biotechnology companies formed between 1991 and 1993.

In addition, the declining availability of public market capital to an ever shortening list of biotechnology companies has had a significant cooling effect upon the private equity sector in 1994. Furthermore, the complexity and cost of establishing and maintaining proprietary product positions is also taking a toll on the industry. In the venture arena, the magnitude of investment -- capital investment -- is high and the duration of biotechnology is long due to both the technology development required and the high product fall-out rate prior to reaching market. As an example, the average biotechnology venture investment runs at least five to seven years in duration. As a result, investors will not finance a company or an entrepreneur idea unless the opportunity for financial return does not far exceed the risk of failure.

Within the venture industry, average annual returns are expected to exceed the Standard and Poors 500 index for the public market by at least

five points. The annual S&P returns for the last 25 years have ranged from 11 to 13 percent. Therefore, venture investors are looking for annual returns of approximately 16 to 18 percent. In other words, a venture investor must double the amount of capital under management or invested approximately every four years to remain competitive.

In addition, venture investors seek opportunities that individually far exceed those returns because when the individual failures have been included the average annual returns are considerably lower. Even the public market investor is seeking to achieve or exceed the annual rate of return of the S&P and, given that the risks of biotechnology investing are substantially greater than with other stock sectors, the initial expectation for return must be higher than the S&P to even consider the investment.

Within the biotechnology sector patents are necessary to allow product pricing stability, generate attractive gross and net profit margins and allow a reasonable period of market exclusivity to overcome all of the risks associated with biotechnology investing. While there agrees some disagreement regarding the ranges of costs associated with pharmaceutical product development, a recent OTA study estimates that the average pretax cost of developing a pharmaceutical product is \$359 million.

In closing, patents have been and will continue to be one of the most critical components to investment decision making and access to capital for the biotechnology industry. Furthermore, there is an inverse relationship between the size of the pools of public and private capital and uncertainty regarding the protection of proprietary technology in products within this sector. In our view, the most constructive course would be to use legislation or regulation to cure some of the existing uncertainty and problems that are present in the patent process today. However, any legislative action that could negatively impact such a critical component of biotechnology and U.S. competitiveness must receive a high level of public attention and comment before alternative policy is established. Thank you.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call Mr. Obenshain; is he here now? From Ernst and Young.

DOUGLAS OBENSHAIN, ERNST and YOUNG

MR. OBENSHAIN: Thank you. My name is Doug Obenshain and I'm an audit partner with the San Diego office of Ernst and Young, LLP. I specialize in serving life sciences' companies and have clients from the conceptual stage of an entrepreneur with a business plan up through a number of public company clients.

E and Y, LLP is the leading professional services firm in the world at serving the biotechnology industry. Each year we publish a national survey of the industry in which we request information from the CEOs of some 1,300 companies nationwide. From this we compile a variety of statistical analyses in everything from financing transactions, strategic alliances, planned actions of the CEOs, executive compensation, and a variety of other matters pertinent to the industry. This report has come to be regarded as the premiere state of the industry publication available. We then present this annual report, which this year is titled "Biotech '95: Reform, Restructure, and Renewal," at a series of approximately two dozen 30- to 45- minute roadshows all across the country.

This morning I'm going to attempt to condense this information into a brief five to seven minute overview of the year in biotech and then I will briefly touch upon my perspectives of the importance of patent and patent protection to this industry.

Each year in our report we identify the defining events of the year and in 1995 there were, as always, both positive and negative defining events. The biotechnology industry is one which requires a significant amount of cash, generally in the form of equity capital, in order to realize its long-term product potential. And, as Kathy indicated, the industry's ability to raise cash has historically been very cyclical. It's been marked by boom periods, such as those experienced in the early 1980s, the late 1980s, and the 1991 and '92 period, and early '93, followed by what are generally considered to be busts or famine periods, and a number of factors combined to make the year end of June 30th, 1994, generally a very difficult year to raise capital.

Certainly the industry was negatively impacted this year by the clinical setbacks of Antril, Thymosin, Respiver and other products. These failures were much ballyhooed in the press and by the investor community and affected not just the valuation of the companies involved but truly the valuations of the industry as a whole. Many companies who have continued to make strong clinical progress saw their valuations plummet due to someone else's travails. However, it should be noted that the major pharmaceutical companies also experience mixed clinical trials all the time and product setbacks all the time. But these, in fact, are expected, are accepted and are therefore not as highly publicized and do not have a significant impact upon the market valuations of either the drug companies individually or the pharmaceutical industry as a whole.

And I must point out that the biotech industry currently has over 50 products in phase three clinical trials, which demonstrates the industry's long-term ability to discover and develop products. Will all 50 of those products be approved? certainly not. And will all of those

approved end up being blockbuster drugs? certainly not. But will they benefit worldwide health care? most certainly they should. And that's the promise and the value of the biotechnology industry.

Speaking of health care. The other factor which significantly negatively impacted the industries' ability to raise cash this year has been concern over health care reform. One need only look at prospectuses of companies issued before mid-1992 and compare them to those issued subsequent to 1992 and see that a very prominent and ominous risk factor appeared labeled "Health Care Reform." Even though reform has now been delayed for at least a year and formal price controls appear to have been removed from most of the popular proposals, uncertainty over health care reform and the fear of price controls has caused the investment community to be extremely skiddish. So the combination of primarily those two factors, as well as rising interest rates, have combined to create a very cautious investor mentality from late 1993 through 1994.

While our report indicates generally positive news on the year ended June 30, 1994, compared to the year ended June 30, 1993, I would tell you that the majority of 1994 has not been a favorable environment for raising cash. The average market capitalization of the industry is down eight to nine percent over a year ago. But for most companies, other than the top 10 or 15 companies in the industry, the drop has been much more precipitous. And, through the first nine months of 1994, initial public offering activity, which is a company's first foray into the public equity markets, has been significantly reduced. Most of the deals are much smaller than companies had envisioned when they originally drafted the prospectus and most deals are being priced at lower than the initial filing range.

Despite all of this, in the year ended June 30, '94, the industry did raise about \$4.5 billion from all sources, which was about a 27-percent increase over the prior year. So, contrary to certain media reports, the capital markets are not completely closed. For a company that's been making good progress and has a good clinical story to tell, the markets are still there. Unfortunately, however, as I mentioned, the average deal size has declined and executives therefore have to spend increasing amounts of their time strategizing about how to raise capital at the expense of spending that time focusing on product development.

Furthermore, the industry ended June 30, 1994, with about \$9 billion in cash, which is \$1 billion more than the year before. The industry is not out of cash; however, much of that is concentrated at the 10 or 15 so-called breakaway companies. So there is some cause for concern; not panic but concern. The industries' burn rate in R&D spending and net loss all continue to increase, and the medium survival index, which we calculate as cash and committed cash divided by their average monthly rate of spending, has dropped from 34 months to 25 months in the last

year. Twenty percent of all biotech companies have less than 12 months of cash in their coffers, and nearly 50- percent have two years or less. But these survival indices were comparable in 1990 and 1992, so hopefully the industry will be able to raise cash in '95 and it hopes to do so at higher valuations than currently exist for most companies.

Now, what will help these companies out? One, a continued improvement in the public and political leaderships' awareness of and understanding of the industry and its issues resulting in a reasonable approach to health care reform.

Two. More product successes, such as those seen this year in Palmazine, Riopro, the flavor saver tomato, and others.

Third. A reasonable consolidation which would reduce the sheer number of companies, and, fourth, a continued influx of funding from the major pharmaceutical companies.

Now let's talk for a moment about patents. If you think about the balance sheet of a typical biotechnology company, the biggest numbers are cash and shareholders' equity. I believe the equity number is simply a reflection of sophisticated investors' perceptions of the value of two things that are either not on the balance sheet or are undervalued: that is the company's people and their ideas. And patents are the primary protection of those ideas; therefore, they're clearly one of the most critical elements of ensuring the future success of the industry.

Legislation was proposed this year, but not passed, to extend patent terms from 17 to 20 years with a patent becoming effective at the application date rather than the issuance date. Given the length of the product development time table in this industry, a sizeable portion of that 20-year period could be consumed by product development rather than product sales. And remember that conventional wisdom suggests it takes 10 to 15 years and as much as \$300 to \$400 million to bring a pharmaceutical to market. If you had made such an investment you would hope to be able to reap the rewards from that effort for as long as a period of time as possible. The shorter the period in which you have patent protection for your developed product, the more you will have to charge on a per dose basis for that therapeutic in order to recap your investment.

I appreciate the opportunity to have made these remarks to you today, and I have copies of our 1995 report available if you wish to see them. And now I'm prepared to respond to any questions that you may have. in the public and political leaderships' awareness of and understand

COMMISSIONER LEHMAN: I think I don't have any and thank you very much.

MR. OBENSHAIN: Okay.

COMMISSIONER LEHMAN: Next I'd like to ask William Epstein from Hoffman-La Roche, Incorporated, to come forward.

WILLIAM EPSTEIN, HOFFMAN-LA ROCHE, INCORPORATED

MR. EPSTEIN: Thank you, Mr. Commissioner, and I wish to thank the members of the Patent Office for having me as a speaker at this meeting. I'm Bill Epstein, associate patent counsel, assistant secretary of Hoffman-La Roche, and I'm speaking today on behalf of BIO's Intellectual Property Committee.

I just want to point out that BIO has prepared a detailed book on --

COMMISSIONER LEHMAN: You'll have to speak into the microphone because the court reporter needs to --

MR. EPSTEIN: Okay. Thank you.

I wish to point out that BIO has prepared a detailed book outlining all of the issues raised at this hearing. We have 55 individuals under the direction and help in editing of Chuck Ludlum, who without this his tireless efforts would not have made this book possible and the reports in this book possible. I read it last night. It was very interesting reading and I would like to present the Commissioner with a copy. It sets out detailed positions on each of the issues raised at this hearing.

I'm here to speak on two issues: One, the question to practical utility and, two, practical utility versus proof of operability. I guess -- I've just been informed I'm the first patent attorney to speak on these subjects here so my remarks are purely that of a patent attorney.

Question two concerns the belief of whether the PTO is correctly applying the legal standards governing the question of practical utility requirements of 35 U.S.C 101. I believe no. I think the legal requirements are clear; however, I do not think the examiners know what the legal requirements are. Today the patent office has a very effective staff of patent examiners. Never before, since I have been practicing, has there been such highly trained, well-understanding patent examiners as for the science; however, for the legal problems that's to me a different story.

The legal standards are well settled as what is practical utility, and there is no distinction. If you satisfy the requirements of practical utility, you don't need any proof of operability. However, the question is: Do the examiners understand what the legal requirements are? and

since they do not, they are not applying them correctly.

A problem arises very quickly from the contrasting, what are the requirements of, let's say, public policy versus the courts? This is where the problem arises. I think public policy statement is very well put out in the Notice of this hearing which says: "Important public policy justifications for the USPTO to review operability of inventions to treat human disorders." A patent provides the public with a high-quality, technically accurate disclosure of new, useful and unobvious inventions -- nonobvious invention. However, the imprimatur of the federal government, a patent can also affect the commercial prospects of the invention in question and can raise or lower expectations of those afflicted with illness the invention is designed to treat. That contrasts very severely with the decisions of the Court of Customs and Patent Appeals, and the CAFC with regard to this item.

One looks at the old case of *In re Anthony*, which is the standard case in this area, here the drug Monase was taken out of public -- off the market because it was toxic, it couldn't be used to treat humans. Yet the court said that this was not sufficient for this drug to meet -- was sufficient for this drug to meet the requirements of U.S. patent law. The fact that the drug was toxic in no way stated that it would not meet the requirements of 31 U.S.C. 101 or 112, and that a practical utility had not been disclosed. They define practical utility as not being a commercial utility. They said that it's the FDA, not the PTO that is responsible for the question of whether a drug can be used in human therapy. And this is what is the distinction; this is the dichotomy that exists between the law and public policy. The Commissioner I think very well stated that the utility requirements is for the purpose of promoting commercialization. I agree with that wholeheartedly, it is not for guaranteeing treatment. That is what some of the examiners' standards in Group 180 are, that the applicant must guarantee treatment, and the patent laws are not to promote commercialization.

Let's look at what is considered practical utility. Practical utility is disclosed in *Nelson v Bowler* as saying that the knowledge of the pharmacological activity of the compound is obviously beneficial to the public. It is inherently faster and easier to combat illnesses and alleviate symptoms when the medical profession is armed with the arsenal of chemicals having known pharmacological activity. Since it is crucial to provide researchers with an incentive to disclose pharmacological activity in as many compounds as possible, we conclude that any proof of any such activity constitutes a showing of practical utility. Therefore, all of it is needed under *Nelson v Bowler*, and in a subsequent case of *Cross v Iizuka* is that it has pharmacological activity, not therapy. Therefore, it isn't -- Therefore, what it is, it does not guarantee treatment.

The requirement of the statute is not -- and as interpreted by the court, is not to guarantee treatment but to promote commercialization. Clearly, this is taken in Cross v Iizuka, in another case, success in in vitro testing will marshal resources and direct the expenditures of efforts to further in vitro testing of most potent compounds, thereby providing an intermediate benefit to the public analogous to the benefit provided by the showing of the In Vivo Act utility. This standard doesn't exist for many examiners, and the question always comes, "What is a therapeutic utility?" "What is pharmacologic activity?" In order to have pharmacologic activity they have to have three things:

One. The activity.

Two. The nexus.

Three. The disease state. This is what the cases hold, you must have all three things. The fact that something burns a hole, a compound or material burns a hole in a piece of paper, that's not utility. What is utility? If you can put what the pharmacological activity is and relate it to the disease state. There must be the nexus in between the pharmacological activity that you're claiming what is your invention and what the -- and a disease state. This I think is clear. The question is, and it always gets to this point: How can we better get the examiners to understand or not understand? Educate the examiners in legal issues, because if we do not the problems are very clear. Basically these rejections will be overturned. Basically all they will do will provide roadblocks. They will not provide, promote commercialization. You will get rejections that are to me are just beating dead horses, and eventually a lot of money is spent in beating the same dead horse, which I feel is wasted.

You take, for instance, typical example with an AIDS drug. Here AIDS is a tremendously important disease to cure and treat. Well, we know that an enzyme -- by inhibiting an enzyme, or certain action of an enzyme we can inhibit the reproduction of the virus. Now, based on that utility, suppose the utility is that, we get back the rejection that anything that inhibits this activity, that's the public policy we can accept as a utility statement. Well, based upon this utility and the fact that the compound has been -- is not toxic millions of dollars have been invested in conducting clinical tests. If this utility is sufficient for investors it should be sufficient for the Patent Office.

Now, the recommendation which I think is very important, to my mind, is that the patent office have a what you would call a control group that would pick up cases at random and act as the inventors advocate.

Today the Patent Office has a quality control but they're only, from what I understand, and I may be totally wrong, that they're only there to

look at rejections, to make other rejections. I once got a rejection at the end of the lot and I saw -- and went to the board of appeals, it was reversed. So they put another barrier, but what about looking into seeing that the applicants, that the attorneys are really benefitting the system -- I mean, not the attorneys, the examiners are benefitting the system by looking at rejections and saying, "Are they being well based?" "Are they really applying the law?" and talking to the examiner, not as a harsh reprimand but as a teaching tool. The teaching comes far better from the Patent Office than it comes from me, because when I tell an examiner something he says, "You're only saying that because you're an attorney. Even the devil can cite our cases." So it should come from the Patent Office and the Patent Office should look at these rejections and see are they really following the law. And this way an examiner -- and I was an examiner -- gets an understanding of what the law is about and appreciates the thing. And this way I think with the technical expertise that we have now in the patent office, and with a little understanding of the law, no system can touch it.

MS. LINCK: Thank you, Mr. Epstein. I'm Nancy Linck. I'm the new solicitor of Patents and Trademarks and we are trying our best I think to follow the cases *Nelson v Bowler* and *Cross v Iizuka*. The *Deuel* case you mentioned is on appeal right now and will be argued in about a week and I think one problem we have is this emerging technology and it's difficult to draw the line. As one of the other speakers mentioned, you know, you don't want us issuing patents that should not issue and, therefore, you know, any help we can get, if you really think that the Board decision is inconsistent with earlier law I urge you to appeal to the Federal Circuit and provide us with additional guidance from the Federal Circuit.

MR. EPSTEIN: I don't think the -- Honestly, I don't think the *Deuel* is inconsistent with law. I don't think it is. Again, there's the nexus. That's a very important thing. I'll never forget a case that I was cited, that I argued, that was argued back to me -- I think it's *Hoffman v Klaus*. I was *Klaus*, but there the nexus was missing. You had the pharmacological activity; you didn't have a nexus. So what was this? There was no nexus involved and that I think is interesting and that's the question. But let's say you cite these cases, whatever they be, you don't get any answer back. There isn't the byplay as if there is to understanding these things. So I think I'm not questioning the *Deuel* case but I am questioning whether the examiners understand the *Deuel* case and the other things.

COMMISSIONER LEHMAN: Well, apparently, though, the very fine report that you suggest does question the *Deuel* case. That's why Ms. Linck made --

MR. EPSTEIN: I'm sorry, if I could just say one thing.

COMMISSIONER LEHMAN: There are two cases that are given as an illustration of --

MR. EPSTEIN: Right.

COMMISSIONER LEHMAN: -- we're not following the CAFC law or where the Board hasn't, are Ex Parte Maizel and Ex Parte Deuel. And I'm not sure that Ex Parte Maizel was ever appealed to the CAFC. So I think the point is we're -- The point of these hearings is obviously we're trying to do what we can do internally, but it also helps us to have the private sector work with the court of appeals for the Federal Circuit to define the law properly, too, or they think that we've made a mistake.

MR. EPSTEIN: Could I just say something about those two cases. First of all I don't think the Maizel case, and maybe I'm wrong, was a 101 case -- a 101 case. I don't think it was. And, if I'm not mistaken, the Deuel case also, that was another rejection which they wanted to re-examine, so I don't -- so they really made an extra finding. I don't think it's part of the holding, and I think the Board asked for the idea of the nexus or to investigate the nexus, so I'm not so sure I agree with the way those cases have been cited in the thing, and they weren't cited -- but I'm saying, look, the document is still a wonderful document. It has certain things, and remember we had very little time to prepare it.

COMMISSIONER LEHMAN: We'll study it very carefully.

MR. EPSTEIN: I appreciate that.

COMMISSIONER LEHMAN: We'll work our way through this. Thank you very much.

MR. EPSTEIN: Thank you.

COMMISSIONER LEHMAN: Next I'd like to call Laura Handley; Weil, Gotshal and Manges.

LAURA HANDLEY, WEIL, GOTSHAL and MANGES

MS. HANDLEY: Good morning, Commissioner Lehman and other distinguished panel members. I'm Laura Handley. I'm an attorney with Weil, Gotshal and Manages. We represent a diverse group of biotechnology clients ranging from universities to agricultural companies to companies designing human therapeutics. I recently completed a clerkship with Judge Plager of the Court of Appeals for the Federal Circuit and on behalf of the BIO Intellectual Property Committee I'll be providing brief summary of the case law concerning the utility requirement. A detailed version of the presentation has been submitted to the record by BIO.

Nine minutes is really not enough time to do justice to over 30 years case law in the utility requirement. Unfortunately, as you'll probably continue to hear today, many here feel that nine minutes is about seven minutes more than the examiners are currently giving the case law.

In any event, I'll now briefly summarize the legal standard for utility; list three common legal errors committed by many examiners, and then describe in detail the case law with regard to each area.

COMMISSIONER LEHMAN: Can I ask when you say that the examiners are spending about two minutes on this, are you talking about all examiners or are you talking about some examiners? In other words, is the problem that we don't -- that we uniformly are misinterpreting the law or that some examiners are misinterpreting it, from your point of view?

MS. HANDLEY: Well, my point of view is based primarily on anecdote from other people. I just left the court about a year ago. I get the impression from my colleagues that it's many, but by no means all examiners. And it may actually be a function of the time constraints that they work under that they don't have time to think through the case law, and they don't have the legal training to think through the case law.

In any event, as for the legal standard for utility. As you know, Section 101 of the patent statute requires that an invention must be useful in order to receive a patent. And that test has been phrased as inquiring whether a patent -- whether an invention has practical utility. What constitutes that practical utility is dictated by controlling case law of the Supreme Court, the Federal Circuit, and the CCPA, the predecessor of the Federal Circuit. Of course all of these are binding precedent upon the PTO.

Brenner v Manson, the Supreme Court's 1966 case on the utility requirement, first set forth this practical utility test. The patent application in Brenner fell far short as the applicants there had completed no testing whatsoever to support the claimed utility. But patent attorneys learn fast.

Brenner v Manson was a 1966 case and today the examiners rarely, if ever see patent applications in which no utility testing whatsoever has been completed. Thus the two cases which provide more helpful guidance on the utility standard rose in the early to mid-eighties and these cases are Nelson v Bowler, decided by the CCPA, and Cross v Iizuka, decided by the Federal Circuit. Both Nelson and Cross apply Brenner to cases in which some testing had been completed and held that practical utility had been demonstrated when the patent applicant provided evidence of pharmacological activity. In Nelson that pharmacological activity was

established by an in vitro assay and also by an in vivo experiment in rats. In Cross the pharmacological activity was established by simply the in vitro assay.

Notably, neither case required clinical data or proof of therapeutic utility. In contrast, the position of the PTO on the utility requirement seems to have shifted dramatically over the last several years and utility has now become a significant procedural hurdle for biotechnology inventors. Applications that do not contain human clinical data, or at the very least mammalian animal data frequently are rejected on utility grounds.

We believe that such rejections deviate from the controlling case law in three respects:

First, the examiners err in shifting the utility inquiry from the claimed invention and instead in focusing on the ultimate therapeutic use which the examiner believes to be the inventor's goal.

Second, the examiners err in routinely rejecting in vitro data.

Third, the examiners err in refusing to accept data that would prove to unskilled in the art that a compound has pharmacological activity.

As for the first error, the examiners cannot shift the focus of the utility inquiry to the product which the examiner surmises is the ultimate goal of the inventor. Instead, the Federal Circuit stated in *Raytheon v Roper Corporation* that the claims of the patent determine the invention to be tested for utility. As it is the claims which dictate the utility inquiry, each case will be fact specific and there can be no uniform utility requirement. If the claims are phrased in terms of the method of treating a disease then the PTO properly requires data relating to treatment of that disease. If one skilled in the art would accept nothing short of human therapeutic data as predictive of success then the PTO may require that that data be presented. If one skilled in the art would accept one mammalian animal model, or even in vitro models as predictive, then the PTO must accept those showings.

However, when the claimed invention is a composition matter and not a method of treating a disease, the CCPA in *In re Bundy* and in *Nelson v Bowler* made quite clear that, quote: "Evidence of any utility is sufficient." Close quote. That utility may relate to a variety of things short of human therapy. For example, pharmacological effects, such as receptor binding; stimulation of immunological reaction; or growth of particular cells. Again, as the court made clear in *Nelson v Bowler*, such showing of pharmacological activity suffice.

In contrast, examiners seem to read the claims that are drawn to

compositions and matter as though the claim instead read, quote: "A method for treating a human disease." This is legal error as it is looking for therapeutic utility rather than pharmacological activity.

COMMISSIONER LEHMAN: Can I ask as question?

MS. HANDLEY: Sure.

COMMISSIONER LEHMAN: If the claimed invention is for human therapy, though, will just mere evidence of pharmacological activity suffice? I mean, doesn't this go partly to what the claimed utility is?

MS. HANDLEY: Right. I would say that the way the examiners approach this is first look at the call of the claim. If the claim is drafted as a method for treating a disease then you look to see if there is evidence in this specification that supports treatment of that disease.

COMMISSIONER LEHMAN: So it's evidence in the specification and then merely shown by, you know, in vitro pharmacological activity would satisfy the test then in that case, in your view?

MS. HANDLEY: I think it all gets back to what would be persuasive to those skilled in the art, and if those skilled in the art would have sufficient confidence in the in vitro assays then that should meet the test. If it's a composition and matter claim, I think the threshold is much lower. I think traditionally in chemical practice composition and matter claims, if you showed any utility whatsoever, that was sufficient because the call of the claim is to the compound itself, and people can later come along and if they have a novel method of using that compound they can then get a method claim. So there's always been a lower standard and broader protection for composition and matter claims.

COMMISSIONER LEHMAN: But my impression, though, is that the problems haven't been with those composition and matter claims as much.

MS. HANDLEY: Well --

COMMISSIONER LEHMAN: Well, that's, you know --

MS. HANDLEY: In my limited experience, I've had almost every one of my composition and matter claims rejected on utility grounds, and the examiners seem to be reading it as if it was a method claim, a method of treatment claim.

As for the second error, examiners frequently refuse to accept the probative value of in vitro or non-human in vivo data, and this, too, is directly contrary to the controlling precedent of *Nelson v Bowler* and *Cross v Iizuka*. As the Federal Circuit specifically stated, in vitro

testing is the accepted practice in the industry and is generally predictive of in vivo results.

For example, in *Cross v Iizuka*, the only data supporting the claimed utility was an in vitro assay showing the effect of the compound in platelets. And I find the *Cross* case to be interesting because it highlights how dramatically the PTO position has changed in recent years. *Cross* was an interferons case which reached the Federal Circuit in 1985 and the party *Iizuka* showing of utility was based on an in vitro assay alone and it was accepted by the PTO. It was then challenged by a disgruntled party, *Cross*, and *Cross's* position on appeal was that, one, regardless of the claims the real utility was as a therapeutic medicine for treating disease and, two, that in vitro data was not sufficiently probative. And, as I've argued above, these are precisely the arguments that the examiners are raising today.

On appeal, the Federal Circuit reject party *Cross's* attempt to shift the inquiry to therapeutic effect and strongly endorsed the probative value of in vitro data. BIO's position then is that the examiners should be following the Federal Circuit and not be reiterating the argument unsuccessfully made by the party *Cross*.

As for the third error, examiners frequently seem to forget that the evidence of utility, be it in vitro or in vivo data, must be persuasive to one skilled in the art and not to the examiner personally. If the examiner raises an objection to a showing of utility, the inventor can overcome that rejection by offering evidence that the showing would be persuasive to one skilled in the art, and that was the case in *In re Jolles* which considered acceptance of animal data.

So to conclude then, nothing in *Brenner v Manson*, which is often the examiners' shibboleth for denying utility, requires submission of human clinical data. The patent applicants in *Brenner* had conducted no testing whatsoever, and the scientific field is acknowledged by all to be unpredictable.

In contrast to those facts, examiners today routinely reject applications that do disclose some testing. The question then becomes one of how much testing and what kind of testing; questions that are answered by both *Nelson v Bowler* and *Cross v Iizuka*. The examiners' conversion of the utility requirement from a minimal threshold into a major administrative hurdle, akin to the showing required by the FDA, is legal error.

Unfortunately, though, many of these erroneous calls simply will not be challenged. Every time the examiner issues a final rejection the inventor is put to the difficult choice of acquiescing or to pursuing costly appeals to the Board and to the Federal Circuit. And,

unfortunately, especially with the increasing tightness of the money market, many clients simply do not have the financial resources to pursue the legal battle. Accordingly, BIO requests that the examiners change current examination practices, study the controlling case law in this utility area and conform with it.

If I may, I'd like to respond very quickly to your point about raising the presumption of utility. I have three points -- three thoughts. One is that a presumption of utility would be consistent with Federal Circuit case law, Tol-o-Matic, Carl Zeiss, and also the old Supreme Court case of Lowell v Lewis that really do seem to treat utility as a minimal threshold.

My second thought on the issue is that it makes --

COMMISSIONER LEHMAN: You mean, you think we don't have a presumption of utility now?

MS. HANDLEY: Right.

COMMISSIONER LEHMAN: We could create one?

MS. HANDLEY: If a presumption of utility were created, it would also mesh very well with -- for human therapeutic inventions -- with Section 271(e)(1) of the patent statute, which basically is a safe harbor provision against patent infringement suits until a company comes out of clinical trials. And that really would allow a company to get its patent, get its ducks in order, get its drug into and out of clinicals and then it would be subject to suit. And, by that point, you would have the clear clinical data to prove the issue one way or the other. Thank you.

MS. LINCK: Ms. Handley, I'd like to ask you one question. It sounds like you've done a fairly in depth review of the case law, and I think it would be helpful for us if you could identify for us any Board decisions or CCPA decisions, but primarily Board decisions, that you think are out of line with controlling case law.

MS. HANDLEY: I am the culprit. I am the author of the section of Deuel and Maizel. My quibble with Deuel and Maizel is that in both cases the Board -- well, in both cases the Board raised the issue sua sponte, and in both cases I didn't think that the Board had focused enough on the claims because in both of those cases there were composition and matter claims, and in both cases all I was working from was the Board opinion itself, and it seemed that the Board in both cases admitted that there was evidence of some utility.

MS. LINCK: Any others?

MS. HANDLEY: Not that come to mind right now.

MS. LINCK: Thank you very much.

MS. HANDLEY: Thank you.

COMMISSIONER LEHMAN: But if we had -- To get back to this presumption question in a situation where we have evidence of some utility then there would be -- if you had adopted some sort of a presumption then that would cross the threshold then?

MS. HANDLEY: Well, as my colleague Elizabeth Enayati will be discussing in a few minutes, however, there is a presumption on the operability issue under Section 112, and the very fact that there's a presumption in place hasn't seemed to have made a practical difference in how these issues are treated by the examiners. So I think better -- a presumption would be a good step but it's only as good as the legal training of the examiners.

COMMISSIONER LEHMAN: So you really think that our -- in many ways an administrative problem is at the core of a lot of this; it's simply the legal training?

MS. HANDLEY: Yes.

COMMISSIONER LEHMAN: I would point out that one of the reforms that our assistant commissioner for patents -- and I'm sorry he's not here today -- he had to be somewhere else now, but we all can't be at different places at the same time, but he's putting in to bring into place a new -- going to be putting into place a new law training program for the examiners so -- where we're bringing the law schools right on site and we're going to try to encourage them to -- but we're not going to give them law degrees because otherwise they'll all join you at Weil, Gotshal and Manges, so we're going to give them -- Larry's idea is we're going to give them a Master of Juridical Science, an MJ degree rather than a JD degree so they won't be able to fly the coop. The only place they'll be able to us it is with us, but we are trying to --

MS. HANDLEY: I think that's an excellent solution for the PTO. As for Weil, Gotshal maybe it's not so good. Thank you.

COMMISSIONER LEHMAN: Our next witness is Timothy Gens from Fenwick and West.

TIMOTHY GENS, FENWICK and WEST

MR. GENS: Good morning Commissioner and other panelists. I am Timothy

Gens. I'm with the -- of counsel with the firm of Fenwick and West which has about 120 attorneys between its Palo Alto and Washington, D.C. offices. Our firm focuses upon providing legal services to high technology companies of which include biotech companies, universities and other types of entities. Personally I've had a little over 16 years of experience practicing before the Patent Office and also before the Federal Circuit Courts with regard to patent issues, and I present these remarks today on behalf of the Biotechnology Industry Organization.

In particular, I'm responding to the first question in the Notice under the issue of "Utility" with regard to whether or not the legal standards governing the requirement for identification of utility developed by the federal courts are clear and concise.

We believe that the legal standards established by the federal courts governing the requirement for the identification of utility are sufficiently clear and appropriate for biotechnology inventions.

We submit that these legal standards are well defined and require a minimum of proof to establish practical utility. The concern over utility requirement for patentability should not be directed to the clarity of the legal standards but rather to the implementation of the standards by the Patent Office. Thus we view the utility issue as one of misapplication of the law rather than incorrect legal precedent.

To resolve the utility issue, we propose that the patent bar be offered the opportunity to understand the content of the examiners' education by the Patent Office on such legal standards and to positively contribute to the content of this legal education. Accordingly, we offer a practical and effective approach to working with the Patent Office in achieving a principal goal of our nation's patent system, that is the promotion of science and innovation.

We commend the patent office for its recent efforts in increasing the number of qualified examiners in the biotechnology area. Consequently, the examiners have evidenced a very positive trend in the technical understanding of the inventions that less than two decades ago were simply pipe dreams.

From the hand-out that was given to us as we came into the room today, with regard to the fact that in Group 1800 over half of the examiners have Ph.Ds, it has been well noted by the practitioners before the Patent Office and we are very thankful for that. But now let's focus on the legal training because that's where we are still running into a major obstacle.

The misapplication of these legal standards governing practical utility is exemplified by the Notice itself. The Notice prominently states that

the Patent Office is interested in ensuring that the practical utility requirement is governed by standards that promote research, development and commercialization of technological advances in biotechnology.

It must be absolutely clear that this laudable interest is of secondary importance. The desire does not grant the Patent Office the right to create policy. Of primary importance to the Patent Office is fulfilling its constitutional obligation to promote science and the useful arts by following and implementing the law decided by the federal courts and legislature. Only when an issue is one of first impression and no direction is otherwise provided by the courts does the Patent Office have the right to extrapolate from the decisions otherwise rendered by the courts. By not adhering to the law, as observed by the inventors and the patent bar, the Patent Office creates confusion in the process of securing a patent which can lead to a variety of deleterious consequences: Notably, the trust and security inherent in the patent system has weakened and our competitive opportunities as a nation are threatened.

The Notice also raises an example of practical utility in the patentability of nucleotide sequences that are produced incident the expression of human gene. There is a concern as to whether the sequence or the gene must be characterized as to its physical, biological or physiological significance in order to establish practical utility. This statement misplaces the focus of the problem. First, the Patent Office must implement the decisions by the federal courts and not respond to concerns by attempting to create policy.

Secondly, the test of utility --

COMMISSIONER LEHMAN: Wait. I completely disagree with you about that. I don't think the federal courts have the right to make policy at all. The federal courts interpret the law. In fact, it's the responsibility of the Commissioner of the Patent and Trademark Office to make the policy regarding the operation of the office, and the responsibility of the Congress to establish statutory policies. I think you've got it all the way around -- completely the other way around. Otherwise, we wouldn't even be here if we didn't have any policy responsibility.

MR. GENS: I don't think that we're necessarily out of sync, Commissioner. The point is that the courts are trying to follow what legislature is giving them as the law. My argument today is that the Patent Office does not have the right to create law. It must follow the law that the court is trying to implement from the legislature that is passed on to them. And from the way that the Notice is written up, it is clear that the focus of the Notice is in terms of what is wrong elsewhere but at the Patent Office. And perhaps what we have to do is -- I'm advocating that we simply focus on trying to reach some common ground as

for what is being decided by the Federal Circuit.

COMMISSIONER LEHMAN: The reason I make that distinction is because I think the Court of Appeals from the Federal Circuit is hardly infallible, and it well may be that the Court of Appeals for the Federal Circuit will make decisions which this industry does not approve of. It may be that we feel that we should even go further than what the Federal Court of Appeals of the Federal Circuit currently is authorizing with regard to the utility standard furthering the industry's direction and that, indeed, we might as a matter of policy decide to test that all the way up to filing a petition for certiorari before the Supreme Court. So I'm just saying don't -- or go to Congress to seek legislative approval, if we feel that we can't, you know, get the court to agree with us. So, I mean, don't -- I'm just saying, don't place too much emphasis on what the Court of Appeals for the Federal Circuit says. That's why we're here.

MR. GENS: I see that my time has expired, but if there's definitely one recommendation that I can make before stopping that is that the PTO concentrate on trying to implement those standards by the Federal Circuit and allow the patent bar access to the legal training that is being made to the examiners. Open it up to the public.

The second point is in terms of opening up what the Patent Office is teaching to the examiners, give the patent bar a chance to influence that. Let's have a two-way communication between us in trying to establish a common ground so that we can avoid unnecessary patent appeals and use as a starting point at least some basis in what we both think the Federal Circuits are trying to say.

COMMISSIONER LEHMAN: Well, that's exactly what these hearings are all about, so thank you very much.

MR. GENS: Thank you.

COMMISSIONER LEHMAN: Next I'd like to ask Bill Kennedy of Morrison and Foerster to come forward, please.

BILL KENNEDY, MORRISON and FOERSTER

MR. KENNEDY: Thank you Commissioner Lehman. I'm Bill Kennedy an associate of Kate Murashige at Morrison and Foerster. I'm in the Palo Alto office.

BIO had asked Doctor Murashige to respond narrowly to question three raised in the context of application of the utility requirement in the USPTO Notice. Doctor Murashige is unable to attend today and asked me to stand in her stead, and I thank you for allowing me to speak today in her place.

Question three asks the following: "Do you believe legal standards and examining practices in foreign systems to assess the patent eligibility of biotechnological inventions, such as those governing industrial applicability and exclusions from patentability, provide a better framework than is available in the United States? Please identify desirable and undesirable practices of foreign offices, particularly in the EPO and Japan." And I will narrowly direct my answer in this limited time.

The utility requirement in the United States is the sole requirement for patentability that addresses the contribution made by the patented subject matter to the well-being and progress to the society as a whole; that is the real world value of the invention. This is addressed in Europe and Japan by requiring that the patented subject matter be susceptible to industrial application.

Additionally, the European Patent Convention, in Article 53, accepts from patentability inventions, the publication, or exploitation of which would be contrary to public order or morality. And also the European Patent Convention excepts from patentability plant or animal varieties or essentially biological processes for production of plants or animals.

Japanese patent law requires that inventions be usable in industry and additionally excludes patentability for inventions liable to be contrary to public order, health or morals.

In neither Japan nor Europe are methods for treating the human body considered to be an industrial application; however, patents may be obtained for the use of compositions whether those compositions be old or new in therapeutic regiments.

It is generally considered that the standard of industrial applicability is a much lower hurdle to overcome than the standard of utility as exercised in the United States Patent Office. This can be particularly apparent where a patent application is being examined in the U.S. Patent and Trademark Office both as a domestic U.S. application and as an international application under Chapter 2 of the Patent Cooperation Treaty. Indeed, it is common experience that claims are rejected in the domestic U.S. case for lack of utility. This comes often, by the way, under the guise of an asserted failure to provide an enabling disclosure. And yet those same claims, on the same specification, are considered to meet the standard of being susceptible to industrial applicability in the international application by a U.S. examiner who, incidentally, is often the very same examiner who authored the utility enablement rejection in the domestic U.S. case. Thus the kinds of data routinely rejected in the United States are routinely accepted in Europe and Japan. This is a scenario but does not bode well for American inventors in a global

economy.

Many problems in attempting to apply the standard of utility, as opposed to a standard of susceptibility to industrial application, are only exasperated by the dictum of the Supreme Court in *Brenner v Manson* to the effect that the invention must be developed to where specific benefit exists in currently available form, that benefit being one derived by the public. The question is: Who is the public? Does the public include that subset of the population actively engaged in research? And what is "currently available form?" Eventually, every member of the public will potentially benefit from the efforts of those members who are engaged in research. Is that benefit sufficiently direct that an immediate benefit to the research community can be considered to be a currently available benefit to the public?

There's no need to answer any of these questions where a standard of industrial application is applied.

The distinction can perhaps best be illustrated by an example. Claims are directed to new and unobvious compounds which tightly bind a known receptor that is present on a particular subset of white blood cells. Because the cells on which the receptor appears are often useful in conducting research to investigate the nature of human disease, the compounds are clearly susceptible of industrial applicability inasmuch as they can be used to purify these cells for a researcher to study. The applicant in this application further states that the compounds can be used to target these cells in therapeutic protocols.

There's no question that if sufficient proof were offered to show that the latter use had therapeutic value, the public would have a currently available benefit. However, showing that this is the case is quite difficult, as others have pointed out earlier today, and the inventor of the claimed compounds in this instance may be a basic researcher employed by a research institution. The inventor is not able personally nor through the research institution to do the necessary animal and clinical studies that would provide the therapeutic parameters and proofs that seem to be required.

On the other hand, of course, commercial entities that may have the capability to carry out such studies will not be interested in pursuing the matter unless they can be assured that the compounds themselves are protected. If the standard of industrial applicability were applied the use of these compounds for purifying the relevant cells for research purposes would be adequate to support the patentability.

In the face of the utility requirement, the question is raised above now introduce a complication -- is the provision of compounds that are, at this moment, of interest only to the research community a currently

available public benefit?

In short, the standard of industrial applicability removes the value judgment dimension of the utility requirement. The standard is easy to apply. The industrial applicability standard is easy to apply. If the material can be used for something that someone is being paid to do it meets the standard. Substitution of this standard for the utility standard would have the benefit of providing clarity in the law as well as removing an unnecessary barrier to the progress of research in biotechnology.

On the other hand, a requirement that the claimed subject matter not be contrary to public morals can introduce its own complications. The presence of this provision in the European patent law has already caused considerable mischief in the form of oppositions to patents granted on transgenic animals. The multiple oppositions that have been filed with respect to these patents have come not from competing commercial interests but rather from animal rights groups and others having particular political agendas. There is considerable concern in Europe that patent opposition provides an inappropriate platform for such agendas. The recent formation by the European Patent Office of a special panel whose approval would be required in addition to the decision of the Examining Division for the grant of a patent on a transgenic animal may have been in response to such pressures. It would clearly be undesirable to add such a provision to the statute in the United States.

A third clear difference between U.S. and Japanese or European law is the exclusion from patentability of methods for treating the human body. As a practical matter, the effects of this prohibition are minimized by the ability to obtain protection for medical use of compounds, and it will come into play only when the method of treatment involves manipulative steps only.

In summary, we feel it would be helpful to substitute for the utility requirement a standard of industrial applicability, but without excluding from patentability inventions that are supposedly contrary to the public order.

COMMISSIONER LEHMAN: Thank you. Is that something that you think would require statutory change or could we do that just by changing our policy protocols in the office?

MR. KENNEDY: Well, you raised earlier the difficulty that if you change your policy within the Patent Office you still may find yourself going up to the courts. It's difficult to say. A statutory change would effectuate it, if that were possible to bring about.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to ask Elizabeth Enayati, of Weil, Gotshal and Manges, to come forward, please.

ELIZABETH ENAYATI, WEIL, GOTSHAL and MANGES

MS. ENAYATI: Well, I was going to say good afternoon. I can't -- it's noon right now so I'll just say, hello, Commissioner Lehman and other distinguished panel members. Welcome back to California. My name is Elizabeth Enayati. I'm a patent attorney at the Silicone Valley office of Weil, Gotshal and Manges. As you may know, Weil, Gotshal is an international firm representing a broad base of both established and start-up technology companies, as well as universities. Our 20-member Silicone Valley office focuses on technology issues including patent prosecution and litigation. My practice includes biotechnology prosecution for universities and members of private industry.

I'm speaking this afternoon on behalf of the Biotechnology Industry Organization -- BIO -- the Intellectual Property Committee, of which my firm is a member. A detailed version of this presentation is submitted for the record by BIO and you have a copy I believe.

I'll be summarizing two sections of that report regarding operability, the introduction, including case law, and question number three regarding the foreign versus U.S. Patent Office practices relating to operability. The next speaker, Bill Scanlon, will be addressing the other two operability questions raised in that formal Notice.

I'll begin by summarizing the testimony you'll be hearing today from the BIO Intellectual Property Committee members on the issue of operability. Since the formation of the Food and Drug Administration, it's that agency and not the PTO that has the responsibility for ensuring that marketed therapeutics satisfy safety and efficacy criteria.

My personal experience, therapeutics and composition inventions that satisfy the statutory requirements for patentability are being rejected by examiners who cite a failure on the part of the inventor to prove operability, including the safety and efficacy of the claimed method and composition inventions. Often the rejection is based on the examiner's disbelief as to operability without supporting evidence despite the inventor's statements and declarations to the contrary. Such a practice is supported by neither the patent statute, nor the case law as I'll describe in a moment.

Operability of composition inventions should be satisfied by reasonable evidence of how to make the composition -- operability of method of using the composition should be satisfied by evidence of how to use the composition in a reasonably predictive model. However, examiners

presently are requiring proof of operability, including efficacy, of how to use a composition when only the composition is being claimed and no method of use is being claimed. By improperly rejecting therapeutics and composition claims, based on unsupported inoperability allegations, the U.S. Patent Office participates both in avoiding the constitutional mandate to promote the arts and useful sciences, and in weakening the efforts of a growing U.S. industry that is important to the competitive efforts of our nation in the world marketplace.

In support of its operability rejections, the U.S. Patent Office relies on a series of cases that were cited in the "Federal Register" notice for this meeting. A complete and accurate summary of those cases is found in the full BIO Intellectual Property Committee report made of record, and I won't be going through each of those cases in detail as I'm sure you're already familiar with them. But as an initial observation, the case law clearly requires that only one object of the invention be shown to satisfy the operability requirement. This could include in vitro or animal data. The burden rests on the examiner to provide evidence inconsistent with the inventor's assertion of operability. This is most clearly stated in *In re Chilowsky*, a 1956 case, and I'll quote: "Applicants for patents in this field --" referring to the biotechnology and human therapeutic arts, "as well as in all others are entitled to specific information as to the grounds on which their applications are rejected and should not be met with anything in the nature of a blanket rejection based on the comparatively recent development of the art and the difficulty which it has been experienced in producing commercial devices." That applies with equal force today as it did in 1956; however, examiners are continuously asserting that the inventor bears the initial burden of showing operability.

Operability is also recognized as an element of the disclosure requirement under 35 U.S.C. Section 112, first paragraph. Again, under that approach, the case law cited by the PTO in this Notice of this meeting supports the premise that the truth and accuracy of statements in a patent application are presumed unless the PTO can establish, using evidence provided to the applicant, that the statements are not true and accurate.

It's become common practice -- and my personal experience from the Patent Office -- to require human clinical data in support of therapeutic claims even when in vitro or animal data is provided in a specification. This frustrates the public policy, often expressed by the courts, in early filing of patent applications. For example, one population of inventors who are significantly impacted by this practice is the population of inventors practicing at universities. If early stage research is not patentable, because they don't have human clinical data, then applications cannot be filed; papers cannot be published; or universities lose their rights. The net effect is a distinctive

disincentive to publish and exchange scientific ideas, which ultimately stifles the progress of useful science instead of promoting the progress of useful science.

In addition, these inventors and universities then cannot find commercial partners to take early stage research into commercial product if that commercial partner has uncertain or no possibility of a proprietary position. Thus products are hindered from reaching the market by the Patent Office practice of requiring human clinical data. By maintaining its position requiring that such human clinical data in support of therapeutics' claims, the Patent Office flies in the face of case law precedents, such as *In re Langer* which held that clinical testing in humans is not required in view of *in vitro* data. In *In re Jolles*, a 1980 case that was discussed earlier, specifically rejected the patent office position that animal data was not relevant to establishing utility, and in *In re Hardtop* the court rejected the Patent Office requirement for human data despite it presented animal data. And *In re Malachowski* non-human data was found by the court to be sufficient to establish utility for use in humans. Although the Board in *Ex parte Balzarini* affirmed the rejection of the claims presented in that case, the Board again stressed that human clinical data would not necessarily be required. Clearly the courts and the Board have continuously stated that human clinical data is not a prerequisite to patenting therapeutic inventions.

Finally, the Patent Office is rejecting claims based on the applicant's failure to present safety and efficacy data from human testing despite case law precedent to the contrary. And *In re Anthony* the court clearly states, and I quote: "Congress has given the responsibility to the FDA, not to the Patent Office, to determine in the first instance whether drugs are sufficiently safe for use that they can be introduced in the commercial marketplace." This is further supported by several cases that due to the time limitations I won't recite.

We respectfully remind the patent office that it is an administrative body charged with issuing patents that satisfy the requirements set forth in the patent statutes. If the patent statutes do not address certain concerns that the public believes are necessary to protect its interests then it's up to Congress to change the statutes which govern the actions of the Patent Office.

Turning now to question three of the public Notice: "Do you believe legal standards and --"

COMMISSIONER LEHMAN: Can I just as a question to clarify something I think is important.

MS. ENAYATI: Sure.

COMMISSIONER LEHMAN: In other words, if we knew for a fact that a particular therapy for which a patent application had been made would have a very toxic effect on individuals and might kill them, that even if that were the case, as long as it otherwise operated against the particular virus or whatever it might be involved and did away with it even though it might kill the patient in the process, it would still meet the utility requirement?

MS. ENAYATI: That's right, and I think that's consistent with what Bill Epstein said previously. And also with the courts, with the case law, where it said if you show one object of the invention then you satisfy the utility requirements for patentability. Now, whether it reaches market or not is a different issue. It also -- Well, I'll leave it at that.

MS. LINCK: I also have a question for clarification. It was my impression from what you said that you've had some experience with examiners requiring human test data but that the Board, in fact, has gone the other way. Is that your experience? Is my understanding --

MS. ENAYATI: That is my experience. I have had examiners say that they won't accept the animal data or they don't accept the animal model that we've presented as being a valid model that shows that this treatment would actually work in humans, and they've required human clinical data in view of a valid animal model data.

MS. LINCK: But that's been reversed whenever you've taken it to the Board?

MS. ENAYATI: I personally have not taken it to the Board, no. So those decisions, we haven't got that far. I haven't had a hearing date yet.

MS. LINCK: All right. Thank you.

MS. ENAYATI: But they are on appeal.

COMMISSIONER LEHMAN: But to follow up on that. What you would like us to do is make it clear to the examiners that animal data are acceptable. Period. That they do not require the human data.

MS. ENAYATI: That's right.

COMMISSIONER LEHMAN: And also to make this other point clear, that it's not their -- that, you know, in effect, the marketplace, the FDA approval being part of the marketplace, will make a determination as to whether the patent is usable or not. Their only purpose -- function -- is is this an invention that kills the particular virus or whatever it may be

that's involved?

MS. ENAYATI: Right.

COMMISSIONER LEHMAN: If it kills that virus it, you know, might kill the person along with it. Might even kill the animal along with it, I suppose. But if it kills the virus then it's, you know --

MS. ENAYATI: It's met the standards of patentability.

COMMISSIONER LEHMAN: Okay. Thank you.

MS. ENAYATI: Okay.

Question three, the legal standards and examining practices in foreign systems, whether they provide a better framework than is available in the U.S. for assessing patentability questions related to operability of inventions for treating human disorders. We believe the answer to that is, "Yes." Both European and, in certain circumstances, Japanese patent laws are applied more favorably and highlight the unnecessarily strict standard of operability imposed by the U.S. Patent Office at this time. Although the laws do not differ significantly in this area, the application of those laws to the examination of biotech patent applications does differ dramatically.

As applied under European patent law, any reasonable statement regarding the operation of an invention is readily accepted. In vitro data generally is sufficient to demonstrate operability of first or second medical uses of a therapeutic composition in human therapy. In some cases a believable, enabling, but "prophetic" description, absent any in vitro or animal data, is acceptable to support claims to a first or second medical use of a therapeutic composition.

Under Japanese patent law inventions to human therapeutics are separated into two classes: Generally, new compounds and then pharmacal compounds with use limitations.

Under the first category, chemical compounds per se, a working example showing that the compound was prepared is required, but no experimental evidence of use is required.

Under the second class, the pharmaceutical compositions with a use limitation, for example, composition for treatment of cancer, in vitro data is acceptable if it's directly supportive of the claimed use. So, for example, if the claim is to pharmaceutical compositions for treating cancer cells then in vitro data demonstrating that the composition kills cancer cells is sufficient.

To conclude, in foreign countries competitors and regulatory agents do not rely on the Patent Office to determine if an invention is operable. The U.S. Patent Office for human clinical data in support of therapeutic use claims prevents the flow of human therapy inventions to the next level of research and ultimately to the marketplace. Consistent with both the European and Japanese patent system, once a threshold of operability has been demonstrated, which may be achieved using in vitro data, the other regulatory agencies charged with regulating the release of drugs and products to the market should make the next level determination of efficacy and safety in operability. Thank you.

COMMISSIONER LEHMAN: I don't want to drag this on, but, you know, all cases -- it seems to me that in all cases you would have in vitro data available, wouldn't you? I mean, otherwise, you wouldn't know whether you had any invention or not.

MS. ENAYATI: Right.

COMMISSIONER LEHMAN: I mean, you wouldn't know whether you had anything that worked, you'd just have an idea.

MS. ENAYATI: That's right. And so what we end up engaging in is a discussion with the examiner whether this is sufficient in vitro data and whether they accept the results of the in vitro data, which is contrary to what the case law seems to be saying.

COMMISSIONER LEHMAN: Thank you very much.

MS. ENAYATI: Thank you.

COMMISSIONER LEHMAN: Next I'd like to call on William Scanlon, from Foley and Lardner.

WILLIAM SCANLON, FOLEY and LARDNER

MR. SCANLON: Thank you, Mr. Commissioner, for the opportunity to speak on this important issue today.

My name is William Scanlon. I am a partner with the firm of Foley and Lardner in its Madison, Wisconsin office, and I'm a member of the firm's biotechnology practice group. The firm represents organizations -- both business organizations and research organizations nationwide.

I've been practicing patent law for 14 years and for the last 12 and a half years the bulk of my practice has been biotechnology. My presentation here is on behalf of the Intellectual Property Committee of BIO, the Biotechnology Industry Organization, not the Association as it says in the program.

I will address briefly questions one and two under part B of the Commissioner's Notice. My presentation is detailed in much greater detail in the BIO paper, which is of record in the hearings here, at pages 61 to 72. Part B of the Notice relates to proof of operability for inventions involving treatment of human disorders.

Question one. Are the legal standards for proof of operability in those situations clear and appropriate?

Question two relates to PTO practices and asks whether the practices are consistent with the law, if not, what are the problems and, if there are problems, what solutions might there be.

As Ms. Enayati just described, the legal standards are clear and we believe appropriate with regard to proof of operability. The problem is not in the legal standards. The problem at present is in the PTO's implementation of the law relating to operability and the imposition of requirements that the courts have rejected long ago under the rubric of operability.

The Commissioner's Notice reflects the problem. The Commissioner's Notice suggests some distinction between, quote, "utility," close quote, and "operability," close quote. This is the distinction that the law does not recognize.

The Notice suggests then, under the rubric of operability, various requirements and policy concerns that are also not part of the law. Safety and clinical efficacy are not proper concerns of the Patent and Trademark Office. Whether the grant of a patent would mislead patients or the public is not a proper concern of the Patent and Trademark Office. The law allocates these concerns to other agencies; the Food and Drug Administration, the Securities and Exchange Commission, the Environmental Protection Agency, the Department of Agriculture, and various state agencies with similar responsibilities, to mention a few.

In the context of inventions for treating human disorders, rejections for inoperability have become routine. I believe it's fair to say that most of these rejections are improper.

Proof of operability is required only if the utility set forth in the application, as of the effective filing date of the application, is incredible or unbelievable to the person of skill in the art. Examiners must be able to establish this credibility or unbelievability with reference to technical data that was available in the art on the date the -- the effective filing date of the application.

The assertions in an application regarding utility must be accepted as true by an examiner unless the examiner has some good reason, again,

based in the technology available on the date -- the effective filing date of the application to prove otherwise.

Now, a claim to a method of curing -- emphasize curing -- a disease, which the art on the effective filing date recognizes as incurable, might well be properly rejected for inoperability, and the applicant in that situation might be put to the test with appropriate data, not necessarily clinical but appropriate data, to show that, in fact, you can cure the disease the art considered to be incurable. But this is different from a method of treating a disease that is incurable. Generally, such a claim would not be inoperable. We treat many incurable diseases: Grave's disease, diabetes, AIDS, to mention a few.

Now, even in cases where proof of operability may be required, human clinical data may not usually be required in accordance with the law, although the PTO itself recognized in the Balzarini case that is addressed in our paper here and that Ms. Enayati mentioned. But now de facto human clinical data are being required in the human therapeutic area.

In our text, at pages 69 to 71, several different practices now in the biotechnology group are described that establish de facto requirement for human clinical data. The requirement for human clinical data is, in fact, wrong as a matter of law in most cases, and it's a travesty as a matter of policy for reasons that have been explained repeatedly here already today in terms of slowing the availability, even denying the availability of new therapeutic methods to the public.

Now, what might we do to correct the problem? Well, we think to begin with PTO policy has to change. The PTO, and Group 180 at least, must recognize its job is to grant patents; it's not to enforce FDA regulations or the SEC rules. Also, we think that better training of the examining corps, particularly with respect to legal standards, would be appropriate. And BIO does support providing to the Patent and Trademark Office the resources it needs to provide such training.

Thank you very much.

COMMISSIONER LEHMAN: Thank you very much. I appreciate that.

Next I'd like to call on Stanley Crooke of Isis Pharmaceuticals to come forward, please.

STANLEY CROOKE, M.D., ISIS PHARMACEUTICALS

DOCTOR CROOKE: Good afternoon. I am Stan Crooke. I'm the founder and chief executive officer of Isis Pharmaceuticals, a development stage, technology based pharmaceutical company. Prior to founding Isis, I was

president of R&D for SmithKline Beecham, and before that a vice president of Mr. Myers. I'm a physician and a scientist and in my career I've been involved in the development of more than 15 drugs that are currently marketed, and numerous other drugs in development. I've published more than 300 scientific papers and 16 books on pharmacology and drug discovery and drug development.

What I want to do today is address a single issue and that is the issue of therapeutic utility. As a practitioner of the science, I'd like to address it from the scientists' perspective and conclude with what I think are some reasonably practical recommendations for what the patent office might do.

The issue reduces to one simple question: What data are sufficient to support claims of potential therapeutic utility? Although there is no universal or, in my view, no absolute right answer to this question, I believe there are precedents that have stood the test of time that can provide guidance and can serve as a basis for reaching agreement. The problem and the reason that we're here on this issue is, of course, that the evaluation of the utility or potential utility of an innovation in the pharmaceutical industry is especially complex.

On average, more than 15 years elapse between the discovery of a new drug and its marketing and, depending on which numbers you accept, somewhere in the range of \$150 to \$350 million has to be invested in that drug before we know whether it works. It would be impossible, and it is impossible, for a company to make this kind of investment without reasonable expectations of patentability, particularly in light of the risks of investments in this area. We know that historically less than one in a thousand compounds synthesized and patented by the pharmaceutical industry has actually succeeded in becoming a drug.

Well, of course, many factors contribute to the risks and uncertainty in drug discovery and development, but I want to focus on one particular problem that I think is particularly germane. And that problem is I think the only way to determine the value of a new pharmaceutical technological innovation is to evaluate the fruits of that innovation. That is to evaluate the activity of drugs that come from that innovation in human beings in detailed clinical trials that require these many years and many millions of dollars.

As an example, let's consider the history of the treatment of ulcer disease. It's been known for many decades that ulcer disease is correlated with stomach acid secretion, and a large number of physiological processes have been identified that influence stomach acid secretion. Early on, the coallergic arm of the autonomic nervous system was shown to increase acid secretion so it was hypothesized that anticoallergic drugs would have therapeutic utility in this disease.

Literally thousands of such compounds were made; they were tested and they were patented. A few of them were even marketed, but the side effects of these drugs were very limiting and so the true therapeutic utility of anticoallergic drugs for ulcer disease was modest at best.

Of course research continued, other factors were identified that influence acid secretion, including histamine. This led to the notion that blockade of a specific receptor for histamine, the H2 receptor, might have therapeutic value. This was controversial. It was hotly debated but research continued and, in fact, the first H2 antagonist that reached the clinic, the Thiamide, failed. But the second succeeded, and that was Tagamet. It revolutionized ulcer treatment. It led to thousands of other H2 antagonist being created and a number of other products. Then came the hypothesis that the inhibition of an enzyme, hydrotopassium ATPaz might be beneficial because this enzyme is thought to actually secrete the acid into the stomach. Again, a very controversial hypothesis. Many compounds were tested, synthesized, patented. All failed, essentially, except for one: Amepresol. Amepresol ultimately was marketed and, again, has made a dramatic improvement in the treatment of ulcer disease.

Well, the points I want to make by reciting this well-known history are several and I think they are very important.

First of all, new concepts and approaches. That is new technologies that might result in therapeutic innovations arise constantly.

Second. Only after a drug, based on a particular mechanism or based on a new concept, has been shown to work in the clinic has the technology been validated.

Third. It is a normal part of the process that some members of a particular class of drugs fail, yet the basic concepts that support the creation of these broad innovations have proven to be valid.

Fourth. Innovations are by definition controversial. And the controversies in this area are not resolved until clinical data -- until the compound is tested thoroughly in the clinic.

Fifth, and perhaps most importantly. Despite these complexities, the traditional practices of the PTO result in effective stimulation of innovation and enormous public benefit. Those traditional practices were to allow claims of reasonable scope long before definitive proof of utility was obtained. This was done by accepting reasonable evidence of potential utility and by maintenance of an attitude that was biased toward rewarding and encouraging innovation by giving the benefit of the doubt to new concepts.

I think, as you've heard from several speakers very recently with regard in particular to patents in biotechnology, we feel there's been a substantial change in the practices of the PTO demands for definitive proof of therapeutic utility that resulted in many patent application rejection.

Let's look at the potential impact of this change in the patent environment. In other words, what will happen if the PTO were to continue to require definitive proof of utility before granting therapeutic use claims? What will happen is absolutely simple and absolutely clear: New drug innovation will be dramatically impeded. So the risks of continuing this practice I believe are very large. I believe the risks of relaxing the policy are really quite negligible. The drug discovery, development and commercialization processes have built-in self-regulating mechanisms that ensure the drugs that should fail do.

So, what harm is done if a patent with appropriate claims is granted to a drug candidate and the drug or the technological concept behind the drug fail? Well, of course, the company that's invested in that drug or that technology loses its investment, but that's the risk it takes. Nothing has been lost by society. In fact, the public actually gained from the exploration of the concept of the technology.

On the other hand, if companies stop investing in new compounds derived from novel concepts or technologies, because of patent uncertainty, everybody loses and I don't think the loss will be made up. So what am I actually recommending?

First. Return to traditional practices with regard to pharmaceutical patents. They worked in the past; they'll work in the future; they will work now.

Second. Return to a positive bias to innovation with an acceptance of reasonable proof of potential -- of potential -- utility.

Third. I think you should treat patents from so-called biotechnology companies and pharmaceutical companies equally. We're the same industry, with the same customers, practicing similar science.

The traditional approaches of the PTO were sufficient to stimulate investment in innovation in both sectors of the drug discovery and development-based industry and they would be going forward.

Fourth. I think you should emphasize consistency across and within technological areas. Just because one approach is labeled "new technology" and another is not, does not mean that the basic approaches or risks are necessarily different. Even the most traditional of

technologies, most of the compounds generated by those traditional technologies will fail.

Fifth. When in doubt, grant therapeutic claims based on specific examples provided in the patent application with scope commensurate with reasonable extrapolation from the examples provided.

Finally, I would urge you to be equally or even more prudent in the allowance of broad concept patents. Allowance of reasonable claims based on examples provided in patent applications is sufficient to stimulate innovation in this area. Granting broad concept patents I think is very rarely justified in the pharmaceutical industry.

With that I'll stop and answer any questions that you have.

COMMISSIONER LEHMAN: Your testimony was so clear that I don't think we have any questions.

DOCTOR CROOKE: Good for you.

COMMISSIONER LEHMAN: Thank you very much.

DOCTOR CROOKE: Thank you.

COMMISSIONER LEHMAN: Next I'd like to ask Vincent Gioia, from Christie, Parker and Hale, to come forward.

VINCENT GIOIA, CHRISTIE, PARKER and HALE

MR. GIOIA: Thank you for giving me the opportunity to make these remarks. My name is Vincent Gioia. I'm a partner with the law firm of Christie, Parker and Hale, in Pasadena, California.

I'm here to request the Patent and Trademark Office to consider recommending amendment to the plant Patent Act, to extent the grant to parts of plants. Under 35 U.S.C. 161 and 163, inventors and discoverers of new plant varieties are granted the right to exclude others from asexually reproducing plants of the new variety or selling or using plants so reproduced.

The law does not expressly apply to cut flowers and fruit of plants of patented varieties. Consequently, fruit or cut flowers of a patented variety may be imported with impunity to the considerable economic disadvantage of plant patent owners, their licensees and the labor force of the industry adversely affected.

It is in the public interest to close this loophole so that owners of U.S. plant patents and those engaged in commerce in patented varieties

will not suffer unfair competitive disadvantage. Correction of this inequity only requires a modest amendment to 35 U.S.C. 163 to make it clear that in the case of a plant patent the grant shall be the right to exclude others from asexually reproducing plants of the patented variety or selling or using the plants so reproduced, or any part thereof.

There's presently very significant importation of parts of plants, such as fruit and cut flowers of plants of patented varieties, into the United States. Illustrative of the extent and impact of importation of products produced by plants of U.S. patented varieties grown outside the United States is the situation with cut roses.

Cut roses was the subject of a report by the United States International Trade Commission in April 1989. In this report -- summarizing the impact of the cut flower importation -- the Executive Summary of the report states: "The U.S. fresh cut rose industry has steadily lost market share to imported roses over the last decade. In 1985 imported roses accounted for 26.5-percent of the U.S. apparent consumption of roses. By 1988 imports had increased their share by over 40-percent accounting for 37.9-percent of apparent consumption."

The Executive Summary goes on to state that, "Although sales of fresh cut roses increased by 10-percent, during 1985 to 1988, the total growing and operating expenses increased faster and, moreover, the number of firms reporting losses increased from 31 in 1985 and 1986 to 36 in 1988." Again, according to the Executive Summary, those firms reporting losses represented almost 38 percent of the growers. It is logical that a similar impact on domestic industry has occurred with respect to other plant parts, such as berries and other fruit, nuts, and fiber.

A copy of the Executive Summary, which I've just referred to, is appended to the report which I submitted for these hearings. A full copy of the ITC report is also available upon request from me or from the ITC.

It is obvious that the impact on domestic industry and those employed by the industry is enormous. Most people working in the agriculture industry are low wage earners without training and skills required for other employment. The adverse impact of the expanding imports of flowers and fruit of U.S. patented varieties is measured by these people in loss of opportunity in jobs and a lower standard of living.

Although the U.S. Supreme Court and U.S. Patent and Trademark Office Board of Patent Appeals and Interferences have agreed that utility patent protection under 35 U.S.C. 101 should be available to plant inventions, in practice, asexually reproducible plants are not able to qualify for utility patents. Even though it has become acceptable to satisfy enabling requirements of 35 U.S.C. 112 by providing a deposit of

biological material in a suitable depository, the fact is that it is not feasible, practical or, indeed, technologically possible at the present time to provide a deposit of biological material of asexually reproduced plants in a manner which would satisfy present U.S. Patent and Trademark Office policies and practices.

Therefore, the Plant Patent Act remains the only viable means for protecting new varieties of plants -- of asexually reproduced plants. Failure to extend this protection to the parts of the plants is not only unjust but would result in a continuation of the severe damage to domestic industries as indicated in the ITC report.

In order to extend full protection of the patent law to inventors and discoverers of new plant varieties, amendment of 35 U.S.C. 163 is required to extend the patent grant to parts of plants. This may be done by a simple modification such as the following:

In the case of a plant patent, the grant shall be the right to exclude others from reproducing plants of the patented variety or selling or using the plants so reproduced, or any part thereof. This suggested amendment not only clarifies that the protection extends to parts of plants but would also make 35 U.S.C. 163 consistent with 35 U.S.C. 161, which states that whoever invents or discovers and asexually reproduces any distinct and new variety of plant may obtain a patent there for.

Clarifying the rights granted the plant patent holders, as suggested, is necessary to avoid unfair competition from abroad and would place owners of plant patents in a position comparable to the holders of utility patents.

Thank you very much.

COMMISSIONER LEHMAN: Thank you. Is there a trade association that represents people that you're talking about?

MR. GIOIA: I've been in the practice of plant variety protection for over 30 years and I've represented breeders of new varieties, almost all phases of the plant industry. There are a couple of trade associations, most particularly the Roses Incorporated, which is a trade association of cut flower growers. There's also a national association of Plant Patent Owners who is expressing a similar position as I've described in this issue, and one which I would also endorse and probably they would endorse my comments as well.

COMMISSIONER LEHMAN: Well, the reason I asked that question is because to the extent that you're talking about statutory change, which you are, and we had recent change in the Plant Variety Protection Act to implement an international treaty just recently. It really helps to have some

group that represents, you know, sort of common interests.

MR. GIOIA: Yeah. I would like to mention in that regard that the Plant Variety Protection Act is applicable to sexually reproducible varieties. Asexually reproducible varieties are protected under the Plant Patent Act so, therefore, the changes that you've mentioned that have been proposed for the Plant Variety Protection Act, primarily to bring us into compliance with the treaty, newly proposed convention of UPOV, won't have any effect at all on the Plant Patent Act.

COMMISSIONER LEHMAN: But we could have used that vehicle, that's what --

MR. GIOIA: Possibly we could have. Your announcement of these hearings, however, raised the opportunity to present this issue to the Patent and Trademark Office along with this request for your assistance in making these recommended changes to the Plant Patent Act.

COMMISSIONER LEHMAN: Is there any opposition that you know of to this?

MR. GIOIA: I know of no opposition to this unless it may be from the importers of the pirated plant patented varieties who are exporting from countries in which no protection is available to breeders the cut flowers and other plant parts into the U.S. market for consumption here.

MS. LINCK: Does the American Rose Society take a position on this issue?

MR. GIOIA: The American Rose Society is an organization of amateur rose growers. They're not typically representing the industry, although industry -- members of the industry are active in the American Rose Society. I am a past president of the American Rose Society and also of the World Federation of Rose Societies, which is the international group composed of the national rose societies and they typically do not take political positions on legislature.

MS. LINCK: Thank you.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to ask Mark Toohey, from Spencer, Frank, and Schneider, to come forward, please.

MARK TOOHEY, SPENCER, FRANK, and SCHNEIDER

MR. TOOHEY: Thank you, Commissioner Lehman.

My name is Mark Toohey. I work with Spencer, Frank, and Schneider. I'm

a registered agent at this point in time finishing up my law degree. I have a doctorate in biochemistry and as part of my legal degree requirements I was studying the issue of practical utility so these hearings came at an opportune time for me. I have extensively studied the case law with respect to both 101 and 112, and I have been particularly interested in the pharmaceutical inventions.

I've come to the conclusion that the Patent and Trademark Office is wrong to maintain the policy they have in rejecting certain classes of biotech inventions on the basis of a lack of practical utility. The Patent Office is wrong because it's narrowly reading the statute under Section 101. The Patent Office is wrong because it's misinterpreting the decisional law and it's wrong because I don't feel it even has the jurisdiction to be in this question.

In misinterpreting the statute, the office is following the Brenner v Manson decision in 1966. That decision is really directed toward a question that was first asked in In re Brenner and the question is whether or not someone must state a utility in their application. The court and the office agreed that the utility must be stated. The Manson applicant did not state the utility and so the invention was found not to be patentable to him. It was unpatentable not because of the class of the invention, which is a pharmaceutical, and not for the use, which is in humans. It was unpatentable because of a procedural error in not alleging utility. Yet the office relies on Manson in its rejection of these biotech inventions.

The Manson majority also narrowly construed Section 101. The court later corrected itself, Diamond v Chakrabarty. In Diamond v Chakrabarty, Judge Rich's appellate decision was followed. There Judge Rich tells us that the House Committee reports say that Section 101 is directed to anything under the sun made by man. The PTO has never corrected itself. Biotech inventions are under the sun and they are made by man. They are useful.

Judge Rich also tells us that Section 101 is the first door to patentability. Section 102 is the second door, and it says the applicant is entitled to a patent unless certain statutorily defined criteria are not met. There is a presumption of patentability. There is a presumption of enablement under Langer. There is a presumption, I believe, under Section 101 of utility also.

With regard to the question of statistically significant clinical trials. That is not the law. Case after case says that is not the law yet the Patent Office or the examiners in the office continue to assert that that is the law. Since the 1961 Krimmel decision, it's consistently been held in the CCPA and also in the Federal Circuit that standard experimental animals and the usefulness there is sufficient proof of

utility. That should be so. That is the industry standard. And now more often in vitro tests is an industry standard. Animal tests are sufficient in the EPO. Animal tests are sufficient in the JPO. I routinely run across the situation where I have applications, the claims of which are allowed in the EPO, allowed in the JPO and blocked in the USPTO. I don't understand why we have this policy.

We would assume the Patent Office would try to promote the industry. We've heard this morning how it's not particularly helping in this utility issue. We would think it would encourage the use of animal models because they're economical, they are reliable -- to an extent -- and they are less dangerous. Why have human data necessary? We think the Patent Office should -- or I think the Patent Office should also encourage in vitro tests. There's a benefit to the public there that goes beyond the animal models. That is there are some parts of the public who are concerned about the ethics of using animal experimentation. In vitro tests would allay those concerns.

The Patent Office has also argued in the past and continues to argue that it must be extremely cautious in issuing patents intended for human use. The office relies on a 1957 decision of *Eisenstadt v Watson*, where the judge expressed concern about the official imprimatur, as is note in the Notice of these hearings, that is associated with a patent, whether rightly or wrongly, and would leave the unsophisticated and the public to believe that the invention works in each and every way described in a specification. But this argument misses the point, which has already been brought up this morning, that the official imprimatur does not give the public anything. The public cannot have access to the drug without approval by the FDA, EPA, USDA and other regulatory authorities. That brings me to the point of jurisdiction. As early as the 1962 case *In re Hardtop*, the court recognized that jurisdiction in the issue of safety and efficacy belongs with primarily the FDA. The Patent Office has never gotten to that decision.

One need only look at Title 21 of the United States Code, the Food, Drug, and Cosmetic Act, and all the implementing regulations under that Act, to see the FDA has jurisdiction on safety and efficacy issues. If the Patent Office is disregarding the court decisions, it's disregarding these statutes and it continues to assert jurisdiction on the basis of a strained interpretation of a single word -- utility -- in the patent statute. I believe the Patent Office should back off in this regard. I think it should leave the question of safety and efficacy to the FDA. They are the ones who have the expertise to determine safety and efficacy, not the Patent Office.

I will stop here in the interest of time.

COMMISSIONER LEHMAN: Thank you very much, Mr. Toohey.

Next I'd like to ask Allen Dow, from Klarquist, Sparkman, Campbell, Leigh and Winston to come forward.

ALLEN DOW, KLARQUIST, SPARKMAN, CAMPBELL, LEIGH and WHINSTON

MR. DOW: Thank you, Mr. Commissioner. I'm an attorney with the law firm of Klarquist, Sparkman, Campbell, Leigh and Winston, in Portland, Oregon. And even as far northwest as Portland our public officials have thought of biotechnology as well as other high technology fields as undergirding economic development for the coming several decades, so this is of great interest to our region as well.

My position in the schedule has reduced my talk, in large part, to a series of ditto marks, so if I stumble and pause for a moment you can utter yourself the ditto. I won't do that myself. That's especially in regards to the citation of case law.

I'd like to respond in part to the Commissioner's comment regarding the presumption of utility and again harken to other areas of technology, like electrical and mechanical cases. In this regard, I do have a cite that I haven't heard before from the attorneys who have preceded me, and others discussing the utility requirement, and that is the dissenting opinion by Judge Smith in *In re Joly* where he said that the application of the practical utility requirement to -- in that case chemical pharmaceuticals -- if that same standard had been applied to mechanical inventions, for example, or electrical inventions, would have rendered unpatentable the Wright Brothers airplane, the Bell Telephone, the Morris Telegraph, and Edison's light bulb at the stage of development at which they actually reached the Patent Office.

Now, I flew here in an airplane. I dozed much of the flight. I found it safe and effective. I may not have had that same experience if I had flown that 120 feet at about 12 feet of altitude with the Wright Brothers in their first airplane.

I also think the case law, Chilowsky, Langer and others, also support the notion that utility should be presumed, at least until the examiner can cite to relevant scientific data that undergirds the argument of asserted utility.

More generally, in cases in which a pharmaceutical utility is asserted, it just blocks patentability altogether. I've heard a couple of times from panelist here, the solicitor in particular, a request that we simply appeal to the Federal Circuit to get clarification of the case law. And what may be, as the gentleman from Hoffman-La Roche indicated, a roadblock for Hoffman-La Roche just stops small biotechnology companies and universities dead in their tracks. They simply cannot mount the

funds to attack through the Board of Appeals to the Federal Circuit.

It's also been stated that the standards of utility, as applied by the Patent Office, may wish to be reserved for the Supreme Court if the Federal Circuit were to find otherwise, and we believed that the likelihood of certiorari being granted by the Supreme Court is simply dismal if we were to try to take the same steps.

MS. LINCK: I guess at this point I'd like to interrupt because, based on what I've heard, it sounds to me like we don't have a lot of Board of Appeals' cases that are inconsistent with the standards that we are trying to apply and we believe that you want applied. So I guess at this point I would say, you know, at least get them to the Board, and if you can cite me cases where the Board has been inconsistent with the standards that you are espousing, you know, I would appreciate that as well.

MR. DOW: I'm happy to do so in my written comments. I haven't really prepared a brief of recent Board decisions. In fact, my basic point was an appeal for the Patent Office to simply apply the standards announced in the CCPA and Federal Circuit cases already discussed here today. It's simply the case -- even an appeal to the Board for clients of ours, such as the Oregon Health Sciences University or Oregon State University and others, is simply an investment that they really feel they can't make and so we really need justice at the level of patent examination. Deep pocket justice won't help us. We've had several cases in which we've gone to the client and actually offered -- in one case in particular -- actually offered to do this as a pro bono effort and, in this case, the university said, "I'm sorry. We just really don't want to go forward. If the Patent Office is going to consistently reject this kind of claim, we're just going to have to stop here."

MS. LINCK: Again you're in an area, emerging technology, and it's difficult to apply case law in well established technologies to cases in emerging technologies, and we do the best we can. But if you stop at the examiner level when we're struggling to do the best job we can, it's very difficult for us to identify the errors that are happening consistently. So that's why --

MR. DOW: We're certainly going to do our best, Madam Solicitor, to encourage our clients to use our services in appealing to the Board.

MS. LINCK: I appreciate that. Thank you.

MR. DOW: You're going to hear some dittos, and I just want to pass through the case law to some experiences I've had in actually trying to prosecute patent applications. I think in one case in particular of a case in which human pharmaceutical utility wasn't asserted but rather a utility as an animal vaccine and, in this case again, we ran into 101,

112 rejection based on lack of utility because we hadn't shown that a herd receiving a vaccine would necessarily be able to mount a response to the contagion. And in this case the claim that was rejected was a DNA claim, and I just -- again, one of the comments from the panel has been that much of the I suppose high-pitch whining of the patent bar has been anecdotal at best.

I just want to toss one more anecdote into the ring. In this case, up through four or five office actions at a cost to our client of tens of thousands of dollars, the claim continued to be rejected as a -- for lack of utility even though it was a DNA claim and other utilities had been asserted in the specification, for example, as probes and so forth. And it just seemed, to me at least, abusive in that particular case.

I will say, too, that it seems to be beyond the level of an individual examiner. In this case, due to restriction requirement, there were at least three applications before the Patent Office and we received almost identical rejections in all three cases, which appeared to me to be less a sign of individual examiners being unreasonable than of examiners of a group representing some kind of policy, if you will. And that troubled me more than individual examiner stories, which I think we're going to encounter in any practice group and on any given day.

Let me simply stop by again making my plea that the decisions of the CCPA and the Federal Circuit be applied consistently and fairly to applications in this field.

Thank you very much.

COMMISSIONER LEHMAN: Can I just ask one question. Are there any circumstances under which you think that -- would you say that human clinical data would never be required to prove utility?

MR. DOW: "Would never be required to prove utility." I had actually prepared a short list which I nixed out but now I have time for that. If I could just run through five instances where I think the legal standard would support not requiring human data.

First. When those skilled in the art attest under oath that the test data offered by the applicant shows pharmaceutical utility, that really should be legally sufficient.

Second. Where there's been proof that the animal model used is one commonly used by those skilled in the art. Now, this is a problem especially in biotechnology. I think in *Cross v Iisuka*, for example, not to require human data is clearly allowed, if you will. And that for many biotechnology inventions there's no such thing really as a standard experimental model, simply because you have a new class of drug. A

brand-new class of drug. And I think there once you simply looked at the literature, what are people using as tests to determine the pharmacological activity of the drug at question. I really don't believe the public will be visited with any great ill as a result of a disclosure, perhaps premature, of pharmaceutical utility where the FDA and the market get to take their shot.

Also, finally, licensing is I think a clear sign of utility; a company being formed to exploit the technology and the fact that a party has undertaken the expensive human trials, notwithstanding the lack of a patent or at least during the pendency of the patent.

COMMISSIONER LEHMAN: Thank you very much.

MR. DOW: Thank you.

COMMISSIONER LEHMAN: Next I'd like to ask Ned Israelsen, of Knobbe, Martens, Olson and Bear, to come forward, please.

NED ISRAELSEN, KNOBBE, MARTENS, OLSON and BEAR

MR. ISRAELSEN: Thank you, Commissioner, distinguished panelists. My name is Ned Israelsen. I'm a patent attorney with the firm of Knobbe, Martens, Olson and Bear. I head up a 12-person biotechnology group and my clients include several biotechnology companies, universities, private research institutions and the National Institutes of Health. In addition to sharing my personal views, two of my San Diego biotechnology clients -- ViCal, Inc., and Alliance Pharmaceutical Corp. -- have asked me to present testimony on their behalf.

As most of the other speakers have, I would first like to touch on the issue of utility and enablement; the rejections that we're getting under the broad heading of operability. And, then, time permitting, I'd like to touch on a few new points that haven't been raised much; the average pendency statistics that the PTO has promulgated; the legal training of examiners; 102(e)/103 rejections; and a new type of In re Katz rejection.

In 1980 the CCPA decided *Nelson v Bowler* which held that mere demonstration of a pharmacological activity was sufficient to establish a patentable utility even without disclosure of any specific therapeutic use. This decision has been followed in *Cross v Iizaku* and other cases that have been discussed today. Also, in the *Brenner v Manson* case, the Supreme Court tied the utility of a method involving a compound to the utility of the compound itself, so I would propose that disclosure of a method for achieving a pharmacological activity is similarly patentable under the *Cross v Iizuka* standard.

Other speakers have pointed out that it is the FDA, not the PTO, that has the expertise and the statutory responsibility to determine safety and efficacy. In re Anthony is a particular case in point. There the FDA actually pulled the drug from the market and yet the CCPA said that it satisfied the statutory utility standard.

In direct contrast to the case law, I believe that Group 1800, in particular, has promulgated an extremely high standard of utility and that inventions dealing with pharmaceuticals and pharmaceutical treatments are held to a much higher standard in Group 1800 than, for example, comparable inventions are held in Group 1200. I believe there is a clear conflict between the two groups in the way the law is applied.

The meeting Notice asked for specific sanitized examples, if available, of some of the horror stories that we're faced with. Let me give you a couple.

My first example deals with a method for treating animals, a method that has general applicability, is not limited to human therapy, the claims -- although that's discussed in the specification -- the claims are not limited to human therapy. This series of applications was filed five years ago. Three and a half years ago the claims were held to be free of the prior art. Since that time, though, a utility/enablement rejection, under Sections 101 and the first paragraph of 112, has been entered and five interviews later, seven declarations later submitting experimental evidence, and 17 publications by third parties who have successfully practiced the invention and reported the results, we still are faced with a utility and enablement rejection because although the industry has accepted the invention, the operability of the invention, the examiner has not. The question of appeal was raised by Ms. Linck. Should we take these cases on appeal, and certainly we struggle with that. My perception is that appeals take several years now in the biotech area; that because there are fewer examiners-in-chief who have biotech training these cases are put on the slow track. We just can't wait.

The next example deals with a gene therapy invention. This application was allowed. The examiner was then reassigned. The case was withdrawn from issue. A utility rejection was put in place and although the examiner cited no affirmative evidence in support of this rejection for lack of utility, it's been maintained. The FDA had determined that evidence of safety is sufficient that -- and efficacy is sufficient that human clinical trials can begin. Apparently the examiner is the only one who is not convinced.

The PTO in its Notice seeks to justify a high standard under the fallacious argument that a patent places the imprimatur of the federal government on the invention, perhaps to the point that it would mislead

patients and others. As other speakers have noted, it's the FDA, not the PTO that determines safety and efficacy. And there's really no harm if a non-efficacious or non-commercializable invention ultimately makes it through the patent system and if it doesn't get FDA approval it will merely fade into obscurity. And if you look at the statistics, out of a handful, out of I should say thousands of pharmaceutical patents that do make it through the gauntlet, there are only a handful of inventions that are -- new drugs that do receive FDA approval in any given year. So if the PTO is measuring its success rate by whether an invention makes it to the market, that's the wrong measure of success.

There are some who say that applications should not be filed until extensive supporting data are in hand, but this ignores reality. An early filing date is essential because except for the U.S. the entire industrialized world is on a "first-to-file" system, and we rely on our U.S. filing date for that purpose. A patent can't wait, in any event, because inventors publish their results and these published results could then be used to reject the later application that has the more extensive data Group 1800 seems to want.

With a time period required of six, to eight, to ten years and over \$100 million to develop these data, small biotech companies simply cannot survive that long, and if they don't have the patent the investment capital will not be available. The problem is even more acute for non-profit research institutions and institutions such as NIH.

Let me jump to a couple of new points. When Congress amended the Section 103 to exempt rejections based on inventions made by people working at the same company, under Section 102(f) and (g), it had a major oversight. That is that it forgot to include Section 102(e). Now, when inventors work together and a CIP is filed, the -- if the inventorship is at all different, the second application is rejected over the first. If the first patent issues the CIP is simply unpatentable. If not, the only cure is to combine the two applications together, file a third application and then split them into fourth and fifth applications all to exalt form over substance and overcome an objection that really never should be made in the first place, that is the rejection of one inventor's invention over co-workers at the same company.

Another solution might be to amend the statute to allow corporations to be applicants for patent and focus on inventions coming out of a corporation rather than just inventions by particular inventive entities.

Next, the push to cut average pendency has been -- of applications in the biotech area has been counterproductive. Although applications average pendency has shortened up, numerous continuations are now necessary with the result that it's taking longer than ever to get our

biotech patents issued.

Thank you.

MS. LINCK: I have one comment, again pushing for the appeals. The Board has a number of new members on it since Mr. McElvy took over and I know that there are at least three that are skilled in the biotech art that have been added to the Board, so perhaps you'll have a little bit speedier action there.

MR. ISRAELSEN: Thanks.

COMMISSIONER LEHMAN: Thank you very much.

That concludes our morning session. We will be returning in approximately 50 minutes, 2:00 o'clock, to begin the afternoon session.

(Whereupon, at 1:10 p.m., the above-entitled matter recessed to reconvene at 2:00 p.m. the same day.)

AFTERNOON SESSION (2:05 p.m.)

COMMISSIONER LEHMAN: We're back on the record and we'll get underway now. I'd like to thank the City of San Diego for being so nice and providing this great room for us and this is a wonderful facility for us to have our meeting in.

Our next witness is Barbara Rae-Venter from Weil, Gotshal and Manges.

BARBARA RAE-VENTER, WEIL, GOTSHAL and MANGES

MS. RAE-VENTER: Commissioner Lehman and distinguished members of the panel, good afternoon. My name is Barbara Rae-Venter. I'm a partner with Weil, Gotshal and Manges and head of the biotechnologies practice for the firm. I'm resident in the Silicon Valley office.

Weil, Gotshal and Manges is an international general practice firm with a diverse array of biotechnology and pharmaceutical clients. My personal specialty is patent prosecution and counseling. I'm speaking on behalf of BIO, of which Weil, Gotshal and Manges is a member. I will testify on the case law relating to technical standards used in measuring nonobviousness and enablement of biotechnology inventions. The full text of BIO's testimony on the subject has already been made of record and a supplemental to testimony by BIO in a previous hearing on nonobviousness.

Nonobviousness and enablement are distinct requirements of the patent code. However, both inquiries are grounded in assessment of what the specification or prior art would teach or suggest to a person of ordinary skill in the art. In many instances, the critical question to be answered in an obviousness inquiry is whether a person of ordinary skill in the art would have had a reasonable expectation of success in performing an experiment suggested by the prior art. By the same token, often the critical question to be addressed in an enablement inquiry is whether in light of the teaching provided in the patent specification a person of skill in the art could make and use the claimed invention without undue experimentation.

Logic dictates that the skill level of this mythical person should not change as we move from Section 103 where examiners tend to see this person as one with genius level skill to Section 112 where examiners tend to see this person as one with very little skill. I will focus my testimony around this apparent dichotomy and the effects that this has had on determining patentability of biotechnology inventions.

As regards the level of skill to be attributed to a person of ordinary skill in the art, during an obviousness inquiry, very few biotechnology cases have focused directly on this issue. For example, in *Bell*, -- and I'm going to omit the cites for the cases -- *Engine*, and *Vaech*, as well as the published cases from the PTO Board, the obviousness issue has turned on what the prior art would have suggested to a person of ordinary skill in the art, or whether that person would have had a reasonable expectation of success in carrying out an experiment, but the scientific attributes of the person of ordinary skill have not been elucidated. Even in *In re O'Farrell*, the case that contains the most discussion of the obviousness issue in the context of a biotechnology invention, specifics concerning the level of skill in the art are addressed solely by reference to the fact that, quote: "Appellants say that in 1976 those of ordinary skill in the arts of molecular biology and recombinant DNA technology were research scientists who had extraordinary skill in the relevant arts and were among the brightest biologists in the world." End of quote. Nonetheless, there is no apparent basis in the case law to suggest that the standards for determining the applicable level of skill possessed by the person of ordinary skill in the art of biotechnology should be any different than those used for making that determination in other arts. Thus it is clear that the person of ordinary skill in the biotechnology field should be considered to be the designer or problem solver in the art, not the user of the invention. While the person of ordinary skill is presumed to be aware of all the pertinent prior art, she is one who thinks along the lines of conventional wisdom in the art and is not innovation oriented. Consequently, the obviousness of an invention to the actual inventor is acknowledged to be irrelevant because inventors are acknowledged as a class to possess skill that sets them apart from the person of ordinary skill in the art.

Though the cases contain very little discussion of the attributes possessed by the person of ordinary skill, the positions taken by the Patent Office in *In re Bell*, *Duel*, and *Movva*, suggests that examiners may be applying under an obviousness analysis a far higher level of skill than that actually possessed by the ordinary person of skill in the art.

An example of the application of this high standard is that examiners are rejecting claims to DNA sequences on obviousness grounds based upon information concerning the amino acid sequence of a protein and a reference describing at most a general cloning method such as the use of probes.

And in *In re Bell*, the court considered such an obviousness rejection. In *Bell* the applicants had isolated the human IGF1 and IGF2 genes and saw it in earlier claims to those compositions having the DNA sequences of the isolated genes and certain DNA genes they would hybridize to the genes. The PTO Board had affirmed the examiner's rejection holding that the examiner had established a prima facie case of obviousness for compositions having the claimed DNA sequences in light of the known amino acid sequence of IGF1, the correspondent link between amino acid sequence and DNA sequences based on the redundancy of the genetic code and a prior art patent describing a general method of isolating a gene for which at least a short amino acid sequence of the encoded protein is known based on constructing nucleic acid probes.

In reversing the PTO's decision holding that a prima facie case of obviousness had been established the Federal Circuit acknowledged that their PTO's decision rested on the assumption that, quote: "Just as closely related homologues analogues and isomers in chemistry may create a prima facie case, the relationship between a nucleic acid sequence and the protein it encodes also makes a gene prima facie obvious over its correspondent protein." The court then held that the PTO had not met its burden of showing that the prior art would have suggested the claimed sequences because the known amino acid sequence, in light of the degeneracy of the code, might have yielded 10 to the 36th sequences but would not have taught one of skill in the art which of those sequences corresponds to the IGF gene which was the claimed invention.

At the same time the court rejected the notion that the prior art patent filled the gap, i.e., when combined with a known amino acid sequence of IGF1, rendered the claimed sequence as obvious. The court noted that when read carefully the reference actually taught away from the claimed invention because it suggested the desirability of designing probes based upon unique code-ons, and IGF had no such unique code-ons.

Finally, the court rejected the PTO's argument that the prior art reference supplied the necessary teaching because the applicant himself

had used the method suggested by the prior art in designing the probes that were used to isolate the cloned gene -- the claimed, excuse me, gene.

Labeling the PTO's focus on Bell's method, quote, "misplaced," end of quote, the court pointed out that Bell's claimed compositions, not the method by which they are made, and cited case law supporting the proposition, that the patentability of a product does not depend on its method of production. This latter statement by the court is quite important in that it is the difference between the subject matter sought to be patented and the prior art to which it is to be compared in the obviousness determination, not the method by which the invention is made that is relevant.

As recognized by the court and is supported by a long line of cases, whether a composition is patentable depends on whether the composition is known in the art or is obvious and not whether the process by which the composition is made is patentable. Unfortunately, the decision in *In re Bell* appears to have done little to dissuade examiners from continuing to reject claims directed to DNA compositions based on some partial amino acid sequence data and the generalized assertion that a particular prior art cloning method would have resulted in a reasonable expectation of isolating the claimed compound.

In *Ex parte Movva* and *Ex parte Deuel*, the PTO again rejected claims as obvious based almost entirely on a prior art method to isolate the claimed compound. In so doing, the PTO is failing to properly consider the statement by the court that it is the claims that define the invention, not the method of making the claimed compositions. A careful reading of the facts in *Deuel* necessitates a conclusion that the focus of the PTO's inquiry is almost solely on the cloning method, not on structural similarity. That focus runs directly counter to the Federal Court statement in *Bell*. In *Deuel*, the prior art supplied only a partial amino acid sequence and only the most general information about cloning. Apparently absent from a general method was any information about the various parameters that one in the art may have needed to vary if attempting to apply the prior art method to a particular desired gene sequence. In fact, this case highlights one of the biotechnology industry's main concerns with the PTO's position that such bare bones information fully satisfies its obligation to make a *prima facie* case of obviousness. Placing the burden on applicants to prove the negative, i.e., that the alleged method would not be expected to yield the claimed composition, is nearly impossible, especially in light of the PTO's consistent refusal to cite relevant experimental parameters.

Returning to the skill level applied by examiners under Section 103, even if the determination that the skill level is high for the art was correct, examiners take a position concerning the level of skill in the

art in connection with an obviousness inquiry that conflicts with the level of skill applied by the examiner in the enablement inquiry. However, as recognized by the PTO in their brief to the Federal Circuit in the Deuel case, quote: "To that end the prior art must describe the compound in such full, concise and exact terms as to enable any person skilled in the art to make and use the compound." End of quote. In fact, in Hybritech the court reversed the district court holding and labeled internally inconsistent findings by the court that the method for producing monocloned antibodies was well known in the art in a Section 103 inquiry, while at the same time holding the patent deficient for lack of enabling disclosure because it fails to teach how to make monocloned antibodies. As a result, it is disingenuous to argue that a claim to a genus is not enabled due to the lack of disclosure of a sufficient number of species while simultaneously arguing that in light of some prior art reference one of skill in the art would have had a reasonable expectation of success in making one of the claimed species.

In summary, it is BIO's position that examiners are not properly construing and applying the statutory requirements for patentability. In the area of nonobviousness, the PTO had misinterpreted the law concerning the test for patentability of biotechnology inventions. In the case of both nonobviousness and enablement requirements, the law is often misapplied and inconsistent positions are taken. Furthermore, all too frequently patent examiners failed to cite any prior art or other evidence in support of their conclusions, relying instead on supposition or unsupported assertion. The practical effect of the PTO's position cannot be understated. Patent Office statistics readily demonstrate that biotechnology patent applications take longer on average to prosecute than applications directed to any other technology. The cases at issue are often extremely narrow and limited to embodiments that have been actually reduced to practice. As a result, the claims are often of little commercial use. Time is money and, unfortunately, far too many precious resources are being needlessly spent by biotechnology companies to secure protection for the results of their research. At the same time, misapplication of the requirements for patentability effectively deprive these same companies of the full return due on their research investment. Thank you.

COMMISSIONER LEHMAN: Thank you. I would say that I think there is something that you said which is incorrect and that is the biotechnology applications take longer to process. They really don't. They're -- Group 2300 has a longer pendency period, for example, than Group 1800. 2300 is the --

MS. RAE-VENTER: If you take into account the fact that biotechnology cases often get refiled several times, I believe it's correct that they, in fact, take a very, very long time.

COMMISSIONER LEHMAN: Well, one of the things that I think we need to do is have better statistics about what really is going on, and we're going to be working on that. Because we seem to be hearing a lot of anecdotal evidence and we need to know whether that is really, you know, reflective of what's going on.

Thank you very much.

Next I'm going to ask Timothy Gens to come back. He only took five minutes of his time this morning and he wanted the other five minutes this afternoon so that he could discuss the issues that we're talking about here. I assume you're going to talk about the obviousness question as well.

TIMOTHY GENS, FENWICK and WEST

MR. GENS: Good afternoon, Mr. Commissioner and distinguished panelists. I am Timothy Gens with Fenwick and West and I am presenting these remarks on behalf of BIO. A complete copy of the text has been previously submitted.

I'm going to address the first question under the issue of "obviousness," and, respectfully, Mr. Commissioner, I don't think you'll like these comments much better than this morning. Rather than start round two just yet, I'd like to focus on a recommendation so that it is not overshadowed by the differences in other areas, and that is that we suggest to the Patent Office that it open the initial and continuing education program of the examiners to the public so that the basis of the examiners' legal education is known to the public. This should provide a consensus starting point for legal arguments to be applied to the facts of the invention under examination. There are clearly many advantages to establishing a legal education for the examiners which reflects a general consensus of both the Patent Office and the patent bar as representatives of the inventors.

Should fewer appeals result as a consequence, the savings and economic and inventive power alone justifies the effort. Accordingly, we recommend allowing the patent bar to cooperate in the education of the examiners by providing comment on the Federal Court case law. As it appears now, the Patent Office relies upon the appeal process to police the legal standards of the federal courts. This is very costly for both the Patent Office and the inventors. In the "Notice for Comments," the Patent Office recognizes that the body of case law is growing and is helpful in giving direction for implementing the requirement for obviousness. Unfortunately, this is a harmful, self-fulfilling prophecy. This growing body of case law is being generated by the misapplication of legal standards which are already well defined, although in different technological areas or factual situations. This is similar to a drug

company doing away with quality control and gauging the quality of its products on survivors who sue or their family members.

The growing number of recent decisions by the Federal Courts on biotechnology patent issues is only the tip of the iceberg. It represents a small fraction of the examiners' decisions that could have, probably should have, been appealed. Very few inventors have the capital and human resources to challenge a misapplied legal standard; first through the Patent Office's examination, then through the Board of Appeals, and then into the Federal Courts. The current implementation of legal standards through successive appeals saps the resources of both the patent office and the inventors. Opening communication between the patent office and its users hopefully would decrease the number of appeals while more accurately and uniformly applying the requirement of nonobviousness.

Examples of misapplying the legal standards governing nonobviousness are in the "Notice for Comments" authored by the Patent Office. The Notice refers to the suggestion that the Patent Office is imposing a per se rule of obviousness for inventions involving sequencing and the expression of genes once any sequence information has been publicly disclosed, whether the sequence information takes the form of a partial amino acid sequence of a protein or DNA sequence information derived from the expression of the gene. The Patent Office asserts that it does not apply per se rules.

To the contrary, however, specific examples exist in the public record where examiners have expressly stated, and I quote: "...the relationship between a gene and the protein it encodes requires a different type of obviousness determination..." The Patent Office clearly changes the nonobviousness requirement when genetic material is involved. The legal standards have been improperly simplified by focusing exclusively on the function of the DNA sequence as a information transfer vehicle while disregarding its chemical structure and the properties and characteristics resulting from its structure. The patentability of a DNA sequence must include the properties and characteristics of its structure as it is inserted in a vector, the vector in a host, and the host grown to produce the desired protein. It must be made absolutely clear that genetic material, and other inventions of biotechnology, are to be judged by the same legal standards as other technologies on the issue of obviousness.

And with that I'll conclude my remarks, and if you have any additional questions I'd be happy to address them.

COMMISSIONER LEHMAN: Have you ever had any -- made any effort to participate in the Biotechnology Patent Institute that we have set up that I described in my opening remarks?

MR. GENS: No, I have not.

COMMISSIONER LEHMAN: Because that was the -- the whole purpose of that is to deal with the problem that you just suggested, and that is to talk about the training that the examiners get, the standards that are used, and it is basically intended -- and consists of people who are companies and people who are in the bar, so you might want to check that out.

MR. GENS: I appreciate that comment, Mr. Commissioner. The only question that I would have is that it does not help for a two-way lines of communication if I have to go into a situation without any basis of understanding how the examiner is being trained. And if we can maybe open up that information that would make that dialogue much more fruitful for both sides.

COMMISSIONER LEHMAN: My point is that there's a mechanism for raising that kind of issue and that's the Biotechnology Patent Institute, so you might want to -- I mean, obviously, there are two mechanisms: One is this hearing and we're listening to what you have to say, and we will -- we may well make modifications based on it, but there's also another mechanism, too, for you to have an ongoing relationship and you might wish to avail yourself of that.

MR. GENS: I'd be more than happy to do so if that is the intent of this other source.

COMMISSIONER LEHMAN: Well, you're a member of the bar and you're out there representing companies and -- presumably in this sophisticated law firm, and I think you, you know, owe it to yourself to know what's going on if you're going to properly represent them.

MR. GENS: Thank you.

COMMISSIONER LEHMAN: Thank you.

Next, Elizabeth Lassen, please, of Calgene.

ELIZABETH LASSEN, CALGENE, INCORPORATED

MS. LASSEN: Good afternoon. My name is Elizabeth Lassen. I am chief patent counsel for Calgene, Inc. I am here today on behalf of Calgene as a member of the Biotechnology Industry Organization and as a concerned member of the patent bar.

Founded in 1981, Calgene is an agriculture biotechnology company which employs approximately 350 full-time employees, about 160 of which are engaged in research and development. Patents are critical to Calgene.

It has taken 12 years for us to commercialize our first genetically engineered product, the Flavor Saver Tomato. In plant biotechnology the product is the factory, patents are all the more important.

I welcome the opportunity to participate in this hearing. I believe that the BIO's written comments which amplify my remarks here are useful in cogent summation of the issues. The fact that it was possible to generate a 100 plus page document, to get a group of patent attorneys with such a diverse clientele in the biotech industry to achieve consensus in this paper underscores the seriousness of the points raised. I've found the process of working with the other BIO members on our written comments to be both comforting and at the same time disturbing. The good news and the bad news is that Calgene is not alone.

My assigned topic relates to the level of skill possessed by persons working in the field of biotechnology. A correct assessment of this level of skill is necessary for both obviousness and enablement determinations. But, before I begin, I would like to make it clear on the record that my purpose in coming here today is not merely to summarize the issues regarding level of skill or other points but to meet Commissioner Lehman and try to help communicate the absolute seriousness of these types of issues to a company such as Calgene.

The Patent Office practices are effecting companies, universities and other research institutions. They're having an impact on the decisions and strategies made by us all today. These issues are creating uncertainty and increased costs. It is changing the way companies and research institutions invest in research. As patent practitioners advise their clients that it's not clear whether they will get adequate protection for biotechnology invention, research dollars are going elsewhere.

Is the PTO properly assessing the level of skill possessed by persons working in the field of biotechnology for obviousness and enablement determinations? Obviousness determinations are made under 35 U.S.C. 103 based upon whether the invention was obvious to one of ordinary skill in the art. For purpose of obviousness, both the suggestion and expectation of success must be found in the prior art.

Enablement determinations are made under 35 U.S.C. 112, first paragraph, based upon whether the written description of the invention is found in the specification enabling any person skilled in the art to practice the invention. Here the test is one of undue experimentation. In the prosecution of biotechnology application, one finds that a unrealistically high standard will be applied for the evaluation of patent claims under Section 103 and a unrealistically low standard applied to the evaluation of patent claims under 112, even though that hypothetical skilled artisan upon which these standards are based is the

same person.

There's an even greater complication in the improper identification of the skilled artisan because in almost every single biotechnology patent application that I have seen, regardless of who prepared the application, the technology or the examiner assigned to the case, both enablement and obviousness issues are presented. The Section 103 rejections and the Section 112, first paragraph, rejections become a game. The level of skill in the art shifts during the course of prosecution depending upon whether the Patent Office is making an argument with respect to obviousness or enablement. This liberty to take inconsistent positions on the part of the U.S. Patent Office creates a disbelief on the part of the scientists who look to the patent examiners kindred spirits. It perpetuates an adversarial atmosphere with patent attorneys because the rejections are offered in sort of a checkmate attitude and it generates a lack of credibility in the entire patent system by the business community.

Biotechnology is a rapidly developing field which employs a great number of highly skilled researchers and also relies upon a significant number of laboratory technicians. Who is one of ordinary skill? That of course depends on the particular facts of a given case, but, as a general rule, the highly educated examiners now in the biotech office are exposed on a daily basis to the very best, newest science in the world of biotechnology and they have forgotten that science is done one step at a time and that much uncertainty always exists before the experiment is completed. They've forgotten that an invention can be obvious to one of greater than ordinary skill and they've forgotten that the published literature only reports successes. They've become the Monday morning quarterbacks of science.

For example, I've observed that the PTO readily asserts that the mere knowledge of an assay for protein is enough to render a purified preparation of such a protein obvious. In many cases methods do exist for every step necessary to go from protein to DNA, but it's random luck to choose the purification procedure from the infinite possible combinations of steps, times, reagents, columns, buffers and detergents, et cetera. The skilled artisan cannot extract from a description of an assay for protein any particular characteristic which would assist the skilled artisan in its purification, such as the isoelectric point, size or shape, the requirements vary so much from protein to protein. But obviousness rejections which pull such prior art together are commonplace. Such simplistic views of the level of skill held by one of ordinary skill in the art is analogous to a software examiner refusing to grant any patents on software programs using existing languages.

It is difficult to discuss the PTO's assessment of level of skill without blending into the test for predictability. The PTO frequently

dismisses the types of arguments and months of laboratory research as routine absent special considerations which are present. Ask the skilled artisan, however, and the work required to obtain nucleic acid sequences from an apparently purified protein will be characterized as arduous and unpredictable, and such an artisan would be, and often are, justifiably insulted to hear that such work is dismissed as routine.

As with the determination of the skilled artisan for Section 103 purposes, the proper identification of the person skilled in the art for Section 112 purposes is also critical to a correct determination of patentability. Under Section 112 a patent specification must include a written description of the invention which will enable any person skilled in the art to make and use the invention. The test for enablement requires that the claimed invention be practiced by the person skilled in the art without undue experimentation. The higher the level of skill which is applied the greater the enabling power of a given patent application. Unfortunately, a patent examiner typically defines the skilled artisan to have no ability to extrapolate away from the patent application when considering such enablement questions.

For example, it's apparently the belief of Group 1800 that once a DNA sequence encoding a protein is disclosed that one skilled in the art is incapable of making any modifications to that sequence without undue experimentation. This is an improper determination of the level of skill. The researchers are able to make and test modifications of gene size, code on substitutions and screen for highly homologous sequences from related sources. The fact that the examiners regularly reject claims on this basis is the subject of many appeal brief now before the Patent Office Board of Appeals.

The imbalance between level of skill applied under 112, first paragraph, as compared with the 103 analysis, goes to the heart of the patent practitioner's difficulty in prosecuting patent applications to biotechnology inventions. How can a claimed subject matter be simultaneously obvious and not enabled, particularly when a Section 112 person has the benefit of the specification while the Section 103 person does not? The confusion is to the level of skill seems to work against the inventor in each case, never in favor of innovation. This results is the anomalous and a logical view that the person having ordinary skill in the art, under Section 103, would have had an expectation of success to practice the technology, but once the patent specification has provided additional information the former competent person is changed to a person skilled in the art who lacks the certainty of what to do in order to practice the invention.

A statement made by an examiner in a recent action highlights how free the examiners are with this type of rationale. We were given the argument that the claims were not enabled because the invention was not

shown to work, yet the claims were obvious over the prior art. We've even seen the same references applied for both 103 and 112, first paragraph, purposes.

Some quick examples further highlight this problem: A protein with activity to a particular substrate was obvious over protein with specificity for a similar but different substrate. Taking the prior art sequence with the known encoding sequence -- with the new encoding sequence, a third substrate was not enabled. The expression of a certain protein in any host cell to effect a common metabolic pathway is obvious yet the specification only enables the change which was exemplified, i.e., the claims must be limited to a particular tissue from a particular construct.

The U.S. Patent office is an agency which is charged with promoting science and the useful arts. Examiners are pressured to meet quotas and, as a result, do not seem to be given much encouragement to fully engage in meaningful dialogue with the patent bar. Moreover, examiners are permitted to become removed from the methods and thinkings of science and permitted to engage in inconsistent arguments which result in improper application of Section 103 and 112 standards. These issues are weakening the patent system we enjoy in the U.S., and the difficulties that I've briefly described are reviewed more fully in the BIO paper.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to ask Daniel Chambers of Viagene to come forward, please.

DANIEL CHAMBERS, VIAGENE, INCORPORATED

MR. CHAMBERS: Commissioner Lehman, other members of the panel, good afternoon. My name is Dan Chambers and I work as in-house patent counsel at Viagene, Inc., a San Diego based gene therapy company.

As it's been said before, the effect of the patent system is critical both to the success of my company and many other biotechnology companies. Today I'm speaking on behalf of BIO's Intellectual Property Committee and will address question four under the nonobviousness section of the --

COMMISSIONER LEHMAN: How big a company is Viagene?

MR. CHAMBERS: What?

COMMISSIONER LEHMAN: How big a company is Viagene?

MR. CHAMBERS: I'm sorry, I still can't hear you.

COMMISSIONER LEHMAN: Your company, how -- can you just tell me what size it is?

MR. CHAMBERS: It has about 160 employees full time and about half that number are employed in terms of their research effort. We're basically developing a variety of different systems to the liver. You know, they're genes basically to people -- presently we have at least three different systems under development. The most advanced system is based on retroviruses.

COMMISSIONER LEHMAN: Do you have any products on the market then at all?

MR. CHAMBERS: Not on the market. Six in Phase One clinical trials -- actually two in six different Phase One clinical trials, both in HIV immunotherapeutic and cancer immunotherapeutic.

Like I said before, I'm going to be addressing question number four under the nonobviousness section of the PTO's "Public Notice." Question four asks: "Are there specific practices of the U.S. Patent Office with regard to determinations under 35 U.S.C. or Section 112 for biotechnology inventions that you believe are inappropriate or inconsistent with legal precedent?" The short answer is, "Yes." In more detail what follows are specific examples of recurring rejections based upon PTO positions that are inconsistent with case law.

Specific examples of case law were obviousness rejections confuse tests of novelty, and nonobviousness include an analysis of the elements of a claim lacking consideration of whether the claim as a whole would have been obvious. As an example, an examiner rejected as obvious a two-step method in view of a single reference stating that the preamble of claim one is taught by the reference. Claim element 1(a), the determining step, is clearly met by figure one of the reference. Claim element 1(b), the step of comparing is taught by the reference. Here the Patent Office dissected a claim and attempted to show that each element was taught or suggested by the cited reference. This is wrong as a matter of law. For example, the Board of Patent Appeals and Interferences explained in *Ex parte Hiyamizu* that citing references which merely indicated that the isolate elements or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious.

In another area the PTO often treats claims directed to DNA molecules as if they are presumptively anticipated. Group 1800 has formulated a policy, apparently, where a novel nucleotide sequence does not render a claimed DNA molecule nonobvious, regardless of whether or not the prior art would have suggested the claimed nucleotide sequence. Following this policy, for example, the Patent Office has rejected claims to novel DNA

sequences encoding tissue specific promoters. In one case, the Patent Office stated that "the fact that the claimed tissue specific promoter has a unique nucleotide sequence does not render it nonobvious." It was not relevant to the PTO that the promoter was characterized by novel nucleotide sequence and that the prior art did not suggest how one could modify the nucleotide sequence of a known tissue specific promoter.

COMMISSIONER LEHMAN: When you have an examiner reject a claim, like in the example that you gave a moment or two ago, and apparently that's a case in progress right now?

MR. CHAMBERS: Well, these are not cases that are pending before Viagene. These were basically put together by the BIO committee here.

COMMISSIONER LEHMAN: I mean, do you request interviews with the patent examiner and try to make your case? I'm trying to get at the process whereby which what are apparently perceived to be errors are corrected and you have -- You can have an examiner interview. It's possible to talk to the supervisor involved. That sort of thing.

MR. CHAMBERS: Yes, Commissioner. In one particular instance which I'm involved with, we have added -- like Viagene has had a case pending before the Patent Office for over seven years and, in the case of that, there have been -- in this case there have been interviews with the examiner and -- but I don't know, over 12 declarations submitted. And we've just had an interview with the examiner and his SPE in the last two weeks and hopefully resolved the issues that were basically outstanding in the case, although we're somewhat apprehensive about the situation that previously we had an interview with the same examiner in the presence of his supervisor and the examiner had indicated his willingness to remove basically all the rejections that were outstanding both under 103 and 112, which were the only issues still remaining in the case. Upon submitting the formal amendment, the examiner came back with a 30-page office action basically putting forth new basis, new grounds for the same rejection and, again, it's just this process of trying to jump through the hoops. I think interviews with the examiners definitely help but, in certain instances, certain examiners, for whatever reason, seem recalcitrant to want deal with --

COMMISSIONER LEHMAN: Do you find this is a problem that -- Is this every application that you make or is this just occasionally that you run into what you call an unreasonable response?

MR. CHAMBERS: It hasn't occurred in every case, although it seems to be more prevalent in what I would consider to be pioneering technology that really enables -- it is a foundation basically upon which companies are built. For whatever reasons, those kind of patents seem to be the ones -- or applications seem to be the ones the Patent Office wants to look at

the hardest, and for good reason. We don't object to that, clearly. But when one is forced to narrow claims down to basically a non-effective level of protection or have to dither in the Patent Office for seven to ten years, just at the examiner level without even having to go up on appeal, in certain instances, that seems to be counterproductive and I think those are the issues that are so important.

COMMISSIONER LEHMAN: What I'm getting at, is there evidence that we have a -- that we have examiners all over the place -- you know, you'll go to one -- It's sort of a Russian roulette as to what examiner you get and that determines the ease of the process and whether or not you get the patent? Or is the problem a problem of overall policy, for example, where you have the -- which is what you seem to be saying that it is -- where you have the pioneering invention and you always have a hard time?

MR. CHAMBERS: I'd say that's pretty much the case with respect to pioneering-type technology, and in certain technology fields as well. I mean, gene therapy is obviously a very limited part of the body of technology industry generally, but -- and I don't know actually how many examiners actually are involved with the examination of those types of applications, although, to my knowledge, there are only a handful. And, as a result of, you know, the concern for whether this is kind of groupwide policy or whether or not it's amongst two or three examiners, it might very well be just amongst two or three examiners. But, if those happen to be the only examiners involved in your art area, it doesn't do you a lot of good if, in fact, it's not a policy that isn't being promulgated by the whole Group 1800.

Moving along. As another example, with regard to the PTO promulgating rejections based upon the obviousness to try standard coupled with implicit hindsight. For example, a claimed gene is often considered to be rendered obvious by the prior disclosure of the isolated protein encoded by the gene. In one case, an examiner rejected claims directed to DNA molecules encoding an enzyme as obvious in view of primary and secondary and tertiary references. The examiner's position was that, one, the primary references describe the purified enzyme; two, the secondary references describe methods for determining the amino acid sequence; and, three, that the tertiary reference described the method for isolating a gene encoding a protein for which a shorter amino acid sequence was known. The examiner concluded that it would have been obvious to one of ordinary skill in the art to have purified the enzyme, to have learned at least part of its amino acid sequence, and to have isolated the enzyme using a method in the tertiary publication.

The examiner's analysis was flawed on two fronts. First, it was factually wrong since the primary reference did not teach methods for obtaining sufficiently pure enzyme to sequence the part of the protein.

Moreover, the inventors, as it was described in their specification, actually described the extra step they had to devise in which to purify the protein to a sufficient level of purity in order to sequence it effectively.

Then on a legal front, the examiner's analysis was also erroneous in view of Ex parte Maizel where the Board of Patent Appeals and Interferences reversed a Section 103 rejection claim directed to DNA molecules and recombinant host cells comprising a DNA fragment encoding B-cell growth factor, also known as BCGF, because the protein BCGF had not been isolated to sufficient purity and in sufficient quantity for amino acid analysis. Consequently, the Board concluded that it would have been virtually impossible to do what the applicant had done.

PTO has also stated that a previously unknown human gene is rendered obvious by the prior disclosure of a homologous mammalian gene. As an illustration, an examiner rejected claims directed to DNA molecules encoding a particular human receptor because the primary reference taught the corresponding rat receptor gene while secondary references taught methods to clone human homologues of various rat genes. The examiner's premise was that there was no suggestion in the art or any evidence of record which would suggest that the receptor gene first identified in rats would not also be present in humans. Clearly, the examiner applied the obvious to try standard since the cited references, at best, only gave general guidance and no specifics about the claimed invention and how to achieve it.

Concerning rejections based upon overuse of hindsight to support an obviousness rejection. One example involves an examiner stating that the primary reference teaches a gene encoding a rat receptor with 91-percent homology to the claimed human receptor. Here, the examiner took the inventor's teachings and the specification and combined this information with the teachings of the primary reference to determine the level of homology that in fact existed. That clearly is an improper combination based on impermissible hindsight.

In another case, an examiner decided the claims to an antiviral composition comprising components A and B would have been obvious, explaining that one skilled in the art seeking to inhibit the virus replication would be motivated to combine a variant of the component A described in the reference with the known antiviral agents, such as component B. For their additive effects, applicant has indicated in the application that a synergistic composition results when component A is combined with component B. Thus, according to the applicant, the mere combination of the two compounds is sufficient to achieve a state of synergy. Again the examiner supported the rejection by improperly referring to a statement in the inventor's specification.

Additionally, he disregarded the significance of the synergistic effect between the two compounds which in and of itself is evidence for the nonobviousness of the invention.

As I can see I'm about out of time here, I'm just going to go ahead and move to the last aspect of what I wanted to talk about which is basically recommendations that we would like to see brought about at the PTO in terms of change.

One mechanism for change is obvious. Provide training for examiners that they can:

One. Appreciate the policy behind legal precedent.

Two. Educate in case law concerning biotech inventions.

Three. Are routinely updated on case law.

If the examiners follow the patent laws interpreted by the courts, patent prosecution for a particular case would be more predictable, making business decisions about filing and prosecution even easier. And I think that's a very important consideration, and particular for small companies like the one that I work for. We don't have a lot of resources to devote to haggling with the Patent Office or spending years trying to get our patents allowed. We need things now because long term our company may not exist. We're more concerned about two or three years down the road, not about the effective life of our patent 15 years from now. It's important to us that we get things moved ahead, and I think that's probably true as well for many other small companies.

So I guess assuming that the Group 1800 has the authority to generate its own internal policies and interpretations of the case law, it would be best if those could be aired, those interpretations and those policies could be aired in a public forum. And, in fact, it's our understanding that the Administrative Procedure Act requires that interpretative rules be published. So that's all. Thanks.

COMMISSIONER LEHMAN: As I understand, you know, your testimony coming from the point of view of a small innovative company which right now is still in basically the venture capital state, somebody's paying all those bills and you don't have a market yet, 160 paychecks every month or two weeks is a lot, and, as we've heard, at very high salary levels in many cases. Clearly, you want to get, you know, in and out the door with your patent application, and hopefully a granted patent as quickly as you can, I assume. And then, you know, get on with the process of commercializing the invention.

MR. CHAMBERS: Right.

COMMISSIONER LEHMAN: So, for you, the optimal system would be, and tell me if I'm wrong about this, would be very clear, bright line tests -- rules that you could understand, patent examiners could understand, a very expeditious examination process and issuance of a patent and then get on with it. And then if the -- you know, if you ended up being stuck in the FDA, and so on and so forth, then you'd probably opt for, I assume, some extended patent term registration legislation that would see you through that problem, and that would be really the optimal system for you.

MR. CHAMBERS: I tend to agree, but I think that with respect to bright line rules and those kinds of things, I'm not so sure that's applicable. I think currently the present standards, as have been discussed recently, are ones that are sufficient and easily understood by people who are educated in those areas, and I think --

COMMISSIONER LEHMAN: And examiners don't understand it?

MR. CHAMBERS: Well, right. And maybe it's because they haven't been educated. I mean, they are clearly highly educated individuals and that's something that we greatly appreciate, but with respect to the law I think it's one of those things where a little more effort could perhaps be put in and it would help everybody, including I think everybody in the biotech business and most patentees as well -- applicants.

COMMISSIONER LEHMAN: Thanks.

MR. CHAMBERS: Thanks.

COMMISSIONER LEHMAN: Next I'd like to call on Thomas G. Wiseman, of Cushman, Darby and Cushman, please.

We're running behind this afternoon and it's partly because we're asking so many questions, so if people can be expeditious it would be appreciated.

THOMAS G. WISEMAN, CUSHMAN, DARBY and CUSHMAN

MR. WISEMAN: I'll keep my remarks brief and to the point.

My name is Thomas G. Wiseman. I'm of counsel with the law firm of Cushman, Darby and Cushman. The organization I'm here representing is the BIO organization. It's a trade organization. My relationship to them is as a member of their Law Committee, their Intellectual Property Committee, and their Emerging Technologies Committee.

I could say a lot of things to you now but I'll give you a sense of what

my background is because you may not be familiar with it. I was a government employee for the period of about 22 years, 19 years of which were at the Patent and Trademark Office in a variety of positions. I was also at the National Institutes of Health at their Office of Technology Transfer. I was their acting patent branch chief.

At the Patent and Trademark Office, I had the honor of serving as a member of the Board of Appeals. I was a supervisor, primary examiner, and I was an examiner. My area as an examiner was in what is now called biotechnology. I was examining these applications when it was still fermentation.

Many things have been said about your particular system but there have been some things that have been done that have been done very well. At one point in time the technology that represents biotechnology was scattered amongst four examining groups. It was brought together over a period of years and put into one examining group. The problems which caused the art to be centered in this one particular group was comments like which you are receiving today; different standards were being applied in different groups, and what you're hearing as a bottom line here is that different standards may be being applied in the different arguments.

I trained many of your SPEs. They were examiners in my art unit. I found them all to be a very motivated group, very well disciplined. I found that if I had any problems I would call the SPE and the SPE would work it out. If I had any problems that the SPE couldn't resolve, I talked to Chuck Warren. Chuck Warren would take care of the problem or he would call me back and say, "Tom, you're on the wrong footing. The person who is working for you is making a mistake." I look into it and I have a tendency to agree with Chuck on that particular issue.

I've had some problems where I've had to go and I've called Barry Richman because it was more in his expertise and he took care of the problems. My bottom line is that the system does work. You have to put effort into it.

I am a member of the Biotechnology Institute. I may even be the chairman this coming year. I think there is some education that has to be addressed, but that's nothing new. It's not horror stories all the time. There are instances when I've had to take over the prosecution of a case from another law firm; it's after final. I've an examiner in Group 180 listen to three responses after final and eventually allowed the case. The examiner saw the merits of the invention, felt it was worth his additional time. Since he is on a counter system it is his additional time, it's his own free time, and this individual did help fashion the claims which eventually resulted in the allowance. It's not all bad. There are many good things. If you look at what Chuck Barry

and the SPEs in this particular group have done, they've assembled from what used to be -- I forget what the size of my original art unit was, but it was about 12 to 16 people. They've taken that particular number of individuals and expanded it to a group size of about 188 examiners. That required training lots of new SPEs. When you consider the short time frame relative to what it takes to educate an examiner so that they're up and coming, they've done a remarkable job and they should be given credit for what they have done.

There are many instances where you've run into an examiner who may not understand the law, but it does not help to tell you that all your examiners don't understand the law, because many of them do. Many of them do a great job. They do a terrific job and they sacrifice. They set up committees on their own so they feel that they are more consistent. Sometimes they set up their committees with the blessing of management, sometimes they don't. Jim Martinell, Bob Benson and some others ran a brown bag seminar. They met on Tuesdays, either every other month or every month, and within those particular brown bag lunches they did try to talk about problems and they did try to work out solutions which were similar.

I'm supposed to answer question five for you today, but, before I do that, I want to add just one more thing.

While I was at the Patent and Trademark Office, I was involved in a number of the harmonization projects we had with the Japanese office and also with the European office. I notice that with the Japanese office and also with the European office they used examination guidelines. These examination guidelines were much more detailed than the manual patent examining procedure.

It is known to me that in the formation of these examining manuals by the Japanese Patent Office, and also by the European Patent Office that they did get some industrial input and some input from the bar in formation of their guidelines, and these guidelines are updated. The formation of guidelines may be quicker and faster than Board of Appeal decision. My particular point of view with regards to the Board of Appeals' decisions which have been coming down of late, they've been getting better and better and more on point all the time. I like the Ex parte Anderson decision. I thought that John Caucasian, M.L. Goldstein -- I can't remember the third member of the panel but that's just because my memory is fading with age -- and it was remarkable what the input they went into.

Bill Smith wrote a very good opinion in Ex parte Balzarini, which if you look at it and you look at the proofs and the burdens that were met and that were not met, and the consequences of it, and what could have been done to have changed the outcome, they were all quite clear and it's a

question of proof, burdens, and the like.

Some of the examiners do work on a per se rule, and they are probably wrong in most instances. They have -- there is a general statement in the utility area with regards to the value that the office must accord to the statement of utility, and if the examiner doubts that utility, it's their burden.

With regards to obviousness, the burden is on the examiner to fashion a prima facie case.

To answer question five which is what I was brought here for. It does not appear that foreign systems provide a better legal framework than does the U.S. system. The U.S. case law system provides a comprehensive, analytical framework for addressing issues such as obviousness, enablement, breadth of claims which are commensurate in scope with the disclosed invention.

We do note that, unlike the U.S. system, the foreign systems explicitly recognize the level of skill does not vary whether the inquiry is based upon the assessment of the prior art or the sufficiency of disclosure. That point has been made many times before and I don't want to dwell on it anymore, but there does seem to be a little bit of disparate treatment in there. But if you read the review article by Trasanski, which is cited in the BIO paper, he has lots of comments which would go along with that.

With regards to the examiners' education system. Dick McGar and other people at the Patent Academy had expended large amounts of resources in trying to provide the best training possible for the examiners under current budget problems, and Dick has done a good job and he's brought in outside trainers; John White, Kim Practice, John Trasanski are just some of the names. These names are also worth noting because they are the same names that appear in the patent resources group training which is given to the private practitioners. NIH has sent many of our patent advisors to the training that was available at the -- by this patent resources group, and the office is making use of the same training.

Do you have any questions of me? I'll gladly answer them based on my upteen years of experience and being probably at fault for many of the policies that many of the people are complaining about today.

COMMISSIONER LEHMAN: Thank you very much. No, I don't really have --

MR. RICHMAN: I have a question. Who put you at the end of the schedule, Tom?

MR. WISEMAN: It was the draw.

MR. RICHMAN: I'd like to just expand on what Tom mentioned. In many talks I've given -- the Director's office has a function and one of the functions of the Director's office is to resolve problems where examiners stray from acceptable practice -- and 308-1223 is my phone number. I give it out wherever I talk. This office needs to hear about these things so that they don't fester and have to surface in a public hearing like this. This is a -- I'm not -- It is a good place for this to occur, but we need to have a chance to resolve these problems before it gets to maybe a stage that it is perceived to be at now.

MR. WISEMAN: I want to let you know that Barry is a person of his word. He was a union representative before he got into management and he usually delivered and he delivered in a very fair fashion. And I've found over my 20 years or more of knowing Barry that he delivers on his word, and if I've ever had a problem, I've gone to Barry and he solved it and he solved it on a real time basis and it wasn't put off, and it was done in an efficacious fashion.

COMMISSIONER LEHMAN: Well, it certainly is true that our -- we have many mechanisms to resolve problems. One of which is to not only the examiner interview but, to where there's a problem of policy, to -- the examiner may not be following office policy, to bring it to the attention of the SPUD or the deputy group director or the group director, and that's something I would encourage people to do.

Obviously, knowing the inside of the office, you know more how to go about that so maybe what we need to do is do a better job of training practitioners before the office as well.

MR. WISEMAN: It's a cooperative effort and if it gets adversarial it shouldn't be there, because it's only by working together that we can make a good system.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to ask George Johnston, the Law Department, Hoffman-La Roche, Incorporated.

GEORGE JOHNSTON, LAW DEPARTMENT, HOFFMAN-LA ROCHE

COMMISSIONER LEHMAN: I should ask you, Mr. Johnson, one of our earlier witnesses produced a little vial of medicine produced by your company in Switzerland that had been I think funded originally by the NIH, and one of the things we're struggling with in the government and the Department of Commerce, what's an American company and what should our policies be with regard to American companies. Hoffman-La Roche is not an American company and looks like we're funding products that are being produced

abroad and taking away jobs from American citizens. You might want to comment on that a little bit.

MR. JOHNSTON: I really don't want to comment on that, Examiner -- Commissioner.

First off, Hoffman-La Roche is a U.S. company. We do have a headquarters in Switzerland but we're red, white and blue just like everybody else, and we have more than 15,000 jobs in the United States.

COMMISSIONER LEHMAN: Well, I think that's important. We wanted to get that on the record so we're not, you know --

MR. JOHNSON: Thank you.

Good afternoon, Mr. Chairman, members of the Patent Office. My name is George Johnston, associate patent counsel at Hoffman-La Roche. I present this testimony on behalf of BIO's Intellectual Property Committee. I'll be speaking on two topics; the first involves question number two on obviousness, and the second involves the experimental use defense. The specifics of my testimony can be found in more detail at pages 95 and 157 of the BIO submission.

Let's focus on the nonobviousness, question number two. Whether the level of skill under Section 112, as developed by the courts is sufficiently clear and appropriate. I know you're not going to be surprised when I say, "yes."

The case law is clear. The speakers before me have gone into that so I will not do that at this moment. The problem is that many of the examiners misapply the standard as requiring the inventor to demonstrate and provide convincing evidence to the Patent Office that the application is, in fact, enabled, and, as we have heard before, that's not the standard that should be applied. If the examiner feels that we have not met our burden then the examiner should provide credible evidence to that effect -- objective evidence, not subjective evidence, not feelings, but specifics, because subjectivity will give rise to a lack of consistency which no one wants.

We recognize that many of the examiners might be frustrated with the standards that are applied. They're good scientists; they believe they're protecting the public. The Patent Office certainly is not attesting that, and there's a lot of work, frankly, in trying to find objective evidence which they feel they have in their head already. Why do they have to go further? I'm sure many of them ask.

But let me digress for a moment. We hired a patent attorney at Roche a few years ago. Young fellow. Ph.D., super biotech type. Great schools. Very, very bright. Came into the office prosecuting applications, and

every time an inventor came to his office he'd find that invention obvious and he'd find that whatever the inventor did was insufficient for purposes of enablement. He couldn't figure out how they could extrapolate beyond the specific experiment. He was a challenge, as we say, a challenge to the supervisor. They started calling him "Doctor No, the unpatent attorney." His supervisor had to be very forceful and go right to the jugular and explain, "Where is your objective evidence? Why is it unpatentable? Where are the references? Why can't you extrapolate? Where is your documentation?" And slowly but surely that patent attorney came around. Actually became a very good patent practitioner. I think this also applies to many of the examiners at the Patent Office that are equally as bright, equally as knowledgeable in the science, and I think they have to be better educated and more definitely educated in the law. I think their supervisors also have to be of support as they have, and perhaps can be a little bit more, with regard to instructing the examiners as to the standards and asking the same type of questions as we have been asking our people under that example.

We talked about before where there was questions as to quality control and going to the Board, and I would respectfully suggest that it's a bit late at the Board level and that quality should be built in from the very beginning; right from the examiner's stage; right through the supervisor's stage; and right up to the Board.

Our recommendations are as follows:

Number one. Simply apply the legal standards consistently. Provide that objective evidence when required, and, if this is not already being done, what I'd suggest is that at the annual reviews of the examiners and supervisors that they specifically be reviewed whether or not there have been instances where this objective evidence has been applied on a case by case basis.

Now, my second topic, as I mentioned, involves experimental use defense focusing on topic C of your Notice of December 27, 1993. Topic C involves whether or not there's a justification for or against a statutory use defense.

The BIO organization is very diverse. There are pharmaceutical companies in it, agricultural companies, research, tool manufacturers, and they all have their idea as to what and what might be included within the experimental use exemption.

The position of BIO is to support the presently created experimental use defense. BIO is against the erosion of this defense; however, we could not reach any consensus on the expansion of the defense by legislative action. You see, sometimes it's very easy to know when an action is research and, therefore, falls within the experimental use defense.

Sometimes it's easier than when it's commercial. Sometimes it's hard. For example, if you're simply verifying whether or not an invention works, as explained in the patent, I think most people would conclude "that's strictly research."

On the other side of the coin, should you be using a third party's patent to perform an assay and screen your library of compounds to identify certain biological active compounds? I think most people would conclude that's not permitted commercial use. But the problem comes "what about in between?" It's not easy to separate out the permitted research from the non-permitted commercial use.

We feel that however it is best to leave it to the courts who created this doctrine and who would best know how to apply it on a case-by-case basis. The standard is clear. In the Roche/Bolar case it was held that the adopting of a patented invention to an experimenter's business constituted a non-permissible use and did not fall within the experimental use defense. But the dilemma of BIO is as follows:

Some of our organizational members will conduct activities which might be perceived differently as falling within or out of this defense. Companies could, therefore, be on either side of a case.

There's a diversity and this diversity I think you can also see in the comments that were submitted to the Patent Office from many other organizations, both within and outside of BIO. But there's one common thread. The common thread, at least among the BIO organization, is that we believe that the experimental use defense involves after patent activities, normally the domain of the courts while the Patent Office's responsibilities involve pre-patent activities. Therefore, our recommendations would be for the Patent Office to concentrate on areas toward pre-issuance of patents, as have been enunciated in these hearings, and leave to the courts the resolution of the experimental use defense cases on a case-by-case basis, because the court would be in a better position than anyone to weigh the equities and, based upon the specific facts of a case, determine whether or not the defense is applicable.

Thank you.

COMMISSIONER LEHMAN: Thank you. The reason that we're interested in the experimental use defense issue is because we clearly have a responsibility to examine and issue patents, but there's no other place to go to make -- advise on legislative policy, excepting us, too. So we have a policy function, and I have been receiving some complaints about the use of patent rights to unfairly prejudice experimentation. And my impression is that one reason that it has not been as much a problem as this -- some of the recent complaints would suggest in the past -- is

because by and large there's a certain tolerance for experimentation in the industry, which, you know, is sort of a culture of pharmaceutical research since everybody sort of has to do it, is not to just rush in and sue everybody else, particularly when you're talking about experimentation which takes place in the not-for-profit sector. Is that -- Would you say that's true?

MR. JOHNSTON: I think that's a fair statement. I think if you look at the number of cases that have actually come down in this area, there's not a lot. And I think there's somewhat of a tolerance, somewhat of a recognition that, as I mentioned before, that with regards to early stage activities you might find yourself on both sides of that coin, and I think there's simply a recognition as to that fact and the fact that it really doesn't make sense to bring someone to court on such a basis.

COMMISSIONER LEHMAN: So, from your point of view, the present system which basically is a case-by-case method, is adequate? We don't need any new legislative experimental use --

MR. JOHNSTON: I think that says it beautifully.

COMMISSIONER LEHMAN: And that reflects basically the view of Biotechnology Industry Organization?

MR. JOHNSTON: I think there's a diversity in BIO where there are certain groups within the BIO association who would like to see it expanded. There are others who are vehemently opposed to that expansion, and, if the Patent Office or any group were to proceed, I would recommend that they proceed with extreme caution.

COMMISSIONER LEHMAN: Okay. And let me say with regard to Hoffman-La Roche, we're more than happy to have more jobs here in the United States and we do everything we can to make our patent system attractive so there won't be hardly a job left except some, you know, just a management job that, you know, a bean counter left in Switzerland, so anyway.

MR. JOHNSTON: Thank you.

COMMISSIONER LEHMAN: We want all the Ph.Ds over here.

Next I'd like to call Richard C. Peet, of Foley and Lardner, please. We've had Foley and Lardner of Madison so this is Foley and Lardner of Washington, D.C.

RICHARD C. PEET, FOLEY and LARDNER

MR. PEET: Thank you, Commissioner Lehman and your colleagues from the Patent Office for giving me the opportunity to speak today.

My name is Richard Peet and I am, as I've mentioned, an attorney with the Washington office of Foley and Lardner. Foley/Lardner represents a diverse array of organizations in the biotechnology industry, including small start-up companies, universities and multinational corporations.

I am submitting testimony today on behalf of the Intellectual Property Committee of the Biotechnology Industry Organization. A detailed version of my presentation has been submitted for the record by BIO and is found on pages 163 to 171 of that book.

I would like to take this opportunity to commend the Patent Office for requesting comment on a frequently overlooked but very important part of the patent statute, and that is the Plant Patent Act. Specifically, I submit testimony today on whether the Plant Patent Act should be amended to permit a holder of a United States plant patent to exercise exclusive rights with respect to parts of a protected plant, such as material harvested from the plant. I would like to make two points, in particular, in my testimony.

First. A court would likely find that unauthorized use of plant parts derived from an asexually propagated plant variety protected by plant patent constitutes infringement under current law. However, this area of the law is very unsettled.

Second. We strongly recommend a legislative effort to amend the Plant Patent Act to explicitly protect plant parts derived from asexually propagated and patented varieties in order to reduce the likelihood of costly litigation, and to provide breeders of asexually reproduced plant varieties with the same explicit protections under the Plant Patent Act as will be afforded plant breeders of sexually produced varieties under the amended Plant Variety Protection Act, signed into law by President Clinton on October 6th, 1994.

Let me give you a little bit of a historical perspective. Since 1930, American plant breeders, American agriculture and the American public have benefitted from the stimulus to innovation in the plant sciences provided by the Plant Patent Act. Reports of the House and Senate Committees that accompanied legislation enacting the Plant Patent Act state that the purpose of the Plant Patent Act was to stimulate invention in the agricultural sector by providing plant breeders with the patent protection equivalent to that available to inventors in industry. The Report of the House Committee contains a statement, for example, by Thomas Edison that, quote: "Nothing that Congress could do to help farming would be of greater value and permanence than to give the plant breeder the same status and the mechanical and chemical inventors now have through the patent law."

Mr. Edison's prediction that patent protection for the inventions of the

plant breeder would stimulate and foster innovation and investment in agriculture was clearly accurate. For example, the Economic Research Service of the United States Department of Agriculture estimated that cash receipts in this country in 1993 for nursery and greenhouse crops alone equaled \$9 billion, nearly 11-percent of all farm crop cash receipts in this country. As a result, this agricultural sector is the sixth largest among all commodity groups. It is even larger than such crops as wheat, cotton and tobacco in terms of farm crop cash receipts.

The plant patent system is not only utilized by plant breeders for the protection of new varieties created for the ornamental plant industry, but is also used to protect new varieties of trees and plants producing fruits, nuts, berries and fiber.

COMMISSIONER LEHMAN: What is your involvement in this? What kind of clients do you represent in this that cause you to --

MR. PEET: Our firm represents a large number of companies involved in the breeding of new varieties, both in the ornamental industry and as well as fruit, crops. Many of these companies are small companies, frequently family owned, and so we represent a diverse array of these companies.

COMMISSIONER LEHMAN: Do they tend to have a big California base? Where would they --

MR. PEET: We represent companies worldwide actually. The United States, the industry is spread all over the country. We represent many companies in Europe and throughout the world.

The Patent Office noted in the "Federal Register" Notice announcing these hearings that, quote: "Growers reproduce and use, outside the United States, plants subject to a U.S. plant patent, and subsequently import products harvested from such plants, to the detriment of U.S. plant patent owner." This very succinctly summarizes a very severe problem in this sector of the plant biotechnology industry.

When competing in the market, against lawfully produced plants, these imported products have a decided competitive advantage because the importer has not invested in the research necessary to develop the plant variety. In contrast, the breeder must sell the product at a price that recoups those research costs. Furthermore, the importer benefits from the breeder's promotional and advertising efforts. The income lost by American plant breeders due to the patent infringement inhibits investment in plant research and development programs which are the foundation of a strong horticultural industry in this country.

The adverse consequences of patent infringement are particularly

apparent in the cut flower industry. For example, from data kindly provided to me by the American Association of Nurserymen, a single domestic rose breeder estimated lost royalty receipts in 1993 of \$1.5 million as a result of unauthorized import of patented imports produced in five Latin American countries.

In my written testimony, already submitted to the Patent Office, I provide the legal rationale underlying why it is likely that a court would find unauthorized sale or use of plant parts derived from any asexually propagated and patented plant variety to be infringing, so I will not go into that in detail here.

However, we strongly recommend a legislative initiative to amend the Plant Patent Act to include the right to exclude others from unauthorized sale or use of any part of the asexually propagated plant variety. The amendment could be achieved by revising 35 U.S.C. 163 of the Plant Patent Act along the lines already recommended by my honorable colleague Mr. Gioia.

This amendment would reduce the need for companies to engage in costly litigation to combat patent infringement by foreign competitors.

The proposed amendment to the Plant Patent Act would also provide clear uniformity in the laws related to the scope of protection available under the Plant Patent Act for asexually propagated plant varieties, and under the amended Plant Variety Protection Act for sexually propagated varieties.

On October 6, 1994, President Clinton signed into law amendments to the Plant Variety Protection Act which provide, in part, that unauthorized use of harvested material obtained from propagating material of a variety protected by Plant Variety Protection Act certificate constitutes infringement. A separate legislative initiative is necessary to provide domestic plant breeders of asexually reproduced varieties with the same explicit protections under the Plant Patent Act as will be afforded plant breeders of sexually reproduced varieties under the Plant Variety Protection Act.

I'd be happy to entertain any questions that you have.

COMMISSIONER LEHMAN: Thank you very much. I don't think we have any.

Next I'd like to ask John Sanders of Mycogen Corporation to come forward, please.

JOHN SANDERS, MYCOGEN CORPORATION

MR. SANDERS: Good afternoon, Commissioner Lehman. My name is John

Sanders. I'm representing the BIO Intellectual Property Committee. I am the general patent and trademark counsel at Mycogen Corporation here in San Diego. I've been an intellectual property lawyer for about 14 years, all within corporations. Most of that time was spent in the chemical area. For the past 18 months I've been with Mycogen and have concentrated on two areas of biotechnology; biopesticides, namely bacilluster and jensus delta antitoxins, which are biopesticides, and plant biotechnology.

I am here today to emphasize the importance of utility patent coverage for inventions relating to plants. After hearing over half of the speakers before me, and from reading in the PTO Notice of this meeting, I'm pretty sure everybody in this room understands how important patents are to all the companies and inventors involved. I will try not to be redundant but I want to re-emphasize this fact with respect to plants.

Because of the obvious value plants have in our industry, and the fact that everybody here knows that, I tried to make up a cute analogy to stress the importance of patents. I couldn't come up with a cute analogy but I did come up with a spokesperson for plant utility patents. That spokesperson is the 1994 all American hero Forrest Gump. If Gump were an attorney, I'm sure he would be a biotech patent attorney and he certainly would be here today.

To say the biotech industry depends heavily on the patent system is quite an understatement. "Biotechnology Journal" reports that yearly agbiotech R&D expenditures are well over \$100 million a year. As a relative newcomer, from the chemical industry, I thought this figure was a little on the low side; however, the same article in "Biotechnology" indicated that there was an over 30-percent increase in R&D spending last year. The private sector is uniquely positioned to comment on the critical role that utility patents play in the agbiotech industry. Staggering R&D investments -- in both time and money -- stringent regulatory review adds unique challenges as well as more time and more money. Without the benefits of a utility patent, there's little hope for recovery to the investor of the substantial costs for R&D. If there's no investor, there's no money. If there's no money, there's no jobs and, consequently, then the research does not get done.

The agbiotech industry desperately clings to the utility patent system to maintain its critical mass from both an investor and science perspective, a critical mass that was shrinking until the expansion that occurred over the last year. The agbiotech is dramatically different from the human health sector.

The human health sector, compared to the agbiotech sector, flourished in the lab, in the marketplace and on Wall Street in the 1980s. As a general rule, the agbiotech industry cannot command the high profit

margins that the drug industry gets. In many cases, human health products provide a solution or a therapy that didn't exist before or, if it did exist, was many orders of magnitude inferior to the therapy or solution offered by the biotech inventions. Erythropoietin, TPA, human insulin, human and animal growth hormones are just a few examples.

The agbiotech industry, on the other hand, faces a perception that there's already adequate technology out there. Let's face it, the average lay person in the industrialized world does not really consider food and fiber production to be a major problem. They do, however, think of AIDS, cancer, heart disease as problems near and dear to them. For all these reasons, a strong utility patent system is the life blood of a successful agbiotech company. Some patent practitioners are concerned that the new amendments to the PVPA to include hybrid seed may result in the PTO relinquishing jurisdiction of plant inventions to the USDA under the PVPA and the examining corps who handles the Plant Patent Act. This should not, and from my perspective cannot, happen. The utility patents have peacefully coexisted with the Plant Patent Act and the PVPA since 1930 and 1970, respectively. Compared to utility patents, the Plant Patent Act and the PVPA provide narrow protection to a particular plant line or variety. Let's remember that the Plant Patent act and the PVPA were enacted in times when plant inventions were only envisioned by using standard plant breeding techniques. These techniques were thought at the time not to comply or be able to coexist with the patent laws at the time. Two issues come to mind:

Namely, the product of nature issue and the written description issue.

COMMISSIONER LEHMAN: Do you think that we should consolidate the administration of the Plant Variety Protection Act under the Patent Office?

MR. SANDERS: I think that would be a good consolidation into an examining corps that has the expertise all located in one location.

Neither the Plant Patent Act, nor the PVPA expressly or impliedly exclude utility patent protection. There's no restriction of utility under 35 U.S.C. 101 in either of the legislative histories of these laws, and is indicated in the landmark case of *Ex parte Hibberd*. To take the position that the PVPA and the Plant Patent Act cover all plant inventions would preclude patent protection to generic inventions, inventions that apply across the board to all plants, process inventions relating to plants, and intermediate composition inventions, all of which are claimable under a utility patent. Overlap of utility versus plant patent versus PVPA doesn't mean irreconcilable conflict. For example, In *re Yardley* it was held that under certain circumstances protection under the copyright law and design patent law was acceptable and coexist. There are other examples also.

Some other important issues relating to plant utility patents are the technology in plant biotech was not contemplated when the Plant Patent Act or the PVPA were enacted. Now that the technology is here, we should be able to protect it.

Plant biotech research is unique in at least one major aspect that I believe is affected in the Patent Office, and that's time. From traditional chemistry and microbiology experiments are measured in chemistry usually on the order of hours or days; in microbiology, in the order of days to weeks, but in plant experiments and plant technology it is not uncommon for an experiment to run up to 10 months in a plant experiment. To make a transgenic plant it involves transformation, tissue culture, selection, regeneration into primary transformance, maturation and progeny preparation and analysis. The research cycle is many more orders of magnitude greater than traditional chemistry. The inventor learns from these experiments and builds upon what he learns in the first experiment for what he does in the next experiment. Because of this long research cycle, and the motivation to file patent applications early, plant utility patents should not be disadvantaged for not having working examples when, in fact, working examples are not required by statute.

The plant biotech invention should not suffer because of the unique attributes. One way they are discriminated against is under 112, in particular, with respect to undue experimentation and breadth of claims. A 10-month experiment with plants is usually quite routine, where in the chemistry lab 10 months may usually result in undue experimentation.

Not only do plants qualify for utility patents, there is no valid general reason that a plant utility patent should be limited to specific plant lines or specific genes. Overly narrow patents are many times useless. Patents stimulate the incentives for agbiotech research. We have seen the revolution in human health sector. Biotech can offer equally exciting results in the agriculture in the abatement of hunger. For the greatest impact, a strong utility patent system is necessary. So if Mr. Gump was here, he'd have very few words. This meeting would probably be over already, but he would have very profound words. He might say, "Transgenic plants happen. Just patent it. Have you patented your new plant today?" or "Say yes to plant utility patents." I agree, and BIO recommends that you make all aspects of utility patents available to the plant technology.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call Micheal Farber of Merchant and Gould. //

MICHEAL FARBER, MERCHANT and GOULD

MR. FARBER: Thank you, Commission Lehman and panelists.

My name is Micheal B. Farber and I'm a patent attorney with Merchant and Gould, in Los Angeles. We are a full-service intellectual property firm, and our biotechnology clients include small start-ups, large Fortune 500 multinational corporations, non-profit research institutions and universities in a broad range of biotechnology areas. I would like to address several issues, particularly with respect to enablement and nonobviousness and the level of ordinary skill in the art.

I think with respect to enablement, which also ties in to some extent with utility, there has been a perceived lack of credibility for biotechnology which has almost put it into the same weird science standard as perpetual motion or cold fusion, and I don't think this is appropriate. I think there's been a failure to realize that this work has built on the advances of the last 40 or 50 years in organic chemistry, genetics, biochemistry, cell biology and other well recognized disciplines.

Specifically, I think in many cases there will be a reference cited on general grounds to yield a rejection under 101 and 112. The rejection will cite generalized reasons, such as, for example, an antiidiotypic response for antibody, a human antimurine antibody response for an antibody that is partially derived from mouse or rat tissues, concerns about lack of bio availability or stability, without an attempt to apply it to the particular case. In other words, this is a general research article which gives possible cautions but there is really no attempt to fit these possible cautions to the specific facts of the claimed invention. Moreover, I think in many cases there is a requirement for virtually 100-percent certainty of efficacy even in conditions in which there is now virtually no effective treatment. An example which I am involved in involves an antibody that may be useful for treatment of liver cancer. As you may know, this is for all intents and purposes 100-percent fatal, although I believe there have been some attempts made to treat it with liver transplants.

The question I think would be: Would a reasonable physician conversant with the research and advances in the art, a mainstream physician not willing to try things that are out of the ordinary, believe that this represents an advance that is worth trying clinically? In other words, we have to go back to the constitutional mandate: Does the claimed invention represent an advance of science in the useful arts?

Along the same lines, I think that there has been a serious lack of consistency with respect to rejections under 101 and 112 and then rejections on 103. In another antibody case, I had a rejection in the same office action, under Section 101, based on lack of utility and, in

the same action, a rejection under 103 based on the recognized and accepted diagnostic utility of an analogue, which according to the examiner provided incentive to modify to produce the claimed compound. This, of course, is completely inconsistent. If one of ordinary skill in the art would believe that there was a sufficiently shown utility to make the modification that same person of ordinary skill in the art would then believe that the -- also believe that the new compound would, in fact, have such utility. This goes back to the point that was raised earlier that when you're considering under Section 103 the person of ordinary skill in the art is a genius, when you're considering 101, and particularly 112, that same person suddenly turns into an idiot. There is a lack of consistency in this area.

A third issue I think is a little bit more specific, and that has to do with the use of a couple of recent cases, notably, Amgen and Fiers, to cut down what I think is the proper standard for enablement that was decided by previous CAFC and CCPA decision in the chemical area. And I'm talking about decisions such as Angstat which says that it is not necessary for an inventor to test all of the potential embodiments of an invention, even in a relatively unpredictable art such as organic chemistry. I think this has come up in two areas that I have seen. One is that an inventor has isolated or discovered a peptide or several peptides and now wants to claim nucleic acid that encode these peptides. The second is that the inventor has isolated or discovered a new antigen or a series of related antigens possibly related by allelic variation and now wants to claim antibodies, either polyclonal or monoclonal to these antigens. And what I have seen is even in situations where the peptides are allowed with reasonable scope or the antigens are allowed with reasonable scope, they're saying that there is undue experimentation required in nucleic acid in putting together nucleic acid sequences or making the antibodies, even though when you know the peptide sequence all of the possible nucleic acid sequences that encode the peptide are automatically available by the genetic code.

In the absence, for example, of evidence that would lead one of ordinary skill in the art to suggest that some of the nucleic acid sequences would be inoperable, for example, is used to insert into vectors and used to transfect bacteria for expression, as a result of code-on choice or instability, or formation of abnormal secondary structure, I don't believe there's any basis for this rejection. The Amgen and Fiers cases hold that if you merely are claiming a nucleic acid that encodes a molecule with a particular function with little or no structural data, you have at most a wish to obtain a product of a particular activity, this comes very close to the long proscribed single means claim.

The situation I'm referring to, though, is you have the protein or the antigen or the peptide and the rest of the experimentation, if any, is routine. In the absence of evidence to suggest that, for example, a

particular nucleic acid sequence would be inoperative as a probe or as an expression vector, or that a particular antigen could not be used to make antibodies, according to the generally accepted procedures in the art, there would appear to be no basis to reject the secondary claims. I think this has the effect of giving the inventor less scope than he or she is properly entitled to under the law.

Thank you.

COMMISSIONER LEHMAN: Thank you very much.

MR. FARBER: Any questions?

COMMISSIONER LEHMAN: I think -- I don't have any, unless someone else does, so I think we'll move along.

Next, Michael Roth of Pioneer Hi-Bred International.

MICHAEL ROTH, PIONEER HI-BRED INTERNATIONAL

MR. ROTH: Good afternoon, my name is Michael Roth. I'm a registered patent attorney and patent counsel for Pioneer Hi-Bred International, on behalf of whom I'm appearing. Pioneer is the world's largest agricultural genetics company with about \$1.8 billion in revenues, most of that in the United States. We currently invest over \$100 million per year in research and development and rely on both Section 101 utility patents and Plant Variety Protection --

COMMISSIONER LEHMAN: Where are your headquarters?

MR. ROTH: Des Moines, Iowa, not Switzerland. And if you were there, you'd notice the difference.

COMMISSIONER LEHMAN: We have to go to Geneva all the time and everybody says, "Oh, wow, it's really glamorous to go to Geneva," but I have to tell you I think Des Moines is a lot more exciting and a lot more interesting than Geneva, Switzerland. The weather is better, too, believe it or not.

MR. ROTH: Well, the last time I was in the Geneva airport I saw someone collecting for the poor of Geneva, and I assume that's a person who has only one BMW.

So, as I said, we invest over \$100 million per year in R&D which means that we invest more in a given year than some companies have in total.

We protect our products with Section 101 patents, Plant Variety Protection Certificates whether they are the product of newer methods of

biotech innovation or earlier methods of genetic manipulation.

We have several comments in response to the questions raised in the official Notice. With regard to the issue of enablement of biotechnological inventions, we believe the legal standards governing assessment of enablement and obviousness, as developed and interpreted by the Federal Courts are sufficiently clear and appropriate for biotechnology inventions. Unfortunately, we have considerable difficulty getting the Patent and Trademark Office to follow those standards. Citations of case law are routinely ignored or explained away without citation of contrary decisions and without specific explanation of why the law as cited by the applicants is inapplicable to the facts of the case.

Now, you asked for specific examples, and the one that I have involves an issue that is not specifically covered in the questions, and that is determination of who is an inventor and who is not. Those who are familiar with the world of scientific publication know that co-authorship of a paper bears no rationale relationship to inventorship of any subject matter disclosed in the paper. It's a highly political process by which academic careers are made and lost and by which debts are incurred and favors repaid. Yet there are examiners on some of our cases who are fond of citing *In re Katz* for the proposition that co-authorship of a journal article raises a presumption of joint inventorship. In fact, in that decision the CCPA specifically rejected -- and those were the words, "We specifically reject the examiner's position that the publication of the subject article provided even a tenuous ground for rejection under 35 U.S.C. 102 (g)," and the court expressly held, and they used those words, "We expressly hold that an authorship of an article does not raise a presumption of inventorship with respect to the subject matter disclosed in the article." Concluding, quote: "Co- authors may not be presumed to be co-inventors merely from the fact of co-authorship." Unquote. Nevertheless, we are being forced to appeal these cases in order to have this highly improper rejection reversed, a process that, as has been mentioned, takes two to three years. And in this business time can be even more important than money.

Let me also observe that an appeal isn't necessarily final. We have a case that has been on file for about five years now. A couple of years ago we took the case to appeal and roughly a year ago we got reversal of the examiner's decision. The case is still awaiting issuance and we understand the examiner is looking for some reason to reopen prosecution and enter new rejections.

The office has noted complaints about the propensity of examiners to use a much higher standard when evaluating enablement than is used when evaluating obviousness. As a specific example, we have a substantial effort to identify and patent proteins that have either anti-insect or

antipathogen activity in plants. If we can express those proteins in plants we can make a genetically engineered plant that has desirable characteristics.

We routinely receive rejections that argue that it would be obvious to screen proteins for anti-insect or antipathogen activity, to sequence those proteins, to assemble synthetic genes coding for those proteins, to insert those sequences into plant expression cassettes, to insert those expression cassettes into plant cells and to regenerate those transformed cells into whole plants.

References are cited in support of each of these propositions. At the same time, the claims are rejected if we do not have actual data showing completion of each of these steps on the basis that the specification is non-enabling even though the specification explains how each of these steps is performed. In these rejections, the examiner refers to, quote, "well known," unquote, uncertainties and difficulties in carrying out these steps.

If references are cited, they're often in conflict with the references cited in the obviousness rejections. This reflects a win by any means or checkmate sort of approach to examination that is inconsistent with the ex parte nature of examination and the proposition that the examiner is there to assist the applicant as well as to represent the public.

Again, a specific example is the routine citation of an article by Potrykus, who is a Swiss scientist -- the article is now several years old -- which purports to set up several criteria for establishing successful plant transformation.

Setting aside for a moment the fact that the Potrykus article sets up unrealistically high standards for proof of plant transformation, we also routinely note that the references cited in rejecting the claims for obviousness do not meet the Potrykus criteria.

Even more specific is an example of a rejection of a patent application directed to a virus-resistant corn plant for non-enablement over Potrykus. The examiner argued that the application failed to show that the Potrykus criteria for proving plant transformation had been met, yet the application showed regeneration of whole plants in the presence of selection for the herbicide resistance marker and the survival of their progeny in field testing in the presence of viral infestation in which the control plants did not survive.

We believe that the in vivo or in vitro results meet the criteria normally applied by commercial companies in advancing their commercial projects, and the pontifications by a bench scientist as to the sort of proof that would be needed to assure academic irrefutability should not

stand in the way.

COMMISSIONER LEHMAN: In that particular case -- Is that still pending? -- did you appeal to the Board of Appeals?

MR. ROTH: Well, the case is still pending and we have to make a choice as to whether to appeal, drop the case, or refile it.

COMMISSIONER LEHMAN: In that sort of a situation, do you talk to the group director where you think that the examiner is being -- is applying an inappropriate standard? Or have you just kind of dealt with it -- relied -- gone no further than an examiner?

MR. ROTH: In general, we rely on the examiner. In many of these cases we have examiners who are not yet primary examiners and so we know that the office actions are being reviewed and signed by their SPE.

COMMISSIONER LEHMAN: Do you talk to the SPE at all or try to contact them to raise these concerns?

MR. ROTH: In most of the cases we talk to the examiner and the examiner talks to the SPE, in effect, on our behalf, if at all. In some cases we have an examiner who agrees with us and the SPE is the one who disagrees. We're getting all this secondhand; the examiners are saying, "There's nothing I can do. My boss won't let me make this change."

The patent grant has traditionally been considered a reward for prompt public disclosure of technology. Yet, under the scenario I've described, we're being penalized for filing our applications as early as possible. In many cases, the work involved in laborious and time consuming, as has been mentioned. Plants have their own schedule for germination and development. It could take months, even years to carry out a project, the results of which have already been foreseen by the inventors; foresight that is confirmed by the hindsight of the obviousness objection. None of the work involved is particularly challenging to carry out using the disclosure as a guide.

What is needed is a consistent standard for assessing what sort of disclosure is needed to put biological inventions in the hands of the person of ordinary skill.

Turning to assessment level of ordinary skill. Many of our patent applications are directed to -- directed to plant inventions are routinely rejected over review articles reviewing improvements and progress in genetic improvement of plants over the last 65 years. The improvements achieved over that time period are cited as establishing that the level of skill in this art is such that the person of ordinary skill in the art can routinely make continued improvements of the sort

that have been made over the past 65 years. This approach is clearly defective for several reasons.

First, it's laden with hindsight. It focuses on the record of successes and ignores millions of failures over the same period. Most important, implicit in this approach is the unsupported assumption that successes reported in the past were routine and obvious. It's akin to reviewing the official "Gazette" for the many patents issued in microelectronics since the first integrated circuit and concluding that such inventions have been made on a regular basis, the level of skill is such that one of ordinary skill can readily make similar improvements in the future.

Before closing, if I could, I'd like to raise one issue not addressed in your Notice and that is deposits of biological material.

In our view, deposits of biological material are currently available on terms inconsistent with the limited nature of the patent grant. For example, the patent is limited to the geographical jurisdiction of the United States while anyone in the world can request a sample of deposited material. The patent gives the right to prohibit others from making, using or selling the patented invention yet the samples, which in many cases are self-replicating embodiments of the invention, are available without any undertaking from the recipients not to make, use or sell the patented invention. The PTO should not require that patentees place their invention at the disposal of the public and then aid and abet circumvention of the patentee's rights, either secretly in the United States or openly in other countries where comparable production is not available.

COMMISSIONER LEHMAN: Are you finding that is a problem? Have you had -- actually had experience where somebody --

MR. ROTH: Well, one of the problems we have, of course, is that the recipient of a sample can transfer that to third parties without notice to us. So what we get from the American-type culture collection is a notice that a law firm has requested a sample. We know they don't want it for themselves and who that ends up in the hands of, we have no way of knowing and so we have no way of finding out in whose research plot that material might be found.

With that, let me conclude and thank you for your time and attention.

COMMISSIONER LEHMAN: Thank you very much.

Our next, Louis R. Coffman, has decided not to testify so that will put us right about on schedule now. In fact, a few minutes ahead.

Now I'll ask Margaret Connor, from the Office of Technology Transfer,

the Agricultural Research Service, U.S. Department of Agriculture, to come forward, please.

MARGARET CONNOR, OFFICE of TECHNOLOGY TRANSFER,

UNITED STATES DEPARTMENT OF AGRICULTURE

MS. CONNOR: Good afternoon. My name is Margaret Connor. I'm a patent advisor for the United States Department of Agriculture, Agriculture Research Service, Office of Technology Transfer, and I'm here today representing the USDA.

The USDA employs approximately 3,000 staff and research scientists and supports agriculture research in agriculture research facilities and land grant universities throughout the nation with a research budget of approximately \$1.5 billion annually.

This department previously submitted written comments strongly advocating an experimental use defense to patent infringement. Today I will emphasize two points in support of our position: The need for and benefits of a research exemption from patent infringement, and our fear that recent use judicial decisions has seriously eroded the experimental use defense.

In addition, I will provide brief comments in support of broadening coverage of the Plant Patent Act to include plant parts and products.

First, a major contributor to the rapid advances in agriculture, medicine and other forms of technology that have benefitted the people of the United States and the world has been public sector scientific research. That is research conducted by or supported by federal and state governments in government, academic and other public and independent research institutions. The success of public sector scientific research has been shown to be critically dependent on the free exchange and use of ideas, research findings and their products.

The experimental use defense of patent infringement has provided protection for scientists in the public sector to pursue research avenues which have added to the knowledge base and led to rapid advances that have been the hallmark of American scientific enterprise.

We believe an experimental use doctrine is critical to the continued advance of agricultural technology. Several representative examples of problem-solving research that have benefitted from the free exchange of plant materials illustrate this. The USDA has been instrumental in the dramatic increase in crop productivity. In the last 40 years the yields of corn, rice, wheat and potatoes have increased an average of 134-percent, with conservative estimates contributing a minimum of

60-percent of these increases to the development of new varieties by plant breeding.

Varietal development requires access to often dozens of ancestral varieties. Typical of this is the rice variety La Mont, whose pedigree covers more than five generations and two dozen varieties. La Mont rice is an important contributor to the 149-percent increase in rice production in the United States since 1950, yet elimination of access to even one unique parental line likely would have destroyed the chain necessary to create this important crop variety. The result would also be reached in other instances of variety development. For example, another important variety developed through public sector research is a wheat variety, Gaines wheat, that was the basis for the grain revolution in wheat. It, too, was critically dependent on unrestricted access to the germ plasm of unique parents. Lack of unrestricted access could have prevented or greatly delayed the development of these varieties. We believe that continued progress in innovation in agricultural science will only be possible with free access.

Second, we no longer are sure what constitutes the present experimental use defense. The extent of experimental use exception has been eroded by several recent judicial opinions. Notably in *Roche v Bolar*, the Federal Circuit dicta has labeled as fatal to the experimental use defense any commercial intent or profit motive whatsoever. This presents difficulties for the continued success of research in the public sector which has been placed under -- strengthens Legislative and Executive Branch mandates to transfer research discoveries to commercial applications. The combined effect of the legislative mandate and the administration's technology policy makes it clear that the intent of the congress and the President is to move technology quickly to resolving the critical needs of the public. Achieving this intended goal should not be hindered by counterproductive restrictions on research use of patented or otherwise legally protected technologies, plants or animals.

In the past, reliance on administrative and industrial practice and policy regarding research use of patented technology, coupled with judicial application of the experimental use defense, has allowed rapid progress. There are recent examples, however, showing that public sector research has been hindered because companies have not permitted use of patented technology for research purposes or have made access to the technology difficult. Thus, the past successes may have no future peers without clarification and/or codification of the experimental use doctrine.

The present system has evolved its own cultural checks and balances over the last 200 years allowing for benefit to the patent holder without undue restriction on further innovation and progress. The Patent and Trademark Office has acknowledged the implicitness of experimental use in

the disclosure requirements of the patent laws. Accepted practice has long included both commercial as well as non-commercial development based on patent disclosures. As an example, descendent patents are commonly applied for and issued during the life of the parent or dominant patent, even though the descendent patent cannot be practiced without backer cross-licensing.

Failure to allow experimental use, whether commercial or non-commercial, could create an artificial barrier to the most promising lines of research. The United States Department of Agriculture supports the codification of a broad application of a research use exemption from infringement of patented technology to include not only research use for understanding the invention and its verification and replication but also to include attempts to improve the patented technologies to make new innovations available to the American people. All of these aspects promote the progress of science for the general welfare of not only the public but of the American industry as well.

The research exemption included in the Plant Variety Protection Act has worked extremely well for agricultural research, both public sector and industrial, and could serve well as the model for our research exemption for utility patents. It reads: "The use and reproduction of a protected variety for plant breeding or other bona fide research shall not constitute an infringement of the protection afforded under this act." In this Act, plant breeding is analogous to new invention or improvement patents. This approach is most consistent with the intent of Congress and with the technology policy of the administration, both of which represent initiatives to boost the commercial competitiveness of U.S. industry and the well-being of the general population.

Finally, in regards to the amendment of the Plant Patent Act. The USDA supports attempts to broaden the extent of protection provided by plant patents to include harvested material, including entire plants and parts of plants.

Current protection can be circumvented by growing the plant outside of the United States and importing harvested materials back into the U.S. for sale. Given recent efforts to extend the protection afforded by both utility patents and Plant Variety Protection Certificates to guard against this kind of act, it is appropriate to guard plant patent owners within this expanded umbrella of owners' rights. The economic effects on the ornamental plant, fruit tree and other industries dependent on plant patent protection warrant this protection.

Thank you.

COMMISSIONER LEHMAN: Thank you. I don't think you gave us any, did you have some specific examples that you witnessed where there has been

discouraging of experimentation based on someone else's patent rights? I mean, that you know of in your USDA experience?

MS. CONNOR: Yes. In our written comments there are some examples, and there was one case where in the breeding area they would not allow the U.S. scientists to have access to what they considered the most important line and, therefore, they had to just go without that and try to do breeding without that line. And then, in the newest instance where there's this patent covering all the lines of genetically engineered cotton, there's an instance that we might not be able to use this without asking for a research license. So it's possible that we may get to use it but it will be -- everything will be made more difficult and that's what we want to guard against, because, for example, in the breeding of the rice and the wheat that I mentioned, if there had been a lot of negotiations and back licensing of those many varieties involved there is a likelihood it just never would have gotten done.

COMMISSIONER LEHMAN: Would you -- In your own technology transfer policies at USDA, do you -- At the time you grant exclusivity to developers, do you include provisions requiring them to issue licenses to other experimenters? If USDA research is involved, you could use your licensing protocols to deal with that problem, couldn't you?

MS. CONNOR: It's my understanding as far as the protected patented property that USDA has control of that we do grant experimental use ability for researchers to use it. Our concern is that the USDA will not get to have experimental use of property protected that belong to other companies, and that's hindering our own research and other public sector research.

COMMISSIONER LEHMAN: Thank you very much.

Next, did you -- Since we missed one witness we'll --

MR. ROTH: Excuse me. I apologize for interrupting. If I had know someone was going to come and speak on this subject from USDA I would have prepared some remarks on this subject. I just want to ask one question that is escaping me and that is, if USDA is part of the U.S. government and rights of eminent domain they don't need a license. They can take it. I don't understand why they need a codified research exemption when the government doesn't worry about patent rights. For example, the Department of Defense issues a contract for an airplane, they don't care who has the patent on the airplane. The only remedy is in the Court of Claims. Why does the Department of Agriculture need a research exemption?

COMMISSIONER LEHMAN: I gather that you disagree with the Department's testimony.

MR. ROTH: Or at least would like an explanation.

COMMISSIONER LEHMAN: Well, then you'll have to address that to them. I think their concern probably goes beyond USDA research to research by others as well who don't have the capacity to violate the law and then just say, "sue me in the Court of Claims."

Next I'd like to call on Steven Brostoff of the Immune Response Corporation, please.

STEVE BROSTOFF, THE IMMUNE RESPONSE CORPORATION

MR. BROSTOFF: I would like to thank you for giving me the opportunity to testify before this hearing. I'm Steven Brostoff, Vice-President of Research and Development, The Immune Response Corporation.

I would like to contrast two different experiences I have had with the Patent Office over the last ten years to illustrate problems that need to be addressed in the patent office. I am the co-inventor on a patent that was filed in 1984. It used a well established animal model of human disease to demonstrate a novel therapeutic approach. After less than three years the patent was issued. The technology was licensed to a biotechnology company and millions were raised to support the development of this approach which is now in Phase II trials for several indications. Five years later, after joining The Immune Response Corporation and using the exact same well established animal model, I was the co-inventor of a new therapeutic approach which was more specific and longer lasting than the first. A patent for this approach was filed by The Immune Response Corporation. The European patent application was filed a year later as was an Australian patent application. This time it took over five years before the Patent Office acknowledged that we had allowable claims. By this time, we had already been issued the European patent and the Australian patent, even though they had been filed a year later.

But wait. The Patent Office changed its mind about issuing the patent because we had not demonstrated utility in humans. This is the Catch 22 of biotechnology patenting. In order to raise the millions in capital needed to pursue clinical trials one needs a proprietary position. However, the Patent Office appears unwilling to grant the patent assuring this proprietary position without clinical data. Moreover, this requirement for demonstration of utility in humans represents a departure from what had been needed just a few years ago. It was not needed to obtain a patent on the previous technology using the same animal model of disease.

In fact, based upon our previous experience that the animal model data would be sufficient The Immune Response Corporation had proceeded into

clinical trials using this technology and expending the millions of dollars needed to do so. The money expended had been raised in the public markets and were not specifically raised to support this technology. The decision by the company to expend this money was based on our assumption that a patent would be granted based upon criteria that had been used in the past by the U.S. Patent Office and that was being used currently by the European Patent Office. We did not know that the rules had changed. Fortunately, however, we had enough significant clinical data to overcome the utility in humans issue and, after another six months, were told once again that we would receive a notice of allowance.

But wait. The supervisor had reviewed the application and had questions about enablement, in spite of the fact that a primary examiner had decided to allow the claims.

I must say this is an experience similar to one we had with an HIV immunotherapeutic that we had developed which took approximately six years for the patent to issue. In that case we did get the patent issued but, once again, had similar experiences and ultimately were required to supply clinical data that fortunately we had and fortunately we had had the resources to expend in doing those clinical trials prior to obtaining the patent.

This kind of behavior on the part of the Patent Office makes it very difficult to make rational decisions on expending the millions necessary to develop new therapeutics. I will use an analogy that I think people here in California can appreciate. Our recent dealings with the Patent Office is like trying to play football in the middle of an earthquake. As we try to execute our game plan the ground keeps shifting under us. Sometimes it takes us sideways. Sometimes backwards, and occasionally forward. What we need is a stable playing field on which to execute our game plan. Moreover, we do not need unreasonable obstacles placed on the playing field. Since our competitors, outside the U.S., do not need to establish utility in humans they have an advantage in establishing a proprietary position in knowing how best to expend their capital in order to develop their technologies. The current policies of the Patent Office can only harm the biotechnology sector and contribute to the loss of the United States dominant position in this field. Most importantly of all, it will impede the development of many therapeutics that will ease pain and suffering and prolong the lives of millions of individuals. Give us reasonable rules to follow, and reasonable criteria to meet, and the biotechnology industry will thrive and we will all benefit by the products that the biotechnology industry will provide for us.

Thank you very much for your attention.

COMMISSIONER LEHMAN: Thank you very much, Mr. Brostoff.

I'd next like to call on Robert Schaffer of Darby and Darby.

ROBERT SCHAFFER, DARBY and DARBY

MR. SCHAFFER: Good afternoon, Commissioner and panel. I am Robert Schaffer. I am a partner with Darby and Darby, in New York. Darby and Darby represents a number of biotech firms ranging from multinational corporations to start-up high tech ventures, and I'm speaking today from the point of view of a practitioner in the Patent Office preparing applications for these clients and responding to office actions.

There has been a consensus developing today that the standards in place, the legal principles in place are appropriate, and I agree with that developing consensus. There also seems to be a developing consensus that those standards are not being properly applied in the Patent Office and that better education for examiners might address that issue.

I think that, however, many of the examiners in Group 1800, in fact, do understand the legal principles or much of them. The examiners understand that in an emerging technology there may be questions about whether the claim of utility in the specification can be accepted on its face. And it is appropriate for an examiner confronted with that doubt to ask the applicant for evidence of utility. The problems arise when the applicant submits his proof, usually in the form of one or more declarations from experts in the field and that response is considered inadequate. I think that the problem is not so much an interpretation or understanding of the law by examiners but, rather, what quantum of proof should be considered sufficient to overcome the examiner's initial doubt that the specification may not provide an adequate basis for utility.

When a declaration is submitted, and I've heard many people here state that they have applications pending for years which have gone through many continuations, and after 12 declarations or so the application is still under rejection. And I've had a similar experience and the response from the examiner to these declarations often takes one of two forms. Either the examiner says the declarations have been carefully considered and are deemed not persuasive -- end of rejection. And that is clearly a misapplication of the law, as has been said earlier.

The examiner should and must set forth specific reasons why that proof was not adequate. But, in many cases, the examiner understands that he or she is supposed to do that and, indeed, attempts to do so, and that usually takes the form of citing other references in the field which express doubts about the state of the art or what the technology can provide today, and so the applicant is in the position of providing sworn testimony that so and so, qualified expert, believes that a person of ordinary skill in the art would find a benefit, a practical benefit from

following the teachings in the specification. And there may be even additional data -- animal data or in vitro data or, in some cases, human data -- supplied but the examiner comes back and says, "Well, nevertheless, I'm not persuaded because here is an article which questions the validity of your animal model." And this basically takes the form of a battle of experts, a battle by the applicant with third-party experts that he can't confront in the Patent Office and this is an insurmountable battle.

It seems to be a position by many examiners that if there is not a consensus within the scientific community or a general agreement in the field that your model is adequate or that this is a disease that can be successfully treated, no declaration that you would present would be sufficient unless, indeed, you then go to the final hurdle of submitting human data which definitively shows utility.

I think that the problem is one of standard and that a more appropriate standard to apply would be that the examiner should review the declarations submitted, the evidence submitted, and if those declarations provide a reasonable basis for finding a utility in the application then, from the Patent Office point of view, it should be accepted that the statutory requirement for utility has been met. And only if defects in the reasoning or logic of the declaration or the scientific underpinnings for the declaration can be shown should those proofs be rejected. It is a standard of reasonableness, and I think that basically the examiner should be asking, "Is the applicant claiming an invention of reasonable scope based on the utility either alleged in the claim itself or in the application, and based on the submitted proofs is it reasonable to believe that this invention is more likely than not to provide some benefit?" And if the answer is, "Yes," then the utility requirement should be deemed satisfied.

There has been a question about whether there should be a presumption of utility and I think to some degree there is already a presumption of utility. The applicant makes a claim of utility and that claim is accepted, and unless there is a reason to doubt it -- and I think that really the examiner should be asking, "Has the applicant put forward a plausible explanation for why his invention should be deemed useful," in the face of the examiner's doubts, and if he does so he's done all that he needs to do.

And the same type of problem applies in enablement rejections which are tied to the utility rejection. Often the examiner will say, "Since you haven't proven that the invention works, you have not enabled a person to practice an invention that works." This is troubling for an additional reason that the applicant can submit proof of utility after the filing date but the applicant cannot supplement his disclosure to satisfy Section 112. And what can happen and has happened is that the examiner

may be satisfied on the utility proof but then will say that the parameters of the experiment that you used to demonstrate utility were not set forth in the specification and, therefore, "Although I am now persuaded that the invention has utility you still don't meet the enablement requirement." And, again there, I think the standard should be one of reasonableness and -- as opposed to a very high standard of definitive proof being required or doubts expressed by others in the field and publications being held against the inventor, and education could help within the Patent Office to some degree, but I agree with the gentleman who proposed that guidelines be promulgated either through the NPEP or through OG notice, and I think that that would be the most helpful mechanism for directing examiners what standards are the most appropriate to apply and how to apply them. I agree also that industry and the bar should review those proposals when possible, and I see that my time is up.

COMMISSIONER LEHMAN: I want to thank you, Mr. Schaffer. I think that was very helpful testimony. There were some specific examples that you gave.

Next I'd like to call Bertram Rowland, from Flehr, Hohbach, Test, Albritton and Herbert.

BERTRAM ROWLAND, FLEHR, HOHBACK, TEST, ALBRITTON and HERBERT

MR. ROWLAND: Before introducing myself I'd like to admonish the members of the Patent and Trademark Office for violating my constitutional rights as well as the other speakers. To request that patent lawyers speak for only nine minutes on these topics I consider cruel and unusual punishment.

COMMISSIONER LEHMAN: I thought you were talking about our constitutional right to not to have to preserve our bottom ends from sitting here through all of these interminable hearings. Sorry.

MR. ROWLAND: Also considering the fact that you're three hours out of phase, I think it admirable that you're still awake and listening to us.

My name is Bertram Rowland of counsel with Flehr, Hohbach, Test. I have the good fortune to have written the first patten in molecular biology in 1974. I've watched the field develop, worked at many academic institutions and start-up companies and I'm still active today.

During this period, we have watched a great industry arise. The industry has not only provided new therapeutic products but also promises to provide us with a continuing stream of new products. Together with the academic community the industry has and is continuing to provide insights into physiological processes which will allow us to understand

life better and to treat diseases better.

This industry has been dependent on the cooperation of academic institutions, venture capital, the drug industry and the dedication of numerous scientists in biological, chemical and electrical fields.

For the most part there has been a common motif in the finding of biotechnology companies. A professor makes a discovery, that opens an opportunity for future development of commercial products, such as drugs and modes of treatment. The professor's institution files a patent application that covers the discovery, and usually claims the use of the discovery toward the product development. Supported by the professor's knowledge and experience, and his colleagues, one or more venture capital companies provide initial funding to carry the development further. At some point the biotech company can raise enough money to do clinical studies or a drug company sponsors the further development for an interest in the product.

The hearing today is not about the exegesis of musty law but whether the development of the biotechnology industry will assume a new course. If academic institutions are unable to obtain patents on their academic discoveries, despite government policies supporting obtaining patents, then the original seed which starts the process of company formation will not be available.

It is essential to academic institutions that their discoveries be promptly disseminated. Patents on the intermediate events leading to the ultimate product, events which may take many years and large investments, will likely not be available to be patented and provide protection for the investment. In deciding what is the basis for Section 101 utility, consideration must be given to the effect such determination will have on the role the Patent Office serves in encouraging investment in technology and who will obtain the reward.

Utility has become a major determinant of patentability in technology, a situation distinctly different from other technologies. This is even true of chemistry where often the purposes for the invention -- diagnosis and treatment of humans -- are the same.

What is the rationale for this divergence? One explanation might be the determination that biotechnology is extremely unpredictable. This was certainly the basis for the decision in *In re Vek*. Expression in *sinobacteria* of a pesticidal protein previously expressed in other procaryotes was found to be unobvious, but the experience with the two strains could not be extended to the genus.

A further consideration, possibly a more important one, associated with the many inventions directed to the development of therapeutics is that

biotechnology companies are focusing their attention on indications for which there are no satisfactory treatments. Frequently no treatments at all. This means that there are no established models, no relationships to through gene and vitro models and animal models with experience in humans, no other compounds with which to compare results.

Proof of efficacy in humans is an insuperable obstacle to patentability. Biotechnology provides a logical approach to the development of therapeutics. It allows us to identify the proteins or other compounds involved in the physiological processes associated with an indication. It permits a determination of the physiological pathway as well as the branches, the regulatory mechanism associated with the pathway and potential points of intervention. In elucidating these pathways, processes and regulatory mechanisms there will be many inventions made before therapeutic compound is developed. None of these inventions is free of uncertainties, and all will require large investments. All of these inventions will aid in the development of a therapeutic and may be essential to the discovery of the therapeutic. The possession of these compositions and processes will greatly aid in the identification of candidates for further investigation. They will be used despite the fact that no prior history of their accuracy in predicting therapeutic activity in humans is available.

In rejecting these inventions as not having practical utility, the Patent Office is asking for a risk free guarantee of probable activity. Asking for proof of the next five or ten years of research and development activity will pay off. In the real commercial world, these efforts are calculated risks where the patent serves to reduce that risk.

Biotechnology has changed dramatically since the early eighties. Most of the early work had direct application. Today the targets are less obvious, require much greater effort toward achieving products, would involve multiple steps where new proteins and physiological processes will be identified, and will only occur over an extended period of time and by large investment. In the absence of obtaining a proprietary position the start-up company must rely on trade secrets. This will prevent the early dissemination of information, may inhibit investment which will impede bringing therapeutics to the marketplace, and could substantially diminish the U.S. leadership in biotechnology, all inimicable to the purposes of the patent system.

I would like to read you one example of the standard of utility in Group 180. For claim of a normal protein involved in the homing of white blood cells to sites of inflammation such as associated with rheumatoid arthritis and reprofusion injury the examiner stated, quote:
"...further, since there was no clear causal relationship between any of the diseases and the proteins of the invention, it is unclear what value

they would have in vitro, e.g., as diagnostic reagents. Knowledge that the proteins are involved in homing and that lymphocytes are involved in a variety of important immunological functions does not demonstrate that the proteins of the invention, which are only one of many components that allow lymphocyte function to occur, can be used to treat or diagnose diseases or other physiologically important conditions." End quote. The invention may not be useful to this examiner, but its discovery required great ingenuity and expense, and may be very import to our effort to treat arthritis and many other disabling diseases.

The issue I have been addressing is what should be the standard of utility for tools which have a reasonable expectation of success but have yet to be established since what is being studied has no precedent. Brenner v Manson does not mandate the present standard. In Brenner it was the compound produced by the process being claimed that had to be researched to find out if it had any activity. Inventions today are intended to screen other compounds for their activity or to be used for scientific research no less than a microscope which may once have been used to look at organisms which had no known utility.

I'm going to skip to my recommendation. Well, I guess I'll try to get through.

One might decide that a practical utility for a tool, product or assay is a reasonable likelihood of aiding and obtaining a commercial disposable. The devil is in the details of how "reasonable" should be defined. By defining the term one way, one could come to the conclusion that any compound associated with an indication for which there are no known or only a few therapeutics is not patentable. Since one cannot show its utility as a predictor of efficacy as a human therapeutic, no intermediate in the development of the therapeutic would be patentable until evidence of efficacy was available. Patents would then go to the developer of the final evidence of efficacy. Regardless of what prior results had shown, any successful Phase III result would be a basis for patentability. This was the result of In re Gangadero. This approach supports the established drug house. It would sound the death nail to the biotechnology industry as we know it today.

I would suggest that a reasonable standard for utility is that the tool does provide some useful information concerning a natural process or can be used to ascertain useful information about a natural process, or the effect of other compounds on the natural process. The availability of the tool does further the needs of mankind.

Without tool patents, there will be reason for academic institutions to file patent applications. For many companies, it has been these applications which have served as a nexus for founding of a biotechnology company. As you can well appreciate, the question of the standard for

utility in biotechnology is not an abstract issue. It is already having an impact and the ruling resulting from this hearing will determine how the industry develops.

Thank you.

COMMISSIONER LEHMAN: Thank you very much, Mr. Rowland. Next I'd like to call John W. Schlicher, of Crosby, Heafey, Roach and May.

JOHN SCHLICHER, CROSBY, HEAFEY, ROACH and MAY

MR. SCHLICHER: Commissioner Lehman, Mr. Van Horn, Mr. Kushan and other members of the panel, thank you for coming to California. Thank you for having these hearings, and thank you for permitting me to come to testify briefly.

I'm John Schlicher. I practice patent law with the firm of Crosby, Heafey, Roach and May, and I'm here to give my personal perspective on these things. I've worked as a patent lawyer for 20 years, some of that time in a biotechnology company, and have worked and thought about biotechnology and its economic significance for most of that time. I also teach patent law from time to time at Stanford Law School.

These hearings are about biotechnology but the issues transcend biotechnology. I have said elsewhere and I will not repeat here, there is not and should not be any separate body of law for biotechnology. Patent law must transcend technical boundaries. Historically it has. The courts insist they do, and we ought to adhere to that in this area as well.

It's also true that throughout the history of patent law there have from time to time --

COMMISSIONER LEHMAN: Could I ask you a question?

MR. SCHLICHER: Of course.

COMMISSIONER LEHMAN: Is that what you think the people have been asking us to do here today is have a separate law for biotechnology? Is that what you have been hearing? If you were here earlier today.

MR. SCHLICHER: I'd prefer not to characterize what other people have in mind. I certainly hear lots of talk about biotechnology patent law and it seems to me there is a perfectly understandable impulse among lawyers, courts and business people for certainty in this area. Bright line rules, as you referred to earlier, shorthand rules of thumb that help you solve your problems quickly and predictably are very attractive in this area. My perception is that -- and I think history bears it out -- it

usually turns out to be a mistake, because patent standards are difficult to define with great precision; it has to do with the economic purposes they have, the facts in which patent laws have to be applied change from time to time in highly unpredictable ways, and unless you adhere to a formulation that says, "Let's apply general principles to the facts of the situation now before us," I think the system is very prone to error.

My second main point is that the Notice that accompanied these hearings ought to be historically a noteworthy event in patent law, because the Notice insist that the information you want is about the economic effects of various rules and their effects on incentives for people to do research, development and commercialize inventions. That is and always has been the right question. The courts and the Congress and the lawyers have not always asked it and that is the reason, in my view, that patent law has for the last 200 years developed an extraordinarily complicated and confusing set of rules. And only by asking it and answering it over and over again will we improve the situation, and that was the impulse for me to spend an unusual amount of time writing a book to try to do that.

My third point is that the Notice talks about the economic role of patents to provide exclusivity to induce investment and risk taking in research, development and commercialization of inventions. I would define the role of patents somewhat differently. The goal of patents is to induce investment and risk taking in producing technological information about new products in processes that in the absence of patents the market would be unlikely to produce or produces early. If someone produces information about the general character and features of a new product or process that distinguish it from prior products and processes, he or she traditionally has been and should be entitled to a patent even if there are large additional expenditures necessary to use that invention commercially, and even if there is no conceivable benefit that's perceptible at the time for consumers and no perceivable commercial potential for the invention. This view of the patent system is consistent with the general history of patent law as it's operated for the last 200 years.

My fourth main point has to do about the operation of the Patent and Trademark Office and its proper role in the patent system, which is the specific subject of these hearings.

The Notice talks about the importance of enforceable patent rights, and the office I think has recently given much attention to trying to focus on the question of quality and it should be applauded for that effort. However, my view of that, there's one danger in pursuing quality. In my view, an economically sensible role for the Patent Office in the system is that the office should screen out and refuse patents for inventions that plainly and clearly do not qualify under one or other standard for

patent issue. Because patent law standards do not draw bright lines, because the facts that underlie applications of those standards are difficult and expensive to ascertain, and because the patent office has limited resources in time, information gathering capability and technological expertise, the Patent Office cannot possibly make a 100-percent accurate assessment of each particular patent application.

Congress set up the system with that in mind. Before anyone ever has to stop doing anything because of a patent, they must get a Federal Court's order ordering someone to stop. And before anyone has to agree to stop or pay a dime because a patent issues, the marketplace permits people to get together and talk about the decision the Patent Office made about the law and the facts and to bargain out a private marketplace assessment of whether or not a patent should have issued and, if it did, the likelihood that a court would agree with the office.

It seems to me there is a terribly important benefit to that system, not the least of which is avoiding a full-fledged costly, factual examination in all cases, and in confining it to the small number of cases where the patents become commercially significant and involve issues that are difficult for the private negotiation process to resolve. Under that view, it is and ought to be of no concern that 10-, 20-, 30- or 40-percent of the patents issued by the patent office are held invalid or otherwise improper by a court in an enforcement action. And if the Patent Office perceives its job as making a 100-percent accurate assessment, my fear would be it would bias incentives of the examiners and the office against granting patents.

Now, you have raised a number of very important substantive questions and I cannot deal with all of them now and I will give you a written paper in which I have and have proposed I think some tests.

My view, if you haven't guessed it already, is that the law in all of these areas is, indeed, unclear, and making it more clear is our job. In some areas I think we could improve it. In others I think probably there are not. And if I had time I'd like to talk about some of those. The only one that I think I will talk about briefly is the utility requirement because it was first on your list and it seems to me it plays an important role in the second.

The law of utility today is that expressed in *Bredner v Manson*, which is deemed to be the controlling case simply because it's the last one from the Supreme Court. In my view, *Brenner v Manson* is one of the low points in patent law. The language in it that causes us so much trouble had no basis in law, no sense in policy, and ought to be utterly and totally disregarded, and, if we have any hesitancy about doing that, I would have no hesitancy to go into Congress on that.

My time has expired. Thank you again for your time.

COMMISSIONER LEHMAN: Can you just sort of complete that thought because, you know, that's very provocative?

MR. SCHLICHER: An invention to talk about Brenner v Manson is an invention to a long discourse.

Brenner v Manson has so many problems it's difficult to know which one of them to pick out first to poke fun at. The language obviously that causes everyone trouble is that until an inventor has developed an invention to the point that a specific benefit exists in currently available form there is insufficient justification to permit the inventor to engross what may turn out to be a broad field indeed. And that language inclines you to believe that a patent ought not issue until specific benefits exists to consumers the day after the patent issues.

Obviousness is applied to a world in which the business and regulatory requirement requires years and hundreds of millions of dollars of investment in development after the fundamental characteristics of a product have been ascertained to some degree of certainty. Brenner v Manson inclines you to try -- inclines you away from granting the patent until that additional development, expenditure, and until that additional time has run. And it seems to me that in this industry, as well as in all others, that's a mistake. That is not a hurdle that Thomas Edison faced when he first went to them with the light bulb, even though there were no transmissions lines and no wires in houses and there were not benefits, and I could go through the whole history of patents and it describes almost every important patent. I think the language is difficult. The decision is easy but I think that it was a fundamentally mistaken proposition that has caused us enormous difficulty.

COMMISSIONER LEHMAN: That completes the thought and I really appreciate that, and I also think it's -- it fits in with my own view that we don't necessarily have to be guided by what, you know, the court's say. I realize that's not what all of our witnesses said, that we have the capacity to develop our own position in the courts from time to time in the Patent and Trademark Office as well.

MR. SCHLICHER: Since you have made that point, I profoundly agree with that, that in this area the Executive Branch has an independent responsibility to determine the policy that will best achieve the economic effects of the system and I would encourage that independence.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call on Alain Schreiber, of Vical Corporation.

ALAIN SCHREIBER, M.D., VICAL CORPORATION

DOCTOR SCHREIBER: Mr. Commission, lady and gentlemen of the panel. I'd like to thank you for the opportunity to testify. My name is Alain Schreiber. I'm the president and CEO of Vical, Inc., a gene therapy biotech company here in San Diego. I'm not a lawyer; I was trained as a physician and in my prior career I was head of preclinical research for a large pharmaceutical company Rompol-Lanke-Rohr.

I first would like to commend you. I don't think biotech CEOs or pharmaceutical executives ever spent a whole day listening to clients and customers and users of their services ventilate frustration or anger and be presumptuous to give you suggestions or recommendations. The other part of this day being very interesting for me, I thought I was being singled out as the only company that couldn't get some patents through the biotech office patent. I see that I have a few other companies that have experienced similar cases.

I'd like to give you the perspective of a CEO of a small and struggling company and the current, difficult capital markets and what I perceive to be the needs of the industry.

We need an efficient system, a fair system. The worst that an investor can face, adding to the uncertainty of clinical development or the drug discovery process, is uncertainty in the proprietary position of his investment. Basically, the longer you drag that uncertainty the less people will be attracted to make speculative investments in, almost by definition, enabling technologies.

My second point is that I would make a plea for reasonableness that there should not be a double standard between how patents can be allowed and obtained for chemical entities, new chemical entities, to which I've been exposed quite a bit, and biotech patents. And I think I'm echoing what some of my colleagues have said, in particular, focusing on utility and enablement and the high standards.

It is difficult to count angels on the pinheads. First you have to show utility in man, that's already difficult to get there. It is even more difficult to teach the skilled practitioner as to dose and regimen, and even FDA approval doesn't always guarantee or ascertain that we know all this. So somewhere finding level playing fields and a dialogue of reason as to what can constitute a reasonable demonstration, in your own minds of a value of an invention, would be very valuable to continue this very exciting industry sector that, for good reasons, is in the United States.

You may have noticed by now my accent is not exactly from San Diego. I am fortunate to come here and benefit from the opportunities of the

American system. The biotech industry is definitely an American endeavor. That was thanks to a number of factors, one of which is availability of capital, and, two, is the general climates encouraging investments, offering protection through your services. It would be ironical if the European Patent Office or the Japanese Patent Office would now provide competitive advantages to this great emerging technology. As a physician and as a businessman, I think we're trying to do exciting things. You've helped so far and I would make that plea that clearly you have already taken a lovely step by coming to America's finest. Thank you.

COMMISSIONER LEHMAN: Thank you very much.

I'd next like to ask Eric Woglom of the Association of the Bar of the City of New York.

ERIC WOGLOM, ASSOCIATION OF THE BAR OF THE CITY OF NEW YORK

MR. WOGLOM: Good afternoon. I'm Eric Woglom. I'm from the firm of Fish and Nieve in New York. I'm here today in my capacity as the chair of the Committee on Patents of the City Bar Association. The City Bar Association is a voluntary association of 20,000 lawyers and judges. It's composed of a number of committees. The Association speaks to the outside through its committees and I'm here today in my capacity as the chair of the Committee of Patents. The views you will hear today are the views of my committee and not necessarily those of myself or my firm.

Mr. Commissioner, I have to applaud you as everybody else has applauded you for holding these hearings. One of the things that you mentioned earlier this morning was that you wanted to hear what your customers think, and I have some good news and some bad news. The good news is we applaud these hearings. The bad news is I suppose is we wouldn't have come all the way from New York City if we were happy in all respects with everything that was happening in Washington, D.C.

The major issue in the eyes of our committee is the question -- is the perception, and I emphasize perception because I don't know what the fact is. The perception is that about four years ago the Patent Office decided to change its internal policy or its internal standards when dealing with the question of utility or operability. This has created some tension between the bar and the office. The people on my side of the bar are concerned because we seem to be in the dark. We don't know exactly what the policy is -- it's just normal anxiety -- although I think we're getting to know what the policy is real quick.

There's also a concern because we're not able to accommodate ourselves to a policy which has not been announced. There's also a concern because

there's a policy, if it really is a policy, has been implemented without public discussion and, frankly, without an opportunity up until today, perhaps, to try to persuade you that the policy is wrong.

Where do we go from here? I think step one is what you're doing right today, is to listen to the bar, listen to industry, listen to the people, listen to your consumers, listen to the people who are effected by what's happening in the Patent Office.

Step two I think would be for us to hear from you, hear from your office, as to whether or not the Patent Office did change its policy, did change its approach to the utility operability question, in at least Group 1800, about four years ago. It may be that the policy hasn't changed. It may be the view of the office that the policy has remained the same, it's just that the kinds of inventions which are being presented to the office have changed over the past four years. That may be the answer and that may quell all the concern. But the concern lingers nevertheless.

It may be that the policy did change four years ago, and if you could tell us that that would clear the air and make a lot of people much more comfortable about the practice.

The question of accommodating ourselves to a policy is much easier accomplished if we know what the policy is. The opportunity to argue against that policy or try to change your mind with regard to that policy is something which would help also, if we knew what the policy was. When I say "you," I'm talking about you as the personification of the Patent Office. Clearly, if I'm talking about something that was instituted four years ago it's not Bruce Lehman who is the person that instituted that policy.

There's speculation in the bar right now as to what is the purpose or what are the motivating forces behind what we perceive to be the new policy as of four years ago. Is the Patent Office trying to carry out the role of the FDA? Is the Patent Office trying to carry out some quasi SEC functions? The Notice of these hearings referred to the imprimatur of the Patent Office on invention, how that may affect the economic value of the invention. It may give rise or may destroy the hopes of people who have illnesses that might be treated by the invention. These are not traditional patent issues. That does not mean that they are irrelevant to the inquiry and it does not mean that they are irrelevant to the formulation of a policy but, again, if the air would be cleared by your office and to tell us whether or not the policy did change four years ago, that would be a big help for all of us.

COMMISSIONER LEHMAN: Maybe I can answer that right now. As I understand it, I don't think the policy changed four years ago. I think

that your characterization is partly correct in that the nature of the -- the nature of the technology started changing four years ago. I think there have been a couple of factors. That is the inventions for which patents are sought have changed, and then the other factor, too, ironically it's the flip side partly of one of the reforms that we made.

In my opening statement I indicated that now over half of our examiners in 1800 are Ph.D.s. Well, I think that initially when a lot of these younger Ph.D.s came in, many of whom had come from the laboratory bench, there was a tendency -- they're very good scientists and there may have been a tendency to look at their work more as peer review rather than as patent examining.

Now, as part of our training process, I think we've changed that a lot. I think there's an internal perception in the Patent and Trademark Office that we're sort of ahead of what we've been hearing here. That, in other words, a lot of the complaints that you've been hearing are complaints that go back to this transition period that I just described when we had examiners perhaps that got away from us, didn't fully -- were well trained technically but not well trained legally, and that given another six months or year you'd start to see a better response. But I don't think -- bottom line, there was no deliberate change of policy four years ago. I think these events that I've just described are what's happened. And, obviously, the purpose of this hearing is to -- we don't want to just wait for this just to gradually fix itself. We want to try to fix it as quickly as we can so that there is a perception of confidence in the Patent and Trademark Office and that's why we're here today, and we're going to move quickly to try to give that sense of confidence following these hearings.

MR. WOGLOM: Just to back up what I was saying with a few specifics. You heard from others today and I could tell the same stories with regard to people who have been practicing in the biotechnical field for 10 years, people who have been practicing in the pharmaceutical field for 10 years, and up until five years ago a certain showing, whatever that showing might would be, would be sufficient to deal with utility issue.

Up until, maybe starting four years ago, maybe starting with the Balzarini decision, that showing has not been accepted. Now, it might have been as a consequence of the examiners on their own exercising some very educated hindsight, or it might have been a policy. And one of the purposes I said was just to come here and try to clear the air in that regard.

A couple of other specifics, if I may. Often the members of our committee, and other people I've talked to in the bar, complain that utility operability rejections are not supported by adequate scientific evidence that in terms of references to receptive articles. What we have

is, more often than not, examiners exercising their own subjective view as to what success might be predicted by one reasonable skilled in the art at the time.

Another problem is the problem with historical inconsistency. Again, one of the earlier speakers referred to what had happened five years ago and then contrasting what's going on today. What we have in situations is maybe an applicant gets a patent, what I'll call the old policy, acknowledging of course that the policy of course hasn't changed, but under the new system the patent doesn't get issued, and it may be on the same invention. You basically have a patent and an application which interfere, in the classic sense, but interference will not be declared because the applicant cannot meet the utility requirement as is now being applied. Would it not be useful to declare interference in those situations? Especially in the situations where the applicant would be the senior party if an interference would be declared, and let those two parties fight it out between themselves, even if the examiner would like to make a sua sponte motion that there is no utility and ought not be a patent issued to either party.

Another question that comes up, of course, is the Section 112/103 issue that some examiners see only divine inspiration when they look at the prior art in diabolical obscurity when they look at the applicant specification. That's an old issue but it's still a current issue.

Lastly, and I see my time is about to expire, I'd like to refer to the issue of the 20-year term. The biotech members of my committee feel that that's something which would be hurtful to the biotech industry and wished there would be more debate on that and not be subject to an or down vote in Congress on the GATT treaty. Likewise, restriction requirement with any term -- 20 years or otherwise -- running from the date of filing something is something which is very difficult for a biotech applicant.

COMMISSIONER LEHMAN: Thank you very much.

MR. WOGLOM: Thank you.

COMMISSIONER LEHMAN: Next I'd like to -- oh, by the way, Stacey Channing, from the Immulogic Pharmaceutical Corporation, we'll not be hearing and so we're going to move directly to T. Andrew Culbert from Drinker, Biddle and Reath. Mr. Culbert is here? Is he here? If not, we're going to move right ahead. Is Ronald Tuttle here from Houghton Pharmaceuticals? Great.

RONALD TUTTLE, HOUGHTON PHARMACEUTICALS, INC.

MR. TUTTLE: While I'm here I had planned to use this 30 minutes to

organize my thoughts so just give me a second to shuffle my papers, if you will.

COMMISSIONER LEHMAN: I thought you were going to say you were going to use the 30 minutes for your --

MR. TUTTLE: No. You'll be grateful to know I don't have any such intentions as that.

Thank you for the opportunity to appear here. My name is Ron Tuttle. I'm not an attorney, I'm a scientist that's been doing R&D in the pharmaceutical industry for the last 28 years. I want to direct my comments to what appears to be to me, as an inventor, a change in standard for utility. It seems that that advent of biotechnology has caused a change in the practice of the patent offices that impacts not only inventions stemming from biotechnology but those drugs stemming from conventional uses in medicinal chemistry, and I contrast my past experience with my present experience; makes me think you're moving in the wrong direction.

I am the inventor of two patents which have turned out well insofar as drugs for the diagnosis and treatment of heart disease. One of those was in 1972, the more recent one in 1992. I think if we'd had the current standard for utility, that is I must at time of making application prove that the drug works not only in animal models of disease but in human models, neither of those drugs would have come to pass and the present patients who are benefitting from those drugs would not have benefitted. The reason for that is simple. It costs a great deal of money to do clinical trials to obtain human data and we get caught in a cycle that I can't finance to get the money to do the clinical trials unless I can assure the investors of a proprietary position which can only come from a patent.

Lastly, in contrasting my experience at the present time with what I regard as good experience in 1972 and 1992 for the advent of those drugs, is a current patent we're trying to obtain, and, although I have supplied a great deal more data on animal models than we did in the past patents, we continue to receive rejections from the examiner based on the lack of human data.

We have supplied what I think are good arguments regarding the relevance of those animal models, and while we all are aware that there's oftentimes failures between animal models and clinical outcome, that generalized principle or finding that there's often failures should not be applied in the specific. I believe if the examiner is going to reject our animal models of diseases irrelevant, specific reasons should be cited and tell us exactly why it's irrelevant in his view.

In the present application, we've gone so far as to supply the toxicology data. We have supplied the institution review committee approvals for testing of the drugs in humans but still we're faced with rejections because of lack of human data on utility.

Lastly, in the Notice to the hearing, I noticed there was concern, which is probably appropriate concern, that some patients suffering from certain diseases for which a patent is issued may have their hopes falsely raised in believing that the issuing of a patent somehow shows the patients that the government believes in this invention. I think that is a confusion of the mission of the Patent Office and the mission of the FDA. And while it's an argument for better public education I don't think it's an argument for changing past practices of the Patent Office which I think past practices have benefitted patients, the industry and the competitive position of the United States. So, obviously, I'm making an argument for the status quo, at least insofar as the advent of biotechnology should not impact how we view inventions from traditional medicinal chemistry.

Thank you.

COMMISSIONER LEHMAN: Thank you very much, Mr. Tuttle.

Next I'd like to call Jeffrey Miller of IXSYS Corporation.

JEFFREY MILLER, IXSYS CORPORATION

MR. MILLER: Thank you for giving me the opportunity to speak here today. My name is Jeffrey Miller. I am currently a postdoctoral scientist at IXSYS, a biotechnology company here in San Diego. I'm co-inventor on patents arising from my work there at IXSYS. However, today Bill Hughes, who's the founder and chief scientific officer of IXSYS thought that my testimony would shed a different light if I were to address this body as a representative of small, independent scientists who endeavor to protect their individual contributions and ideas in the arena of biotechnology. Therefore, I am here as an individual patent applicant today.

In December of 1992, prior to coming to IXSYS, I filed a patent application for methods and compositions for in vivo sedensynthesis. This is a new and -- In the new terms, terms I'm starting to learn to use, a basic enabling technology. The initial application has been through two office actions and I filed both PCT and continuation applications as well as related trademark applications.

During these two years, I have learned some of the terms and workings of the Patent Office and these experiences are the basis of some of the concerns that I voice here today.

First, it appears that a final action is frequently rendered by the patent office in response to the second office action or following the second office action. This response is advanced without regard or consideration of progress which may be evident in the prosecution of the patent. Now, this action appears to be -- on the outside to be a mechanism for restarting the patent examiner's clock and obtaining filing fees for the Patent and Trademark Office, rather than action necessary or desired for expediting patent review.

Second, I'll say I've noted a lot of people have voiced their views today that there appears to be an inconsistency in the evidentiary standards required to determine the utility in patents related to biotechnology. It is clearly impossible to expect an individual or a small biotechnology company to garner data required to determine efficacy for an invention in a primary patent application.

Current mechanisms for addressing this issue, from the scientist's or patent attorney's perspective, I understand include to continue to the costly and unproductive prosecution of the patent application, basically keeping the ball in the air and claims active while awaiting efficacy data from collaborators with deep pockets. Unfortunately, run into a Catch 22 situation where any potential deep pocket collaborators generally require proof of proprietary position prior to advancing the funds to initiate the costly efficacy trials.

I think that the small inventor in biotechnology would like to see a return to normalized evidentiary standards where simple, reproducible, practical utility meets the basic requirements. Typically, I'll say that patent applications are not requests for a hunting license, rather we're trying to protect ourselves from the large corporations which can turn the crank and circumvent our initial applications with refinements which require resources beyond our reach. Therefore, I think you should let the market forces determine the efficacy or utility of the method or composition.

And one of the points that was brought up earlier is a good point and that is if you can get licensing money for your patent application, your method or your composition, that's a pretty good indication that somebody thinks that -- somebody who's got a good scientific background thinks your patent application, your patent has merit. This approach allows a reasonable extrapolation of claims which will protect you downwind down stream and protect potential uses and serves to protect both the small innovative scientist and the large biotechnology company. This consistent application of existing standards -- existing standards -- will allow exploitation and marketing of ideas and innovation without doing harm to the public. Thank you.

COMMISSIONER LEHMAN: Thank you. Were you here earlier when Mr. Schaffer testified, the lawyer from New York who testified that one of the solutions might be for us to, in effect, establish a presumption in favor of declarations from experts as opposed to, you know, having any --

MR. MILLER: I think --

COMMISSIONER LEHMAN: Would this solve the problem for your company?

MR. MILLER: If you're asking whether expert testimony would --

COMMISSIONER LEHMAN: That's right.

MR. MILLER: -- provide that, I think his point was that that might be useful. However, you might also run into the problem where you would have two schools of thought, both of which are expert witnesses and they could be in disagreement.

COMMISSIONER LEHMAN: In this case the only witnesses would be the witnesses that you produced.

MR. MILLER: That would help.

COMMISSIONER LEHMAN: You don't have oppositions in --

MR. MILLER: I don't have any problem with that.

COMMISSIONER LEHMAN: -- the U.S. Patent Office as you do in Europe.

MR. MILLER: That would be great.

COMMISSIONER LEHMAN: So basically the -- The answer is "Yes," that would solve your problem?

MR. MILLER: Yes, I think that would solve the problem. Thank you very much. Appreciate it.

COMMISSIONER LEHMAN: Next I'd like to call Gail Kempler of Regeneron Pharmaceuticals.

GAIL KEMPLER, REGENERON PHARMACEUTICALS

MS. KEMPLER: My name is Gail Kempler. I'm the patent counsel for Regeneron Pharmaceuticals. We're located in Tarrytown, New York, so I'd first like to thank you for coming to these hearings in San Diego. When I had the opportunity to meet you a couple of weeks ago, at a Council on Foreign Affairs breakfast, I told you that I thought current practices in Group 1800 were paralyzing the biotech industry. You said you thought

that that had all been resolved but, if not, I should testify today -- so here I am.

Regeneron Pharmaceuticals in a biotechnology company that was established in 1989 by two neurologists who were fed up with available therapies for treating neurological diseases, such as Lou Gehrig's Disease. The founders recognize the potential of neurotrophic factors which are naturally occurring proteins which can be used to promote the and/or survival of neurons, and they had the concept that if these factors could be used to help neurons to survive that they would be useful for the treatment of neurological diseases where you have degeneration of neurons.

Although we hoped when the company was founded that we would be the first to identify and clone these new factors, as it turns out, there are a lot of other companies that had the same idea and many of these companies have armies of cloners which we don't have. Unfortunately, we only have currently 200 people at Regeneron. So, more often than not, we lose out to the armies of cloners and other companies are the first to get the patents on the genes and the proteins. However, since our roots are in neurobiology, we do pride ourselves in being able to figure out what a lot of these new proteins do and we do develop both in vitro assays in house, as well as novel animal models. So often we do determine therapeutic utilities that have not been discovered by the companies that have cloned the new gene. Accordingly, we have a patent portfolio that's filled with human therapeutic applications.

I spoke at a forum about a month ago where Charlie Warren was asked -- and I'm quoting the question: "Sometimes an applicant must come up with data sufficient to persuade the FDA to approve a drug in order to persuade your department to issue a patent." In response Charlie said, and I quote: "That is not true. I will say we have had examiners who have taken that tack in the past and hopefully the past is behind us and they don't do that anymore. "However --" and I stress this "however," and I'm still quoting -- he said, "as I did say, there are some disease states where one skilled in the art, given contemporaneous knowledge in the art, will not accept anything less than human clinical data to establish utility and enablement."

Well, obviously, the diseases that we're working on at Regeneron are part of these kind of diseases and basically Group 1800 has held us to this requirement for human clinical data. I think it's common knowledge, at least I've heard this, that there are certain disease states that fall into this category; they seem to be cancer, and they seem to be AIDS treatments, they seem to be neurological diseases. And it seems to be the diseases for which there are not very many therapies. It also seems to be the diseases for which the therapies are most needed and most necessary.

Although Group 1800 says that animal model data will suffice, the current standard requires that such animal models be art recognized. This means not only must the animal models have symptoms that mimic the diseases that you're going to treat but there also has to be a demonstrated correlation between the data derived in the animal model and human utility. So, in other words, if you're a company like Regeneron where you've developed animal models that have never been utilized before, you're effectively punished because you cannot possibly correlate data obtained in these animal models with human data. So, in effect, you have to run a human clinical trial to prove your models. And, so, you know, if you're going to go to that you may as well just run your human clinical data -- human clinical trial and submit your human clinical data. We've tried this, too, in a recent application that we've been prosecuting and have found that trying to submit human clinical data is wrought with even more problems.

The data comes continuously. You don't have just one piece of data that you can submit to the patent office. You have a continuous stream of data and so every time you get more data out of your clinical trial you run into the problem of: "Do I have to continuously update the Patent Office every time I have some new information?" You also have the problem that you have several treatment groups in your human clinical trial so you have to decide: "Do I have to submit to the Patent Office all of the treatment groups or just the ones that work? Do I have to average them together?" and then you're accused of withholding certain data and only submitting the good data if you only submit the data from the treatment groups that work.

We are also told by Group 1800 that the data has to be statistically significant at the same level as required by the FDA.

And, finally, our biggest problem, which I really haven't heard anyone mention here today, is we are a small biotech company and any data that we derive from a human clinical trial is material. So the SEC requires that we make public any information that we have regarding our human clinical trial. So when we submit data to the Patent Office to satisfy Group 1800's requirements, we also have to disclose the information to the public in some kind of a disclosure; press release, and also in our prospectus.

When you make such a disclosure in your prospectus, you generally have to put in some kind of disclaimer because you don't want to be accused by the SEC of hyping your drug before its time. So we generally put a statement in our prospectus that says: "The results of Phase I and Phase II clinical trials do not necessarily predict the results of the Phase III trial or any further trial or potential regulatory approval of or success of the drug." And we've recently had this statement from our

prospectus cited against us in an examiner's rejection. So not only is Phase I and Phase II clinical data insufficient, you now have to have Phase III clinical data or any disclaimer that you put into any of your SEC-related statements will be used against you.

So the bottom line is that, despite Group 1800's indications otherwise, for diseases such as neurodegenerative diseases only data sufficient for FDA approval will suffice to obtain patent protection. And small biotechnology companies, like Regeneron, just simply cannot afford to run these human clinical trials absent some form of patent protection. The result is only the companies that -- the only companies that can run clinical trials are the ones with the army of cloners. And I think that even though I would say in some cases we'd be happy to take all the works home because we're first to clone a protein, I think it's a problem when a company clones a protein and really hasn't discovered many of the utilities and therapeutic applications of that protein but yet they take home all the bacon because the companies that discover alternative therapies for those compounds simply cannot compete. They simply can't run clinical trials.

We continue to believe that the understanding of novel proteins and what they do is as valuable and as deserving as protection as the cloning of the molecules. The standard used to examine applications relating to the tough diseases such as cancer, AIDS and neurodegenerative diseases shouldn't be so tough that it discourages discoveries of therapies in these areas.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call on Andrew D. Fortney of Oblon, Spivak, McClelland, Maier and Nestadt.

ANDREW FORTNEY, OBLON, SPIVAK, McCLELLAND, MAIER and NESTADT

MR. FORTNEY: Good afternoon. My name is Andrew Fortney. I'm a patent agent with Oblon, Spivak, McClelland, Maier and Nestadt. We have offices in Arlington, Virginia, literally right across the street from the building in which Group 1800 is located, and we have an office in San Jose. I'd also like to thank the Commissioner for informing me of the teleconferencing office to be set up in Sunnyvale. I'm sure our firm will take full advantage of that.

I guess last night when I should have been thinking about what I was going to say today I was watching 60 minutes and if anybody else saw it there's a big story on this cloning of a gene from an Italian family who have really very little, if any, signs of cardiovascular disease even though a number of members of this family have life-threatening cholesterol conditions. Since it didn't cost me anything, I'll submit

the front page of "U.S.A. Today" as evidence of this discovery.

The point of the story I guess is hopefully there is some relevance to everybody -- to the public -- that now biotech has some really exciting offerings that can effect everyone directly.

Doctor Shaw, who I guess is a doctor at Cedars Sinai who did the rabbit testing studies in this case, he was, you know, on TV telling the public this technology exists. We can bring it to you now. This is what biotechnology has to offer to you. So, with that in mind, I'd like to actually go into a couple of things that I'm kind of curious about from a policy perspective which maybe we haven't really talked much today. I guess I should also read the papers and listen to the news more often than I do, but from what I gather the Clinton Administration is interesting -- or interested in maybe three policy goals which the Patent Office can have a direct effect on.

One is advancing the business atmosphere in high technology. This is an area in which the U.S. is concerned about losing its competitive advantage, biotech being one of these fields.

In the area of health care reform, they're interested in lowering the cost of pharmaceutical agents most prominently.

And another policy goal of the Clinton Administration, as I understand it, is minimizing the duplication of effort and of overlap between the different agencies. I think some of the ideas we've heard today sort of address these things, but particularly the need by the biotech industry for early issuance of patents and getting some kind of reasonable claims' scope. At least scope a little bit broader than the working examples.

I'd like to address this last issue that the industry wants, getting reasonable claims' scope. Earlier we heard some testimony that over the last 10 or 12 years biotech has become somewhat more predictable than it was earlier. I think this supports position of allowing somewhat broader claims' scope at the Patent Office, and, if necessary, maybe the Patent Office can consider looking into some kind of preponderance of the evidence standard for determining what the level of ordinary skill in the art is under 112 in order to evaluate what sort of claims' scope is allowable. Over the long term this will also help to lower the cost of pharmaceuticals and primarily in the fact that it will help to give a little bit more certain atmosphere for conducting the business transactions necessary for the smaller companies to get the capital to do the studies later on. It will help establish the proprietary positions with greater certainty, and it will kind of minimize the reliance on the doctrine of equivalence, if a patent ever needs to be enforced. It will make it easier to establish direct infringement I think for very minor modifications. Overall, that's going to lead to lowering the cost of

pharmaceuticals.

I think under the utility issue, again we still would like to see your early issuance of patents with reasonable claim scope. I think one -- although this will be the only comment I have about any direct practices of the PTO. I think the issues for compound claims specifically, and it is proteins and polynucleic acids in biotech, should be addressed separately from the issues under -- or relevant to composition -- pharmaceutical composition claims and method of treatment claims. For pharmaceutical composition claims, typically you have to recite an effective amount to achieve some desired medical result. In method claims, typically you are counting on some sort of human treatment as the utility. So I think the issues are different.

For compound claims specifically, there are a number of different utilities which are often asserted and I think the case law is fairly clear that if a compound claim has a utility other than for human treatment then it's met the utility statute. However, as far as the pharmaceutical composition claims and method claims go, and this may be somewhat professional suicide for saying it, I think the Patent Office has legitimate concerns about whether the public is actually being served by issuance of a patent if there's some question about whether the thing is going to be effective or not.

I think again the suggestion to simply accept at face value a declaration tying whatever results are there to prediction of in vivo effectiveness I'm also hesitant to support, because I think if there is evidence that the Patent Office is aware of that maybe the test isn't accepted, or that may challenge directly the evidence that was submitted by applicants in that case, then it raises some questions perhaps of the validity of the patent. And it's certainly going to be a lot cheaper and easier to resolve this at the Patent Office than it will be to try to resolve it later on in litigation. So I'd like to suggest in this case that maybe a preponderance of the evidence standard also be looked at as a possibility for resolving utility issues in applications where pharmaceutical compositions and methods of treatment are being claimed.

I guess that's pretty much it. I do have a couple more quick comments. You asked for specific cases, and even though my firm really doesn't want me to speak about any specific cases for reasons of, you know, possibly generating estoppel or any bad will at the Patent Office, and believe me we regard our good will with the Patent Office with the utmost respect, we did have one case in the AIDS treatment area where we had a notice of allowability for a number of years. I think it was there for about two years and every once in a while we'd check and see when we were going to receive the notice of allowance and it never came. You know, we were real curious about why that was but I don't think we ever got a straight answer. And I think it kind of underlies the perception that maybe there

was some policy change at the Patent Office, even though, you know, we hear that there wasn't any. Whether there was or wasn't, you know, I think is almost beside the point because it's -- it's obvious that some things have changed and, you know, at least personally I'm glad we're having this hearing to try and resolve the issues.

Also I think we heard some comments earlier about educating examiners on the cases, and I'm not so sure that's the best use of time and resources. My experience in interviewing examiners is that it doesn't really matter too much what the cases say, it depends on whether their supervisor is going to agree with what they say in the office action that counts. And I think at least the preponderance of evidence standards that I suggested earlier, that's a pretty easy test to look at and see whether, you know, the evidence is on one side or another.

If I can make one last suggestion to you, Mr. Commissioner. You were concerned about the length of prosecuting biotech applications relative to those in other areas of technology. I think that can be resolved pretty quickly just by looking at the front cover page of most patents issuing from Group 1800. It's pretty rare when there aren't any earlier filed applications cited in support of it, but I think in other areas, and I think most particular in polymer areas, it's pretty rare to see that there was an earlier filed application. At least looking at the front page of each issued patent, that should give you some idea of how many continuation applications were filed in the prosecution of a particular case. And, at least on our side, we look at cases rather than applications. You know, we consider continuation applications to be part of the same case.

With that, I see my time is up.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call Robert Benson of Genelabs, Incorporated.

ROBERT BENSON, GENELABS, INCORPORATED

MR. BENSON: Thank you. My name is Robert Benson. I'm vice-president and general counsel for Genelabs Technologies, in Redwood City. We have about 220 employees and we deal primarily with viral diseases. We isolate novel nucleic acids. From those we do assays for detecting the virus. We do vaccines and therapeutic agents, and I think there's a tremendous need for isolating new viruses. Quite a few of them are adapting themselves to the human host and we need to stay very active in this area.

The patent issues in this area of viral isolation are very critical to my company, and I think to all small companies in that the FDA regulatory

testing required cost a great deal of money. And it's been said already many times today, and I'm not going to belabor it, but delays in the patent process creates a tremendous sense of insecurity on the part of our investors and our corporate collaborators.

It's very important that we have a sense of patent stability in order to move these discoveries through to commercial products. In many instances, the lack of a clear patent position undermines the ability of small companies to attract the needed resources.

In the interest of having listened to almost seven hours to other people talk, I'm going to summarize some of the things I was going to say, sort of a short ditto.

In the area of FDA data -- FDA-like data in order to get a patent allowed, that is human clinical testing, I think it's beyond the scope, or should be beyond the scope of what the examiners require and I think a practical utility basis is all that should be necessary.

I'd also like to comment on something that hasn't been brought up too much today and that is the patenting of novel nucleic acid sequences and their use, particularly in the area of reductions of practice either actual or constructive. It's our position that nucleic acid sequence composition claims should not require a description of the best final utility, be it dependent upon human clinical data or any other kind of data; that what you need to have is a statement of practical utility together with enabling application.

There seems to be an awful lot of flux in the area of what a partial nucleic acid sequence qualifies you for in the U.S. Patent Office. I would like to just drop back and say you should get whatever you disclose in your patent application, and that is if you can show that you can do something with it that is practical and that has a real utility then you should be able to get a claim for it, because others in the future will find improvement patents or other uses for it, that's fine. We shouldn't foreclose that kind of improvement either.

Next I'd like to address briefly what I like to refer to as the double standard that the Patent Office practices in the area of 103 versus 112 enablement issues. It's always been hard to explain to an inventor how if he published something it becomes prior art against them that's going to block them, but if he puts the same information in a patent application it won't be sufficient for him to get a patent. I have never been able to quite get that information across to an inventor. In fact, sometimes I have trouble understanding it myself. And I would like to see some type of reduction in this type of double standard where the ability of a patent applicant in -- to publish his research or to file a patent application is not made difficult.

And, finally, I would like to emphasize the importance of training and keeping a well experienced examining corps. I know that the corporations and the law firms bear an awful lot of responsibility for their recruiting efforts on the trained examiners. It's a problem that is largely economic until the Patent Office has the money necessary to retain the examiners the problem will continue, or until the world is saturated with qualified patent practitioners, but that's I think quite a ways off right now.

I was very upset to find out that \$30 million of our fee money was going into the general fund as opposed to, as I had been led to understand, to support the Patent Office and the maintenance of a qualified examining corps.

I would like to go into just one little piece of what I would call sort of technical argument here and it has to do with the patenting of partial nucleic acid sequences, which I've recently seen in the press a lot where some are upset about patent applications containing partial sequences. I would just like to put in the record what I think are a few utilities which I think should support the patentability of a nucleic acid sequence.

One example would be encoding a unique antigenic peptide that provides an antigenic site for detecting the presence of antibody of clinical significance, particularly in the area of being able to test for a virus -- something like that.

Another example would be encoding a polypeptide that provides a unique receptor, binding lignin, that modulates the activity the receptor stimulated.

A third example would be providing a unique hybridizing sequence that permits detection of a target nucleic acid sequence having clinical value.

And still another would be nucleic acid primers that give specificity to a polymer chain reaction assay of clinical value.

These are all examples of ways in which a nucleic acid sequence can be used in a very real way but that do not necessarily mean that the patent applicant knows the entire sequence or necessarily what the entire sequence will be able to do. That I would leave to future inventors to go into the laboratory and discover.

Finally I would like to sort of digress a little and that is when I first entered patent law I was in a Washington law firm and a man left the U.S. Patent Office named Irving Marcus. He'd been there for I think

about 30 years. He was in the office next to me and I used to go over to him all the time because I couldn't understand why the Patent Office was doing stuff and he would try to explain to me how the patent office worked, and that sometimes there were champions within the patent office for certain positions, and how he had been a champion for certain issues two or three times and he had been right and it had worked out pretty well for him and he managed to move up within the Patent Office. And I think one of the things I'd like to see is some champions today who take positions for improving the patent system and particularly resolving problems like our current dilemma involving human clinical testing in order to get patent applications. I would like to see more of an ex parte situation and less of an adversarial proceeding in the securing of patent applications.

Thank you.

COMMISSIONER LEHMAN: Thank you very much, Mr. Benson.

Next I'd like to call our second witness from Isis Pharmaceuticals, Lynne Parshall.

LYNNE PARSHALL, ISIS PHARMACEUTICALS

MS. PARSHALL: Thank you Commissioner Lehman and other members of the hearing board, good afternoon. My name is Lynne Parshall. I'm senior vice-president of Isis Pharmaceuticals and Doctor Crooke from our company spoke with you earlier today. Among my responsibilities at Isis is to supervise our patent prosecution. I appreciate the opportunity to talk to you today. I'm going to focus on just one item in connection with our patent prosecution that's affected us significantly, which is something that you've heard a lot about today, rejections that we've received based on incredible utility.

As a development stage research based pharmaceutical company, for many years our most significant asset has been and will continue to be our patent estate. We've invested extremely heavily in this area. Our major focus at Isis has been on the development of antisense technology and the commercialization of drugs based on this technology. We're the leading company in our area and have invested more than \$100 million in this technology during the past five years. Many major pharmaceutical companies and other development stage companies also have antisense programs.

In many respects, we think antisense technology epitomizes some of the special challenges that the Patent Office faces in dealing with new technologies. It also epitomizes some of the potential harm that can be done by inconsistent activities with regard to the evaluation of patent applications.

Antisense technology is a true innovation in drug discovery and development, as are many of the technologies you've heard about today. It's very broadly applicable to many different therapeutic areas. It's a technology that has extraordinary promise and importance and it's a technology we believe that needs and deserves the protection of the patent system.

We have focused our technology on a new target for drug discovery, RNA, and used a new class of chemicals, modified nucleotides to approach this target. At Isis, we've simultaneously invented new minuscule chemistry and the basic pharmacologic framework to apply this technology. Even for practitioners remaining abreast of this technology is very difficult. In this way the antisense field is really though no different from any other important field in which rapid innovation is occurring. What is different, however, appears to be how the patent office analyzes the growing body of literature concerning antisense technology.

In reviewing this literature, as you would expect, you will find work of varied quality; good and bad, and work expressing a variety of approaches to and views of the field. In addition, you'll find work which due to the rapid pace of innovation is no longer state of the art. It's the selective use of this literature by the Patent Office to doubt rather than support innovations in antisense that hinders our patent prosecution and which we believe provides an obstacle to patenting innovations in the field.

Not surprisingly, in the beginning of this technological area there were more questions than answers about the potential for antisense compounds to be drugs. Today we believe most of those questions have been answered and the overwhelming wealth of current evidence supports the contention that our technology will, in fact, yield important therapeutic advances. This evidence includes efficacy data from many animal models of disease and from many different laboratories. It also includes clinical data on two antisense drugs that we've studied in humans. Despite this wealth of data, we're continually facing rejections based on utility for our compositions of matter and therapeutic use patents.

In the last year, we've received office action rejections in approximately 70 different applications that included some sort of incredible utility or lack of utility rejection. Responses to these office actions has cost us hundreds of thousands of dollars in legal fees, which probably makes some members of the audience more happy than others.

Patent applications of ours have been rejected for compounds for which we've shown significant inhibition of viral replication in well recognized models, including models that the government's using in its

agencies for therapeutic screening. In the face of the fact that more traditional chemical showing activity in these models have, in fact, been granted patents, patent applications have been rejected for compounds for which we've shown significant activity in animal models of disease when more traditional chemical showing activity in these models have also received patents. Even patent applications for compounds for which we've shown evidence of activity in humans have been rejected. For example, one of our pending patent application claims composition of matter to certain phosphorothioadal alignin nucleotides used to treat a debilitating viral infection. Today patients are being treated with one of these alignin nucleotides to help the spread of this disease.

In the latest office action we received for this application, the examiner maintained a previous 101 rejection for lack of patentable utility. This rejection has been maintained in the face of declarations from two experts in support of this application. One of these declarations was from a clinician actually treating patients with the drug in our ongoing clinical trial. The declaration detailed the positive activity of the drug in patients. The other declaration came from a well respected scientist who reviewed the current state of the antisense field. These declarations from well known, well respected clinicians and scientists were dismissed by the Patent Office.

COMMISSIONER LEHMAN: How were they dismissed? On what grounds?

MS. PARSHALL: We were told that they were conclusionary and anecdotal.

COMMISSIONER LEHMAN: Can I ask you a question about that, because, you know, I earlier referred to Robert Schaffer's testimony recommended basically that we adopt a policy of providing a presumption to such declarations. And then we heard another witness just a few minutes ago, Mr. Fortney who opposed that because he said: Well, you know, what's going to happen there then is that on the basis of sometimes inadequate declarations a patent is going to issue and then, you know, you'll be out in the marketplace with a potentially invalid patent and, you know, you'll have to spend then not just \$200,000 but you'll have to spend, you know, \$1 million on a patent infringement case. It will, you know, put you out of business.

What's your response to that? We hear Mr. Schlicher say basically that we should err in favor of getting the patents out the door and literally leave it to the litigation system to sort of round off the edges.

MS. PARSHALL: My feeling is you're talking about two different issues here. One of them is whether or not there's an invention that's truly different from what's both in the literature and what's been claimed in other patent applications. And the other issue is whether or not the Patent Office believes that there's utility associated with this

particular invention to give it -- to grant it the status of something that's patentable. I think the Patent Office's job is to do the former and not second guess experts in the field with regard to the latter. I think the Patent Office needs to look at the existing literature to make sure that something put in front of it is truly inventive. I think that when the Patent Office is faced with physicians and well respected scientists who say, in fact, "In this area this is likely to work," -- the Patent Office I know is composed of, you know, very good scientists as well, but I think they can't hope to be experts in every single area, particularly with the type of innovations going on among the companies that you've heard talk today.

Our frustration is that our science is being second guessed by people who, although they try and be as expert as they can, aren't experts. Not that the Patent Office is making errors in looking at what's out there and what's been invented in the past.

COMMISSIONER LEHMAN: We can certainly say they are experts in the prior art. Probably the best experts because they work with it every day, but they, as you point out, are not experts in what's coming down the pike.

MS. PARSHALL: That's right.

To continue with just another example. We face similar Patent Office rejections in basic chemistry cases. Just a few days ago we received an office action on a process patent application. The invention claimed in the application as an improved process for preparing phosphorothioadal alignin nucleotides, and in the office action the examiner rejected all of the claims as lacking patentable utility. The examiner stated, and I quote: "The antisense activity of the instantly claimed compounds borders on the incredible and such an asserted utility is deemed unlikely to be correct in view of contemporary knowledge in the art." End of quote. It's difficult to understand the examiner's position. The recent scientific literature does not indicate that the activity of phosphorothioadal alignin nucleotides is incredible and deemed unlikely to be correct. In fact, ongoing clinical trial of these compounds do not indicate either that the activity of phosphorothioadal alignin nucleotides is incredible and deemed unlikely to be correct. The declarations that we've submitted from well respected clinicians and scientists do not indicate that the activity of the phosphorothioadal alignin nucleotides is incredible and deemed unlikely to be correct.

I'm not trying to suggest that drug discovery is not fought with uncertainty. We all know -- you probably heard a lot about how long it takes to come up with drugs and how long it takes and how expensive it is. What I do urge you to see, though, is that antisense compounds merely represent yet another example of a new area of innovation similar to those that have been encountered numerous times in pharmaceutical

patenting new classes of chemicals designed to intervene in disease processes in a new way. Support of this type of innovation in the pharmaceutical industry by granting patent protection of reasonable scope has been and continues to be necessary to simulate continuing innovation.

With regards to patents in biotechnology, we feel that there's a different standard applied than that applied to traditional pharmaceuticals. Demands for definitive proof of therapeutic utility which seems to equate to pivotal controlled human clinical trials have resulted in many patent application rejections. As Doctor Crooke said to you earlier today, there's no harm done and, in fact, significant public policy is served by giving pharmaceutical innovators the benefit of the doubt in terms of the utility of their inventions.

I'd like to reiterate the recommendations Doctor Crooke made to you earlier today:

We recommend that the Patent Office return to traditional practices with regard to pharmaceutical patents. This would include a return to a positive bias towards innovation with the acceptance of reasonable proof of potential utility for inventions.

We request that you treat patents from so-called biotechnology companies and pharmaceutical companies as equivalent. We're the same industry with the same customers practicing the similar science.

We request that you act consistently across and within technology areas. Just because one technology is labeled a new technology and another is not does not mean that the basic approaches or risks are necessarily different.

We further request that when in doubt you grant therapeutic claims based on the specific examples provided with scope commensurate with reasonable extrapolation from the examples that have been provided.

Let me thank you for this opportunity to speak to you today. I think this issue is a very critical importance to us and to innovation in health care. I'm confident that as you consider the issue, and hear all that you've heard today and will continue to see in the record, that you will agree that it is in the public interest to continue to encourage innovation in the pharmaceutical industry, not to discourage it.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to call on Robert Sobol of the San Diego Regional Cancer Center. Mr. Sobol? Guess he's not here.

Susan Perkins of Cambell and Flores.

If Mr. Sobol comes back we'll try to fit him in. He might be hanging around because we're about a half hour out of time. I'll call him later.

SUSAN PERKINS, CAMBELL and PERKINS

MS. PERKINS: I was going to make that specific request before I got into my testimony. If he Doctor Sobol -- given that we are about a half hour ahead -- could go after my testimony.

COMMISSIONER LEHMAN: I think we've told people that there was a half hour either way so we're right at that cutoff, you know, at that far edge.

MS. PERKINS: My name is Susan Perkins and I'd first like to start my testimony by echoing many of Tom Wiseman's positive comments he made today. Having worked as a patent examiner for over four years in the biotechnology group, I too am familiar with the fine and hard working people at the Patent Office who are trying to make this system work.

I'm currently in private practice for Campbell and Flores, a San Diego based law firm that specializes in biotechnology patent law. We work primarily for small start-up companies, not-for-profit organizations and universities. You have heard today from a number of our clients -- and hopefully Doctor Sobol. Universally these clients have commented on the more stringent and unrealistic standards that are being applied in Group 1800, particularly as to utility. Our clients have also commented, and I'd like to further emphasize, just how critical patent protection is for the continued research and development to these companies. I would like to add briefly to those comments already provided by our clients. While the views I express are my own, they are derived not only from my recent experiences with the patent office but also those of other practitioners at my firm as well as and, more importantly, the experiences of our clients.

In one application in our office we successfully interviewed the application. It was deemed allowable, having removed all the 103 and 112 rejections. We then got a call from the examiner that prosecution was going to need to be reopened for new utility rejections and new enablement issues. When asked if we could reinterview the case. We were told by the examiner that we could argue the utility issues until we were blue in the face, that this examiner was taking direction from his superiors and that the examiner, and I quote, "feels like a robot in following the mandate of his supervisors."

I cannot help but wonder how happy this examiner must be in their job,

and I'm very familiar with what an enjoyable job patent examining can be. But more importantly, it appears to me that this application has been prejudged and finally determined without us even having had the opportunity to interview or submit a response because of the current utility standards being applied today. This case is not unusual for what we are seeing in our office. We are often having cases reopened for prosecution based on utility and enablement issues which I think speaks directly to the changed standards over the last couple of years. And it is also not uncommon that where we submit sufficient legal arguments or supporting technical data, this does not appear to be enough.

In this particular case, the inventor's response to the utility rejection was he'd be happy to do human clinical trials. If only the examiner would give his right arm for that injection, he'll do the human clinicals. This may seem like a ridiculous statement -- I laughed myself -- but I think it certainly speaks to the frustration that the inventors are feeling today with the Patent and Trademark Office and these enhanced standards of utility.

In other cases, we are now seeing restrictions drawn along in vitro and in vivo lines. Claims to in vitro procedures or uses are held to be patentably distinct from in vivo uses or procedures. We even get this restriction where we have not specifically claimed in vitro or in vivo uses or procedures. The examiners argue that the claims are generic to in vivo and in vitro. One problem with this is that you must realize that restrictions drawn along in vivo and in vitro lines can result in more groups in a single restriction than there are claims in an application. I think these restrictions are really so the examiner doesn't have to deal with the in vivo utility issues until you file a divisional application, not that in vivo and in vitro inventions are patentably distinct. And I don't think a restriction for utility purposes and those additional uses of utility for in vivo inventions warrant a restriction.

Now, there is a positive side to these in vitro/in vivo restrictions. Now, it would appear that the Patent Office cannot apply prior art disclosing in vitro data only as rendering obvious in vivo claims. One of the largest downsides I see to these restrictions is I'm not sure how, where an applicant elects the in vitro group, to prosecute that application where we don't even have an in vitro use or procedure disclosed. And it's not uncommon that we have cases that get these restrictions between in vitro and in vivo lines where we don't even have in vitro inventions disclosed, yet the examiner has made this restriction and indicates we have some in vitro invention. I don't know how we're supposed to prosecute those applications.

On another issue regarding the double standard, which has been talked at great length today, but I'd like to focus specifically there also on in

vitro and in vivo. The examiner is holding in vitro data disclosed in a publication as rendering obvious applicant's invention, but that in vitro data or animal models are not sufficient to meet the current utility standards. While I as a former examiner appreciate the need for compact prosecution and that we make all possible rejections in the first office action, and I even appreciate that there's different standards between enablement and obviousness, it seems to me that the Patent Office needs to really make a decision about whether or not in vitro data in a disclosure renders something obvious or whether applicant's in vitro data and animal model is going to be sufficient for utility. These two inconsistent positions cannot both be correct, and yet we're seeing these rejections vigorously maintained until we refile the application and argue it again in a new continuing application.

When I was an examiner, I routinely made these apparent inconsistent rejections of 103 and 112 and 101, but generally one of those was wrong and had to fall, and I think that's particularly true where you've got in vitro data supporting an obviousness rejection but in vitro data fails to support enablement and utility.

Many people also have spoken on the need for more legal education. I would strongly agree. And as I understand it, Howard Shane's legal precedent course has not been taught since the time when I was back at the Patent Office which is now over two years ago. I know myself and many other examiners considered Howard Shane's legal precedent class to be invaluable, and I would just strongly recommend that he or other superiors in Group 1800 again take up teaching a legal precedent course within Group 1800.

My last comment is that I understand there's been consideration of other time for interviews between applicants and examiners, to give the examiners other time for the interviews. I hope that goes through. I think many people have spoken today about how much interviews help facilitate dialogue, and certainly cuts down on the pendency of applications, which I know is of real concern to the Patent Office.

I thank you very much for the opportunity to testify.

COMMISSIONER LEHMAN: Thank you very much. You know, I just would make the point that we are, indeed, considering giving examiners additional time for interviewing, but, in addition to the \$30 million Congress has taken out of our pocket, basically out of your pocket, we also have restrictions that are being imposed upon us from above by -- regarding the number of people that we can hire at the Patent and Trademark Office even if we have the business to do and even though we have the money to pay them, and this makes it extremely difficult to give examiners some of this time. We'll probably do it anyway but we can only stretch so far, and I think it's incumbent upon our constituents to follow these things

carefully; to let Congress know; to let the Office of Management and Budget know, and so on, because, otherwise, we can't provide you with the kind of service that we would like to.

These hearings are two-way streets. They give us a chance to tell you what's going on and hear what you're thinking.

It looks to me like Mr. Sobol has arrived, and so why don't you come forward -- from the San Diego Cancer Center.

ROBERT SOBOL, M.D., SAN DIEGO CANCER CENTER

DOCTOR SOBOL: I'm a physician and have never been on time to anything in my life and today I thought for sure I was going to break that, but here I am late. I apologize.

COMMISSIONER LEHMAN: I want you to know that, you know, in the Bill Clinton Administration that we're over Clinton time now. We're a half hour early, we're not a half hour late.

DOCTOR SOBOL: Oh, I see. I won't feel so badly then.

My name is Robert Sobol. I'm a medical oncologist and a clinical investigator at the San Diego Regional Cancer Center where I serve as the Director of Clinical Science. I appreciate having the opportunity to testify before you today concerning the use of animal models to obtain patent approvals. I provide the perspective of a current patent applicant from an academic non-profit research institution.

In the past, animal data was sufficient to obtain patent approvals. The patent approval process, based upon animal efficacy data, resulted in expenses for research and patent prosecution costs that were within the means of most non-profit research institutions. These issued patents protected the intellectual property of investigators at non-profit institutions which have very strained overall budgets.

In addition, these approvals fostered the clinical development of novel therapies by providing the private sector with the confidence to make the considerable capital investments required to obtain FDA marketing approvals. This process based on patent approvals from animal data has been responsible for the rapid growth and preeminence of the biotechnology industry in the United States.

I would like to share with you from our own experience how the development of novel treatments may be threatened by the recent unwillingness of patent examiners to accept animal data as sufficient for patent approval. We had demonstrated in an animal tumor model the effectiveness of a novel approach for the treatment of cancer; however,

the statistically significant data was rejected by the patent examiner who requested evidence in human subjects that the therapy would be efficacious. The animal data rejected by the examiner was, however, deemed satisfactory to justify clinical application of the approach in human subjects by federal review boards containing scientists and physicians skilled in the art of our developed therapy.

The further development of this particular technology is threatened by the patent examiner's request for human data. This would entail significant expenditures requiring hundreds of thousands or possibly millions of dollars, depending on the manufacturing requirements to generate the clinical grade materials required for human study. These costs are well beyond the means of most non-profit research institutions and the private sector support to develop this technology would be enhanced by an approved patent.

An alternative is to appeal the patent examiner's ruling. However, this process is also formidable as appeals may take several years of litigation with resulting legal expenses beyond the means of many academic institutions which are becoming increasingly strained financially.

We are frustrated by the apparent requirement to expend significant amounts of capital and time to protect our intellectual property if human clinical data is required for approvals. The trend in current policy to demand human data will discourage patent filings from our non-profit academic institutions where many novel technologies originate.

It would be detrimental to the progress of medical research in our country if academic research institutions can no longer afford the costs of obtaining issued patents. Animal models are predictive of effects in humans and the results of these studies should remain sufficient for patent approvals as they are for the clinical application of novel therapies in human subjects. Thank you.

COMMISSIONER LEHMAN: Is there any circumstance under which you think human testing should be required for proof of utility? Can you imagine any?

DOCTOR SOBOL: Well, I think to those skilled in various elements of medical research where certain animal models would be known not to be predictive of what would happen in humans, that under those circumstances evaluation of humans would be required.

COMMISSIONER LEHMAN: I think that's probably exactly what's happened in some cases. We've probably had those kinds of situations and that's what causes --

MR. RICHMAN: That's exactly correct. You have, in essence, competing literature where on one hand someone is saying it is predictive and on the other hand people are saying animal models are not predictive of that disease state, and that's what we have to deal with.

DOCTOR SOBOL: That's always a difficult situation, but I believe that in most circumstances the animal data has been predictive of what does occur in humans and it's on that basis that many clinical trials are approved for humans and therapies are developed for humans. And I think it's perhaps more the exception rather than the rule and that that may be what we need to have guiding the decisions.

COMMISSIONER LEHMAN: Your organization, is it primarily a research organization or is it a treatment organization doing some research?

DOCTOR SOBOL: It's really dedicated to research, translational research, taking the studies that are developed in vitro and in animals and apply them in clinical settings in humans.

COMMISSIONER LEHMAN: And it's not for profit?

DOCTOR SOBOL: It's a non-profit; that's correct. So we really base all of our work on moving things rapidly into clinical trials with humans, and that's all predicated on results that we obtain in in vitro systems and animal models. And that's --

COMMISSIONER LEHMAN: You're of course a user of research as well then and so you would have a sense of -- since you're not really a profit making company -- of the implications possibly of issuing patents that shouldn't be issued, and I assume that you basically don't see much of a problem there?

DOCTOR SOBOL: I think that that's less of a concern than the opposite: Having something not issue and get protected that really requires it to be developed, and I think if one had to err one would prefer to err on the side of making certain that we were able to provide protection for intellectual property, provide the stimulus for development. I think we will harm more people, slow the development of novel treatments for patients that suffer from diseases by being too conservative in what we allow to be patented, and if errors have to be made I think it's better to err on the side of granting and issuing patents.

COMMISSIONER LEHMAN: Have you had any problems with experimental use of other people's patented technology? We heard at least one witness call for the need for a statutory experimental use exception. Is that a problem for you at all?

DOCTOR SOBOL: Not encountered it personally, no.

COMMISSIONER LEHMAN: Thank you very much.

Next I'd like to ask if David Lowin of Syntex is here?
it's perhaps more

DAVID LOWIN, SYNTEX, INC.

MR. LOWIN: Mr. Commissioner, members of the panel, and die-hard members of the audience. It's been about two hours since somebody congratulated you on still being here and listening to us so it's probably time for another counter to that effect. Thank you for staying and listening. It's important.

I'm David Lowin, Assistant Director, Patent Law Department, at Syntex. I also teach patent law at Stanford Law School and at U.C. Berkeley's Bolt Hall. My testimony is offered as a matter of personal opinion, not on behalf of any organization.

Suffice it to say that nine minutes isn't long enough to address the full subject matter of this hearing so I'm also working on a written submission that will hopefully be of a broader scope. My prepared remarks are addressed to the somewhat different approach on the policy behind the utility requirement and the environment in which that policy must be carried out.

The Notice setting this hearing started by reference to the Supreme Court's decision in *Brenner v Manson*. There the court upheld the rejection of the chemical process patent application for failing to establish a substantial utility. Now, *Brenner* may not be the greatest decision the Supreme Court's ever handed down and I think a lot of that has to do with the underlying facts, what the application had in it and what was trying to be argued. But I think there are some things within *Brenner* that can still be applicable today and that can help the Patent Office accomplish what's been requested by the industry here, and that's what I'll turn my attention to.

I think there are two key aspects to the majority's conclusion in *Brenner v Manson*. First, the majority concluded a patent is not a hunting license; it is not a reward for the search but compensation for its successful conclusion.

Now, whether an invention represents the successful conclusion of the search kind of depends on what it is that you're searching for in the first place. Biotechnology has changed what we're searching for in the first place. It's made it possible for us to understand the complexity of life and to intervene with disease at a level far more precise than was possible when the Supreme Court considered *Brenner v Manson*.

The successful conclusion in today's search for cellular mechanisms and

ways to modulate them would have probably been considered only an invitation to further experimentation back in 1966, and what we consider to be the successful conclusion today is going to be different 10 years from today. The point is that the legal principles, such as the successful completion of the search, have to be applied in the manner consistent with the progress of technology.

The second key aspect of the Court's decision in *Brenner v Manson* is the quotation they took from the CCPA decision in application of *Rushut*. A patent system must be related to the world of commerce rather than to the realm of philosophy. So what's the world of commerce to which the successful conclusion of today's search must relate? It's a different world of commerce than it was in 1966. Just as the patentable subject matter, definition of Section 101, had to expand to cover Chakrabarty's bacteria as part of anything under the sun that is made by man, so now our definition of the world of commerce must expand to cover the state of the art in the commercial impact of today's biotechnology inventions.

When we clone the DNA for a protein that's known to be involved in a disease; when we develop that protein into an assay for screening potential therapeutic drugs; when potential therapeutic drugs are identified as active in that model, and when those drugs are tested in clinical trials, all of these efforts involve hundreds of scientists, thousands of other people performing related tasks in hospitals, in banks, in shipping companies, in equipment manufacturers, in accounting firms, and even in the United States Patent and Trademark Office. Every step along the way entails enormous investment. The successful conclusion of each of these searches triggers even more investment. Ultimately, hundreds of millions of dollars change hands during the development of a single drug, and that's commerce. That's commerce at a level that the founding fathers couldn't even have imagined.

Today's hearing is taking place because the U.S. Patent and Trademark Office has, seemingly more often than not, refused to allow our applications for patents on the DNA, on the proteins and on the screening models, allegedly because they aren't the successful conclusion of the search and they don't relate to the world of commerce. The patent office has refused to issue patents on the drugs because they haven't been proven safe and effective in statistically powered human clinical trials even though, in this first-to-file world, the patent applications cannot be filed containing such data because they have to be filed years before the clinical trials can be started.

And now, if eventually granted, the terms of these patents will have been expiring since the day they were filed, and the GATT implementing legislation appears to require an applicant to make a choice between an extension for appeals or interferences versus extension under the provisions of Waxman-Hatch. It's under 156(a)(2). This is Waxman-Hatch

says if the patent has previously been extended, it doesn't say under this provision that you can't extend it under this provision. That's something that will just get cleared up after the implementing legislation goes through. But that's something that we're facing today.

COMMISSIONER LEHMAN: I assume you favor a revisitation of the patent term registration legislation to make it more favorable to the --

MR. LOWIN: Yes, I definitely do. Particularly in the context of the new 20-year term that's soon to be.

COMMISSIONER LEHMAN: But you also correctly point out that for all practical purposes most global competitors already live under the 20-year term.

MR. LOWIN: Absolutely. I mean, it's a practical reality of the world we're living in.

COMMISSIONER LEHMAN: So we could fashion an equitable solution by -- because the biotech industry is unique in this regard really. By fashioning -- we already have built in of course to the legislation five-year extension for interferences and appeals, and so on. But we also -- Then we have the existing patent term with extension legislation as well, but we could expand on that. That's not violative of any of our GATT obligations, I don't believe, and then we can have hopefully a win/win situation when we get better external access in foreign markets such as Japan. We please other aspects of the patent industry that don't want patent terms going on forever and then we solve your problems at the same time.

MR. LOWIN: That would be just great. Good luck. I will be happy to help with that. We've got some ideas.

If you would put up the first slide. I wanted to put things in perspective on the standard of utility. On the mechanical side, the Patent Office is regularly granting inventions on anything up to and including the kitchen sink, and witness U.S. Patent 53494708 issued September 27th for the foldable kitchen sink.

Next slide, please. Not to mention also issued on the same day the patent on the nail file protector device.

Next slide, please, and the last of these that I'll take your time with. The air cooled umbrella.

COMMISSIONER LEHMAN: I thought you were going to do the paper clip.

MR. LOWIN: No, that one's good.

COMMISSIONER LEHMAN: I still don't understand that one, I must say.

MR. LOWIN: Depends on when it was issued. I'm sure all three of these inventions were worthy of patent protection, but I'm also that even combined these inventions covered by these three patents are not going to effect the world of commerce to the same degree as even one of the legally useless biotech inventions we've been talking about today.

I think the office needs to apply the utility requirement consistent with the current commercial and scientific standards. The utility standards should resolve doubts in favor of the patent applicant, and when an applicant pleads for allowance of its application because its competitors are already practicing the claimed invention, it doesn't make sense to reject the application as lacking utility. We've got room in this area of the law for the principle, "no harm, no foul." The courts are not being clogged and the progress of science in the useful arts is not being blocked by patents covering technology that just doesn't work.

Very briefly. I know I'm out of time. I thought a great suggestion was made earlier and that was establishing a group within the office that occasionally picks up an application and looks at it from the standpoint of the applicant and says: Gee, how would I feel about this? That would be a great balance to the other group that you have.

COMMISSIONER LEHMAN: What would your view be about the suggestion about giving greater weight to declarations to substitute for clinical data?

MR. LOWIN: I think that's fine. I think the procedures already provide for that. If there has been a declaration, then there needs to be some evidence, under oath, to counterbalance it. And when there isn't counterbalancing evidence then the declaration has to have the weight.

COMMISSIONER LEHMAN: Thank you very much.

MR. LOWIN: Thank you.

COMMISSIONER LEHMAN: Sarah Adriano of Merchant and Gould will not be here this evening so that moves us up a little bit further. We're going to probably end up finishing -- looks to me like we'll finish probably about 50 minutes, 45 minutes ahead of time.

Is Jeffrey Sheldon from Sheldon and Mak here?

JEFFREY SHELDON, SHELDON and MAK

MR. SHELDON: Honorable Commissioner, thank you for the opportunity to address you and your colleagues, and my colleagues that are still in the

audience.

I am the founding partner of Sheldon Mak. We're an intellectual property law firm with offices in Pasadena, California; Palo Alto, and San Bernardino. We have a diverse biotechnology practice representing individual inventors; the University of California; non-profit organizations; and some Fortune 500 companies. I'm here on behalf of the firm and myself. I'm also president of the Los Angeles Intellectual Property Law Association, and past chairman of the State Bar of California Intellectual Property Section. I teach advanced patent at Loyola University, but I'm not here on behalf of any of those organizations, just to add some import to the weight of my comments.

Initially I was only going to talk on the 20-year term; however, you've asked some questions which I'd like to address on two other issues. One is the utility requirement asking should the Patent Office err in favor of issuing patents or continue its present system of insisting on some utility. I believe strongly it should err on issuing patents. If, for some reason, a mistake is made that the patent invention in fact has not utility, who cares? Simple as that. If it doesn't work nobody is going to infringe. The few reported cases, and I think one is at the Court of Appeals -- I use it in my patent law course but I don't remember the cite -- where somebody challenged the patent on utility grounds but when shown to have infringed the patent the court disposed of that automatically. It said: "How can you at the same time infringe the patent and claim it has no utility?" So go ahead and issue all the useless patents you want. David Lowin just showed you three of them. Who cares? So if you're going to err, err on the side of getting these patents out there so that if they do have utility people can invest.

The other point, and, again, I didn't --

COMMISSIONER LEHMAN: One of the concerns, though, is that, you know, foldable kitchen sink or whatever, is an area where somebody can have something very similar. It's a crowded field of technology, and one of the problems of biotechnology is that it isn't, particularly where you are focusing on the more breakthrough inventions. It's not necessarily a crowded field and obviously the concern is that you will place roadblocks to alternative developments because of the power of the patent grant if you issue indiscriminately certain patents. We certainly are hearing here that there doesn't seem to be much of a concern about that here, at least the industry doesn't seem to share that concern.

MR. SHELDON: Again, the marketplace will take care of that. If, in fact, it is a roadblock it must have some utility and generally it's some sort of license negotiation will develop. I -- In my practice, I've not seen a roadblock of a patent who should not have issued because of utility.

COMMISSIONER LEHMAN: Well, of course, you know, if it's a valid patent it's a valid patent and I think the concern is that we would issue invalid patents and also they would be a roadblock to other people and other people's inventions.

MR. SHELDON: In my practice, the roadblocks I've seen are invalidity on 102 and 103 grounds and not utility grounds, and that has been a problem but that's not what you're here to listen to.

I didn't plan on addressing the inconsistency of the Patent Office between enablement and obviousness; however, I happen to have in my briefcase to review in breaks an amendment written by one of my associates on a simple technology, its capillary zone electrophoresis. The patent application discloses six, seven applications for it. It has specific examples. On one of them we have two rejections: One rejection is we're entitled to only the one claim where we disclose how to use this invention for uric acid analysis.

By the same token, I have a 103 rejection. The Patent Office says: "The selection of appropriate anilities, enzymes, coenzyme, and wavelength is conventional and within the skill of the art to which the invention pertains." An obvious inconsistent position, so I can't get anymore current than that since we're responding to the amendment.

I'm not going to take up the suggestion of going over the examiner's head because whenever that happens the next time I have the same examiner life gets very difficult, and it would just be as if somebody went over my head or one of my subordinates' heads in the office. It's politically not a good idea.

Now, let me get to the main points I wanted to deal with was the 20-year term. The 20-year term -- and I have some solutions to the problem. The 20-year term creates problems in view of the Patent Office's position on utility because if I've got to prove the utility by clinical data it's going to take me ten years at best and I'm left with ten years at best, and it also provides problems with the restriction requirement. The current system in the Patent Office strongly encourages examiners to give restriction requirements. It's my understanding there's a quote system, there's a bonus system based on dispositions. If you can examine one application and do all the hard work and do the search and then you can give a restriction requirement and get eight divisionals, you're going to look awfully good when it comes bonus time. So the current system in the Patent Office is encouraging restriction requirements. An example -- and I have a book how to write patent applications, and we talk about in the book how to claim a new protein isolated and purified from natural sources. There's 15 different ways to claim that at least, and the Patent Office in many cases will give you 15 restriction requirements,

but it's really only one basic invention.

The one suggestion I have is, if we are going to have a 20-year term, I think the legislation that's pending that says the longer of 20 years or 17 years from date of issuance should be what goes through. I believe that would comply with all requirements that the Patent Office currently has, so that is one possible solution to a lot of the issues raised on the 20-year term.

COMMISSIONER LEHMAN: When we had a hearing like this in Silicon Valley earlier this year we heard lots of complaints that were just the exact opposite, they were that we should have shorter patent term, not longer patent term. We really have this sort of dichotomy between the two California interest groups; biotechnology and computer software. So how would you respond to that?

MR. SHELDON: Well, if I were probably up there representing my computer software clients and they have a different view -- but I think a longer term overall is best, particularly for the biotechnology. And I have not seen a problem in the fast advancing arts in the computer --

COMMISSIONER LEHMAN: The problem basically, as I gather -- I mean, the problem really boils down to not that a 17-year or 20-year term is inherently inadequate. The problem has to do with the unique relationship of biotechnology to both the regulatory review process and the exceptionally long development period from the invention -- from inception of the invention to, you know, practical application. That's really the problem here; isn't it?

MR. SHELDON: Well, let me suggest other alternatives. A second alternative is if you're -- the Patent Office -- is requiring utility by human testing then built into the statute, much as we have a patent extension term for FDA regulatory delay, a delay in meeting the utility requirement of the Patent Office. Consider as a third alternative a new reissue proceeding where I issue my patent where I claim utility in rabbits, if I can later on show that I have utility in humans get me a reissue patent on that. And it will be a broader claim so it would take a new reissue statute. It's a creative approach. Consider that. Definitely we need less strict application of the restriction requirement and what will go a long way for that is reducing the examiners' incentive for restriction requirements. Either give them no credit for divisionals or less credit for a divisional than they get for original application.

Another way would be to allow -- like in the Trademark Office we can examine in a single application all the so-called separate inventions by paying an extra fee, if necessary, and give the examiner a little bit of extra credit for that. And when the patent actually issues, if we're going to enforce the interpretation of the patent statutes that you only

get one patent per invention and one invention per patent, then require divisionals. Divide it out at that time, at least it will reduce the cost. I don't have to prosecute nine applications and get nine different office actions. I can do everything at one time. I think that will be to the benefit of the Patent Office.

My time is up and I thank you for listening to my remarks.

COMMISSIONER LEHMAN: Thank you, sir. Very interesting suggestion, the one you just made.

Just to give you an example of the problem from some other end that we're getting a few. Tune into the Internet, you'll find on it the following: For those of you who are fans of Jerome Lemuelson, here's yet another of his submarine patents that issued with a continuation division chain back to 1954. For those of you who hate submarine patents your fears of lawsuits will be well fed by the claims to this patent which are infringed by most U.S. industries so, you know, that's the kind of thing that we're hearing loud and clear complaints about in terms, and the 20-year term from filing has attempted to address that kind of concern. But that doesn't mean that there aren't very real concerns of biotech industry and we will do what we can to try to address those.

MR. SHELDON: If I may, the suggestion that all the divisionals are prosecuted at the same time with dividing them out right before issuance would address the submarine patent concern and still meet the objections of the 20-year term.

COMMISSIONER LEHMAN: Thank you very much.

We're getting down near the end. Is Ted Green of Amylin Pharmaceuticals here?

TED GREEN, AMYLIN PHARMACEUTICALS

MR. GREEN: You're actually running a little ahead of schedule according to my chart.

COMMISSIONER LEHMAN: Yes, we are. We had a couple of people that canceled that's why. We had three cancellations and we're just about that far ahead of schedule.

MR. GREEN: All right. Well, my name is actually Howard Edward Green, Junior, and people know me by the nickname Ted. I am chairman and chief executive officer of Amylin Pharmaceuticals, Incorporated, a seven-year old biotech company working to develop novel medicines for treating diabetes. Before becoming a full-time employee of Amylin, I was a venture capitalist and helped start six medical technology companies;

five of which have gone public. Earlier I was chief executive of Hybritech, Incorporated, which was acquired by Eli Lilly in 1986.

I am a named inventor on two patents, both of which have broad method of use claims. The first patent covers Hybritech's product technology for two-site monoclonal antibody assays and is I think considered somewhat of a landmark among biotech patents.

The second patent covers one of Amylin's technologies for treating diabetes and is the focus of a major development program in collaboration with Glaxo, Britain's largest pharmaceutical company.

I'm speaking today on behalf of myself and other inventors in the pharmaceutical field who have made discoveries with broad medical potential. My message is this: We need broad method of use claims in order to raise the investment capital needed to develop our products. Moreover, these method of use claims should not be held to a higher standard of clinical utility than have the composition claims traditionally pursued by drug companies.

Recent pharmaceutical history shows why broad use claims are so important. Two pioneering inventions stand out. The first is the discovery of H2 antagonists for treating stomach ulcers, and the second is the discovery of ace inhibitors for lowering blood pressure. The companies that pioneered these products have now been merged out of existence. Why? Well, partly because they did not have broad enough patent coverage. Within a few years of the first H2 antagonist, that was Tagamet from SmithKline, and the first ace inhibitor, Capatin from Squibb, competitors Glaxo and Merck launched "me too" medicines that treat the same diseases by the same mechanisms of action. In my opinion, SmithKline and Squibb were unable to reap the full rewards of their inventions because they did not have the right patent coverage. SmithKline and Squibb probably would be independent companies today if they had been issued broad methods of use claims for H2 antagonists and ace inhibitors.

Now we read in the newspapers about drug firms buying distribution companies rather than investing in pharmaceutical research. In effect, the industry and Wall Street are now questioning the value of research expenditures which run to hundreds to millions of dollars for pioneering medicines. Why? Because competition from "me too" products within therapeutic classes is killing the investment returns from pioneering products because traditional pharmaceutical patents protection has been narrowly focused on composition claims, because the most important inventions, the sites of drug action, have not been claimed in patents.

We all know that recent discoveries in biology combined with modern pharmaceutical technologies are replacing random screening and blind luck

in the development of new medicines. The disclosure of a novel site of action, such as the H2 receptor or the ace enzyme now teaches competitors how to make their own drug. Just ask any competent medicinal chemist. Without patent claims to protect these important discoveries inventors and entrepreneurs will not receive the protection to which they are entitled and they will have difficulty attracting sufficient risk capital to carry their inventions all the way to market.

I also wish to state it is very important to receive patent protection quickly for these discoveries rather than after years of negotiating with the PTO.

First, potential equity investors want to know that they will be protected and ongoing rejections from the PTO make investors very nervous, to say the least. I remember when our patent counsel calmly announced that we had received a, quote, "final rejection," for one of our applications, which engendered raw panic in our management ranks. We have since gotten that patent issued.

Second, the big drug companies often don't pay much attention to patent applications preferring to wait to see what will actually issue. Consequently, it's harder to do deals with just patent applications.

Third, the lack of issued claims means the potential competition can't define the boundaries clearly. A situation which may encourage considerable economic waste.

At my company Amylin, we are pioneering new concepts for explaining why diabetes happens and why the disease is so difficult to treat. So far we have dedicated seven years to this effort, recruited over 150 highly skilled employees, and raised over \$130 million in capital. I believe we have spent more money on diabetes research than either the American Diabetes Association or the Juvenile Diabetes Foundation have since their inceptions.

For our key products we have received U.S. patents with broad methods of use claims and these claims have played an important role in attracting both investor and corporate partner support. In short, without patent protection there would be no Amylin Pharmaceuticals in San Diego.

Our patent laws have played a central role in making America the world's technological leader in general and in biotechnology. Our patent laws have encouraged innovation and risk taking that is the envy of the world. Our patent laws have helped make pharmaceuticals one of America's strongest industries and have encouraged the birth of our biotech industry. It would be a tragedy to strangle pharmaceutical innovation by denying the breadth of patent coverage for methods of use to which our scientific pioneers are entitled. Thank you.

COMMISSIONER LEHMAN: Thank you very much for your pioneering work that you've done and for your statement.

We're down to the bitter end now and we're going to hear I believe from a witness who's already testified before. Came all the way from Wisconsin so he's still here. If it wasn't for the fact that he's from Madison, which is my hometown, I'd say, you know, we're done, but I can't possibly say that now.

WILLIAM J. SCANLON, WISCONSIN BIOTECHNOLOGY ASSOCIATION

MR. SCANLON: Thank you very much for letting me testify again. I appreciate it very much and so does the party that I'm testifying for here. As indicated in my earlier testimony, my name is William Scanlon and I am a partner with the firm of Foley and Lardner in it's Madison, Wisconsin, office, and other biographical details you can obtain from the earlier testimony. I do have a written statement which I'll give to Mr. Kushin at the end, along with a floppy disk with everything on it.

I'm testifying today on behalf of the Wisconsin Biotechnology Association. This association has over 70- member organizations which are involved with biotechnology and are either based in Wisconsin or have operations there. Membership has tripled since 1992 and continues to grow rapidly. Testimony has been presented earlier today by representatives of several members of the association, including Mycogen, Pioneer Hi-Bred, Hoffman-La Roche, Ernst and Young, Merchant and Gould, and my own firm Foley and Lardner.

We are here today simply to make of record our concern over the patent system of the United States, our concern that it function effectively with respect to biotechnological inventions. I won't take time here to detail these concerns. We will submit a lengthily written statement in accordance with the Notice, the Commissioner's Notice.

Biotechnology is a major industry in our state. Businesses, universities and non-profit research organizations in Wisconsin are heavily involved in biotechnology research and development and diagnosis and treatment of human disease, dairying and agriculture generally, forestry and papermaking, environmental remediation, brewing and food processing, specialty chemical production and other areas. The Commissioner, who we note is a son of Wisconsin, is surely familiar with many of these activities.

These activities are greatly affected by how well the patent system as it relates to biotechnology functions. Our concern that the patent system function effectively involves several issues relating to the Patent Office's handling under present law of patent applications on

biotechnological inventions. Some of these issues are raised in the Commissioner's Notice of this hearing.

Our concern also involves effects that changes in the law pursuant to the GATT, or otherwise, as indicated in the Notice, or failure to enact such changes in the law might have on the patent system generally both in and outside the Patent Office as the system relates to biotechnology.

An effective patent system provides important incentives for the major investment required for advances in biotechnology and commercialization of biotechnological products and processes.

An effective patent system provides legal and economic bases for strategic alliances among business organizations or between such organizations and academic or governmental institutions, and these alliances spur progress in biotechnology and are often essential for commercialization of products and processes of the science.

An effective patent system provides legal and economic bases for economic development, that is for the creation of businesses to develop biotechnological inventions made in academic or governmental laboratories.

To function effectively for biological inventions, the patent system must -- like it must for all technologies -- reward with a grant of exclusive rights of appropriate breadth for a reasonable time discoveries in all fields relevant to biotechnology including, of course, plants and animals.

To function effectively, the patent system must function efficiently in granting such rights promptly and at a reasonable cost when such a grant is warranted, and we are concerned on both counts.

We understand that the Commissioner's responsibilities extend to all of the great range of technologies of concern to the Patent and Trademark Office and we thank him for the special concern for biotechnology he has demonstrated in holding this hearing today and, indeed, in sitting through the entire hearing.

We believe it would be appropriate for the Commissioner to hold another hearing, perhaps in six months or so, to determine how much progress will have been made in implementing the various changes in the law and Patent Office procedures recommended in today's proceedings.

And finally we recommend that this next hearing be held in Wisconsin. A beautiful place. A wonderful place, especially in the spring. But also a hot bed of biotechnology and at the center of major biological activity throughout the midwest.

Thank you very much.

COMMISSIONER LEHMAN: Thank you very much, Mr. Scanlon.

MR. SCANLON: Do you have any questions?

COMMISSIONER LEHMAN: I think not. And it's unusual that we would have this many people staying over that have such an interest in this hearing so I want to thank you all for sitting with us here and keeping us company all day long.

That concludes our hearing and we will be announcing I'm sure, in the not too distant future, a number of policy initiatives will flow out of this.

(Whereupon, at 6:41 p.m., the above-entitled matter was concluded.)

REMARKS SUBMITTED FOR THE RECORD

TESTIMONY PRESENTED BEFORE

PATENT AND TRADEMARK OFFICE HEARINGS OF BIOTECHNOLOGY INVENTIONS

San Diego, CA

October 17, 1994

Stanley T. Crooke, M.D., Ph.D.

Chairman and Chief Executive Officer

Isis Pharmaceuticals

Good afternoon. I am Stan Crooke. I am the founder and Chief Executive Officer of Isis Pharmaceuticals, a development stage, technology-based pharmaceutical company. Prior to founding Isis, I was President of Research and Development for SmithKline Beckman and, before that, a Vice President at Bristol Myers. I am a physician and scientist. In my career, I have been involved in the development of more than 15 drugs

that are currently marketed and numerous other drugs in development and have published more than 300 scientific papers and 16 books on pharmacology, drug discovery and development.

I believe that we can all agree that it is in the public interest to encourage innovation in the pharmaceutical industry by prompt issuance of patents of appropriate scope. We can also agree that public policy, or institutional behavior that discourages pharmaceutical innovation, is not in the public interest and will likely reduce the international competitiveness of one of America's most important industries.

Where we may diverge - and where I think we need to work together to find common ground is the topic I want to discuss today: What data are sufficient to support claims of potential therapeutic utility? Although there is no universal or absolutely right answer to this question, I believe that there are precedents that have stood the test of time that can provide guidance and can serve as a basis for reaching agreement.

The evaluation of the potential utility of an innovation in the pharmaceutical industry is especially complex. On average, more than 15 years elapse between discovery of a new drug and its marketing. Even after marketing, we may gain additional information that indicates that the product does not have sufficient therapeutic utility to remain available to the public. Furthermore, prior to marketing, on average more than \$350 million must be invested. It would be impossible for a company to make this kind of investment without reasonable expectations with regard to patentability, particularly in light of the risk of investments in this area. We know that, historically, less than 1 in 1000 of the compounds synthesized and patented by the pharmaceutical industry become products.

Many factors contribute to the risk and uncertainty in drug discovery and development, but there is one that I particularly want to emphasize today: the only way to determine the value of a new pharmaceutical technological innovation is to evaluate the fruits of that innovation, i.e. the drugs that are based on that innovation, in man.

As an example, let's consider the history of the treatment of ulcer disease. It has been known for many decades that ulcer disease is correlated with stomach acid secretion and a large number of physiological processes that influence acid secretion were identified. Early on, the cholinergic arm of the autonomic nervous system was shown to increase acid secretion, so it was hypothesized that anticholinergic drugs would have therapeutic utility in this disease. Literally thousands of compounds of this type were synthesized, tested and patented. A few were even marketed, but the side effects of these drugs were very limiting, so the true therapeutic utility of anticholinergic drugs for ulcer disease was modest at best. Research continued and other

involved factors, including histamine, that increase acid secretion were identified. This led to the notion that a blockade of a specific receptor for histamine, the H2 receptor might be of therapeutic value. This concept was hotly debated, but research continued. The first H2 antagonist to enter clinical trials, Metiamide, failed, but the next, Tagamet, worked and revolutionized ulcer treatment. Then came the hypothesis that the inhibition of an enzyme, H/K ATPase, might be beneficial as the enzyme was thought to result in the secretion of acid. Again, this was highly controversial. Many compounds were synthesized and patented and most failed. Omeprazole ultimately was marketed and has improved ulcer treatment.

The points that I want to make by reciting this well-known history are several and they are important. First, new concepts and approaches (technologies) that might result in therapeutic innovations arise constantly. Second, only after a drug of a particular mechanism has been shown to work in the clinic have these new concepts (technologies) been validated. Third, it is a normal part of this process that some members of a particular class of drugs fail and yet the basic concepts that supported the creation of these broad innovations have proven to be valid. Fourth, innovations are controversial and the controversies are not resolved till drugs based on this innovation are thoroughly tested. Fifth, despite these complexities, the traditional practices of the U.S. Patent and Trademark Organization resulted in effective stimulation of innovation and enormous public benefit.

Those traditional practices were to allow claims of reasonable scope long before definitive proof of utility was obtained. This was done by accepting reasonable evidence for potential utility and by maintenance of an attitude that was biased toward rewarding and encouraging innovation by giving the benefit of the doubt to new concepts.

Very recently, with regard in particular to patents in "biotechnology", we feel there has been a substantial change in the practices of the U.S. Patent and Trademark Office. Demands for definitive proof of therapeutic utility (which I remind you can truly only come after a drug has been marketed for 2-3 years to assure that it is safe with broad use) have resulted in many patent application rejections.

Now, let's look at the potential impact of this change in the patent environment. In other words, what will happen if the Patent and Trademark Office were to continue to require definitive proof of utility before granting therapeutic use claims? What will happen is simple: new drug innovation will be dramatically impeded.

So the risks of continuing this practice are very large, while the risks of relaxing the policy are really quite negligible. The drug discovery, development and commercialization processes have built-in self-regulating

mechanisms that ensure that drugs that should fail, do. What harm is done if a patent with appropriate claims is granted to a drug candidate and the drug or the technological concept behind it fails? The company loses its investment, but that's the risk it takes. Nothing has been lost by society. In fact, the public actually gains from the exploration of the concept or the technology. On the other hand, if companies stop investing in new compounds derived from novel concepts or technologies because of patent uncertainty, everyone loses. The loss cannot be made up.

So what am I recommending?

¥ Return to traditional practices with regard to pharmaceutical patents. They work.

¥ Return to a positive bias to innovation with the acceptance of reasonable proof of potential utility.

¥ Treat patents from so-called biotechnology companies and pharmaceutical companies equally. We are the same industry with the same customers, practicing similar science. The traditional approaches are sufficient to stimulate investment in innovation in both sectors of the drug discovery and development-based industry.

¥ Emphasize consistency across and within technological areas. Just because one approach is labeled a "new technology" and another is not, does not mean that the basic approaches or risks are necessarily different.

¥ When in doubt, grant therapeutic claims based on the specific examples provided with scope commensurate with reasonable extrapolation from the examples provided.

¥ Finally, be even more prudent in the allowance of broad "concept" patents. Allowance of reasonable claims based on the examples provided in the application is sufficient to stimulate innovation. Granting of broad "concept" patents is very rarely justified in the pharmaceutical industry.

Again, let me thank you for this opportunity. I believe this issue is of critical importance to the viability of innovation in health care, and I am confident that as you consider this issue, you will agree that it is in the public interest to continue to encourage innovation, not destroy it.

I'll be glad to respond to any questions you may have.

3061

TESTIMONY PRESENTED BEFORE
PATENT AND TRADEMARK OFFICE HEARINGS
OF BIOTECHNOLOGY INVENTIONS

San Diego, CA

October 17, 1994

B. Lynne Parshall

Senior Vice President

Isis Pharmaceuticals, Inc.

Commissioner Lehman and other members of this hearing board, good afternoon. My name is Lynne Parshall. I am Senior Vice President of Isis Pharmaceuticals. Dr. Crooke from our Company spoke with you earlier. Among my responsibilities at Isis is to supervise our patent prosecution.

I very much appreciate the opportunity to talk with you today. I plan to focus on one item in connection with our patent prosecution that has affected Isis significantly: rejections we have received based on "incredible utility."

As a development stage, research-based pharmaceutical company, for many years Isis' most significant tangible asset will be our patent estate. We have invested heavily in this area.

Our major focus at Isis has been on the development of antisense technology and the commercialization of drugs based on this technology. We are the leading company in our area and have invested more than \$100 million in this technology during the past five years. Many major pharmaceutical companies and other development stage companies also have antisense programs. In many respects, antisense technology epitomizes some of the special challenges that the patent office faces. It also epitomizes some of the potential harm that can be done by inconsistent activities with regard to the evaluation of patent applications.

Antisense technology is a true innovation in drug discovery and development. It is broadly applicable among therapeutic areas. It is a technology that has extraordinary promise and importance. It is a technology that needs and deserves the protection of our patent system.

Antisense technology focuses on a new target for drug discovery, RNA, and uses a new class of chemicals, modified oligonucleotides, to approach this target. At Isis we have simultaneously invented a new medicinal chemistry and the basic pharmacological framework to apply this technology. Even for practitioners, remaining abreast of this technology is difficult. In this way, the antisense field is no different than any other important field in which rapid innovation is occurring. What is different however, appears to be how the patent office analyzes the growing body of literature concerning antisense technology. In reviewing this literature, as you would expect, you will find work of varied quality (good and bad) and expressing a variety of approaches to and views of the field. In addition, you will find work which, due to the rapid pace of innovation, is no longer state-of-the-art. It is the selective use of this literature by the PTO to doubt rather than to support innovations in antisense that hinders our patent prosecution and provides an obstacle to patenting innovations in the field.

Not surprisingly, in the beginning there were more questions than answers about the potential for antisense compounds to be drugs. Today, most of the questions have been answered and the overwhelming wealth of current evidence supports the contention that antisense technology will yield important therapeutic advances. This evidence includes efficacy data from many animal models of disease, from many different laboratories and it also includes clinical data on two antisense drugs that Isis has studied in humans. Despite this wealth of data, we are continually facing rejections based upon "utility" for our compositions of matter and therapeutic use patents. In the last year we have received office action rejections in approximately 70 applications that included some sort of "incredible utility" or "lack of utility" rejection. Responses to these office actions has cost us hundreds of thousands of dollars in legal fees.

Patent applications have been rejected for compounds for which we have shown significant inhibition of viral replication in well recognized models (including models used by government agencies for therapeutic screening) when more traditional chemicals showing activity in these models have been patented. Patent applications have been rejected for compounds for which we have shown significant activity in animal models of disease, when more traditional chemicals showing such activity have received patents. Even patent applications for compounds for which we have shown evidence of activity in humans have been rejected.

For example, one of our pending patent applications claims compositions

of matter to certain phosphorothioate oligonucleotides used to treat a debilitating viral infection. Today, patients are being treated with one of these phosphorothioate oligonucleotide to halt the spread of this disease. In the latest office action we received for this application, the examiner has maintained a previous 101 rejection for lack of patentable utility. This rejection has been maintained in the face of declarations from two experts in support of this application. One of these declarations was from a clinician treating patients with this drug in our ongoing clinical trial. This declaration detailed the positive activity of the drug in patients. The other declaration came from a well-respected scientist who reviewed the current state of the antisense field. These declarations from well-known, well-respected clinicians and scientists were dismissed.

We face similar patent office rejections in basic chemistry cases. Just a few days ago, we received an office action on a process patent application. The invention claimed in the application is an improved process for preparing phosphorothioate oligonucleotides. In the office action the Examiner rejected all of the claims as lacking patentable utility. The Examiner stated (quote) "... the antisense activity of the instantly claimed compounds borders on the incredible and such an asserted utility is deemed unlikely to be correct in view of contemporary knowledge in the art." (end of quote)

It is difficult to understand the Examiner's position. The recent scientific literature does not indicate that the activity of phosphorothioate oligonucleotides is "incredible and... deemed unlikely to be correct." Ongoing clinical trials of these compounds do not indicate that the activity of phosphorothioate oligonucleotides is "incredible and... deemed unlikely to be correct." The declarations we have submitted from well-respected clinicians and scientists do not indicate that the activity of phosphorothioate oligonucleotides is "incredible and... deemed unlikely to be correct."

I am not suggesting that drug discovery is not fraught with uncertainty or that the road from drug discovery to commercialization is not long. What I do urge you to see is that antisense compounds merely represent yet another example of a new area of innovation, similar to those that have been encountered numerous times in pharmaceutical patenting: new classes of chemicals designed to intervene in disease processes in a new way. Support of this type of innovation in the pharmaceutical industry by granting patent protection of reasonable scope has been and continues to be necessary to stimulate continuing innovation.

With regard to patents in "biotechnology", we feel there is a different standard applied than that applied to traditional pharmaceuticals. Demands for definitive proof of therapeutic utility (which the PTO seems to equate with pivotal, controlled human clinical trials), have resulted

in many patent application rejections. As Dr. Crooke said to you earlier today, there is no harm done and, in fact, significant public policy is served, by giving pharmaceutical innovators the benefit-of-the-doubt in terms of patenting.

I would like to reiterate the recommendations Dr. Crooke made to you earlier today. We recommend that the PTO return to traditional practices with regard to pharmaceutical patents. This would include a return to a positive bias toward innovation with the acceptance of reasonable proof of potential utility for inventions. We request that you treat patents from so-called "biotechnology" companies and pharmaceutical companies equivalently; we are the same industry with the same customers, practicing similar science. We request that you act consistently across and within technological areas. Just because one technology is labeled a "new technology" and another is not, does not mean that the basic approaches or risks are necessarily different. We further request that, when in doubt, you grant therapeutic claims based on the specific examples provided with scope commensurate, with reasonable extrapolation, from the examples provided.

Again, let me thank you for this opportunity to speak to you today. I believe this issue is of critical importance to the viability of innovation in health care, and I am confident that as you consider this issue, you will agree that it is in the public interest to continue to encourage innovation in the pharmaceutical industry, not discourage it.

I'll be glad to respond to any questions you have.

Honorable Commissioner and panelists, my name is Michael B. Farber. I am a patent attorney at Merchant & Gould in Los Angeles, specializing in biotechnology. Merchant & Gould is a full-service intellectual property law firm. My clients include Fortune 500 companies in pharmaceuticals and diagnostics, smaller start-ups, universities, and research institutions.

I would like to address three points in particular. The first of these points is that the Patent and Trademark Office seems to see a lack of credibility of the science on which biotechnology is based, leading to an inappropriately high standard for utility under 35 U.S.C. § 101 and enablement under 35 U.S.C. § 112. The second point is inconsistency in issuing rejections under 35 U.S.C. § 101 or § 112 and under 35 U.S.C. § 103, even in the same office action. The third point is more narrow and is what I believe is a misinterpretation of the Amgen v. Chugai and Fiers v. Sugano cases to narrow the scope of enablement under 35 U.S.C. § 112 in certain situations, a narrowing which I believe is contrary to previous case law.

I. LACK OF CREDIBILITY GIVEN TO BIOTECHNOLOGY

Recent actions by the Patent and Trademark Office have suggested that biotechnology is almost placed in the same "weird science" category as cold fusion and perpetual motion. This is a distortion of the basis of biotechnology, which is an outgrowth of the work of the last 40 years in genetics, cell biology, organic chemistry, biochemistry, microbiology, and other disciplines. This seeming lack of credibility given the science has led to an enhanced standard both for utility under 35 U.S.C. α 101 and for enablement under 35 U.S.C. α 112. This is contrary to case law on "incredible utility", such as *In re Chilowsky*, 229 F.2d 457, 108 U.S.P.Q. 321 (C.C.P.A. 1956). The science on which biotechnology is based is not contrary to recognized physical laws or is of such a nature that it cannot be tested by known scientific principles.

In particular, this reluctance to credit the soundness of the science involved has led Examiners to strain to make rejections under α 101 and the first paragraph of α 112. A frequently used tactic has been to find a research article or review that cites a potential problem with a treatment method, such as the possible existence of a human anti-murine antibody response ("HAMA"), an anti-idiotypic response, or concerns about the stability or bioavailability of a reagent such as a monoclonal antibody, and then use this article or review to reject the claims to the reagent or to methods for its use. This rejection is made even though there is no specific basis for believing that the problems set out in the article would apply to the claimed reagent or method; the concerns are general in nature.

Another aspect of this reluctance to credit the science on which biotechnology is based is a seeming requirement for a cure in 100%, or nearly 100%, of the cases when human treatment is contemplated. I had one application, involving a monoclonal antibody for which one of the possible indications was renal cancer, in which a rejection was made because the Examiner considered that the success rate was not likely to be more than 20%. This rejection was made on general concerns of the type discussed above, and there was again no specific basis for asserting that these concerns would limit the success rate to 20% in the use of this particular antibody. However, even accepting the soundness of these assertions, a 20% success rate in a disease now almost completely untreatable and uniformly fatal represents a significant advance in the art. I believe that we must go back to the constitutional mandate of "progress in science and the useful arts," and consider the situation from the standpoint of a well-informed physician who is conversant with recent medical advances, not a quack or a fringe practitioner, but one who is willing to try new advances in an otherwise hopeless case. If such a practitioner would accept a 20% success rate in an otherwise untreatable condition such as renal cancer as an advance in the art, the Patent and Trademark Office should do likewise.

II. INCONSISTENCY

The second point that I would like to raise is inconsistency in issuing rejections under α 101 or α 112 and under 35 U.S.C. α 103. I recently received an office action in an application for a monoclonal antibody directed against a CD antigen (i.e., an immune system antigen) in which a rejection was made under α 101 for lack of utility, and, in the same office action, under α 103 based on the accepted diagnostic utility of a prior art antibody considered analogous by the Patent and Trademark Office. This is inconsistent and illogical. If one of ordinary skill in the art would believe that there was a sufficiently shared utility to provide an incentive to make the modification resulting in the claimed antibody, that same person of ordinary skill in the art would also accept that the claimed antibody would have a similar diagnostic utility. These rejections should not be based on inconsistent reasoning. When the Patent and Trademark Office seeks to make a rejection under α 103, the "person of ordinary skill in the art" seems to be a genius; when the rejection is sought to be made under α 101 or α 112, that "person of ordinary skill in the art" suddenly turns into an idiot.

III. IMPROPER USE OF AMGEN AND FIERIS TO NARROW SCOPE OF ENABLEMENT

The third issue on which I would like to speak is narrower and more technical. It concerns the use by the Patent and Trademark Office of *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 U.S.P.Q. 2d 1016 (Fed. Cir.), cert. denied, 112 S.Ct. 169 (1992), and *Fiers v. Sugano*, 984 F.2d 1164, 25 U.S.P.Q. 2d 1601 (Fed. Cir. 1993) to narrow the scope of enablement and thus the scope of claims.

This arises particularly in two situations:

(1) An inventor isolates or discovers a protein or peptide and determines its complete amino acid sequence, and now wants to claim nucleic acid sequences encoding the protein, for use as probes or in a recombinant expression system.

(2) An inventor has isolated or discovered a new antigen, such as a protein or a peptide, that occurs in several different forms, as a result of allelic variation or other factors, and wants to claim antibodies (monoclonal or polyclonal) that bind the different forms of the antigen, after actually making one or more antibodies.

In either of these cases, the claims should be allowed absent specific evidence of inoperability or specific reasoning that compels a conclusion that species within the claims are inoperative. In the first case, once the primary amino acid sequence is known, all possible nucleic acid sequences can be determined by the genetic code. Unless there is evidence that certain nucleic acid sequences would be inoperable, e.g.,

as probes or for expression vectors, as a result of peculiar secondary structure, abnormal codon utilization in hosts for the vectors or other factors, the claims should be allowed. In the second case, in the absence of specific evidence that any of the forms of the antigen would be non-immunogenic, the claims to the antibodies should be allowed even if the inventor has not prepared antibodies to all possible antigens.

The Patent and Trademark Office is wrong to reject claims of this type as lacking enablement under the first paragraph of α 112 by analogy to cases such as *Amgen v. Chugai* and *Fiers v. Sugano*. These situations are in no way analogous to a claim that seeks to encompass all nucleic acids having a particular function without any structural information. Here, a specific structure or structures are known. The situation in cases such as *Amgen v. Chugai* and *Fiers v. Sugano* resembles a "single means claim," a type of claim long proscribed. Here, by contrast, a defined structure or structures are recited, and the inventor is seeking additional protection for molecules produced by generally well-understood processes using the information contained in the original, completely defined, structure.

Therefore, it is improper to reject claims of this sort as "broader than the disclosure" or for "undue breadth" under the first paragraph of 35 U.S.C. α 112. Rejections of this sort, which are sometimes purportedly justified by recitations of the potentially large number of structures encompassed by the claim, are contrary to much Court of Customs and Patent Appeals case law in the organic chemical field, such as *In re Angstadt*, 537 F.2d 498, 190 U.S.P.Q. 214 (C.C.P.A. 1976). The inventor need not make or test every possible species within the scope of claims in order for enablement to exist under the first paragraph of 35 U.S.C. α 112 under this line of cases. These rejections, therefore, are improper.

IV. CONCLUSION

In conclusion, I believe that the Patent and Trademark Office must examine biotechnology applications in accordance with the principles that the courts have developed for organic chemical and pharmaceutical inventions, paying due heed to the constitutional mandate. This will resolve many of the problems encountered by applicants in biotechnology at the present time.

October 24, 1994

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

Attention: Jeff Kushan, Office of Legislation and International Affairs

RE: Patent and Trademark Office

Notice of Public Hearings and

Request for Comments on Patent

Protection for Biotechnological Inventions

Dear Sir:

My name is Andrew D. Fortney, Ph.D. I am a patent agent for Oblon, Spivak, McClelland, Maier & Neustadt, where I have actively prosecuted patent applications in all areas of chemistry, biotechnology and medical technologies, and to a more limited extent in mechanical and electrical technologies. I am also a student in the fourth year of the evening program at the George Washington University National Law Center. The following remarks, which represent my personal views, are offered in response to the notices in the Federal Register and the Official Gazette. My views have been influenced by numerous discussions with members of Oblon, Spivak, McClelland, Maier & Neustadt, particularly with Dr. Richard Chinn and Mr. Steven Kelber, and with persons (both clients and non-clients) active in the biotechnology industry and in related fields.

RELEVANT POLICY OBJECTIVES OF THE CLINTON ADMINISTRATION, AND HOW THE PTO CAN MORE EFFICIENTLY ADVANCE PRESIDENTIAL POLICIES

President Clinton has advocated at least three policy-related goals affecting the biotechnology industry which the U.S. Patent and Trademark Office (the "PTO") may affect through its actions, including:

- (1) Fostering the growth of "high-technology" industries, including biotechnology;
- (2) As a part of an overall program to reform health care, reducing the costs of medical care and particularly of pharmaceuticals; and
- (3) Minimizing the size and expenses of the Federal bureaucracy, including resolving conflicts between Federal agencies and eliminating or minimizing duplication of effort among Federal agencies.

As an administrative agency, the PTO can have an effect on executive policies by the manner in which it investigates questions of

patentability (e.g., utility, operability and obviousness). However, the analysis of patent applications is necessarily fact-based, and is performed on a case-by-case basis. Thus, it may be difficult or inappropriate to apply general rules of patentability to examination of biotechnological patent applications, such as granting a presumption of inherent utility to all biotechnological inventions. Such a presumption may result in patents being obtained from applications which may not disclose the utility of the invention. This would circumvent the "quid pro quo" of the patent system, full disclosure of the invention, including disclosure of contemplated uses.

In my opinion, the PTO can more effectively accomplish President Clinton's policy objectives by:

- (A) Allowing claims with reasonably broad scope;
- (B) Permitting Examiners to accept applicants' assertions of utility and operability unless objective evidence questioning such assertions is provided;
- (C) Where assertions of pharmaceutical utility are raised, consistently treating claims to compounds separately from claims to pharmaceutical compositions and methods of use; and
- (D) Where pharmaceutical utility is relied upon for patentability, adopting a preponderance-of-the-evidence standard for establishing pharmaceutical utility.

Adoption of proposed policies (A)-(D) by the PTO above will result in (i) earlier issuance of patents and (ii) greater patent protection for inventors. The proposed policies will achieve the President's policy goals by more adequately rewarding inventors for the knowledge which they provide, thus encouraging further research and development of biotechnological products and services, in turn fostering the growth of the biotechnology industry.

Earlier issuance of patents and broader patent protection will reduce the costs of prosecuting patent applications, and thus contribute directly to reduced costs for pharmaceuticals and medical services. Earlier issuance of patents will also result in an earlier end to the patent right, thus allowing competition to enter the marketplace at an earlier date, further resulting in lower costs through competition.

Finally, where the PTO has challenged asserted utilities and broad statements of operability in an application, adopting a preponderance-of-the-evidence standard for resolving questions of pharmaceutical utility and of predictability in the art will reduce the amount of evidence which Examiners must review, and thus, the amount of

time spent reviewing such evidence. As a result, the costs of running Group 1800 will be reduced, and duplication of effort between the PTO and other regulatory agencies, such as the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA), which must independently analyze data supporting assertions of safety and efficacy, will be minimized. Consequently, potential conflicts between such agencies can also be avoided.

THE BIOTECHNOLOGY INDUSTRY PERCEIVES PTO ACTIONS AS NOT MOST EFFICIENTLY FOSTERING ITS GROWTH

The Public Hearing on Patent and Protection for Biotechnological Inventions held by the PTO in San Diego, California on October 17, 1994 demonstrated the effects of perceived practices by Group 1800 of the PTO on the biotechnology industry. Many industry representatives believe that the PTO requires clinical data in many cases asserting pharmacological utility. Many of those in the industry also perceive Group 1800 as viewing the levels of ordinary skill under 35 U.S.C. 103 and 35 U.S.C. 112 quite differently (and in the opinion of some, almost antithetically). Such practices are viewed by the biotechnology industry as impeding the progress of the industry, and thus, as not advancing President Clinton's policy of encouraging the growth of biotechnology. For example, many representatives of "start-up" biotechnology companies and of non-profit research organizations explained that, due to rejections under 35 U.S.C. 101 and 35 U.S.C. 112, first paragraph, for lack of utility and lack of enablement, they cannot obtain patent protection unless clinical data are submitted. Typically, the start-up company or non-profit research organization does not have the capital necessary to conduct clinical studies, and must look to larger pharmaceutical companies or venture capital firms for funding. However, unless the patent is granted, they are unable to make business agreements (e.g., licenses) and thus obtain the capital necessary to conduct clinical studies. Thus, the PTO is perceived by the biotechnology industry as pursuing a policy which impedes further growth and development in the industry.

Furthermore, patents which are issued by the PTO are too often restricted in scope to working examples (e.g., to a protein having a particular sequence, to a particular deposited strain of microorganism or cell, etc.). As a result, it becomes fairly easy for competitors to design around the patented invention. In addition, patent owners must rely to a greater degree on enforcement under the Doctrine of Equivalents (as opposed to direct infringement), thus increasing the costs of enforcing a patent. The increased costs of litigation in turn increase the costs of any products or services covered by the patent. Consequently, considering the value of the contributions offered by innovators in biotechnology, the value of the patent is frequently not as

high as it should be to justify the costs of bringing a product to the marketplace.

PROPOSED POLICIES IN GREATER DETAIL:

ALLOWING CLAIMS HAVING REASONABLY BROAD SCOPE

The proposals stated above will achieve the President's policy objectives in a manner both consistent with the law and more to the satisfaction of the biotechnology industry. First, I would like to address the topic of allowing reasonably broad claim scope in biotechnological patent applications. Of the issues detailed in the Official Gazette and Federal Register Notices, the degree of disparity in the levels of ordinary skill under 35 U.S.C. 103 and 35 U.S.C. 112 may be the most important because it affects every application filed in Group 1800. (On the other hand, the perceived requirement for clinical data in applications asserting pharmacological utility affects only those applications asserting pharmacological utility). As described above, allowing claims with reasonably broad claim scope increases the value of the patent. This will directly increase the reward for innovators, thus directly fostering the growth of the industry.

Such a policy on the part of the PTO is consistent with the law and with President Clinton's policy objectives. Reasonably broad claim scope will also provide greater certainty of patent protection with regard to modifications of the disclosed invention. As a consequence, one's patent position will be established to a greater degree of certainty, and one's business position will be more stable. In turn, more reliable business agreements can be made based on the protection provided by the patent. As a result, business agreements can be made at an earlier stage and with less effort, thus lowering the cost of the effort involved and the amount of time needed to develop and market the product. These factors all contribute to (1) a healthy business atmosphere for biotechnology and (2) an overall, long-term lowering of the costs of biotechnological products.

Allowing claims with reasonably broad scope is legally acceptable where the technology is reasonably predictable. As we heard in a number of comments at the hearing, biotechnology is in general, becoming more predictable. An noteworthy example of the level of predictability in biotechnology as it exists today was provided on October 16, 1994, by CBS' 60 MINUTES. A story on the cloning of a particular mutant gene from an Italian family was reported. Many members of this family had life-threatening cholesterol conditions, but showed little or no signs of coronary disease. Doctors identified the gene, and Pharmacia apparently cloned it in a recombinant microorganism. Sufficient quantities of the mutant protein now appear to be available to conduct the studies necessary to bring the protein to the marketplace.

Tests with the mutant protein in rabbits displaying coronary disease conditions (i.e., fatty tissue deposits in arteries) showed a 70% reduction in fatty tissue deposits. Dr. Shah of Cedars-Sinai Medical Center and UCLA explained to the American public that this technology presents a potential cure to coronary disease the like of which has not been seen before. Further, Dr. Shah also explained to the public that the means and the technology for bringing therapies based on the protein (including gene therapy) to the public exists now. In the absence of human clinical data, this assertion is strong evidence of the predictability of the biotechnological arts in bringing useful, effective products to the marketplace once they have been identified and once preliminary test data in an acceptable model indicate a reasonable likelihood of effectiveness.

Furthermore, the leading cases interpreting predictability in the art of biotechnology are based on technology that is generally from 7 to 15 years old. Thus, the state of the law regarding predictability of biotechnology a level of skill considerably lower than that which exists today. In fact, the most recently decided cases indicate that there is some predictability in modifying biotechnological inventions. Thus, where the level of ordinary skill is such that one can make minor modifications to the disclosed invention reasonably predictably and without undue experimentation, one should be entitled to claims having a scope commensurate with (1) that supported by the application and with (2) the level of ordinary skill.

What can the examining corps in Group 1800 do to achieve the President's goals? One low-cost, low-effort option is to accept patent applicants' statements at face value, unless there is a reason to question such statements (and preferably, where such questions are supported by objective evidence). However, as an administrative agency, the PTO may have a reasonable public policy interest in challenging statements of operability, particularly where they are not believable on their face. Where legitimate questions of operability exist, a preponderance-of-the-evidence standard (as explained above) should be acceptable for establishing the level of ordinary skill.

Thus, at the Examiner level, the Examiner should be permitted to accept assertions in the application at face value, particularly in the absence of objective evidence challenging such assertions. However, where objective evidence challenging operability assertions exists, the Examiner may shift the burden onto the applicant to provide evidence supporting operability for the scope of protection sought. However, once applicants rebut such a challenge with objective evidence establishing the level of ordinary skill and/or the level of predictability in the art, the Examiner should weigh all of the evidence on the record objectively. Where the weight of the evidence supports operability and

predictability of modifications commensurate in scope with applicants' assertions and with the claimed invention, the Examiner should rule in the applicants' favor. However, where the weight of the evidence does not support the applicant's assertions, the Examiner may properly reject an application for lack of operability over the entire scope of protection sought.

THE UTILITY ISSUE

With regard to the perceived requirement for human clinical data, again I would like to encourage the Group 1800 examining corps to accept applicants' assertions at face value. Thus, where the assertions are believable on their face, an Examiner should be permitted to find the application and claims acceptable under 35 U.S.C. 101. However, where an application asserts a medical or pharmaceutical use, there may be a public concern regarding whether the assertion is true on its face, particularly where supporting evidence is scant or not yet widely accepted in the art. In such cases, the PTO should consistently treat compound claims separately from pharmaceutical composition and method of use claims.

Compound claims (e.g., drawn to a protein or polynucleic acid) often rely on non-pharmaceutical utilities, such as use in an in vitro diagnostic assay. As a result, compound claims often do not rely solely on pharmaceutical utility to comply with 35 U.S.C. 101. Thus, challenges to compound claims on the basis of failure to demonstrate medical or pharmaceutical effectiveness in humans should be made only when only pharmaceutical or medical utility is asserted utility for the compound.

On the other hand, pharmaceutical composition claims typically recite an effective amount of an active agent. Where the desired effect may be in a human, the claims may rely on effectiveness in humans for utility. Naturally, claims to methods of treating medical conditions and/or administering pharmaceutical agents necessarily rely on pharmaceutical utility to comply with 35 U.S.C. 101. Consequently, questions regarding effectiveness in humans are more easily raised in claims to pharmaceutical compositions and methods.

However, the biotechnology industry perceives an over-reliance by the PTO on clinical data to resolve such questions. As explained above, this perceived over-reliance is causing some difficulty for the biotechnology industry and appears to impede rather than advance President Clinton's stated policy objectives.

In resolving questions regarding claims which rely on effectiveness in humans for utility, I propose that a preponderance-of-the-evidence standard is appropriate.

The leading case on pharmaceutical utility is *Brenner v. Manson*. In *Manson*, a method for preparing a compound structurally related to, but distinct from, a certain steroid known to have pharmaceutical utility was claimed. *Manson* relied on homology of the product of the claimed process to a steroid have tumor-inhibiting effects in mice, and the potential usefulness of product of the claimed process for patentability. Although the C.C.P.A. held that a novel chemical process is patentable so long as its yields the intended product and so long as the product is not itself "detrimental," the Supreme Court held that until the product of a process claim has been shown to be useful, the metes and bounds of the monopoly of knowledge encompassed by the process patent are not capable of precise delineation. The process patent would grant its owner the right to prevent others from finding the first use for the product of the process, and thus, the patent may block off whole areas of scientific development without compensating benefit to the public (i.e., the knowledge of the usefulness of the products of the process).

As a result of their holding, the Supreme Court struck down the concept that an invention is useful so long as it is not frivolous or detrimental. I believe this would make any presumption that biotechnological inventions are inherently useful to be inconsistent with the Supreme Court's interpretation of 35 U.S.C. 101 regarding chemical process or pharmaceutical inventions.

Manson failed the proposed utility evaluation tests explained above. First, the application apparently made no assertions of utility for the products of the process. Accordingly, the usefulness of the process itself was not readily apparent on its face, and could be challenged. Secondly, *Manson* provided no evidence that the products of his claimed process were useful. Consequently, after the PTO shifted the burden onto *Manson*, *Manson* failed to provide sufficient evidence to outweigh the PTO's concerns.

Subsequent cases decided by the Federal Circuit and the Patent Office Board of Appeals are also consistent with the suggested tests above. For example, in *Nelson v. Bowler*, claims to prostaglandins shown to be effective in rat blood pressure and gerbil colon smooth muscle stimulation tests were found to be useful under 35 U.S.C. 101. Although the Board of Appeals upheld the rejection for not showing adequate proof of practical utility because the tests were "rough screens, uncorrelated with actual utility," the Federal Circuit found that tests evidencing pharmacological activity may manifest a practical utility even though they may not establish a specific therapeutic use. Thus, in weighing the evidence, *Nelson's* evidence, which was held to be reasonably indicative of the desired response, and which was challenged on the basis of statistical significance rather than on the basis of objective evidence, were found to have a utility under 35 U.S.C. 101. The weight of the evidence in this case was clearly on the side of the applicants. In *In re Jolles*, a pharmaceutical composition and a method for the

treatment of acute myeloblastic leukemia were claimed. Declarations reporting the results of clinical treatment were submitted. A rejection of the claims under 35 U.S.C. 101 and 112, first paragraph, for lack of proof of utility were based on assertions of incredible utility and that the clinical data were not convincing. The rejection was reversed by the C.C.P.A. because the Board of Appeals dismissed the evidence as not being relevant to human utility. Again, the weight of the objective evidence was on the side of the applicant.

In *In re Langer*, claims to a dentifrice and method for reducing enamel solubility were rejected under 35 U.S.C. 101 for lack of proof of utility. Langer provided both in vitro and in vivo animal test data showing that the claimed composition reduced enamel softening and erosion. The Examiner cited a number of references disclosing in vitro test data supporting an assertion that those skilled in the art would not accept applicant's allegation [of utility] as obviously valid and correct. The animal test data were attacked on the bases that (i) the tested material appeared to be a denture adhesive rather than an emollient dental paste, (ii) the concentration tested was insufficient for the claimed range, and (iii) the test animals may have received fluoride in their drinking water. However, the unsupported assertions by the PTO regarding the in vivo data were rejected by the C.C.P.A., holding that the evidence submitted was sufficient for compositions and methods reciting Sn2EDTA, but insufficient for generic claims. The logic of the C.C.P.A. was based largely on the weight of the evidence submitted.

In *Ex parte Balzarini*, the Examiner established with objective evidence a lack of correlation between applicants' in vitro test method and a reasonable expectation of effectiveness in vivo. In response, Balzarini made the unsupported assertion that the test conditions resembled circumstances in vivo as much as possible. Thus, in this case, the PTO provided the greatest weight of evidence regarding the utility on which the claimed invention relied (treating retroviral diseases in an animal or patient and treating human cells in a manner effective to inhibit the replication and effects of HIV).

In *Ex parte Deuel*, the application apparently failed to disclose the use of the claimed protein. The Board of Appeals recognized that this application necessarily fails to comply with the requirement set forth in *Brenner v. Manson* that at least one use of the claimed product be disclosed. As a result, the Board of Appeals issued a new ground of rejection under 35 U.S.C. 101 and 112, first paragraph. Again, in this situation, any asserted utility could be properly challenged, and in the absence of evidence, applicants fail the proposed weight-of-the-evidence test.

Similarly, *Ex parte Stevens* also concerned an application claiming a therapeutic or prophylactic composition and a method for treatment of

cancer, rejected under 35 U.S.C. 101 and 112. The rejection was affirmed because Stevens expressly acknowledged that no actual evidence of the effectiveness existed. As a result, Stevens necessarily failed a weight-for-the-evidence test after having the burden of proof shifted onto them.

In *Ex parte* Heicklen, a composition and method for retarding the aging process were claimed. A rejection under 35 U.S.C. 101 for incredible utility was made (i.e., questioning the assertion of utility on its face). The evidence submitted by Heicklen merely showed that mice receiving the active agent lived longer than mice which did not receive the active agent. In the Board's opinion, insufficient evidence was submitted to establish that the increased longevity resulted from a retardation of the aging process (the utility relied upon by Heicklen for patentability). The rejection was thus maintained by the Board. Naturally, Heicklen failed the weight-of-the-evidence test for establishing the relied-upon utility.

In *Ex parte* Aggarwal, a method for the treatment of tumors was claimed. A rejection for lack of utility under 35 U.S.C. 101 and a lack of enablement rejection under 35 U.S.C. 112 were issued by the Examiner, based on inoperability over the broad range of cancers and tumors claimed. The Examiner provided a list of reasons why treatment of tumors is essentially unpredictable. The prior art relied upon by the Examiner and appellants confirmed the Examiner's skepticism regarding the relied upon utility. Furthermore, in the Board's opinion, the evidence submitted Aggarwal by was not shown to have been recognized by the art as being predictive of success in the treatment of tumors. Consequently, the rejection was maintained by the Board. Coincidentally, Aggarwal also failed the proposed weight-of-the-evidence test.

In *Ex parte* Rubin, claims to a method for improving the effectiveness of interferon in the treatment of neoplastic conditions and a composition containing interferon and an agent for inhibiting tyrosinase were rejected under 35 U.S.C. 101 and 112, first paragraph for incredible utility. However, the evidence in the case showed that the anti-neoplastic utility of interferon was known. Further, the Examiner failed to provide any objective evidence to support the challenge to the asserted utility. Thus, Rubin passed the proposed weight-of-the-evidence test. Furthermore, the rejection under 35 U.S.C. 101 was reversed by the Board.

In *Ex parte* Busse, claims to a method for reducing metastasis and neoplastic growth were rejected under 35 U.S.C. 101 and 112. The specification expressly asserted utility in humans. Consequently, the claims were held to rely upon effectiveness in humans for utility. Evidence submitted by Busse established only that further study of a compound tested for suitability as a human anti-neoplastic and anti-metastatic drug was warranted, rather than any likelihood of effectiveness for the claimed method. Thus, the Board maintained the

rejection, and regarding the proposed weight-of-the-evidence test, no evidence was provided supporting the asserted utility.

Consequently, I believe that a preponderance- or weight-of- the-evidence test is appropriate where assertions of pharmacological activity are (1) not believable on their face and (2) relied upon for patentability.

WHAT POLICY OBJECTIVES ARE ACHIEVED BY THE PERCEIVED PRACTICES OF GROUP 1800?

Looking at the issues from the PTO perspective, what policy objectives are furthered by the perceived practices of the PTO regarding biotechnological inventions? There may be a general public concern with the effects of biotechnological inventions. Many, if not, most of the members of the public are unfamiliar with biotechnology. In the wake of recent, highly publicized environmental disasters and increased health risks associated with the products of high technology, a significant section of the public may be uncomfortable with biotechnology, and may wish to suppress advances in the field until the technology receives wide public acceptance.

The PTO may also be concerned with the imprimatur of the Federal Government, which may raise or lower expectations of those afflicted with illnesses which biotechnological inventions may be designed to treat. Further, some consumer organizations and government representatives have expressed concern over the costs of pharmaceuticals. Patents are typically cited as one cause of excessive pharmaceutical prices. Thus, by reducing the availability of patents or by discouraging obtaining patents as a means of protecting intellectual property, the PTO may be effecting a control on the pharmaceutical industry to keep expensive products out the market.

There are a number of problems with such policy objectives. First, the effort exerted by the PTO to examine data regarding the ultimate effectiveness of pharmaceuticals in humans and to address concerns over safety and efficacy of biological products unnecessarily duplicates the effort of the FDA and/or the USDA, who must review such data independently in order to evaluate whether such products should be allowed into the marketplace. The patent right concerns only the right to exclude others from making, using or selling the patented invention, rather than any right to enter the marketplace. On the other hand, one cannot market a new pharmaceutical drug or a new biological product without obtaining approval from the FDA or the USDA, respectively. In response to public concerns, the FDA has evaluated several recent, highly-publicized biotechnological products quite closely. Thus, public concerns regarding safety and/or acceptability of biotechnological products are best represented by the FDA and the USDA.

Furthermore, in the conventional medical marketplace, medical doctors and patients typically look to the FDA as the ultimate authority on safety and efficacy of pharmaceutical compounds. However, in the "underground" medical marketplace, actual examples of efficacy in patients are the basis upon which judgments to use a particular pharmaceutical agent are made. Sources of information regarding actual examples of efficacy are readily available through scientific publications, approval notices by the FDA at an introductory or intermediate level (e.g., Phase I or Phase II trials) and word-of-mouth stories of successful treatment therapies. Approval of a patent application may indicate some probability of effectiveness, but ultimately, actual examples of effectiveness are the most persuasive evidence of effectiveness, both to those in the FDA-regulated marketplace and in the "underground" marketplace.

The policy also conflicts with Securities and Exchange Commission and EPA regulations, which may prohibit inventors from asserting pharmacological utility or from testing recombinant organisms in the field prior to their approval. Furthermore, if the Clinton administration is concerned over the appearance of unfair prices established by a particular company for a particular pharmaceutical agent, it seems reasonable to have the SEC investigate and resolve the situation. Increasing the difficulty level in obtaining patent protection as a means to reduce the price of pharmaceuticals appears to punish all practitioners in biotechnology for the perceived sins of a few.

With regard to pricing of pharmaceuticals, the SEC may be the most appropriate agency for evaluating such concerns. The current PTO policies actually result in an increase in the cost of pharmaceuticals and of other biotechnological inventions. As explained above, the perceived practices of Group 1800 result in increases in the costs of application prosecution and of patent enforcement. Further, relatively narrow claims are less valuable and result in increased uncertainty as to the scope of protection. As a result of this uncertainty, licensing arrangements are riskier and take longer to consummate, thus causing delays in taking the steps necessary to further develop and market the product. As a result, approval by other regulatory agencies and the entry of competition into the marketplace is delayed. Competition may very well be the key to reducing the costs of pharmaceuticals. By delaying issuance of the patent, the end of the patent term is extended, thus increasing the period of time under which a marketing company may price its product without competition. Thus, the perceived PTO policies serve to increase the costs of pharmaceuticals.

In summary, other agencies, such as the FDA, USDA, SEC and EPA, may be more appropriate for addressing policy concerns regarding the effects of individual products entering the marketplace.

CONCLUSIONS

President Clinton has advocated at least three policy- related goals affecting the biotechnology industry, including (1) Fostering the growth of biotechnology, (2) reducing the costs of medical care and of pharmaceuticals; and (3) minimizing the size and expenses of the Federal bureaucracy, including minimizing duplication of effort among Federal agencies. As an administrative agency, the PTO can more effectively advance the President's policies by

- (A) Allowing claims with reasonably broad scope;
- (B) Permitting Examiners to accept applicants' assertions of utility and operability unless objective evidence questioning such assertions is provided;
- (C) Where assertions of pharmaceutical utility are raised, consistently treating claims to compounds separately from claims to pharmaceutical compositions and methods of use; and
- (D) Where pharmaceutical utility is relied upon for patentability, adopting a preponderance-of-the-evidence standard for establishing pharmaceutical utility.

Adoption of proposed policies (A)-(D) by the PTO above will result in (i) earlier issuance of patents and (ii) greater patent protection for inventors. The proposed policies will achieve the President's policy goals by more adequately rewarding inventors for the knowledge which they provide, thus encouraging further research and development of biotechnological products and services. Earlier issuance of patents and broader patent protection will reduce the costs of prosecuting patent applications and enforcing patents, will increase the certainty of patent positions and license agreements, thus increasing the speed with which further product development occurs, and will result in an earlier end to the patent right, thus allowing competition to enter the marketplace at an earlier date, further resulting in lower costs through competition. Finally, where the asserted utilities and statements of operability in an application are challenged, adopting a preponderance-of-the- evidence standard for resolving questions of pharmaceutical utility and of predictability in the art will reduce the effort of Examiners on reviewing such evidence. As a result, the costs of running Group 1800 will be reduced, and duplication of effort between the PTO and other regulatory agencies, including the FDA and the USDA, will be minimized, and potential conflicts between such agencies can also be avoided.

Respectfully submitted,

Andrew D. Fortney, Ph.D.

ADF:dsf

Mr. Commissioner, members of the panel and the audience:

I'm David Lowin, Assistant Director of the Patent Law Department at Syntex. I also teach Patent Law at Stanford Law School and at U.C. Berkeley's Boalt Hall. My testimony is offered as personal opinion, not on behalf of any organization.

Suffice it to say that 9 minutes isn't long enough to address the full subject matter of this hearing, so I am also working on a written submission covering a broader scope. My prepared remarks are addressed to a somewhat different approach on the policy behind the utility requirement, and the environment in which that policy must be carried out.

The notice setting this hearing started by reference to the Supreme Court's decision in *Brenner v. Manson*, where the Court upheld the rejection of a chemical process patent application for failing to establish a substantial utility. Now, this may not have been a great decision, but that is mostly due to the underlying facts. I believe that the decision is not, however, inconsistent with the positions urged at today's hearing, and it is to this that my remarks are focused.

I think there are two key aspects to the majority's conclusion in *Brenner v. Manson* which shed light on practical utility problem faced by biotech inventions today.

First, the majority concluded that "...a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion."

Whether an invention represents the successful conclusion of a search depends on what you're searching for in the first place. Biotechnology has changed what we're searching for. It has made it possible for us to understand the complexity of life, and to intervene with disease at a level far more precise than was possible when the Court considered *Brenner v. Manson*. The successful conclusion in today's search for cellular mechanisms and ways to modulate them would have probably been considered only an invitation to further experimentation back in 1966. And what we consider to be a successful conclusion today is going to be different again ten years from now. The point is that legal principles

such as "successful completion of the search" have to be applied in a manner consistent with the progress of technology.

The second key aspect of the decision in Brenner v. Manson is the quotation taken from the CCPA's decision in Application of Ruschig, "[A] patent system must be related to the world of commerce rather than to the realm of philosophy."

So, what is "the world of commerce" to which the successful conclusion of today's search must relate? It's a different world of commerce than it was in 1966.

Just as the "patentable subject matter" definition of Section 101 had to expand to cover Chakrabarty's bacteria as part of "anything under the sun that is made by man," now, our definition of the "world of commerce" must expand to recognize the state of the art and the commercial impact of today's biotechnology inventions.

When we clone the DNA for a protein known to be involved in a disease, ... when we develop that protein into a model for screening potential therapeutics, ... when drugs are identified as active in that model, ... and when those drugs are tested in clinical trials, these efforts involve hundreds of scientists and thousands of others performing related tasks in hospitals, banks, shippers, equipment manufacturers, accountants, and even in the United States Patent and Trademark Office. Each step along the way entails enormous investment. The successful conclusion of each of these searches triggers even more investment. Ultimately hundreds of millions of dollars change hands during the development of a single drug. Now that's commerce, commerce at a scale our founding fathers couldn't have even imagined.

Today's hearing is taking place because the United States Patent and Trademark Office has more often than not refused to allow our applications for patents on the DNA, on the proteins, and on the screening models (allegedly because they are not the successful conclusion of the search, and do not relate to the world of commerce). The PTO has also refused to issue patents on the drugs (because they haven't been proven safe and effective in statistically powered human clinical trials) even though, in this first to file world, the patent applications cannot contain such data because they have to be filed years before clinical trials could even begin. And now, if eventually granted, the terms of these patents will have been expiring since the day they were filed. [At this point erroneous mention was made to a perceived problem in the GATT implementing legislation that could preclude Waxman-Hatch extensions. The error was brought to my attention by Mr. Van Horn upon conclusion of the hearing. I regret any inconvenience or misimpression that may have resulted.]

Just to put things in perspective, the same United States Patent and Trademark Office regularly grants patents on mechanical inventions for anything up to and including the kitchen sink, such as U.S. Patent No. 5,349,708, issued September 27, 1994 for "Foldable Kitchen Sink," not to mention the patents on the "Nail File Protector Device" and the "Air Cooled Umbrella" both issued the same day. Now I'm sure that all three of these inventions were worthy of patent protection. I'm also sure that even combined, the inventions covered by these three recently issued mechanical patents will not effect "the world of commerce" to the same degree as any of the "legally useless" biotech inventions we're talking about today.

I think that the Office needs to apply the utility requirement consistent with current scientific and commercial standards. The utility standard, as applied, should resolve doubts in favor of the patent applicant. When an applicant pleads for allowance of its application because its competitors are already practicing the claimed invention, it doesn't make sense to reject the application as lacking utility.

There is room here for the common sense principle, "no harm - no foul."

The courts are not being clogged, and the progress of the useful arts and science is not being blocked, by patents covering technology that just doesn't work.

Thank you for your attention.

BIOTECHNOLOGY PATENT POLICY

A Consumer's Perspective and Recommendations

Testimony before the Commissioners of the U.S. Patent & Trademark Office

October 17, 1994

Eugene P. Schonfeld, Ph.D.

President and Chief Executive Officer

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1234 Sherman Avenue

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By way of introduction, I am a kidney cancer patient. Chemotherapy and radiation are not effective in treating kidney cancer. Biological response modifiers created through biotechnology are helpful, but these agents are far from perfect and most patients are not cured despite rigorous therapy.

I am also President and Chief Executive Officer of the National Kidney Cancer Association, a non-profit charity which provides information to patients and physicians, sponsors biomedical research, and acts as an advocate on behalf of the nation's 75,000 kidney cancer patients.

I hold a Ph.D. in Management from the J. L. Kellogg Graduate School of Management at Northwestern. I have also worked as a new product consultant in the Advanced Methods Group of N.W. Ayer, a major advertising agency. In addition, I have started five high tech computer-related companies, including one that has been publishing economic information on research and development expenditures for 15 years.

I have no financial interest in any drug, biotech or health care company. However, I am a kidney cancer patient whose life depends upon private industry efforts to find a cure for my disease.

General Perspective

Patent policy in biotechnology is extremely relevant to the well-being of millions of Americans who suffer from cancer, AIDS, Alzheimers, and other diseases for which there are no effective treatments. Patent policy can speed scientific progress or retard it, accelerate products to patients or delay cures. Therefore, as health care consumers, patients cannot be indifferent to the work of the Patent and Trademark Office.

If patent policy creates high hurdles, companies will be granted few patents or have to expend extraordinary resources to get a patent. If too few patents are granted, incentives for invention will be diminished and the public will get fewer medical advances. Similarly, if tremendous resources are consumed obtaining a few patents, the public will get fewer medical advances as dollars are shifted from laboratories to legal offices.

Similarly, a patent policy which is too lax would generate too many patents. These patents would represent a cheap currency. It would create intellectual assets devoid of real economic value with limited

protections. It would also water down the incentives for legitimate discovery. The public would enjoy fewer advances.

When patent policy is either too restrictive or too lax, corporate management and outside investors would be less willing to commit capital to research intensive ventures. No manager or investor wants capital consumed by an overly complicated patent process that adds little value to products delivered to the public. No manager or investor wants capital committed to creating intellectual property which has no value and limited protection even though it is patented.

From this perspective, the Patent & Trademark Office and the patent process itself should add economic value to inventions. An optimal patent policy should maximize this value. The public will be served because maximized value will stimulate investment and the development of more life saving inventions.

Making Life Saving Inventions Special

In addition to maximizing value, the Patent & Trademark Office should speed the processing of patent applications so life saving advances reach the public more quickly. As I understand current operations of PTO, patent applications are supposed to be processed within eighteen months. However, this goal is not always achieved.

The public has a unique and special interest in life saving inventions in contrast to inventions which are primarily commercial. Therefore, as a matter of public policy, the Patent & Trademark Office should automatically make "Special" all patent applications for inventions which diagnose and treat life threatening illnesses.

There is significant precedent for accelerating the processing of important patent applications. During the energy crisis of the 1970's, the PTO embraced a policy of making "Special" all patent applications for energy conservation inventions. Public health and the lives of the nation's cancer, Alzheimers, and AIDS patients are no less important than energy conservation.

Many life saving inventions also reduce the cost of health care. Too often, I see cancer patients go from therapy to therapy in search of a drug which will stop their disease. Great sums of money are expended on treatment after treatment which does not work. Accelerating the patent process for life saving inventions will help control health care costs by bringing new, more effective treatments to market faster.

To implement the policy which I have recommended, the Patent & Trademark Office should expand its core of biotechnology patent examiners through new hiring and retention of its existing staff. Congress should

appropriate the funds to support the needed staffing.

The eighteen month goal should be a "hard target" for life saving inventions both on the part of the PTO and the applicant. Additional computerized systems and other resources may be needed to expedite and support the processing of patents. Investment in PTO infrastructure is probably money well spent and Congress should be urged to make the required investment.

Practical Utility and Clinical Trials

As we all know, Practical Utility is an essential criteria in patent decision making. While human clinical trial data are valuable for documenting Practical Utility of a new invention, the standard for determining Practical Utility should not be a human clinical trial.

In clinical care, many intervening factors may determine clinical efficacy or safety. It is often impossible for an inventor to control or even anticipate these factors, such as:

1. The characteristics of an affliction such as a tumor, bacteria, genetic damage, or injury. For example, you and I can both have the same type of cancer, but biologically yours is different from mine because genetically our tumors are different even though we share the same diagnosis.
2. The characteristics of the host which bears the affliction. For example, biologically you and I are unique and different from other individuals suffering from the same illness.
3. The effect of a drug on both the affliction and the host may depend upon unique characteristics of the drug or agent, the amount of substance administered, the route of administration, the method of administration, the timing of administration, and where it is administered. For example, it is recognized that many living organisms, including people, have circadian biological rhythms which are extremely important to health, yet we know little about these rhythms except that we know that the same drug can produce different side effects and different benefits depending upon the timing of its administration.

From this perspective, there is a biological and clinical gestalt which must be understood in order for the utility doctrine to be meaningfully and reliably implemented from the perspective of human clinical trials. Unfortunately, it is often impossible to forecast the gestalt itself let alone control it. The development and clinical use of Interleukin-2 provides interesting proof of this point.

Interleukin-2: A Clinical Case History

IL-2 was first identified as an anti-cancer agent in 1976. It was first given to humans about 1984. It came up for review before the FDA in July of 1990 and was not approved even though it had been approved in nine European countries and had been shown to be safe and effective when tested in thousands of patients with advanced kidney cancer.

The reason that it was turned down was that it produced only a 15 percent response rate and the side effects of IL-2 were so severe, patients were put in intensive care when they received the drug. Eventually, with more research and a push from the National Kidney Cancer Association, IL-2 was approved by the FDA in early 1992.

During its review, the FDA focused on IL-2 as a single agent given in high doses by IV. However, that is not the way the drug is used today. One of the most effective ways for a cancer patient to get IL-2 today is to inhale the drug using an inhalator like asthmatics use. Since metastatic kidney cancer occurs most frequently in the lungs, inhalation of the drug delivers high concentrations of the drug where it is needed while avoiding the side effects of systematic therapy.

In German clinical trials for metastatic kidney cancer in the lungs, inhalation therapy has produced a 65 percent response rate and is an outpatient therapy with almost no side effects. Many clinicians now believe that the human body produces and uses IL-2 locally rather than systemically. Inhalation therapy may be effective because it more closely approximates what the body itself is doing.

The lesson in this case history is that nobody ever envisioned that IL-2 would be inhaled when it was invented, or when it went through U.S. clinical trials, or when it came before the FDA. In fact, initial clinical trials shed little light on Practical Utility and the initial trial data almost led the FDA not to approve the drug at all.

Accelerated Patent Processing and Human Clinical Trials

In addition, requiring lengthy human clinical trials is completely at odds with a policy of accelerating the patent process for life saving inventions. Animal experiments are one substitute for human clinical trials, but even more is possible.

Surrogate end points are often needed and used as precursors to human clinical end points. In fact, modern clinical practice itself is moving away from a blind reliance on average response rates derived from clinical trials. For example, in vitro drug tests using a patient's living tumor tissue are now being used to determine a specific individual's drug sensitivity and resistance, and to design "patient

specific" therapies.

The FDA has adopted a policy of using surrogate end points in its evaluation of AIDS drugs. T-cell counts and other markers have been used by the FDA as a basis for the approval of new drugs.

Many of the same scientific advances which allow gene fragments and other tiny biological components to be evaluated, also enable the Patent Office to adopt surrogate end points for patent decision making. What is required is the motivation by the Patent Office to develop and use an evaluation system composed of valid surrogates.

In this regard, it may be wise for the Patent & Trademark Office to develop "advisory boards" as the FDA has. These advisory boards, however, would not advise on any specific patent applications. Their responsibility would be confined only to the system of evaluation and assist the Patent Office in the selection of appropriate surrogate markers used in evaluating biotechnology patent applications.

Summary

To sum up my recommendations:

1. Develop a patent policy which maximizes the value of patented inventions, a policy which is neither too restrictive nor too lax.
2. Adopt a policy of making "Special" all patent applications which involve life saving inventions, and in so doing, accelerate the patent process for these inventions.
3. Beef up the corp of biotechnology patent examiners.
4. Do not rely on human clinical trial data for decision making. It is helpful but is not the proverbial "gold standard" for decision making for clinical practice or for the FDA.
5. Develop a system of surrogate end points for use in evaluating new life saving inventions and in evaluating biotechnology patents.
6. Build an advisory board to help the Patent & Trademark Office develop and update its system of surrogates and decision making criteria.

I urge you to consider these recommendations because, if adopted, they will enable the Patent & Trademark Office to better serve the public, particularly those of us who are suffering from life threatening illnesses. Thank you.

STATEMENT OF JOHN W. SCHLICHER

CROSBY, HEAFEY, ROACH & MAY

OAKLAND, CALIFORNIA

BEFORE THE UNITED STATES PATENT AND TRADEMARK OFFICE

CONCERNING NOTICE OF PUBLIC HEARINGS AND REQUEST FOR COMMENTS ON PATENT PROTECTION FOR BIOTECHNOLOGICAL INVENTIONS

ON OCTOBER 17, 1994

Commissioner Lehman, Mr. Van Horn, Mr. Kushan, and the other members of the panel, thank you for the opportunity to appear and give my personal views on these issues. I am a patent lawyer with the firm of Crosby, Heafey, Roach & May in its Oakland, California office. I also teach patent law at Stanford Law School as a part-time Lecturer.

My comments are arranged in the following order:

1. Patent Law Should Be Based On Sound Economic Analysis
2. The Economic Purpose Of Patent Law
3. There Is Not And Should Not Be A Separate Set Of Patent Law Doctrines For Biotechnology
4. Practical Utility For Biotechnological Inventions
5. Proof Of Operability For Human Therapeutic Inventions
6. Standards Used In Measuring Nonobvious And Enablement Of Biotechnological Inventions
7. Experimental Use Defense To Patent Infringement
8. Implications Of Pending Legislative Reform On PTO Operations And Examination Procedures
9. Other Issues
10. The Most Efficient Use Of The PTO's Resources In The Overall Patent System

These hearings focus on patent law and biotechnology. Patents are important to the biotechnology industry. The advances of the past twenty years have created vast opportunities for future research. However, the policy and legal issues transcend the biotechnology industry. The policy and substance of patent law should apply to all technologies in the same way. Biotechnology inventors should be treated no less and no more favorably than any others.

1. Patent Law Should Be Based On Sound Economic Analysis

I commend you for holding these hearings and for preparing a thoughtful notice to focus the issues. The notice is a model of good sense in one critical respect. The notice focuses on the economics of the patent system. The notice asks for information about the effect of the law on the amount or rate of investment in attempts to create the technological information about new products and processes that patent law calls "inventions." Patent law has not always developed to best serve the United States economy because the people responsible for making and interpreting the law failed to ask that question or to answer it correctly. A classic example is the Supreme Court's decision in *Brenner v. Manson*. By asking the right question, these hearings have improved the likelihood that patent law will develop to better serve the country. My concern about the economic effects of patent law lead me to write a book to focus attention on this question and to try to help answer it. Much of what I say here is described in greater detail in that book.

2. The Economic Purpose Of Patent Law

The notice is fundamentally correct when it says the patent system exists to induce investment and risk-taking in research, development and commercialization of biotechnology inventions. The notice is right on target by focusing on increasing incentives to invest in inventing and take the risk of failure. There are other theories of patent law. The notice provides a valuable insight by ignoring them.

For purposes of developing patent law standards, I would define the role of patents only slightly differently. Patent law exists to alter the private incentives for use of resources that the market would otherwise provide. By "resources," I mean anything that is scarce and that, if put to one use, may not also be put to another. A market may misdirect resources, if people do not expect to capture all the benefits their investments provide to other people. In the absence of corrective laws, potential producers of technical information are likely to spend too few resources over a given period attempting to produce technical information. They will do that, because they anticipate being unable to capture all of the value of the information they produce.

I would say the economic goal of patents is to induce investment and risk-taking in producing technological information about new products and processes that, in the absence of patents, the market would be unlikely to produce or produce as quickly. Whether this information-generating activity is called "research" or "development," the goal is to identify and grant rights in those situations where the cost and risk of production would likely have been sufficiently large that potential profit-motivated producers would likely have shunned the effort in the absence of patent rights.

Patent law seeks to assist markets to induce the owners of resources to use more of them in producing new technical information about potential new products and processes. This means patent law also induces the owners of resources to use fewer of them in producing the products and processes available from existing technical information. United States consumers benefit from this better use of the country's scarce resources. The improved supply of technology may permit different or better products to be produced in the future or to be produced more cheaply.

If the level or rate of information production increases due to patents, the level or rate of commercialization of new products and processes will also increase. However, patent law leaves commercial use to the market, not to legal regulation. Like all other property rights systems, the patent system relies on self-interested decisions by producers, consumers and the market to decide which inventions to try to make, which to develop and use commercially, and how much to pay for that use. Patent rights were historically granted by the government without regard to their potential benefits to consumers or their potential commercial value. The patent system leaves it to producers and consumers to make those judgments, however wise or foolish they might appear to the government. It is entirely likely that a rational patent system may induce many patentable inventions and only a small percentage of them will be used in a commercial product. That fact is and ought to be no cause for concern.

There is one decision by the Supreme Court in 1966, *Brenner v. Manson*, that can be read to say the patent system exists to induce the development and bringing of new products to the market. If the goal is to induce developing and bringing new products to market, the standards for patent availability would be quite different than they have been for the last 200 years and are today.

3. There Is Not And Should Not Be A Separate Set Of Patent Law Doctrines For Biotechnology

United States consumers benefit from advances in biotechnology and all other technologies. For that reason, patents apply to all areas of technology where there are opportunities for profit-motivated people to

conduct research and produce technical information. Because the policy of patent law applies to all technologies, patent law doctrines must apply across the board to all technologies.

There is not or should not be a special subset of patent law doctrines for biotechnology. One risk is that a separate set of biotechnology standards may develop in a way that makes patents for biotechnology inventions more difficult to obtain or less valuable than patents in other areas. Patent law would be unwise if it created greater relative incentives to invent in the 10,000th mouse trap design than the first therapy for a previously untreatable human disease.

In general, I find the notice to be consistent with that view. However, the notice refers to the Court of Appeals for the Federal Circuit "refining" the law of nonobviousness for biotechnological inventions. I do not understand the Court of Appeals for the Federal Circuit to have created a subset of patent law doctrines for biotechnology. While the decisions cited in the notice discuss patent law in the context of biotechnology, the legal standards found in the decisions are the same standards applied to all types of technologies.

In the patent area, people sometimes confuse legal principles with decisions applying legal principles to particular facts. It is important for patent lawyers and the Patent and Trademark Office ("PTO") to understand that a decision by a court to find a particular invention unpatentable or a patent invalid for lack of utility, obviousness, or lack of an enabling disclosure does not establish a new legal principle. For example, if the PTO issued a patent on an invention based on research that identified a gene that encodes a particular protein (by identifying the protein sequence, creating sets of probes and probing DNA libraries), and a court were to declare that invention obvious and unpatentable, this decision does not change the law of nonobviousness one iota. That decision is and ought to be irrelevant to the obviousness of a different invention based on research that identified a different gene that encodes a different protein (again by identifying the protein sequence, creating sets of probes and probing DNA libraries). The decision declaring the first invention obvious and unpatentable does not and ought not provide a basis for the same decision in a second case that looks superficially similar.

People who work with patent law often try to find certainty in the law where certainty does not exist and to simplify the process of applying the law to the facts, where simplicity is not possible or wise. The easiest way to achieve certainty (and predictability) and to reduce the cost of decision-making is by applying formal or informal (that is secret) rules of thumb across all fact situations that appear at some general level to be similar. Lawyers, patent examiners, courts and others need to resist that impulse. They need to apply the same general

legal standards to the particular facts in each particular situation. The notice affirms that is the operating procedure in the PTO. The notice, borrowing a phrase from antitrust law, affirms there are no per se rules. Each application is examined based on applying general legal principles to the unique facts surrounding the application. That declaration is very important. The challenge is to make this approach effective.

4. Practical Utility for Biotechnological Inventions

The notice asks about the purpose of the utility requirement. That is the right question. Unfortunately, the Supreme Court in *Brenner v. Manson* gave the wrong answer. The Court said:

The basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility. Unless and until a process is refined and developed to this point -- where specific benefit exists in currently available form -- there is insufficient justification for permitting an applicant to engross what may prove to be a broad field.

In my view, the Court was wrong.

In *Brenner v. Manson*, Justice Fortas, and six other Justices, implied that inventors seeking a patent must first show that the invention had been developed to the point where "specific benefit exists in currently available form." Justice Fortas seemed to believe it undesirable to issue a patent on the process of producing a chemical, if the only known use for the chemical was to conduct further research into uses for it. Justice Fortas also seemed to believe that research is useless and researchers do not count as consumers in need of better products and processes. I find it startling to suggest that all of the instruments and all of the reagents sold by numerous companies to the biotechnology industry for conducting research are beyond the pale of the patent system, because research is deemed an inherently useless activity. If someone might be willing to pay for a material, whether an isolated strand of DNA, an isolated polypeptide, an isolated protein, or an isolated microorganism, that material has, in my view, sufficient utility to be patentable.

In my view, *Brenner v. Manson*'s language is unclear and, if taken literally, inappropriate for a well-functioning patent system. I have explained why elsewhere. If someone produces information about the general character and features of a new product or process that distinguishes it from earlier products and processes, he or she traditionally has been and should be entitled to a patent even if (1) there may be no perceivable benefits to consumers or others and no commercial potential for that invention, or (2) there is commercial

potential, but there are large additional development expenditures necessary to use that invention commercially. In my view, Brenner v. Manson should also be of limited importance. That decision is inconsistent with more recent Supreme Court decisions defining the purpose of patent law. The Brenner v. Manson majority decision is premised on a "quid pro quo" theory that diverts attention from the real issue - incentives to invent. The dissenters, Justices Harlan and Douglas, asked about the effect of that decision on the incentives to do research into new processes, and reached the correct result. I believe the dissenter's approach consistent with the theory the Court applied in its later patent decisions in the 1970's and 1980's, such as Chakrabarty, Dawson, Kewanee Oil, and Diehr and that the Federal Circuit often applies.

Rather, the test articulated by Justice Joseph Story (who sensibly defined most fundamental patent law rules in the early part of the last century) made clear that the criteria for issuing patents had nothing whatsoever to do with the anticipated benefits of the invention. Under Justice Story's test, that prevailed until 1966, any invention was patentable if it might be put to a beneficial use rather than being useful only in harmful enterprises such as poisoning people. If the invention might be put to some use that was not "mischievous or immoral", it was patentable even though consumers and the market might attribute to that invention an economic value of zero. Under that standard, one asked only whether the patented invention might be useful for any lawful purpose, and was invalid only if it could not possibly be used for any such purpose.

Are there desirable changes to legal standards? If the Brenner v. Manson language is the legal standard, change is plainly desirable. Under a better rule, the whole question ought to be whether an invention may or might be used by anyone for any lawful purpose. Among the lawful purposes for which an invention might be useful is the conduct of additional research.

I should note that many of the points I have made here and elsewhere were articulated by Judge Rich in his dissenting decision in In Re Kirk, 376 F.2d 936, 946 (CCPA 1967).

5. Proof Of Operability For Human Therapeutic Inventions

The notice characterizes the next substantive issue as the requirement that an invention be "operative." "Operativeness" is a word sometimes used as a synonym for the utility requirement and for the enabling description requirement in Section 112. The notice directs attention to application of these requirements for inventions whose sole use is the treatment of human disorders. While I do not believe that the law in this area is as clear as it might be, I do not propose to discuss it in

any detail. I offer these thoughts.

First, I believe much of the confusion in applying the utility requirement to that type of invention derives directly from the misstatement of the utility requirement in *Brenner v. Manson*. Assume the law is that an invention is not useful, until a "specific benefit exists in currently available form" presumably to consumers. If the regulatory reality of development and introduction of human therapeutic agents requires many years of development before anyone may lawfully make a product available to consumers, this utility rule seems to preclude a patent until all testing is completed.

Because I do not believe that the utility standard should have anything to do with potential commercial significance, it is an error to require that an applicant have in hand all of the information that will be necessary to immediately introduce a commercial therapeutic product. While some decisions expressly renounce tests that would require possession or disclosure of all commercially significant technical information, the language in *Brenner* creates a danger that the rules will be applied by decision-makers to require such information. The Supreme Court uttered those words in 1966 and it is difficult to put that genie back in the bottle. However, I think it is necessary to utterly ignore *Brenner v. Manson* for purposes of applying the utility requirement to human therapeutic inventions. That is a proper thing to do because the applicant in *Brenner v. Manson* did not assert that its process produced a human therapeutic agent.

Second, while patent law, as I understand it, formally rejects application of Food, Drug and Cosmetic Act standards for safety and efficacy as having anything to do with patent standards of utility, the danger is that in practice they will be applied. If the law insists that the PTO and the courts make judgments about whether "specific benefit exists in currently available form," it is likely that many decision-makers will be tempted to take that regulatory reality into account in applying and developing patent standards. The law and the PTO would do well, in my view, to repeatedly and emphatically make clear that the requirements for distributing a drug under the Food, Drug and Cosmetic Act, or under any other regulatory regime, have no applicability at all to patent law.

Patent law seeks to increase the rate of research about potential new products and leaves it to people and market forces to choose the nature, type, timing and commercial use of the results of the research. The Food, Drug and Cosmetic Act seeks to decrease the rate of research about potential new products, and to replace private, market-driven decisions with government decisions. Adopting Food, Drug and Cosmetic Act standards for patent law (whether formally or informally) would be economic folly.

Third, the utility requirement in this area raises an issue that patent law does not address with much specificity. The question is whether the patent law does, or ought to, require some minimum level of certainty that the technical assertions in a patent application are true before a patent should issue. Should the patent law permit inventors to patent their guesses? Should an inventor be permitted to patent the use of a certain agent for treatment of a certain disease because he or she has some theory that, while somewhat plausible, is more likely than not to be untrue? Should Linus Pauling have been able to patent the use of Vitamin C for treatment of the common cold in the early 1970's? In my view, the law does and must insist that the information in a patent meet some minimum level of accuracy and correctness. I would impose that requirement not out of concern that some apparently goofy idea will in fact turn out to be goofy and an utterly useless patent issue. Rather, I would be concerned that issuing a patent to the first person to take a wild stab, or even an educated guess, at a potential therapeutic invention decreases the incentives of other inventors, who are undertaking the costs and risks of determining whether such a therapeutic invention in fact will work. By "work", I mean have some biological or pharmacological activity in humans that might potentially be useful in treatment of disease.

The difficult question is how certain one must be before an application is filed. I do not have a good answer. For the near term, I can not do much better than suggest a "reasonable degree" of certainty, recognizing that test is not very meaningful. I would be inclined today to ask whether there is sufficient certainty that we would be willing call-off all research by others on the same invention and rely on the person who developed the basis for that degree of certainty to carry on the work. It is clear to me is that absolute certainty is the wrong standard, and that certainty to the extent required by the Food, Drug and Cosmetic Act is the wrong standard. Again, I understand the law to endorse those two views. The more difficult, but necessary, task is for us to decide how and where to draw that line.

Fourth, if the law imposes a requirement that there be some degree of certainty about the correctness of an assertion of therapeutic utility, what is the standard that should trigger the PTO's ability to ask for proof and what is the proof that an applicant must provide? Those are difficult questions. I have no clear answer. However, there are two things I would caution against.

The notice asks about "incurable" diseases. This is an area in which it is unwise to attempt to develop a particular legal standards for each type of disease and each type of therapy. I do not believe it is wise to automatically require proof or require more persuasive types of proof for proposed therapies for "incurable" diseases than for any others. The

standards for requiring proof and the nature of the proof should be the same regardless of one's judgment of the history of success or failure in the past. With the exception of a person's ability to patent a machine that violates the Second Law of Thermodynamics, I do not believe that patent law has historically applied any different standard in other areas of technology with a long histories of failure. Bell, Edison, Marconi and the others faced no special obstacle when they pioneered new fields. I would not treat medicine differently.

I also believe it important the law avoid placing patent applicants in this area in a substantive and, perhaps, procedural disadvantage that does not exist in other areas. My concern is with the operation of rules that shift some type of "burden" from the PTO to the applicant. First, if the law says some "burden" has shifted to the applicant, the law may unnecessarily bias the decision-making process against issuing patents in this area. In the close or difficult cases, and there are many, there is a temptation to say the person with the burden loses. Second, the law may bias the process against issuing patents if the threshold for requiring proof is low and the threshold for satisfying the burden is high. For example, assume the rule (1) requires the PTO to insist on proof if the assertion of utility is not "likely to be true" or if there is a "reasonable" basis for doubt, and (2) requires that the proof must be "convincing," "persuasive" or some other word meaning very highly likely to be true. This is also an area where there is often a reasonable scientific basis for some uncertainty about therapeutic effects. In an area where the legal standard is not easily defined and there is always some inherent uncertainty about the facts, these rules may operate to require proof in all situations and then require very highly probative evidence, such as substantial human clinical trials, before a patent application may be filed or a patent issued. I believe we should be cautious about such a rule.

6. Standards Used In Measuring Nonobvious And Enablement Of Biotechnological Inventions

The law governing the application of the nonobviousness requirement, Section 103, (and its predecessor nonstatutory invention requirement) has been the most difficult patent doctrine to define and apply, in an area of the law with many serious contenders. The enablement requirement has been less difficult in practice, but no less difficult in theory. These rules defy brief explanation. I would offer only these comments. First, the notice refers to many decisions in this area by the Court of Appeals for the Federal Circuit and the Board of Patent Appeals and Interferences and notes that they provided "much needed guidance" when applying those standards to "biotechnology inventions." That statement prompts me to repeat what I said earlier. There is not as I understand the courts, and should not be, as I understand the purpose of the law, a separate body of biotechnology nonobviousness or enablement law. Nor

should the actual decisions in particular factual settings be extrapolated in decision-making into other settings that look superficially similar.

This is not the first technical field in which the urge to develop shorthand formulas for deciding obviousness has been exhibited. The law books are full of cases that tried to articulate rules of thumb for determining obviousness. Perhaps the most famous was the so-called synergism test for determining the obviousness of "combination" inventions. These rules of thumb have been repudiated. We should be careful not to repeat those experiences in this important technical field.

Second, the notice frames the discussion of nonobviousness in terms of one of its elements, namely, determining the level of skill of an ordinary person at the time the invention is made. In my view, the law has not articulated wonderfully clear standards for decision-making about that subpart of the Section 103 analysis. The Court of Appeals for the Federal Circuit has pointed us to six factors to take into account, but it is far from clear how to do so. On another one occasion, the Court of Appeals for the Federal Circuit announced that a person of ordinary skill is one who "thinks along the line of conventional wisdom in the art and is not one who undertakes to innovate, whether by patient, often expensive, systematic research or by extraordinary insight, it makes no difference which." However, that standard does not seem to uniformly run through all the that court's decisions.

One of the factors the Court of Appeals for the Federal Circuit has said should be considered in determining the level of ordinary skill is whether the act is "advancing rapidly." It has never been clear to me how to employ that consideration. The fact that technology in a particular area is moving rapidly does not suggest to me that, if patents are eliminated, the same advances will continue at the same rate. The patent system must be applicable to all industries and technologies, whether the pace of technical change appears to be fast, slow or nonexistent. Of course, the pace of technical change does alter the administrative burdens for the PTO in ways that Congress may dimly or slowly perceive in budget considerations. However, it is important that the PTO avoid any effort or tendency to micromanage the rate of technical change. If technical change in a particular technology appears to be slow, that is no reason to try to issue more patents to speed it up. Conversely, if technical change appears to be very fast, that is no reason to issue fewer patents to try to slow the pace.

Third, the notice refers to changes in the state of the art affecting determinations as to the level of skill possessed by individuals working in the field. While it is true that skilled people are deemed to have available to them the state of the art, I would caution against taking

that notion very far. The danger is that, because the state of the art may, for example, include many examples of researchers successfully identifying and isolating genes for particular proteins, all similar efforts are deemed to be within the level of ordinary skill and all such activities deemed obvious and unpatentable. That is very unwise economic policy. The fact that many success stories are reported in the scientific and patent literature does not indicate to me that there are not many failures we can not read about or that the successes came easily and with little risk of failure.

My reading of the decisions in this area over the past about 150 years is, stated generally, that the economic purpose of the unobviousness requirement is to identify and eliminate from the realm of patentable inventions, those that involve such little cost and so little risk that it is highly likely, if not certain, that private producers of products (and today those who make a living as researchers) would have produced those inventions and done so at about the same time. In short, the market would yield those inventions without the additional incentives provided by a patent. If that is the general economic purpose, the fact that many people have used generally similar strategies to, for example, identify and clone genes for particular proteins in many prior situations, tells me little about whether it is economically sensible to grant patents for people investing effort and taking risks in attempting to identify and isolate other genes. If the costs and risks of that activity in a particular situation are significant, any resulting invention should be a serious candidate for a patent. The fact that many other people have followed similar strategies earlier and many had succeeded does not indicate that patents do not have an important role in inducing researchers dependent on the market from continuing to invest effort and take risks in that activity.

Viewed this way, decisions by the PTO and the courts under Section 103 are not merely technical decisions. They are technical and economic decisions of vast importance. If we lose sight of the economic purpose of the rule in the overall system, we may apply the rule in a way that is inconsistent with achieving the goals of the system.

The difficult issue is that the law has not defined with much precision the minimum levels of cost and riskiness that we require before we say that an invention is not obvious. Perhaps, we will do somewhat better in the future. However, the law does not and should not find inventions unpatentable, because a researcher sitting somewhere in an office would probably have recognized that (1) the invention was one of many possible ones to which attention might be given and (2) the most likely way of making it was a strategy sometimes used successfully in the past on similar problems. That those thoughts would have passed through the mind of an ordinary person should not render the invention unpatentable, if there were significant risks and costs of carrying out the project. The

reason is that simply because someone recognizes the possibility of making an invention and a potential way of doing it, does not mean that, in the absence of likely patent protection, researchers operating in the private marketplace, attempting to make money from that endeavor, would in fact have undertaken the program at about the same time.

7. Experimental Use Defense to Patent Infringement

The December 27, 1993 notice about hearings on the experimental use issue asked many important questions about the economic purpose of patent law and an experimental use doctrine. However, it frames analysis in two respects that I find less helpful than the current notice.

In my view, the purpose of the patent system to promote innovation requires that a person who receives the patent for a particular product or process should not be able to preclude others from using that invention (and making embodiments of it) for the sole purpose of making improvement inventions or substitute inventions. The basic reason is that, if we permit an inventor to capture the value of commercial embodiments of the product or process embodying his or her invention (namely, those supplied to consumers or used to make products supplied to consumers) and the value of all subsequent potential inventions whose development depends on use in research of the patented product or process, we would permit an inventor to acquire a patent whose value exceeds the value of that inventor's particular contribution. Hence, I have always assumed that a patent did not give the owner a right to exclude others from making or using the invention in order to make complimentary or substitute inventions. While I believe there is room for disagreement about my view of the economic consequences, my understanding of the development of patent law over the years by Congress and the courts is that this view is the prevailing one.

Until the early 1980s, I believe there was a doctrine which generally permitted others to use an invention in conducting research. I believe that the reason there was so little litigation to test that proposition and so few cases, as the earlier notice indicated, was that there was universal consensus about that rule. While the words in the cases seemed somewhat narrower, most patent owners either understood (or operated on the implicit assumption) that others could conduct research using their inventions, when that research was designed to make other inventions, whether complementary or substitute, or to do research simply for the sake of doing research, historically the function of our universities. The Federal Circuit's Roche decision in 1984 and Congress' response to it, as well as other cases, have created confusion and uncertainty. The problem with the Congressional response is that it left unclear whether experiments other than those protected by that section are non-infringing activities under the general experimental use doctrine.

Therefore, while I find nothing implicit in the public disclosure of the invention in the patent on the day it issues or in the quid pro quo theory to lead me to believe that use of an invention for experimental purposes should not be infringement, I believe it generally should not be. There are two situations where experimentation may be infringement. The first is experimentation involving the use (or making) of an embodiment of an invention, that is useful only in research and is incapable of any other use, such as use by ordinary consumers. Hence if someone invents and patents scientific instruments or reagents useful only by researchers, the making and use of such inventions for their intended purposes should be infringement. However, their use (and making) for the purpose of inventing improved or substitute versions of those instruments and reagents should not be infringement. The second is research conducted only for the purpose of developing a commercial product or process that would itself embody the patented invention. In other words, research undertaken solely and, perhaps, principally to implement a business decision to make, use or sell an infringing product may be called infringement. Such research is no different than building a plant to make the infringing product. It is logically infringement, since the principal value of that activity depends upon some subsequent infringing use of the invention in commercially used products or processes. An alternative is to reach the same result by permitting an owner to bring an action for threatened patent infringement, whenever it appears that someone else has made a definite decision to infringe and has begun to invest in projects or facilities (including research projects) whose only use would be as a step toward the make, use or sale of an infringing product. However, the courts have limited that availability of that action to such an extent that it may be useful to alter infringement doctrines to make clear that the activity is an act of infringement. The hard cases will be where a company undertakes research that might logically be designed (1) to improve an invention or to find a substitute for it or (2) to use the invention commercially.

I do not find it helpful in these contexts to describe one kind of research as commercial or for business reasons, and another kind experimental or for philosophical reasons. Those distinctions seem to me to bias decision-making against private sector research enterprises that may be conducting research primarily for the purpose of developing improvements or substitutes, and yet, by virtue of their very nature, operate with a commercial and business-oriented goal.

I do not have a view at this time about whether this is a problem best taken to Congress, to the courts, or for patent owners and users simply to work out among themselves. My impression is that legislation might be appropriate in this area. If legislation is proposed, I do not believe that the legislation enacted after the Roche decision was necessarily a happy model.

8. Implications Of Pending Legislative Reform On PTO Operations And Examination Procedures

The notice invites comments about potential changes to the patent system, including a 20-year term measured from the United States filing date and automatic publication of application 18 months after the earliest effective filing date. I am not a proponent of these so-called reforms. My view is that, on balance, this 20-year patent term diminishes the expected economic value of a patent and hence the incentives to do research. The effective term of a patent now depends directly on the length of prosecution. For complex inventions, prosecution is likely to exceed three years, and the value of patents for such inventions declines. The length of prosecution for all inventions depends in part on the resources available to the PTO. Those resources depend on Congress, and the political process. If the political process allocates too few resources to the PTO, the system is devalued. I am not aware of sufficient benefits to offset that cost and risk. I would much rather have dealt with the trivial number of so-called "submarine" patent holders by extensions of estoppel and laches defenses to conduct before the PTO. My view is that these changes are unwise and that, if we were the only country in the world, no one would have dreamed of suggesting them. However, we are not and the events of the last ten to twenty years cannot be rewritten.

If I am correct that the 20-year term will have some effect in reducing incentives to invent, this will in the long run reduce the number of patent applications filed. Of those that are filed, the term rule will have some effect of inducing applicants to respond more promptly to the PTO and generate increased pressure on the PTO to promptly process applications. My principal concern is that, if the net burdens on the PTO increase, Congress will not provide the additional funds that will permit the PTO to effectively meet these increased demands that rule will place on the system.

The change to a 20-year term from filing, particularly if it is ultimately coupled with a rule that the filing date is used to determine priority, will force applicants to make very difficult decisions about when to file. These will be particularly difficult depending upon how the courts apply the utility requirement. In order to win priority contests, one's incentives will be to file quickly. In order to sustain the basis for a patent under the utility requirement, one's incentives are to file after the proof is in hand.

The notice directs attention to the restriction practice. I believe a first step in reconsidering restriction practice is to ask what restriction practice is designed to accomplish? There seem to me two possible candidates. One is to confine an application to an invention or group of inventions so closely related that the fees reasonably relate to

the costs the Office anticipates with respect to searching, examining and other procedures on that application. The second is to confine an application to an invention or group of inventions that are sufficiently closely related to that, if a single patent issues, it will be possible for the PTO and other people who wish to know about patents to identify its subject matter from the title, the abstract and the classifications that are assigned to it. For both purposes, a standard of "patentable distinctness," whatever it means, is one that I do not find terribly helpful. This is an area in which I believe the Commissioner has considerable discretion and some modifications would be helpful. I cannot today suggest what they are.

9. Other Issues

The notice invites comments on other issues. There are a number of changes to patent law that I believe would have a positive impact on the patent system. I have described many of them elsewhere. Among other substantive issues I would focus on are (1) the patent misuse doctrine that overregulates agreements between owners and users, (2) the Lear doctrine and related rules that limit potential agreements that reduce the risks and costs of litigation, (3) the interpretation of Section 102(b), (4) the application of Sections 102(e) and 102(g) as sources of "prior art" (in the latter case where the prior inventor is not himself or herself seeking a patent), (5) the rules determining the scope of a patent, (6) the inequitable conduct doctrine and (6) the damage standards.

10. The Most Efficient Use of the PTO's Resources in the Overall Patent System

These hearings are, in part, about the application of patent law in the Patent and Trademark Office. The notice refers to the importance of "enforceable patent rights" to the value of those rights to inventors. Throughout the history of patent law, there have been those who criticized the performance of the PTO in its review and patent-issuing responsibilities. That criticism has often focussed on the frequency with which the courts in enforcement actions found patents to be invalid under one or another of the criteria for a patent to issue. This type of criticism is largely misplaced and may bias the PTO's decision-making process against issuing patents.

Because (1) patent standards do not draw bright lines, (2) the facts that underlie application of those standards are difficult and expensive to ascertain and (3) the PTO has limited resources in terms of time, information-gathering capability and technical expertise, the PTO cannot possibly make one hundred percent "accurate" assessments of each particular application. Congress set up the system with that reality in mind. Before a patent permits an inventor to capture any of the value of

the invention by excluding someone from using one or more of the rights, the owner of the patent must bring an action in a federal court. In that action, the person accused of infringement may seek to prove that the PTO incorrectly issued the patent. Moreover, before anyone will agree to be excluded or pay for the privilege of using a patented invention, the market place permits patent owners and users of inventions to review the PTO's decision and make a private assessment of the likely application of legal standards in a judicial proceeding and make a financial accommodation based on that assessment.

There are several important benefits to that system Congress adopted, not the least of which is confining a full-fledged, costly factual inquiry to those small percentage of patents that become commercially significant and involve issues that are difficult for the private negotiation process to resolve. This concept of the role of the PTO in the system is that the PTO should screen out and refuse patents for inventions that plainly and clearly do not qualify under one or another standard for a patent to issue. Under this view, it is and ought to be no cause for concern that ten, twenty, thirty or forty percent of patents litigated in the courts would be found invalid under one or the other of the criteria applied by the PTO. If the PTO perceives its job as making a one hundred percent "accurate" assessment of the facts and application of the law (meaning that only a small percentage of its decisions will be overruled by a court), it seems to me the PTO may operate with a bias against granting patents. While one should commend the PTO for its focus on doing a quality job, I think it would be unfortunate if that important goal translated into a bias against issuing patents. Rather, it seems to me the system is designed to operate most efficiently if the PTO were to issue patents unless it is not clear that the facts and the law require that a patent not issue.

[FOOTNOTES:]

[1] I have worked as a Research Fellow in chemistry at Stanford University, a scientist with Syntex Corporation, an attorney with Fish & Neave in New York City, and in-house counsel for Genentech, Inc., a leading biotechnology company. I am the author of the book Patent Law: Legal and Economic Principles (Clark, Boardman, Callaghan 1992).

[2] "Molecular genetics has made a beginning; a wealth of detail about many biological systems is already available. But the successes do not amount to a complete or even a very profound understanding. on the contrary, current ignorance is vaster than current knowledge." Paul Berg and Maxine Singer, Dealing With Genes (1992), Epilogue, p. 241.

[3] I refer to the notice appearing in the September 1, 1994 Federal Register p. 45267.

Schlicher, J., Patent Law, Legal and Economic Principles (Clark Boardman Callagan 1992).

The notice also refers to patents as a device to facilitate relationships between "government, university, and private sector partners by providing an impetus and a mechanism for commercializing advances at the cutting edge of biotechnology research." Patents provide a mechanism for a researcher in any type of institution, and even one working alone at home, to capture part of the commercial value of the invention he or she makes. If the producer is incapable of using the rights commercially or is less capable than others, the law permits transfer of the rights to a person able to better put them to commercial use. However, the purpose of patent law is not to shift income from private sector businesses to universities or government or to encourage commercial use of inventions that a university or government producer is incapable of using. The purpose is to increase the value of inventing whether performed by a private sector, university or government entity. While it is probably true in many cases that the existence of patent rights lowers the transaction costs of licensing between any two entities, I do not regard that effect as a principal purpose for a patent law.

[4] Research by the government or a university, whose principal endeavors are presumably not to develop and sell products commercially, is important in one respect. To the extent the patent law standards are interpreted to encourage development and commercial production of products, the law will bias the system against the ability to patent inventions produced by university or government researchers. Indeed, one of the principal advantages of the patent system is that it permits a number of different people, acting separately, to make inventions that may ultimately be used together to produce or sell a commercial product. This permits those best able to make certain parts of a total bundle of technical information more efficiently than other parts to focus their energies on the parts they are best able to produce. The market in rights will permit these separate to be combined to yield a commercial product. The ability of patent law to permit researchers to specialize in different parts of a total technological puzzle has that important benefit and operates best if each part of a commercial technological puzzle is separately patentable. Again, to the extent that patent law is perceived as devised to induce investment in the development and commercialization of products, the law will tend to bias decision-making against issuing patents that contain less than a total bundle of information necessary for the commercial production and sale of a product.

[5] Schlicher, Patent Law, chapter 2 and particularly ¶ 2.18.

[6] I refer here to the notice appearing in the September 1, 1994

[7] Those effects on others are sometimes called "external effects" or "externalities."

[8] For a more detailed explanation, see Schlicher, Patent Law, chapter 2.

[9] Many people own resources (such as their time and energy, and the resources they may buy with their savings) that they may devote to making and selling an existing product or to trying to design a better product. If they make and sell the existing product (or invest their savings in companies that do so), others can not use the products unless they pay for them. If those people try to design the new and better product (or invest their savings in companies that do so), others may use the design without paying. There are many ways a producer of a new design may lose exclusive possession of it. The design may become available to others who contribute nothing to producing it and who benefit from it without being required to pay. In that situation, people may devote to many resources to making existing products and too few resources to trying to design new and better products.

[10] Schlicher, Patent Law α 3.02.

[11] Brenner v. Manson, 383 U.S. 519 (1966).

[12] Today, the standards of patentability are not directed exclusively to whether anyone previously introduced the same product into the market. If an invention is described in an article on a library shelf and has never been developed or marketed, it may not be patented even though the patent would increase the private profitability of undertaking development and marketing. Patent law standards do not even ask whether an inventor intends to market a product or thinks there is a market for the product. Indeed, a person may obtain and enforce a patent even though he or she plans not to use the invention commercially. Continental Paper Bag Co. v. Eastern Paper Bag Co., 210 U.S. 405, 426-30 (1908); cf., Special Equip. Co. v. Coe, 324 U.S. 370, 371-77 (1945).

[13] Brenner v. Manson, 383 U.S. 519, 534-35 (1966). The utility requirement, as interpreted by the Supreme Court in 1966, has not been part of the patent system for 200 years. Brenner v. Manson was a totally unexpected and significant change in the formulation and application of that standard.

[14] Schlicher, Patent Law α 3.02[3].

[15] Id. $\alpha\alpha$ 1.05, 2.18[1], 3.03[5][c], and, e.g., Paulik v. Rizkalla, 760 F.2d 1270, 1276 (Fed.Cir. 1985). An added feature of Brenner v.

Manson is that the Supreme Court said that it should not permit a patent to issue, unless the court could determine that Congress's intent was to "clearly command" that a patent issue in that situation. The Supreme Court during that period often invoked this notion that patents should be made unavailable and construed narrowly unless Congress clearly commanded otherwise. The Court's later decisions in *Chakrabarty*, *Diehr* and others expressly reject that approach to interpreting the Patent Act.

[16] *Id.* at 3.02[2].

[17] While Section 112 focuses on the nature of the specification in the application, the Court of Appeals for the Federal Circuit, as noted in the notice, has interpreted the "how to use" language of Section 112 to incorporate the utility requirement of Section 101. In other words, if the application does not disclose a "practicable utility" for the invention, then the invention is not patentable under Section 101 and Section 112.

I do believe it is legitimate to ask whether people skilled in the art would consider the historical difficulty of developing a treatment for a particular disorder as bearing on the likelihood that a particular proposed therapy will yield a biological response that might be useful in treatment.

[18] *Id.* at 5.03.

[19] Schlicher, *Patent Law*, at 5.03[13] and 5.03[18].

[20] *Id.* at 5.04[2].

[21] *Environmental Designs v. Union Oil Of California*, 713 F.2d 693, 696-98 (Fed. Cir. 1983).

[22] *Standard Oil Co. v. American Cyanamid Co.*, 774 F.2d 448, 454 (Fed.Cir. 1985).

[23] *Environmental Designs v. Union Oil Of California*, 713 F.2d 693, 696-98 (Fed. Cir. 1983)("...(4) rapidity with which innovations are made ...").

[24] *Graham v. John Deere Co.*, 383 U.S. 1, 10 (1966)("The difficulty of formulating conditions for patentability was heightened by the generality of the constitutional grant and the statutes implementing it, together with the underlying policy of the patent system that "the things which are worth to the public the embarrassment of an exclusive patent," as Jefferson put it, must outweigh the restrictive effect of the limited patent monopoly. The inherent problem was to develop some means of weeding out those inventions which would not be disclosed or devised but for the inducement of a patent."). The Court said one should weed out

the inventions that would not be "disclosed or devised" but for the inducement of the patent. The issue is how to do that. One way is to focus on the costs and risks of making the particular invention. If they are significant, the invention should be patented. If they are not, it should not be.

[25] First, the notice asks about "the balance of rights" between patent owners and "the general public regarding research oriented use of patent protected technology." That notice inferentially includes as part of the "general public," the United States government and the country's universities. My concept of a patent system is that it exists to increase the level of investment in producing inventions by increasing the value of those inventions to their producers. The general public benefits from that reallocation of resources because, in the absence of rules, people would have too little incentives to direct their efforts toward that activity. When too little resources are devoted to inventing, the products and processes ultimately used to provide goods and services to consumers do not improve as quickly as they should or at all. If an experimental use doctrine is justified, and I think it is, the reason for it does not lie in finding some proper trade-off between patent owners and the general public. Rather, it can be found only by asking whether the rule will induce patent owners and others in the long run to behave in ways that would benefit the general public.

Second, and more importantly, the earlier notice, unlike this notice, says there are two primary objectives of the patent system. One is the promotion of innovation that provides the frame of reference in the current notice. The second is the "public disclosure of inventions." This is the quid pro quo theory, a variant of which I criticized earlier and which I have criticized elsewhere. Schlicher, Patent Law, ¶ 2.18[3]. While I recognize that the courts have from time to time tried to explain the patent system on that basis, I do not find it a satisfactory or sufficient explanation for the system, and I find it uniquely unhelpful in developing sensible patent law.

[26] Schlicher, Patent Law, ¶ 8.04.

[27] Schlicher, "If Economic Welfare Is the Goal, Will Economic Analysis Redefine Patent Law?" in 4 The Journal of Proprietary Rights 12 (Prentice Hall, 1992); "Department of Justice Antitrust Policy, Economic Growth, and Intellectual Property Licensing," Intellectual Property/Antitrust 1993 (Practicing Law Institute, 1993); "A Lear v. Adkins Allegory", 28 Journal Of The Patent And Trademark Office Society 427 (1986).

TESTIMONY OF MARK G. TOOHEY BEFORE THE U.S. PATENT AND TRADEMARK OFFICE

October 17, 1994

San Diego, CA

Honorable Commissioner and Distinguished Panel:

I thank you for the opportunity to testify today.

I am a registered practitioner working with the private firm of Spencer, Frank & Schneider. Our firm is a multifaceted intellectual property firm covering all aspects of intellectual property practice. The views I will express today are my own and are not necessarily espoused by Spencer, Frank & Schneider.

I hold a doctorate in Biochemistry and am currently completing the J.D. degree. As part of my legal training, I have extensively studied the 35 U.S.C. 101 and 112, first paragraph, so-called "practical utility" requirements. Thus, when notice of these hearings was recently published, I felt more than obliged to testify upon the issues presented.

Having thoroughly studied the issue of "practical utility" under the patent statute, I come to the conclusion that the Patent and Trademark Office is wrong to maintain a policy of rejecting certain classes of Biotech inventions on the alleged basis of a lack of "practical utility."

The Patent Office is wrong in narrowly reading the statute.

The Patent Office is wrong in misinterpreting the decisional law.

The Patent Office is wrong in asserting jurisdiction that it does not rightly have.

That is my view.

Let us first look at the statute. In misinterpreting section 101, the Patent Office follows the 1966 Supreme Court decision *Brenner, Comm. Pats. v. Manson* (148 USPQ 689 (U.S. 1966)). This reliance is, at best, misplaced.

Starting with the case of *In re Bremner* (86 USPQ 74 (CCPA 1950)), the court and the Patent Office agreed that a patent applicant must state the usefulness of his invention in his application. Contrary to this doctrine, the *Manson* applicant, however, failed to assert any utility for

his invention. For this reason alone, the result in the Manson decision was correct. The invention was not disclosed in compliance with well-settled case law and was therefore unpatentable to the applicant.

Unpatentable, however, NOT because of the class of invention - a pharmaceutical. Unpatentable NOT because of its intended use - as a treatment for, inter alia, humans. Rather, unpatentable for a procedural error on the part of the applicant.

In dicta, the Manson majority indicated that section 101 should be construed narrowly. The Court later corrected itself in the ground-breaking Chakrabarty (206 USPQ 193 (U.S. 1980)) decision in 1980. The Patent Office has never corrected itself accordingly.

The Chakrabarty Court followed the eloquent exposition of the Honorable Judge Giles Rich in the appellate decision in that case. Judge Rich, a co-drafter of the 1952 Patent Act, set forth in that decision the statutory scheme of the Act. Judge Rich reminds us, and the Supreme Court echoed, that the Committee Reports accompanying the 1952 Act states that section 101 is intended to include "anything under the sun made by man."

Are Biotech inventions encompassed in "anything under the sun made by man"? Certainly, certainly YES!

There has been some discussion this morning as to whether the Patent Office should adopt a policy holding a presumption of "practical utility." I would like to briefly address that issue.

In *In re Langer* (183 USPQ 288 (CCPA 1974)), the C.C.P.A. ruled that a patent application "must be taken as sufficient to satisfy the utility requirement of section 101 for the entire claimed subject matter unless there is reason for one skilled in the art to question the objective truth of the statement of utility or its scope." (emphasis in the original). That would seem to be a clear statement that a presumption of "utility" already exists, though perhaps unrecognized by the Patent Office.

As further evidence of a presumption of "utility," one need only look to the presumption of patentability embodied in section 102. That statutory section states that the patent applicant is entitled to a patent unless certain statutorily defined conditions are met. I argue that a presumption of patentability, expressly stated in the statute, necessarily implies a presumption of "utility" under the Patent Act.

Another focus of these proceedings is the Patent Office policy, whether intentional or not, of requiring applicants with certain types of Biotech inventions to show "statistically significant" results of "clinical

trials" in humans. The Office often takes the position that anything short of completely successful human trials is insufficient to show a "practical utility" for the invention.

The court, however, has consistently held since the 1961 Krimmel (130 USPQ 215 (CCPA 1961)) decision, that proof of "utility" ("operability") in "standard experimental animals" is sufficient to satisfy the requirements of section 101. One would think that the Patent Office would embrace such a position. Animal tests are less expensive than human tests. Animal tests are less time consuming than human tests. And most importantly, animal test are less dangerous than human tests. Again, one would think that the Patent Office would embrace such a position. It does not. But the Patent Office does not justify why it would require testing in humans for patentability when the statute has no such requirement.

More and more these days, animal tests are being replaced by in vitro models of human disease. These tests have all the benefits of animal models with the additional desirability of not using animals. This is a particular policy concern for that section of the public that questions the ethical propriety of animal experimentation. One would think that the Patent Office would embrace such a policy, whereby the needs of science are met and animals are spared. But the Patent Office does not. I respectfully submit, something is amiss.

One also questions why industry accepted standards, be they animal models or in vitro models, would not be acceptable to the Patent Office. Certainly the fact that the industry relies on these models ought to carry significant weight with the Patent Office, absent compelling policy reasons to the contrary. The courts have long recognized the realities of the pharmaceutical industry and the nature of its research. It is high time that the Patent Office make a similar recognition.

At this point, I note that I have routinely had client's claims allowed in the European Patent Office and the Japanese Patent Office with data in the disclosure showing "utility" in laboratory animals. In other cases before those Patent Offices, no showing of "utility" has been required because the foreign examiner recognizes the clear "utility" of the invention. Yet counterparts to these same applications, claiming substantially the same subject matter, are forever blocked in the U.S. Patent and Trademark Office.

The Patent Office has argued in the past (and continues to argue) that it must be extremely cautious in issuing patents for inventions intended for use in humans. The Patent Office relies on the rationale of the 1957 decision *Isenstead v. Watson*, Comm'r. Pats. (115 USPQ 408 (D.D.C. 1957)), where Judge Holtzoff expressed concern that the "official imprimatur" associated with a patent, rightly or wrongly, would lead the

unsophisticated in the public to believe that the patented invention works in all ways disclosed in the specification, including use in humans. Without reference to the Isenstead decision, the Official Notice of these proceedings brings this argument to the table.

What the Patent Office must recognize is that regardless of an "official imprimatur," if one exists, the public cannot legally obtain the pharmaceutical in question without that product first complying with the requirements of the Food, Drug and Cosmetic Act. The issuance of a patent is thus neutral with respect to public safety and/or drug efficacy.

This brings us to the issue of jurisdiction. As first pointed out in *In re Hartop* (135 USPQ 419 (CCPA 1962)), and subsequently reaffirmed, the issue of drug "safety" and "efficacy" is squarely within the jurisdiction of the Food and Drug Administration (and the EPA, OSHA, SEC, etc. to a much, much lesser extent). To discover the proper jurisdictional basis in this regard, one need only look to the pages upon pages of detailed statute in Title 21 of the United States Code - the Food, Drug and Cosmetic Act, as amended - and the hundreds of pages of implementing regulations. Jurisdiction on the issues of drug "safety" and "efficacy" is certain.

Yet the Patent Office disregards the courts; disregards the proper interpretation of the statutes; and asserts jurisdiction on the strained interpretation of a single word - "useful" - in the Patent Act. This jurisdictional assertion by the Patent Office is clearly wrong.

The Biotechnological industry needs valid patents to protect its intellectual property. We have already heard this common theme from early on in these proceedings. The Biotechnological industry needs patents to spur investor interest and fund further research. We have heard this also this morning. The public needs the speedy issuance of valid Biotechnical patents to promote research and hasten valuable new products based upon this technology to the market.

I conclude that the Patent Office should discontinue its practice of slowing the progress of Biotechnical inventions to the market place and impeding the Biotech industry under the policy of issuing legally baseless "practical utility" rejections.

Thank you for your time.

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EXHIBIT 3

Electronics, Inc. from Arlington, Virginia to McLean, Virginia; and Rockwell International Corporation from El Segundo, California to Seal Beach, California.

A copy of the amended certificate will be kept in the International Trade Administration's Freedom of Information Records Inspection Facility, Room 4102, U.S. Department of Commerce, 14th Street and Constitution Avenue, N.W., Washington, D.C. 20230.

Dated: July 11, 1995.

W. Dawn Busby,

Director, Office of Export Trading Company Affairs.

[FR Doc. 95-17353 Filed 7-13-95; 8:45 am]

BILLING CODE 3510-DR-P

National Institute of Standards and Technology

Judges Panel of the Malcolm Baldrige National Quality Award

AGENCY: National Institute of Standards and Technology Department of Commerce.

ACTION: Notice of closed meeting.

SUMMARY: Pursuant to the Federal Advisory Committee Act, 5 U.S.C. app. 2, notice is hereby given that there will be a closed meeting of the Judges Panel of the Malcolm Baldrige National Quality Award on Wednesday, August 9, 1995. The Judges Panel is composed of nine members prominent in the field of quality management and appointed by the Secretary of Commerce. The purpose of this meeting is to review the 1995 Award applications and to select applications to be considered in the site visit stage of the evaluation. The applications under review contain trade secrets and proprietary commercial information submitted to the Government in confidence.

DATES: The meeting will convene August 9, 1995, at 8 a.m. and adjourn at 5 p.m. on August 9, 1995. The entire meeting will be closed.

ADDRESSES: The meeting will be held at the National Institute of Standards and Technology, Administration Building, Gaithersburg, Maryland 20899.

FOR FURTHER INFORMATION CONTACT: Dr. Curt W. Reimann, Director for Quality Programs, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, telephone number (301) 975-2036.

SUPPLEMENTARY INFORMATION: The Assistant Secretary for Administration, with the concurrence of the General Counsel, formally determined on March 3, 1994, that the meeting of the Panel of

Judges will be closed pursuant to Section 10(d) of the Federal Advisory Committee Act, 5 U.S.C. app. 2, as amended by Section 5(c) of the Government in the Sunshine Act, P.L. 94-409. The meeting, which involves examination of records and discussion of Award applicant data, may be closed to the public in accordance with Section 552b(c)(4) of Title 5, United States Code, since the meeting is likely to disclose trade secrets and commercial or financial information obtained from a person and privileged or confidential.

Dated: July 7, 1995.

Samuel Kramer,

Associate Director.

[FR Doc. 95-17316 Filed 7-13-95; 8:45 am]

BILLING CODE 3510-13-M

Patent and Trademark Office

[Docket No. 950706172-5172-01]

Utility Examination Guidelines

AGENCY: Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: The Patent and Trademark Office (PTO) is publishing the final version of guidelines to be used by Office personnel in their review of patent applications for compliance with the utility requirement. Because these guidelines govern internal practices, they are exempt from notice and comment and delayed effective date rulemaking requirements under 5 U.S.C. 553(b)(A).

EFFECTIVE DATE: July 14, 1995.

FOR FURTHER INFORMATION CONTACT: Jeff Kushan by telephone at (703) 305-9300, by fax at (703) 305-8885, by electronic mail at kushan@uspto.gov, or by mail marked to his attention addressed to the Commissioner of Patents and Trademarks, Box 4, Washington, DC 20231.

SUPPLEMENTARY INFORMATION:

I. Discussion of Public Comments

Forty-four comments were received by the Office in response to the request to public comment on the proposed version of utility guidelines published on January 3, 1995 (60 FR 97). All comments have been carefully considered. A number of changes have been made to the examining guidelines and the legal analysis supporting the guidelines in response to the comments received.

Many of the individuals responding to the request for public comments suggested that the Office address the relationship between the requirements

of 35 U.S.C. 112, first paragraph, and 35 U.S.C. 101. The Office has amended the guidelines to provide a clarification consistent with these requests. The guidelines now specify that any rejection based on a "lack of utility" under section 101 should be accompanied by a rejection based upon section 112, first paragraph. The guidelines also specify that the procedures for imposition and review of rejections based on lack of utility under section 101 shall be followed with respect to the section 112 rejection that accompanies the section 101 rejection.

A suggestion was made that the guidelines should be modified to provide that an application shall be presumed to be compliant with section 112, first paragraph, if there is no proper basis for imposing a section 101 rejection. This suggestion has not been followed. Instead, the guidelines specify that section 112, first paragraph, deficiencies other than those that are based on a lack of utility be addressed separately from those based on a lack of utility for the invention.

Several individuals suggested that the guidelines address how section 101 compliance will be reviewed for products that are either intermediates or whose ultimate function or use is unknown. The Office has amended the guidelines to clarify how it will interpret the "specific utility" requirement of section 101.

Some individuals suggested that the guidelines be amended to preclude Examiners from requiring that an applicant delete references made in the specification to the utility of an invention which are not necessary to support an asserted utility of the claimed invention. The guidelines have been amended consistent with this suggestion.

One individual suggested that the legal analysis be amended to emphasize that any combination of evidence from *in vitro* or *in vivo* testing can be sufficient to establish the credibility of an asserted utility. The legal analysis has been amended consistent with this recommendation.

A number of individuals questioned the legal status of the guidelines, particularly with respect to situations where an applicant believes that a particular Examiner has failed to follow the requirements of the guidelines in imposing a rejection under section 101. The guidelines and the legal analysis supporting the guidelines govern the internal operations of the Patent and Trademark Office. They are not intended to, nor do they have the force and effect of law. As such they are not substantive rules creating or altering the

rights or obligations of any party. Rather, the guidelines define the procedures to be followed by Office personnel in their review of applications for section 101 compliance. The legal analysis supporting the guidelines articulates the basis for the procedures established in the guidelines. Thus, an applicant who believes his or her application has been rejected in a manner that is inconsistent with the guidelines should respond substantively to the grounds of the rejection. "Non-compliance" with the guidelines will not be a petitionable or appealable action.

Some individuals suggested that the guidelines and legal analysis be amended to specify that the Office will reject an application for lacking utility only in those situations where the asserted utility is "incredible." This suggestion has not been adopted. The Office has carefully reviewed the legal precedent governing application of the utility requirement. Based on that review, the Office has chosen to focus the review for compliance with Section 101 and Section 112, first paragraph, on the "credibility" of an asserted utility.

Some individuals suggested that the guidelines be amended to address how a generic claim that covers many discrete species will be assessed with regard to the "useful invention" requirements of sections 101 and 112 when one or more, but not all, species within the genus do not have a credible utility. The guidelines have been amended to clarify how the Office will address applications in which genus claims are presented that encompass species for which an asserted utility is not credible. The legal analysis makes clear that any rejection of any claimed subject matter based on lack of utility must adhere to the standards imposed by these guidelines. This is true regardless of whether the claim defines only a single embodiment of the invention, multiple discrete embodiments of the invention, or a genus encompassing many embodiments of the invention. As cast in the legal analysis and the guidelines, the focus of examination is the invention as it has been defined in the claims.

Some individuals questioned whether the guidelines and the legal analysis govern actions taken by Examining Groups other than Group 1800 or the Board of Patent Appeals and Interferences. The guidelines apply to all Office personnel, and to the review of all applications, regardless of field of technology.

In addition to the changes made in response to comments from the public,

the Office has amended the guidelines to clarify the procedure to be followed when an applicant has failed to identify a specific utility or an invention. The guidelines now provide that where an applicant has made no assertion as to why an invention is believed useful, and it is not immediately apparent why the invention would be considered useful, the Office will reject the application as failing to identify any specific utility for the invention. The legal analysis has also been amended to address evaluation of this question.

II. Guidelines for Examination of Applications for Compliance With the Utility Requirement

A. Introduction

The following guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any application for compliance with the utility requirements of 35 U.S.C. 101 and 112. The guidelines also address issues that may arise during examination of applications claiming protection for inventions in the field of biotechnology and human therapy. The guidelines are accompanied by an overview of applicable legal precedent governing the utility requirement. The guidelines have been promulgated to assist Office personnel in their review of applications for compliance with the utility requirement. The guidelines and the legal analysis do not alter the substantive requirements of 35 U.S.C. 101 and 112, nor are they designed to obviate review of applications for compliance with this statutory requirement.

B. Examination Guidelines for the Utility Requirement

Office personnel shall adhere to the following procedures when reviewing applications for compliance with the "useful invention" ("utility") requirement of 35 U.S.C. 101 and 35 U.S.C. 112, first paragraph.

1. Read the specification, including the claims, to:

(a) Determine what the applicant has invented, noting any specific embodiments of the invention;

(b) Ensure that the claims define statutory subject matter (e.g., a process, machine, manufacture, or composition of matter);

(c) Note if applicant has disclosed any specific reasons why the invention is believed to be "useful."

2. Review the specification and claims to determine if the applicant has asserted any credible utility for the claimed invention:

(a) If the applicant has asserted that the claimed invention is useful for any

particular purpose (i.e., a "specific utility") and that assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility. Credibility is to be assessed from the perspective of one of ordinary skill in the art in view of any evidence of record (e.g., data, statements, opinions, references, etc.) that is relevant to the applicant's assertions. An applicant must provide only one credible assertion of specific utility for any claimed invention to satisfy the utility requirement.

(b) If the invention has a well-established utility, regardless of any assertion made by the applicant, do not impose a rejection based on lack of utility. An invention has a well-established utility if a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties of a product or obvious application of a process).

(c) If the applicant has not asserted any specific utility for the claimed invention and it does not have a well-established utility, impose a rejection under section 101, emphasizing that the applicant has not disclosed a specific utility for the invention. Also impose a separate rejection under section 112, first paragraph, on the basis that the applicant has not shown how to use the invention due to lack of disclosure of a specific utility. The sections 101 and 112, rejections should shift the burden to the applicant to:

- Explicitly identify a specific utility for the claimed invention, and
- Indicate where support for the asserted utility can be found in the specification.

Review the subsequently asserted utility by the applicant using the standard outlined in paragraph (2)(a) above, and ensure that it is fully supported by the original disclosure.

3. If no assertion of specific utility for the claimed invention made by the applicant is credible, and the claimed invention does not have a well-established utility, reject the claim(s) under section 101 on the grounds that the invention as claimed lacks utility. Also reject the claims under section 112, first paragraph, on the basis that the disclosure fails to teach how to use the invention as claimed. The section 112, first paragraph, rejection imposed in conjunction with a section 101 rejection should incorporate by reference the grounds of the corresponding section 101 rejection and should be set out as a rejection distinct from any other

rejection under section 112, first paragraph, not based on lack of utility for the claimed invention.

To be considered appropriate by the Office, any rejection based on lack of utility must include the following elements:

(a) *A prima facie showing that the claimed invention has no utility.*

A *prima facie* showing of no utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific utility asserted by the applicant for the claimed invention. A *prima facie* showing must contain the following elements:

(i) A well-reasoned statement that clearly sets forth the reasoning used in concluding that the asserted utility is not credible;

(ii) Support for factual findings relied upon in reaching this conclusion; and

(iii) Support for any conclusions regarding evidence provided by the applicant in support of an asserted utility.

(b) *Specific evidence that supports any fact-based assertions needed to establish the prima facie showing.*

Whenever possible, Office personnel must provide documentary evidence (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) as the form of support used in establishing the factual basis of a *prima facie* showing of no utility according to items (a)(ii) and (a)(iii) above. If documentary evidence is not available, Office personnel shall note this fact and specifically explain the scientific basis for the factual conclusions relied on in sections (a)(ii) and (a)(iii).

4. *A rejection based on lack of utility should not be maintained if an asserted utility for he claimed invention would be considered credible by a person of ordinary skill in the art in view of all evidence of record.*

Once a *prima facie* showing of no utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a printed publication, that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, Office personnel shall review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments and any new reasoning or evidence provided by the applicant in support of an asserted utility. It is essential for Office personnel to recognize, fully

consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under section 101, withdraw the section 101 rejection and the corresponding rejection imposed under section 112, first paragraph, per paragraph (3) above.

Office personnel are reminded that they must treat as true a statement of fact made by an applicant in relation to an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

III. Additional Information

The PTO has prepared an analysis of the law governing the utility requirement to support the guidelines outlined above. Copies of the legal analysis can be obtained from Jeff Kushan, who can be reached using the information indicated above.

Dated: July 3, 1995.

Bruce A. Lehman,

*Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks.*
[FR Doc. 95-17304 Filed 7-13-95; 8:45 am]
BILLING CODE 3510-16-M

COMMITTEE FOR PURCHASE FROM PEOPLE WHO ARE BLIND OR SEVERELY DISABLED

Procurement List; Additions

AGENCY: Committee for Purchase From People Who Are Blind or Severely Disabled.

ACTION: Additions to the Procurement List.

SUMMARY: This action adds to the Procurement List services to be furnished by nonprofit agencies employing persons who are blind or have other severe disabilities.

EFFECTIVE DATE: August 14, 1995.

ADDRESSES: Committee for Purchase From People Who Are Blind or Severely Disabled, Crystal Square 3, Suite 403,

1735 Jefferson Davis Highway,
Arlington, Virginia 22202-3461.

FOR FURTHER INFORMATION CONTACT:
Beverly Milkman (703) 603-7740.

SUPPLEMENTARY INFORMATION: On July 22, 1994, April 28, May 12 and 19, 1995, the Committee for Purchase From People Who Are Blind or Severely Disabled published notices (59 FR 37466, 60 FR 20971, 25695 and 26876) of proposed additions to the Procurement List.

After consideration of the material presented to it concerning capability of qualified nonprofit agencies to provide the services, fair market price, and impact of the additions on the current or most recent contractors, the Committee has determined that the services listed below are suitable for procurement by the Federal Government under 41 U.S.C. 46-48c and 41 CFR 51-2.4.

I certify that the following action will not have a significant impact on a substantial number of small entities. The major factors considered for this certification were:

1. The action will not result in any additional reporting, recordkeeping or other compliance requirements for small entities other than the small organizations that will furnish the services to the Government.

2. The action does not appear to have a severe economic impact on current contractors for the services.

3. The action will result in authorizing small entities to furnish the services to the Government.

4. There are no known regulatory alternatives which would accomplish the objectives of the Javits-Wagner-O'Day Act (41 U.S.C. 46-48c) in connection with the services proposed for addition to the Procurement List.

Accordingly, the following services are hereby added to the Procurement List:

Administrative Services for the following locations:
Fleet and Industrial Supply Center, San Diego, California
Fleet and Industrial Supply Center, Long Beach, California
Janitorial/Custodial for the following locations:
Federal Building, 525 Water Street, Port Huron, MI
Social Security Administration Building, 142 Auburn Street, Pontiac, MI
Janitorial/Custodial, Carl Albert Federal Building and U.S. Courthouse, 301 E. Carl Albert Parkway, McAlester, Oklahoma
Janitorial/Custodial, IRS Service Center Complex, Memphis, Tennessee

EXHIBIT 4

USPQ2d 1016, 1021 (Fed. Cir. 1991) (“A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated.”) (citations omitted). In such instances the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor’s idea of the invention. *Burroughs Wellcome Co. v. Barr Laboratories Inc.*, 40 F.3d 1223, 1229, 32 USPQ2d 1915, 1920 (Fed. Cir. 1994). Reduction to practice in effect provides the only evidence to corroborate conception (and therefore possession) of the invention. *Id.*

49. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

50. See, e.g., *Eli Lilly*.

51. For example, in the genetics arts, it is unnecessary for an applicant to provide enough different species that the disclosure will permit one of skill to determine the nucleic acid or amino acid sequence of another species from the application alone. The stochastic nature of gene evolution would make such a predictability nearly impossible. Thus, the Federal Circuit could not have intended that representative number requires predictability of sequences.

52. See *Wertheim*, 541 F.2d at 263, 191 USPQ at 97 (“[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.”). See also MPEP § 2163.05.

53. See MPEP § 714.02 and 2163.06 (“Applicant should * * * specifically point out the support for any amendments made to the disclosure.”).

54. See, e.g., *In re Wright*, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989) (Original specification for method of forming images using photosensitive microcapsules which describes removal of microcapsules from surface and warns that capsules not be disturbed prior to formation of image, unequivocally teaches absence of permanently fixed microcapsules and supports amended language of claims requiring that microcapsules be “not permanently fixed” to underlying surface,

and therefore meets description requirement of 35 U.S.C. 112.).

55. See, e.g., *In re Robins*, 429 F.2d 452, 456–57, 166 USPQ 552, 555 (CCPA 1970) (“[W]here no explicit description of a generic invention is to be found in the specification * * * mention of representative compounds may provide an implicit description upon which to base generic claim language.”); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily implicitly described by a genus encompassing it and a species upon which it reads).

56. See, e.g., *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950–51 (Fed. Cir. 1999) (“To establish inherency, the extrinsic evidence ‘must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.’”) (citations omitted).

57. When an explicit limitation in a claim “is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation.” *Hyatt v. Boone*, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998).

58. See, e.g., *Johnson Worldwide Associates Inc. v. Zebco Corp.*, 175 F.3d at 993, 50 USPQ2d at 1613; *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d at 1479, 45 USPQ2d at 1503; *Tronzo v. Biomet, Inc.*, 156 F.3d at 1159, 47 USPQ2d at 1833; and *Reiffin v. Microsoft Corp.*, 48 USPQ2d at 1277.

59. See, e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

60. *Wertheim*, 541 F.2d at 262, 191 USPQ at 96.

61. See *In re Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326.

62. See *In re Alton*, 76 F.3d 1168, 1176, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996).

Dated: December 16, 1999.

Q. Todd Dickinson,

*Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks.*

[FR Doc. 99–33053 Filed 12–20–99; 8:45 am]

BILLING CODE 3510–16–P

DEPARTMENT OF COMMERCE

Patent and Trademark Office

[Docket No. 991027289–9289–01]

RIN 0651–AB09

**Revised Utility Examination
Guidelines; Request for Comments**

AGENCY: Patent and Trademark Office,
Commerce.

ACTION: Notice and request for public
comments.

SUMMARY: The Patent and Trademark Office (PTO) requests comments from any interested member of the public on the following Revised Utility Examination Guidelines. The PTO is publishing a revised version of guidelines to be used by Office personnel in their review of patent applications for compliance with the utility requirement based on comments received in response to the Request for Comments on Interim Guidelines for Examination of Patent Applications. Under the 35 U.S.C. 112, ¶ 1 “Written Description” Requirement; Extension of Comment Period and Notice of Hearing. 63 FR 50887 (September 23, 1998). These Revised Utility Guidelines will be used by PTO personnel in their review of patent applications for compliance with the “utility” requirement of 35 U.S.C. 101. This revision supersedes the Utility Examination Guidelines that were published at 60 FR 36263 (1995) and at 1177 O.G. 146 (1995).

DATES: Written comments on the Revised Utility Examination Guidelines will be accepted by the PTO until March 22, 2000.

ADDRESSES: Written comments should be addressed to Box 8, Commissioner of Patents and Trademarks, Washington, DC 20231, marked to the attention of Mark Nagumo, or to Box Comments, Assistant Commissioner for Patents, Washington, DC 20231, marked to the attention of Linda S. Therkorn. Alternatively, comments may be submitted to Mark Nagumo via facsimile at (703) 305–9373 or by electronic mail addressed to

“mark.nagumo@uspto.gov”; or to Linda Therkorn via facsimile at (703) 305–8825 or by electronic mail addressed to “linda.therkorn@uspto.gov.”

FOR FURTHER INFORMATION CONTACT: Mark Nagumo by telephone at (703) 305–8666, by facsimile at (703) 305–9373, by electronic mail “mark.nagumo@uspto.gov,” or by mail marked to his attention addressed to the Commissioner of Patents and Trademarks, Box 8, Washington, DC 20231; or Linda Therkorn by telephone at (703) 305–9323, by facsimile at (703) 305–8825, by electronic mail at “linda.therkorn@uspto.gov,” or by mail marked to her attention addressed to Box Comments, Assistant Commissioner of Patents and Trademarks, Washington, DC 20231.

SUPPLEMENTARY INFORMATION: The PTO requests comments from any interested member of the public on the following Revised Utility Examination Guidelines. As of the publication date of this notice, this revision will be used by PTO personnel in their review of patent

applications for compliance with the "utility" requirement of 35 U.S.C. 101. Because this revision governs internal practices, it is exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Written comments should include the following information: (1) Name and affiliation of the individual responding, and (2) an indication of whether the comments offered represent views of the respondent's organization or are respondent's personal views.

Parties presenting written comments are requested, where possible, to provide their comments in machine-readable format in addition to a paper copy. Such submissions may be provided by electronic mail messages sent over the Internet, or on a 3.5" floppy disk formatted for use in a Macintosh, Windows, Windows for Workgroups, Windows 95, Windows 98, Windows NT, or MS-DOS based computer.

Written comments will be available for public inspection on or about April 19, 2000, in Suite 918, Crystal Park 2, 2121 Crystal Drive, Arlington, Virginia. In addition, comments provided in machine readable format will be available through the PTO's Website at <http://www.uspto.gov>.

I. Discussion of Public Comments

Comments received by the Office in response to the request for public comment on the Interim Written Description Guidelines regarding the patentability of expressed sequence tags (ESTs) suggested the need for revision or clarification of the final Utility Examination Guidelines as published at 60 FR 36263 (1995) and 1177 O.G. 146 (1995). All comments have been carefully considered. Many comments stated that sufficient patentable utility has not been shown when the sole disclosed use of an EST is to identify other nucleic acids whose utility was not known, and the function of the corresponding gene is not known. Moreover, several comments opined that ESTs are genomic research tools that should be available for unencumbered research to advance the public good. One comment stated that asserted utilities for ESTs, such as mapping the genome or tissue typing, would probably not satisfy the requirements of 35 U.S.C. 101 if the length of the attached DNA sequence were greatly extended. Other comments stated that the disclosure of a DNA sequence alone is insufficient to enable scientists to use ESTs for mapping or tissue typing. Some comments suggested that PTO examination procedures would result in granting

patents based on nonspecific and nonsubstantial utilities, contrary to established case law. See *Brenner v. Manson*, 383 U.S. 519, 534-35, 148 USPQ 689, 695 (1966) (requiring disclosure of "specific utility," and of "substantial utility," "where specific benefit exists in currently available form"); accord, *In re Ziegler*, 992 F.2d 1197, 1201, 26 USPQ2d 1600, 1603 (Fed. Cir. 1996) (requiring that a specific and substantial or practical utility for the invention be disclosed as a condition of meeting the practical utility requirement of § 101). Consequently, a number of changes have been made to the Utility Examination Guidelines to clarify the position of the Patent and Trademark Office. Updated training material will be developed in the examination corps to address technology-specific issues.

II. Guidelines for Examination of Applications for Compliance With the Utility Requirement

A. Introduction

The following guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any patent application for compliance with the utility requirements of 35 U.S.C. 101 and 112. These guidelines have been promulgated to assist Office personnel in their review of applications for compliance with the utility requirement. The guidelines do not alter the substantive requirements of 35 U.S.C. 101 and 112, nor are they designed to obviate the examiner's review of applications for compliance with all other statutory requirements for patentability.

B. Examination Guidelines for the Utility Requirement

Office personnel are to adhere to the following procedures when reviewing patent applications for compliance with the "useful invention" ("utility") requirement of 35 U.S.C. 101 and 112, first paragraph.

1. Read the claims and the supporting written description.

(a) Determine what the applicant has claimed, noting any specific embodiments of the invention.

(b) Ensure that the claims define statutory subject matter (i.e., a process, machine, manufacture, composition of matter, or improvement thereof).

2. Review the claims and the supporting written description to determine if the applicant has asserted for the claimed invention any specific and substantial utility that is credible.

(a) If the invention has a well-established utility, regardless of any

assertion made by the applicant, do not impose a rejection based on lack of utility. An invention has a well-established utility if a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties or applications of a product or process).

(b) If the applicant has asserted that the claimed invention is useful for any particular practical purpose (i.e., it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility.

(1) A claimed invention must have a specific and substantial utility. This requirement excludes "throw-away," "insubstantial," or "nonspecific" utilities, such as the use of a complex invention as landfill, as a way of satisfying the utility requirement of 35 U.S.C. 101.

(2) Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record (e.g., test data, affidavits or declarations from experts in the art, patents or printed publications) that is probative of the applicant's assertions. An applicant need only provide one credible assertion of specific and substantial utility for each claimed invention to satisfy the utility requirement.

(c) If no assertion of specific and substantial utility for the claimed invention made by the applicant is credible, and the claimed invention does not have a well-established utility, reject the claim(s) under section 101 on the grounds that the invention as claimed lacks utility. Also reject the claims under § 112, first paragraph, on the basis that the disclosure fails to teach how to use the invention as claimed. The section 112, first paragraph, rejection imposed in conjunction with a section 101 rejection should incorporate by reference the grounds of the corresponding section 101 rejection.

(d) If the applicant has not asserted any specific and substantial utility for the claimed invention and it does not have a well-established utility, impose a rejection under section 101, emphasizing that the applicant has not disclosed a specific and substantial utility for the invention. Also impose a separate rejection under section 112, first paragraph, on the basis that the applicant has not disclosed how to use the invention due to the lack of a specific and substantial utility. The sections 101 and 112 rejections shift the

burden of coming forward with evidence to the applicant to:

(1) Explicitly identify a specific and substantial utility for the claimed invention; and

(2) Provide evidence that one of ordinary skill in the art would have recognized that the identified specific and substantial utility was well established at the time of filing. The examiner should review any subsequently submitted evidence of utility using the criteria outlined above. The examiner should also ensure that there is an adequate nexus between the showing and the application as filed.

3. Any rejection based on lack of utility should include a detailed explanation why the claimed invention has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the *prima facie* showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

(a) Where the asserted specific and substantial utility is not credible, a *prima facie* showing of no specific and substantial credible utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention.

The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that the asserted specific and substantial utility is not credible;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record.

(b) Where no specific and substantial utility is disclosed or known, a *prima facie* showing of no specific and substantial utility must establish that it is more likely than not that a person skilled in the art would not be aware of any well-established credible utility that is both specific and substantial.

The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that there is no known well established utility for the claimed invention that is both specific and substantial;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record.

4. A rejection based on lack of utility should not be maintained if an asserted utility for the claimed invention would be considered specific, substantial, and credible by a person of ordinary skill in the art in view of all evidence of record.

Office personnel are reminded that they must treat as true a statement of fact made by an applicant in relation to an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under section 101, withdraw the § 101 rejection and the corresponding rejection imposed under section 112, first paragraph.

Dated: December 16, 1999.

Q. Todd Dickinson,

*Assistant Secretary of Commerce and
Commissioner of Patents and Trademarks.*

[FR Doc. 99-33054 Filed 12-20-99; 8:45 am]

BILLING CODE 3510-16-M

DEPARTMENT OF EDUCATION

Submission for OMB Review; Comment Request

AGENCY: Department of Education.

SUMMARY: The Leader, Information Management Group, Office of the Chief Information Officer invites comments on the submission for OMB review as required by the Paperwork Reduction Act of 1995.

DATES: Interested persons are invited to submit comments on or before January 20, 2000.

ADDRESSES: Written comments should be addressed to the Office of Information and Regulatory Affairs, Attention: Danny Werfel, Desk Officer, Department of Education, Office of Management and Budget, 725 17th Street, NW, Room 10235, New Executive Office Building, Washington, DC 20503 or should be electronically mailed to the internet address DWERFEL@OMB.EOP.GOV.

SUPPLEMENTARY INFORMATION: Section 3506 of the Paperwork Reduction Act of 1995 (44 U.S.C. Chapter 35) requires that the Office of Management and Budget (OMB) provide interested Federal agencies and the public an early opportunity to comment on information collection requests. OMB may amend or waive the requirement for public consultation to the extent that public participation in the approval process would defeat the purpose of the information collection, violate State or Federal law, or substantially interfere with any agency's ability to perform its statutory obligations. The Leader, Information Management Group, Office of the Chief Information Officer, publishes that notice containing proposed information collection requests prior to submission of these requests to OMB. Each proposed information collection, grouped by office, contains the following: (1) Type of review requested, e.g., new, revision, extension, existing or reinstatement; (2) title; (3) summary of the collection; (4) description of the need for, and proposed use of, the information; (5) respondents and frequency of collection; and (6) reporting and/or recordkeeping burden. OMB invites public comment.

Dated: December 15, 1999.

William E. Burrow,

*Leader, Information Management Group,
Office of the Chief Information Officer.*

**Office of Special Education and
Rehabilitative Services**

Type of Review: Extension.

EXHIBIT 5

DEPARTMENT OF COMMERCE**National Oceanic and Atmospheric Administration****Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries**

AGENCY: Office of National Marine Sanctuaries (ONMS), National Ocean Service (NOS), National Oceanic and Atmospheric Administration (NOAA), Department of Commerce (DOC).

ACTION: Notice of availability.

SUMMARY: Notice is hereby given that NOAA is requesting comments on the report "Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries" and two peer reviews of this report. The report and peer reviews are available for download at <http://www.sanctuaries.nos.noaa.gov/news/newsboard/newsboard.html> or by requesting an electronic or hard copy. Requests can be made by sending an email to submarine.cables@noaa.gov (subject line "Request for Fair Market Value Analysis") or by calling Matt Brookhart at (301) 713-3125 x140.

DATES: Comments on this notice must be received by January 18, 2001.

ADDRESSES: Address all comments regarding this notice to Matt Brookhart, Conservation Policy and Planning Branch, Office of National Marine Sanctuaries, 1305 East-West Highway, 11th Floor, Silver Spring, MD 20910, Attention: Fair Market Value Analysis. Comments may also be submitted by email to: submarine.cables@noaa.gov, subject line "Fair Market Value Analysis."

FOR FURTHER INFORMATION CONTACT: Helen Golde, (301) 713-3125 x152.

SUPPLEMENTARY INFORMATION: The Office of National Marine Sanctuaries has issued several special-use permits to companies seeking to install fiber optic cables in National Marine Sanctuaries. The Sanctuary statute allows ONMS to permit the presence of cables on the sanctuaries' seafloor should it decide to do so. If an application is approved, ONMS may collect certain administrative and monitoring fees. In addition, ONMS is entitled to receive fair market value for the permitted use of sanctuary resources.

The report "Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries" presents an assessment of fair market value for the use of National Marine Sanctuary resources for a fiber optic cable. Proper stewardship of sanctuary resources and open and equitable

relations with telecommunication industry interests require a clear and consistent policy in this matter. The content of this report is based on dozens of industry and government sources and draws on the collaboration and review of numerous experts in the business, legal and technical arenas.

Once finalized, the fee structure proposed in this report will be used to assess fees (as stated in their respective special use permits) for cables already installed in the Olympic Coast and Stellwagen Bank National Marine Sanctuaries. In addition, this structure will provide the basis for future fair market value assessment of submarine cable permit applications in National Marine Sanctuaries. Comments on the report and peer reviews should focus on the methodology employed and the conclusions that it reached.

Dated: December 29, 2000.

John Oliver,

Chief Financial Officer, National Ocean Service.

[FR Doc. 01-387 Filed 1-4-01; 8:45 am]

BILLING CODE 3510-08-P

DEPARTMENT OF COMMERCE**United States Patent and Trademark Office**

[Docket No. 991027289-0263-02]

RIN 0651-AB09

Utility Examination Guidelines

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: The United States Patent and Trademark Office (USPTO) is publishing a revised version of guidelines to be used by Office personnel in their review of patent applications for compliance with the "utility" requirement of 35 U.S.C. 101. This revision supersedes the Revised Interim Utility Examination Guidelines that were published at 64 FR 71440, Dec. 21, 1999; 1231 O.G. 136 (2000); and correction at 65 FR 3425, Jan. 21, 2000; 1231 O.G. 67 (2000).

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Mark Nagumo by telephone at (703) 305-8666, by facsimile at (703) 305-9373, by electronic mail at "mark.nagumo@uspto.gov," or by mail marked to his attention addressed to the Office of the Solicitor, Box 8, Washington, DC 20231; or Linda Therkorn by telephone at (703) 305-9323, by facsimile at (703) 305-8825, by

electronic mail at "linda.therkorn@uspto.gov," or by mail marked to her attention addressed to Box Comments, Commissioner for Patents, Washington, DC 20231.

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "utility" requirement of 35 U.S.C. 101. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

I. Discussion of Public Comments

The Revised Interim Utility Examination Guidelines published at 64 FR 71440, Dec. 21, 1999; 1231 O.G. 136, Feb. 29, 2000, with a correction at 65 FR 3425, Jan. 21, 2000; 1231 O.G. 67, Feb. 15, 2000, requested comments from the public. Comments were received from 35 individuals and 17 organizations. The written comments have been carefully considered.

Overview of Comments

The majority of comments generally approved of the guidelines and several expressly stated support for the three utility criteria (specific, substantial, and credible) set forth in the Guidelines. A few comments addressed particular concerns with respect to the coordinate examiner training materials that are available for public inspection at the USPTO website, www.uspto.gov. The comments on the training materials will be taken under advisement in the revision of the training materials. Consequently, those comments are not specifically addressed below because they do not impact the content of the Guidelines. Comments received in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 'Written Description' Requirement," 64 FR 71427, Dec. 21, 1999; 1231 O.G. 123, Feb. 29, 2000, which raised issues pertinent to the utility requirement are also addressed below.

Responses to Specific Comments

(1) *Comment:* Several comments state that while inventions are patentable, discoveries are not patentable. According to the comments, genes are discoveries rather than inventions. These comments urge the USPTO not to issue patents for genes on the ground that genes are not inventions. *Response:* The suggestion is not adopted. An inventor can patent a discovery when the patent application satisfies the statutory requirements. The U.S.

Constitution uses the word “discoveries” where it authorizes Congress to promote progress made by inventors. The pertinent part of the Constitution is Article 1, section 8, clause 8, which reads: “The Congress shall have power * * * To promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries.”

When Congress enacted the patent statutes, it specifically authorized issuing a patent to a person who “invents or discovers” a new and useful composition of matter, among other things. The pertinent statute is 35 U.S.C. 101, which reads: “Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.” Thus, an inventor’s discovery of a gene can be the basis for a patent on the genetic composition isolated from its natural state and processed through purifying steps that separate the gene from other molecules naturally associated with it.

If a patent application discloses only nucleic acid molecular structure for a newly discovered gene, and no utility for the claimed isolated gene, the claimed invention is not patentable. But when the inventor also discloses how to use the purified gene isolated from its natural state, the application satisfies the “utility” requirement. That is, where the application discloses a specific, substantial, and credible utility for the claimed isolated and purified gene, the isolated and purified gene composition may be patentable.

(2) *Comment:* Several comments state that a gene is not a new composition of matter because it exists in nature, and/or that an inventor who isolates a gene does not actually invent or discover a patentable composition because the gene exists in nature. These comments urge the USPTO not to issue patents for genes on the ground that genes are products of nature. Others state that naturally occurring DNAs are part of our heritage and are not inventions. Another comment expressed concern that a person whose body includes a patented gene could be guilty of patent infringement. *Response:* The comments are not adopted. A patent claim directed to an isolated and purified DNA molecule could cover, e.g., a gene excised from a natural chromosome or a synthesized DNA molecule. An isolated and purified DNA molecule that has the same sequence as a naturally occurring gene is eligible for a

patent because (1) an excised gene is eligible for a patent as a composition of matter or as an article of manufacture because that DNA molecule does not occur in that isolated form in nature, or (2) synthetic DNA preparations are eligible for patents because their purified state is different from the naturally occurring compound.

Patenting compositions or compounds isolated from nature follows well-established principles, and is not a new practice. For example, Louis Pasteur received U.S. Patent 141,072 in 1873, claiming “[y]east, free from organic germs of disease, as an article of manufacture.” Another example is an early patent for adrenaline. In a decision finding the patent valid, the court explained that compounds isolated from nature are patentable: “even if it were merely an extracted product without change, there is no rule that such products are not patentable. Takamine was the first to make it [adrenaline] available for any use by removing it from the other gland-tissue in which it was found, and, while it is of course possible logically to call this a purification of the principle, it became for every practical purpose a new thing commercially and therapeutically. That was a good ground for a patent.” *Parke-Davis & Co. v. H. K. Mulford Co.*, 189 F. 95, 103 (S.D.N.Y. 1911) (J. Learned Hand).

In a more recent case dealing with the prostaglandins PGE₂ and PGE₃, extracted from human or animal prostate glands, a patent examiner had rejected the claims, reasoning that “inasmuch as the ‘claimed compounds are naturally occurring’ * * * they therefore ‘are not ‘new’ within the connotation of the patent statute.’” *In re Bergstrom*, 427 F.2d 1394, 1397, 166 USPQ 256, 259 (CCPA 1970). The Court reversed the Patent Office and explained the error: “what appellants claim—pure PGE₂ and PGE₃—is not ‘naturally occurring.’ Those compounds, as far as the record establishes, do not exist in nature in pure form, and appellants have neither merely discovered, nor claimed sufficiently broadly to encompass, what has previously existed in fact in nature’s storehouse, albeit unknown, or what has previously been known to exist.” *Id.* at 1401, 166 USPQ at 261–62. Like other chemical compounds, DNA molecules are eligible for patents when isolated from their natural state and purified or when synthesized in a laboratory from chemical starting materials.

A patent on a gene covers the isolated and purified gene but does not cover the gene as it occurs in nature. Thus, the concern that a person whose body

“includes” a patented gene could infringe the patent is misfounded. The body does not contain the patented, isolated and purified gene because genes in the body are not in the patented, isolated and purified form. When the patent issued for purified adrenaline about one hundred years ago, people did not infringe the patent merely because their bodies naturally included unpurified adrenaline.

(3) *Comment:* Several comments suggested that the USPTO should seek guidance from Congress as to whether naturally occurring genetic sequences are patentable subject matter. *Response:* The suggestion is not adopted. Congress adopted the current statute defining patentable subject matter (35 U.S.C. 101) in 1952. The legislative history indicates that Congress intended “anything under the sun that is made by man” to be eligible for patenting. S. Rep. No. 1979, 82d Cong., 2d Sess., 5 (1952); H.R. Rep. No. 1923, 82d Cong., 2d Sess., 6 (1952). The Supreme Court interprets the statute to cover a “naturally occurring manufacture or composition of matter—a product of human ingenuity.” *Diamond v. Chakrabarty*, 447 U.S. 303, 309, 206 USPQ 193, 197 (1980). Thus, the intent of Congress with regard to patent eligibility for chemical compounds has already been determined: DNA compounds having naturally occurring sequences are eligible for patenting when isolated from their natural state and purified, and when the application meets the statutory criteria for patentability. The genetic sequence data represented by strings of the letters A, T, C and G alone is raw, fundamental sequence data, i.e., nonfunctional descriptive information. While descriptive sequence information alone is not patentable subject matter, a new and useful purified and isolated DNA compound described by the sequence is eligible for patenting, subject to satisfying the other criteria for patentability.

(4) *Comment:* Several comments state that patents should not issue for genes because the sequence of the human genome is at the core of what it means to be human and no person should be able to own/control something so basic. Other comments stated that patents should be for marketable inventions and not for discoveries in nature. *Response:* The comments are not adopted. Patents do not confer ownership of genes, genetic information, or sequences. The patent system promotes progress by securing a complete disclosure of an invention to the public, in exchange for the inventor’s legal right to exclude other people from making, using, offering for sale, selling, or importing

the composition for a limited time. That is, a patent owner can stop infringing activity by others for a limited time.

Discoveries from nature have led to marketable inventions in the past, but assessing the marketability of an invention is not pertinent to determining if an invention has a specific, substantial, and credible use. "[D]evelopment of a product to the extent that it is presently commercially salable in the marketplace is not required to establish 'usefulness' within the meaning of § 101." *In re Langer*, 503 F.2d 1380, 1393, 183 USPQ 288, 298 (CCPA 1974). Inventors are entitled to patents when they have met the statutory requirements for novelty, nonobviousness and usefulness, and their patent disclosure adequately describes the invention and clearly teaches others how to make and use the invention. The utility requirement, as explained by the courts, only requires that the inventor disclose a practical or real world benefit available from the invention, i.e., a specific, substantial and credible utility. As noted in a response to other comments, it is a long tradition in the United States that discoveries from nature which are transformed into new and useful products are eligible for patents.

(5) *Comment*: Several comments state that the Guidelines mean that anyone who discovers a gene will be allowed a broad patent covering any number of possible applications even though those uses may be unattainable and unproven. Therefore, according to these comments, gene patents should not be issued. *Response*: The comment is not adopted. When a patent claiming a new chemical compound issues, the patentee has the right to exclude others from making, using, offering for sale, selling, or importing the compound for a limited time. The patentee is required to disclose only one utility, that is, teach others how to use the invention in at least one way. The patentee is not required to disclose all possible uses, but promoting the subsequent discovery of other uses is one of the benefits of the patent system. When patents for genes are treated the same as for other chemicals, progress is promoted because the original inventor has the possibility to recoup research costs, because others are motivated to invent around the original patent, and because a new chemical is made available as a basis for future research. Other inventors who develop new and nonobvious methods of using the patented compound have the opportunity to patent those methods.

(6) *Comment*: One comment suggests that the USPTO should not allow the

patenting of ESTs because it is contrary to indigenous law, because the Supreme Court's *Diamond v. Chakrabarty* decision was a bare 5-to-4 decision, because it would violate the Thirteenth Amendment of the U.S. Constitution, because it violates the novelty requirement of the patent laws, because it will exacerbate tensions between indigenous peoples and western academic/research communities and because it will undermine indigenous peoples' own research and academic institutions. The comment urges the USPTO to institute a moratorium on patenting of life forms and natural processes. *Response*: The comments are not adopted. Patents on chemical compounds such as ESTs do not implicate the Thirteenth Amendment. The USPTO must administer the patent statutes as the Supreme Court interprets them. When Congress enacted § 101, it indicated that "anything under the sun that is made by man" is subject matter for a patent. S. Rep. No. 1979, 82d Cong., 2d Sess., 5 (1952); H.R. Rep. No. 1923, 82d Cong., 2d Sess., 6 (1952). The Supreme Court has interpreted § 101 many times without overturning it. *See, e.g., Diamond v. Diehr*, 450 U.S. 175, 209 USPQ 1 (1981) (discussing cases construing section 101). Under United States law, a patent applicant is entitled to a patent when an invention meets the patentability criteria of title 35. Thus, ESTs which meet the criteria for utility, novelty, and nonobviousness are eligible for patenting when the application teaches those of skill in the art how to make and use the invention.

(7) *Comment*: Several comments state that patents should not issue for genes because patents on genes are delaying medical research and thus there is no societal benefit associated with gene patents. Others state that granting patents on genes at any stage of research deprives others of incentives and the ability to continue exploratory research and development. Some comment that patentees will deny access to genes and our property (our genes) will be owned by others. *Response*: The comments are not adopted. The incentive to make discoveries and inventions is generally spurred, not inhibited, by patents. The disclosure of genetic inventions provides new opportunities for further development. The patent statutes provide that a patent must be granted when at least one specific, substantial and credible utility has been disclosed, and the application satisfies the other statutory requirements. As long as one specific, substantial and credible use is disclosed and the statutory requirements are met, the USPTO is not

authorized to withhold the patent until another, or better, use is discovered. Other researchers may discover higher, better or more practical uses, but they are advantaged by the starting point that the original disclosure provides. A patent grants exclusionary rights over a patented composition but does not grant ownership of the composition. Patents are not issued on compositions in the natural environment but rather on isolated and purified compositions.

(8) *Comment*: Several comments stated that DNA should be considered unpatentable because a DNA sequence by itself has little utility. *Response*: A DNA sequence—i.e., the sequence of base pairs making up a DNA molecule—is simply one of the properties of a DNA molecule. Like any descriptive property, a DNA sequence itself is not patentable. A purified DNA *molecule* isolated from its natural environment, on the other hand, is a chemical compound and is patentable if all the statutory requirements are met. An isolated and purified DNA molecule may meet the statutory utility requirement if, e.g., it can be used to produce a useful protein or it hybridizes near and serves as a marker for a disease gene. Therefore, a DNA molecule is not *per se* unpatentable for lack of utility, and each application claim must be examined on its own facts.

(9) *Comment*: One comment states that the disclosure of a DNA sequence has inherent value and that possible uses for the DNA appear endless, even if no single use has been worked out. According to the comment, the "basic social contract of the patent deal" requires that such a discovery should be patentable, and that patenting should be "value-blind." *Response*: The comment is not adopted. The Supreme Court did not find a similar argument persuasive in *Brenner v. Manson*, 383 U.S. 519 (1966). The courts interpret the statutory term "useful" to require disclosure of at least one available practical benefit to the public. The Guidelines reflect this determination by requiring the disclosure of at least one specific, substantial, and credible utility. If no such utility is disclosed or readily apparent from an application, the Office should reject the claim. The applicant may rebut the Office position by showing that the invention does have a specific, substantial, and credible utility that would have been recognized by one of skill in the art at the time the application was filed.

(10) *Comment*: Several comments stated that the scope of patent claims directed to DNA should be limited to applications or methods of using DNA, and should not be allowed to

encompass the DNA itself. *Response:* The comment is not adopted. Patentable subject matter includes both “process[es]” and “composition[s] of matter.” 35 U.S.C. 101. Patent law provides no basis for treating DNA differently from other chemical compounds that are compositions of matter. If a patent application claims a composition of matter comprising DNA, and the claims meet all the statutory requirements of patentability, there is no legal basis for rejecting the application.

(11) *Comment:* Several comments stated that DNA patent claim scope should be limited to uses that are disclosed in the patent application and that allowing patent claims that encompass DNA itself would enable the inventor to assert claims to “speculative” uses of the DNA that were not foreseen at the time the patent application was filed. *Response:* The comment is not adopted. A patent on a composition gives *exclusive* rights to the composition for a limited time, even if the inventor disclosed only a single use for the composition. Thus, a patent granted on an isolated and purified DNA composition confers the right to exclude others from *any* method of using that DNA composition, for up to 20 years from the filing date. This result flows from the language of the statute itself. When the utility requirement and other requirements are satisfied by the application, a patent granted provides a patentee with the right to exclude others from, *inter alia*, “using” the patented composition of matter. See 35 U.S.C. 154. Where a new use is discovered for a patented DNA composition, that new use may qualify for its own process patent, notwithstanding that the DNA composition itself is patented.

By statute, a patent is required to disclose one practical utility. If a well-established utility is readily apparent, the disclosure is deemed to be implicit. If an application fails to disclose one specific, substantial, and credible utility, and the examiner discerns no well-established utility, the examiner will reject the claim under section 101. The rejection shifts the burden to the applicant to show that the examiner erred, or that a well-established utility would have been readily apparent to one of skill in the art. The applicant cannot rebut the rejection by relying on a utility that would not have been readily apparent at the time the application was filed. See, e.g., *In re Wright*, 999 F.2d 1557, 1562–63, 27 USPQ2d 1510, 1514 (Fed. Cir. 1993) (“developments occurring after the filing date of an application are of no

significance regarding what one skilled in the art believed as of the filing date”).

(12) *Comment:* Several comments stated that DNA should be freely available for research. Some of these comments suggested that patents are not necessary to encourage additional discovery and sequencing of genes. Some comments suggested that patenting of DNA inhibits biomedical research by allowing a single person or company to control use of the claimed DNA. Another comment expressed concern that patenting ESTs will impede complete characterization of genes and delay or restrict exploration of genetic materials for the public good. *Response:* The scope of subject matter that is eligible for a patent, the requirements that must be met in order to be granted a patent, and the legal rights that are conveyed by an issued patent, are all controlled by statutes which the USPTO must administer. “Whoever invents or discovers any new and useful * * * composition of matter * * * may obtain a patent therefor.” 35 U.S.C. 101. Congress creates the law and the Federal judiciary interprets the law. The USPTO must administer the laws as Congress has enacted them and as the Federal courts have interpreted them. Current law provides that when the statutory patentability requirements are met, there is no basis to deny patent applications claiming DNA compositions, or to limit a patent’s scope in order to allow free access to the use of the invention during the patent term.

(13) *Comment:* Several comments suggested that DNA sequences should be considered unpatentable because sequencing DNA has become so routine that determining the sequence of a DNA molecule is not inventive. *Response:* The comments are not adopted. A DNA sequence is not patentable because a sequence is merely descriptive information about a molecule. An isolated and purified DNA molecule may be patentable because a molecule is a “composition of matter,” one of the four classes of invention authorized by 35 U.S.C. 101. A DNA molecule must be *nonobvious* in order to be patentable. Obviousness does not depend on the amount of work required to characterize the DNA molecule. See 35 U.S.C. 103(a) (“Patentability shall not be negated by the manner in which the invention was made.”). As the nonobviousness requirement has been interpreted by the U.S. Court of Appeals for the Federal Circuit, whether a claimed DNA molecule would have been obvious depends on whether a molecule having the particular *structure* of the DNA would have been obvious to one of

ordinary skill in the art at the time the invention was made. See, e.g., *In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995) (“[T]he existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious.”); see also, *In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993).

(14) *Comment:* One comment suggested that genes ought to be patentable only when the complete sequence of the gene is disclosed and a function for the gene product has been determined. *Response:* The suggestion is not adopted. To obtain a patent on a chemical compound such as DNA, a patent applicant must adequately describe the compound and must disclose how to make and use the compound. 35 U.S.C. 101, 112. “An adequate written description of a DNA * * * requires a precise definition, *such as* by structure, formula, chemical name, or physical properties.” *Univ. of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1556, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997) (emphasis added, internal quote omitted). Thus, describing the complete chemical structure, *i.e.*, the DNA sequence, is one method of describing a DNA molecule but it is not the only method. In addition, the utility of a claimed DNA does not necessarily depend on the function of the encoded gene product. A claimed DNA may have a specific and substantial utility because, *e.g.*, it hybridizes near a disease-associated gene or it has a gene-regulating activity.

(15) *Comment:* One comment stated that the specification should “disclose the invention,” including why the invention works and how it was developed. *Response:* The comment is not adopted. The comment is directed more to the requirements imposed by 35 U.S.C. 112 than to those of 35 U.S.C. 101. To satisfy the enablement requirement of 35 U.S.C. 112, ¶ 1, an application must disclose the claimed invention in sufficient detail to enable a person of ordinary skill in the art to make and use the claimed invention. To satisfy the written description requirement of 35 U.S.C. 112, ¶ 1, the description must show that the applicant was in possession of the claimed invention at the time of filing. If all the requirements under 35 U.S.C. 112, ¶ 1, are met, there is no statutory basis to require disclosure of why an invention works or how it was developed. “[I]t is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works.” *Newman v. Quigg*,

877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989).

(16) *Comment*: One comment suggested that patents should "allow for others to learn from and improve the invention." The comment suggested that claims to patented plant varieties should not prohibit others from using the patented plants to develop improved varieties. The comment also stated that uses of plants in speculative manners should not be permitted. *Response*: By statute, a patent provides the patentee with the right to exclude others from, *inter alia*, making and using the claimed invention, although a limited research exemption exists. See 35 U.S.C. 163, 271(a), (e). These statutory provisions are not subject to revision by the USPTO and are not affected by these Guidelines. Where a plant is claimed in a utility patent application, compliance with the statutory requirements for utility under 35 U.S.C. 101 only requires that a claimed invention be supported by at least one specific, substantial and credible utility. It is somewhat rare for academic researchers to be sued by commercial patent owners for patent infringement. Most inventions are made available to academic researchers on very favorable licensing terms, which enable them to continue their research.

(17) *Comment*: Two comments suggested that although the USPTO has made a step in the right direction in raising the bar in the Utility Guidelines, there is still a need to apply stricter standards for utility. *Response*: The USPTO is bound by 35 U.S.C. 101 and the case law interpreting § 101. The Guidelines reflect the USPTO's understanding of § 101.

(18) *Comment*: Several comments addressed specific concerns about the examiner training materials. *Response*: The comments received with respect to the training materials will be taken under advisement as the Office revises the training materials. Except for comments with regard to whether sequence homology is sufficient to demonstrate a specific and substantial credible utility, specific concerns about the training materials will not be addressed herein as they will not impact the language of the guidelines.

(19) *Comment*: Several comments suggested that the use of computer-based analysis of nucleic acids to assign a function to a given nucleic acid based upon homology to prior art nucleic acids found in databases is highly unpredictable and cannot form a basis for an assignment of function to a putatively encoded protein. These comments also indicate that even in instances where a general functional assignment may be reasonable, the

assignment does not provide information regarding the actual biological activity of an encoded protein and therefore patent claims drawn to such nucleic acids should be limited to method of use claims that are explicitly supported by the as-filed specification(s). These comments also state that if homology-based utilities are acceptable, then the nucleic acids, and proteins encoded thereby, should be considered as obvious over the prior art nucleic acids. On the other hand, one comment stated that homology is a standard, art-accepted basis for predicting utility, while another comment stated that any level of homology to a protein with known utility should be accepted as indicative of utility. *Response*: The suggestions to adopt a *per se* rule rejecting homology-based assertions of utility are not adopted. An applicant is entitled to a patent to the subject matter claimed unless statutory requirements are not met (35 U.S.C. 101, 102, 103, 112). When the USPTO denies a patent, the Office must set forth at least a *prima facie* case as to why an applicant has not met the statutory requirements. The inquiries involved in assessing utility are fact dependent, and the determinations must be made on the basis of scientific evidence. Reliance on the commenters' *per se* rule, rather than a fact dependent inquiry, is impermissible because the commenters provide no scientific evidence that homology-based assertions of utility are inherently unbelievable or involve implausible scientific principles. See, e.g., *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (rejection of claims improper where claims did "not suggest an inherently unbelievable undertaking or involve implausible scientific principles" and where "prior art * * * discloses structurally similar compounds to those claimed by the applicants which have been proven * * * to be effective").

A patent examiner must accept a utility asserted by an applicant unless the Office has evidence or sound scientific reasoning to rebut the assertion. The examiner's decision must be supported by a preponderance of all the evidence of record. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). More specifically, when a patent application claiming a nucleic acid asserts a specific, substantial, and credible utility, and bases the assertion upon homology to existing nucleic acids or proteins having an accepted utility, the asserted utility must be accepted by the examiner unless the Office has sufficient evidence

or sound scientific reasoning to rebut such an assertion. "[A] 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' is sufficient." *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565, 39 USPQ2d 1895, 1900 (Fed. Cir. 1996). The Office will take into account both the nature and degree of the homology.

When a class of proteins is defined such that the members share a specific, substantial, and credible utility, the reasonable assignment of a new protein to the class of sufficiently conserved proteins would impute the same specific, substantial, and credible utility to the assigned protein. If the preponderance of the evidence of record, or of sound scientific reasoning, casts doubt upon such an asserted utility, the examiner should reject the claim for lack of utility under 35 U.S.C. 101. For example, where a class of proteins is defined by common structural features, but evidence shows that the members of the class do not share a specific, substantial functional attribute or utility, despite having structural features in common, membership in the class may not impute a specific, substantial, and credible utility to a new member of the class. When there is a reason to doubt the functional protein assignment, the utility examination may turn to whether or not the asserted protein encoded by a claimed nucleic acid has a well-established use. If there is a well-established utility for the protein and the claimed nucleic acid, the claim would meet the requirements for utility under 35 U.S.C. 101. If not, the burden shifts to the applicant to provide evidence supporting a well-established utility. There is no *per se* rule regarding homology, and each application must be judged on its own merits.

The comment indicating that if a homology-based utility could meet the requirements set forth under 35 U.S.C. 101, then the invention would have been obvious, is not adopted. Assessing nonobviousness under 35 U.S.C. 103 is separate from analyzing the utility requirements under 35 U.S.C. 101. When a claim to a nucleic acid supported by a homology-based utility meets the utility requirement of section 101, it does not follow that the claimed nucleic acid would have been *prima facie* obvious over the nucleic acids to which it is homologous. "[S]ection 103 requires a fact-intensive comparison of the [claim] with the prior art rather than the mechanical application of one or another *per se* rule." *In re Ochiai*, 71 F.3d 1565, 1571, 37 USPQ2d 1127, 1132 (Fed. Cir. 1995). Nonobviousness must be determined according to the analysis

in *Graham v. John Deere*, 383 U.S. 1, 148 USPQ 459 (1966). See also, *In re Dillon*, 919 F.2d 688, 692, 16 USPQ2d 1897, 1901 (Fed. Cir. 1990) (in banc) (“structural similarity between claimed and prior art subject matter, * * * where the prior art gives reason or motivation to make the claimed compositions, creates a prima facie case of obviousness”) (emphasis added). Where “the prior art teaches a specific, structurally-definable compound [] the question becomes whether the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention.” *In re Deuel*, 51 F.3d 1552, 1558, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

(20) *Comment*: Several comments indicated that in situations where a well-established utility is relied upon for compliance with 35 U.S.C. 101, the record should reflect what that utility is. One comment stated that the record should reflect whether the examiner accepted an asserted utility or relied upon a well-established utility after dismissing all asserted utilities. Another comment stated that when the examiner relies on a well-established utility not explicitly asserted by the applicant, the written record should clearly identify this utility and the rationale for considering it specific and substantial. *Response*: The comments are not adopted. Only one specific, substantial and credible utility is required to satisfy the statutory requirement. Where one or more well-established utilities would have been readily apparent to those of skill in the art at the time of the invention, an applicant may rely on any one of those utilities without prejudice. The record of any issued patent typically reflects consideration of a number of references in the prior art that the applicant or the examiner considered material to the claimed invention. These references often indicate uses for related inventions, and any patents listed typically disclose utilities for related inventions. Thus, even when the examiner does not identify a well-established utility, the record as a whole will likely disclose readily apparent utilities. Just as the examiner without comment may accept a properly asserted utility, there is no need for an examiner to comment on the existence of a well-established utility. However, the Guidelines have been revised to clarify that a well-established utility is a specific, substantial, and credible utility that must be readily apparent to one skilled in the art. Most often, the closest prior art cited and applied in the course of examining the

application will demonstrate a well-established utility for the invention.

(21) *Comment*: Several comments stated that the Guidelines erroneously burden the examiner with proving that a person of skill in the art would not be aware of a well-established utility. One comment states that this requires the examiner to prove a negative. Another comment states that the Guidelines should direct examiners that if a specific utility has not been disclosed, the applicant should be required to identify a specific utility. *Response*: The comments have been adopted in part. The Guidelines have been revised to indicate that where the applicant has not asserted a specific, substantial, and credible utility, and the examiner does not perceive a well-established utility, a rejection under § 101 should be entered. That is, if a well-established utility is not readily apparent and an invention is not otherwise supported by an asserted specific, substantial, and credible utility, the burden will be shifted to applicant to show either that the specification discloses an adequate utility, or to show that a well-established utility exists for the claimed invention. Again, most often the search of the closest prior art will reveal whether there is a well-established utility for the claimed invention.

(22) *Comment*: Several comments suggested that further clarification was required with regard to the examiner's determination that there is an adequate nexus between a showing supporting a well-established utility and the application as filed. The comments indicated that the meaning of this “nexus” was unclear. *Response*: The Guidelines have been modified to reflect that evidence provided by an applicant is to be analyzed with regard to a concordance between the showing and the full scope and content of the claimed invention as disclosed in the application as filed. In situations where the showing provides adequate evidence that the claim is supported by at least one asserted specific, substantial, and credible or well-established utility, the rejections under 35 U.S.C. 101 and 112, first paragraph, will be withdrawn. However, the examiner is instructed to consider whether or not the specification, in light of applicant's showing, is enabled for the use of the full scope of the claimed invention. Many times prior patents and printed publications provided by applicant will clearly demonstrate that a well-established utility exists.

(23) *Comment*: One comment states that the Office is using an improper standard in assessing “specific” utility. According to the comment, a distinction

between “specific” and “general” utilities is an overreaching interpretation of the specificity requirement in the case law because “unique” or “particular” utilities have never been required by the law. The comment states that the specificity requirement concerns sufficiency of disclosure, *i.e.*, teaching how to make and use a claimed invention, not the utility requirement. The comment states that the specificity requirement is to be distinguished from the “substantial” utility requirement, and that the *Brenner v. Manson* decision concerned only a “substantial” utility issue, not specificity. *Response*: The comment is not adopted. The disclosure of only a general utility rather than a particular utility is insufficient to meet statutory requirements. Although the specificity requirement is relevant to § 112, it is not severable from the utility requirement.

[S]urely Congress intended § 112 to presuppose *full satisfaction* of the requirements of § 101. Necessarily, compliance with § 112 requires a description of how to use presently useful inventions, otherwise an applicant would anomalously be required to teach how to use a useless invention. As this court stated in *Diederich*, quoting with approval from the decision of the board:

‘We do not believe that it was the intention of the statutes to require the Patent Office, the courts, or the public to play the sort of guessing game that might be involved if an applicant could satisfy the requirements of the statutes by indicating the usefulness of a claimed compound in terms of possible use so general as to be meaningless and then, after his research or that of his competitors has definitely ascertained an actual use for the compound, adducing evidence intended to show that a particular specific use would have been obvious to men skilled in the particular art to which this use relates.’ As the Supreme Court said in *Brenner v. Manson*:

‘* * * a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.’

In re Kirk, 376 F.2d 936, 942, 153 USPQ 48, 53 (CCPA 1967) (affirming rejections under §§ 101 and 112) (emphasis in original).

II. Guidelines for Examination of Applications for Compliance With the Utility Requirement

A. Introduction

The following Guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any patent application for compliance with the utility requirements of 35 U.S.C. 101 and 112. These Guidelines have been promulgated to assist Office personnel in their review of applications for compliance with the utility

requirement. The Guidelines do not alter the substantive requirements of 35 U.S.C. 101 and 112, nor are they designed to obviate the examiner's review of applications for compliance with all other statutory requirements for patentability. The Guidelines do not constitute substantive rulemaking and hence do not have the force and effect of law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

B. Examination Guidelines for the Utility Requirement

Office personnel are to adhere to the following procedures when reviewing patent applications for compliance with the "useful invention" ("utility") requirement of 35 U.S.C. 101 and 112, first paragraph.

1. Read the claims and the supporting written description.

(a) Determine what the applicant has claimed, noting any specific embodiments of the invention.

(b) Ensure that the claims define statutory subject matter (*i.e.*, a process, machine, manufacture, composition of matter, or improvement thereof).

(c) If at any time during the examination, it becomes readily apparent that the claimed invention has a well-established utility, do not impose a rejection based on lack of utility. An invention has a well-established utility (1) if a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (*e.g.*, properties or applications of a product or process), and (2) the utility is specific, substantial, and credible.

2. Review the claims and the supporting written description to determine if the applicant has asserted for the claimed invention any specific and substantial utility that is credible:

(a) If the applicant has asserted that the claimed invention is useful for any particular practical purpose (*i.e.*, it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility.

(1) A claimed invention must have a specific and substantial utility. This requirement excludes "throw-away," "insubstantial," or "nonspecific" utilities, such as the use of a complex invention as landfill, as a way of satisfying the utility requirement of 35 U.S.C. 101.

(2) Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record (*e.g.*, test data, affidavits or declarations from experts in the art, patents or printed publications) that is probative of the applicant's assertions. An applicant need only provide one credible assertion of specific and substantial utility for each claimed invention to satisfy the utility requirement.

(b) If no assertion of specific and substantial utility for the claimed invention made by the applicant is credible, and the claimed invention does not have a readily apparent well-established utility, reject the claim(s) under § 101 on the grounds that the invention as claimed lacks utility. Also reject the claims under § 112, first paragraph, on the basis that the disclosure fails to teach how to use the invention as claimed. The § 112, first paragraph, rejection imposed in conjunction with a § 101 rejection should incorporate by reference the grounds of the corresponding § 101 rejection.

(c) If the applicant has not asserted any specific and substantial utility for the claimed invention and it does not have a readily apparent well-established utility, impose a rejection under § 101, emphasizing that the applicant has not disclosed a specific and substantial utility for the invention. Also impose a separate rejection under § 112, first paragraph, on the basis that the applicant has not disclosed how to use the invention due to the lack of a specific and substantial utility. The §§ 101 and 112 rejections shift the burden of coming forward with evidence to the applicant to:

(1) Explicitly identify a specific and substantial utility for the claimed invention; and

(2) Provide evidence that one of ordinary skill in the art would have recognized that the identified specific and substantial utility was well established at the time of filing. The examiner should review any subsequently submitted evidence of utility using the criteria outlined above. The examiner should also ensure that there is an adequate nexus between the evidence and the properties of the now claimed subject matter as disclosed in the application as filed. That is, the applicant has the burden to establish a probative relation between the submitted evidence and the originally disclosed properties of the claimed invention.

3. Any rejection based on lack of utility should include a detailed explanation why the claimed invention

has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence regardless of publication date (*e.g.*, scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the *prima facie* showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

(a) Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial. The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is not both specific and substantial nor well-established;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(b) Where the asserted specific and substantial utility is not credible, a *prima facie* showing of no specific and substantial credible utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention.

The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that the asserted specific and substantial utility is not credible;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(c) Where no specific and substantial utility is disclosed or is well-established, a *prima facie* showing of no specific and substantial utility need only establish that applicant has not asserted a utility and that, on the record before the examiner, there is no known well-established utility.

4. A rejection based on lack of utility should not be maintained if an asserted utility for the claimed invention would be considered specific, substantial, and credible by a person of ordinary skill in the art in view of all evidence of record.

Office personnel are reminded that they must treat as true a statement of fact made by an applicant in relation to

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

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DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027288-0264-02]

RIN 0651-AB10

Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Stephen Walsh by telephone at (703) 305-9035, by facsimile at (703) 305-9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therkorn by telephone at (703) 305-8800, by facsimile at (703) 305-8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therkorn@uspto.gov."

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications

Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site (www.uspto.gov). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers v. Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,

EXHIBIT 6

2106.02 ****>Mathematical Algorithms<** **[R-5]**

****>**Claims to processes that do nothing more than solve mathematical problems or manipulate abstract ideas or concepts are complex to analyze and are addressed herein.

If the “acts” of a claimed process manipulate only numbers, abstract concepts or ideas, or signals representing any of the foregoing, the acts are not being applied to appropriate subject matter. *Gottschalk v. Benson*, 409 U.S. 63, 71 - 72, 175 USPQ 673, 676 (1972). Thus, a process consisting solely of mathematical operations, i.e., converting one set of numbers into another set of numbers, does not manipulate appropriate subject matter and thus cannot constitute a statutory process.

In practical terms, claims define nonstatutory processes if they:

- consist solely of mathematical operations without some claimed practical application (i.e., executing a “mathematical algorithm”); or
- simply manipulate abstract ideas, e.g., a bid (*Schrader*, 22 F.3d at 293-94, 30 USPQ2d at 1458-59) or a bubble hierarchy (*Warmerdam*, 33 F.3d at 1360, 31 USPQ2d at 1759), without some claimed practical application.

Cf. *Alappat*, 33 F.3d at 1543 n.19, 31 USPQ2d at 1556 n.19 in which the Federal Circuit recognized the confusion:

The Supreme Court has not been clear . . . as to whether such subject matter is excluded from the scope of 101 because it represents laws of nature, natural phenomena, or abstract ideas. See *Diehr*, 450 U.S. at 186 (viewed mathematical algorithm as a law of nature); *Gottschalk v. Benson*, 409 U.S. 63, 71-72 (1972) (treated mathematical algorithm as an “idea”). The Supreme Court also has not been clear as to exactly what kind of mathematical subject matter may not be patented. The Supreme Court has used, among others, the terms “mathematical algorithm,” “mathematical formula,” and “mathematical equation” to describe types of mathematical subject matter not entitled to patent protection standing alone. The Supreme Court has not set forth, however, any consistent or clear explanation of what it intended by such terms or how these terms are related, if at all.

Certain mathematical algorithms have been held to be nonstatutory because they represent a mathematical definition of a law of nature or a natural phenome-

non. For example, a mathematical algorithm representing the formula $E = mc^2$ is a “law of nature” — it defines a “fundamental scientific truth” (i.e., the relationship between energy and mass). To comprehend how the law of nature relates to any object, one invariably has to perform certain steps (e.g., multiplying a number representing the mass of an object by the square of a number representing the speed of light). In such a case, a claimed process which consists solely of the steps that one must follow to solve the mathematical representation of $E = mc^2$ is indistinguishable from the law of nature and would “pre-empt” the law of nature. A patent cannot be granted on such a process.<

2107 **Guidelines for Examination of Applications for Compliance with the Utility Requirement**

I. INTRODUCTION

The following Guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any patent application for compliance with the utility requirements of 35 U.S.C. 101 and 112. These Guidelines have been promulgated to assist Office personnel in their review of applications for compliance with the utility requirement. The Guidelines do not alter the substantive requirements of 35 U.S.C. 101 and 112, nor are they designed to obviate the examiner’s review of applications for compliance with all other statutory requirements for patentability. The Guidelines do not constitute substantive rulemaking and hence do not have the force and effect of law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

II. EXAMINATION GUIDELINES FOR THE UTILITY REQUIREMENT

Office personnel are to adhere to the following procedures when reviewing patent applications for compliance with the “useful invention” (“utility”) requirement of 35 U.S.C. 101 and 112, first paragraph.

(A) Read the claims and the supporting written description.

(1) Determine what the applicant has claimed, noting any specific embodiments of the invention.

(2) Ensure that the claims define statutory subject matter (i.e., a process, machine, manufacture, composition of matter, or improvement thereof).

(3) If at any time during the examination, it becomes readily apparent that the claimed invention has a well-established utility, do not impose a rejection based on lack of utility. An invention has a well-established utility if (i) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties or applications of a product or process), and (ii) the utility is specific, substantial, and credible.

(B) Review the claims and the supporting written description to determine if the applicant has asserted for the claimed invention any specific and substantial utility that is credible:

(1) If the applicant has asserted that the claimed invention is useful for any particular practical purpose (i.e., it has a “specific and substantial utility”) and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility.

(i) A claimed invention must have a specific and substantial utility. This requirement excludes “throw-away,” “insubstantial,” or “nonspecific” utilities, such as the use of a complex invention as landfill, as a way of satisfying the utility requirement of 35 U.S.C. 101.

(ii) Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record (e.g., test data, affidavits or declarations from experts in the art, patents or printed publications) that is probative of the applicant’s assertions. An applicant need only provide one credible assertion of specific and substantial utility for each claimed invention to satisfy the utility requirement.

(2) If no assertion of specific and substantial utility for the claimed invention made by the applicant is credible, and the claimed invention does not have a readily apparent well-established utility, reject the claim(s) under 35 U.S.C. 101 on the grounds that the

invention as claimed lacks utility. Also reject the claims under 35 U.S.C. 112, first paragraph, on the basis that the disclosure fails to teach how to use the invention as claimed. The 35 U.S.C. 112, first paragraph, rejection imposed in conjunction with a 35 U.S.C. 101 rejection should incorporate by reference the grounds of the corresponding 35 U.S.C. 101 rejection.

(3) If the applicant has not asserted any specific and substantial utility for the claimed invention and it does not have a readily apparent well-established utility, impose a rejection under 35 U.S.C. 101, emphasizing that the applicant has not disclosed a specific and substantial utility for the invention. Also impose a separate rejection under 35 U.S.C. 112, first paragraph, on the basis that the applicant has not disclosed how to use the invention due to the lack of a specific and substantial utility. The 35 U.S.C. 101 and 112 rejections shift the burden of coming forward with evidence to the applicant to:

(i) Explicitly identify a specific and substantial utility for the claimed invention; and

(ii) Provide evidence that one of ordinary skill in the art would have recognized that the identified specific and substantial utility was well-established at the time of filing. The examiner should review any subsequently submitted evidence of utility using the criteria outlined above. The examiner should also ensure that there is an adequate nexus between the evidence and the properties of the now claimed subject matter as disclosed in the application as filed. That is, the applicant has the burden to establish a probative relation between the submitted evidence and the originally disclosed properties of the claimed invention.

(C) Any rejection based on lack of utility should include a detailed explanation why the claimed invention has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence regardless of publication date (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the *prima facie* showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

PATENTABILITY

2107.01

(1) Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial. The *prima facie* showing must contain the following elements:

(i) An explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is not both specific and substantial nor well-established;

(ii) Support for factual findings relied upon in reaching this conclusion; and

(iii) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(2) Where the asserted specific and substantial utility is not credible, a *prima facie* showing of no specific and substantial credible utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention. The *prima facie* showing must contain the following elements:

(i) An explanation that clearly sets forth the reasoning used in concluding that the asserted specific and substantial utility is not credible;

(ii) Support for factual findings relied upon in reaching this conclusion; and

(iii) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(3) Where no specific and substantial utility is disclosed or is well-established, a *prima facie* showing of no specific and substantial utility need only establish that applicant has not asserted a utility and that, on the record before the examiner, there is no known well-established utility.

(D) A rejection based on lack of utility should not be maintained if an asserted utility for the claimed invention would be considered specific, substantial, and credible by a person of ordinary skill in the art in view of all evidence of record.

Office personnel are reminded that they must treat as true a statement of fact made by an applicant in relation to an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to

doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under 35 U.S.C. 101, withdraw the 35 U.S.C. 101 rejection and the corresponding rejection imposed under 35 U.S.C. 112, first paragraph.

2107.01 General Principles Governing Utility Rejections [R-5]

35 U.S.C. 101. *Inventions patentable*

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof may obtain a patent therefor, subject to the conditions and requirements of this title.

See MPEP § 2107 for guidelines for the examination of applications for compliance with the utility requirement of 35 U.S.C. 101.

The Office must examine each application to ensure compliance with the “useful invention” or utility requirement of 35 U.S.C. 101. In discharging this obligation, however, Office personnel must keep in mind several general principles that control applica-

tion of the utility requirement. As interpreted by the Federal courts, 35 U.S.C. 101 has two purposes. First, 35 U.S.C. 101 defines which categories of inventions are eligible for patent protection. An invention that is not a machine, an article of manufacture, a composition or a process cannot be patented. See *Diamond v. Chakrabarty*, 447 U.S. 303, 206 USPQ 193 (1980); *Diamond v. Diehr*, 450 U.S. 175, 209 USPQ 1 (1981). Second, 35 U.S.C. 101 serves to ensure that patents are granted on only those inventions that are “useful.” This second purpose has a Constitutional footing — Article I, Section 8 of the Constitution authorizes Congress to provide exclusive rights to inventors to promote the “useful arts.” See *Carl Zeiss Stiftung v. Renishaw PLC*, 945 F.2d 1173, 20 USPQ2d 1094 (Fed. Cir. 1991). Thus, to satisfy the requirements of 35 U.S.C. 101, an applicant must claim an invention that is statutory subject matter and must show that the claimed invention is “useful” for some purpose either explicitly or implicitly. Application of this latter element of 35 U.S.C. 101 is the focus of these guidelines.

Deficiencies under the “useful invention” requirement of 35 U.S.C. 101 will arise in one of two forms. The first is where it is not apparent why the invention is “useful.” This can occur when an applicant fails to identify any specific and substantial utility for the invention or fails to disclose enough information about the invention to make its usefulness immediately apparent to those familiar with the technological field of the invention. *Brenner v. Manson*, 383 U.S. 519, 148 USPQ 689 (1966); *In re Fisher*, 421 F.3d 1365, 76 USPQ2d 1225 (Fed. Cir. 2005); *In re Ziegler*, 992 F.2d 1197, 26 USPQ2d 1600 (Fed. Cir. 1993). The second type of deficiency arises in the rare instance where an assertion of specific and substantial utility for the invention made by an applicant is not credible.

I. SPECIFIC AND SUBSTANTIAL REQUIREMENTS

To satisfy 35 U.S.C. 101, an invention must be “useful.” Courts have recognized that the term “useful” used with reference to the utility requirement can be a difficult term to define. *Brenner v. Manson*, 383 U.S. 519, 529, 148 USPQ 689, 693 (1966) (simple everyday word like “useful” can be “pregnant with ambiguity when applied to the facts of life.”). Where an applicant has set forth a specific and substantial

utility, courts have been reluctant to uphold a rejection under 35 U.S.C. 101 solely on the basis that the applicant’s opinion as to the nature of the specific and substantial utility was inaccurate. For example, in *Nelson v. Bowler*, 626 F.2d 853, 206 USPQ 881 (CCPA 1980), the court reversed a finding by the Office that the applicant had not set forth a “practical” utility under 35 U.S.C. 101. In this case the applicant asserted that the composition was “useful” in a particular pharmaceutical application and provided evidence to support that assertion. Courts have used the labels “practical utility,” “substantial utility,” or “specific utility” to refer to this aspect of the “useful invention” requirement of 35 U.S.C. 101. The Court of Customs and Patent Appeals has stated:

Practical utility is a shorthand way of attributing “real-world” value to claimed subject matter. In other words, one skilled in the art can use a claimed discovery in a manner which provides some immediate benefit to the public.

Nelson v. Bowler, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980).

Practical considerations require the Office to rely on the inventor’s understanding of his or her invention in determining whether and in what regard an invention is believed to be “useful.” Because of this, Office personnel should focus on and be receptive to assertions made by the applicant that an invention is “useful” for a particular reason.

A. Specific Utility

A “specific utility” is *specific* to the subject matter claimed and can “provide a well-defined and particular benefit to the public.” *In re Fisher*, 421 F.3d 1365, 1371, 76 USPQ2d 1225, 1230 (Fed. Cir. 2005). This contrasts with a *general* utility that would be applicable to the broad class of the invention. Office personnel should distinguish between situations where an applicant has disclosed a specific use for or application of the invention and situations where the applicant merely indicates that the invention may prove useful without identifying with specificity why it is considered useful. For example, indicating that a compound may be useful in treating unspecified disorders, or that the compound has “useful biological” properties, would not be sufficient to define a specific utility for the compound. >See, e.g., *In re Kirk*, 376 F.2d 936, 153 USPQ 48 (CCPA 1967);

In re Joly, 376 F.2d 906, 153 USPQ 45 (CCPA 1967).< Similarly, a claim to a polynucleotide whose use is disclosed simply as a “gene probe” or “chromosome marker” would not be considered to be *specific* in the absence of a disclosure of a specific DNA target. >See *In re Fisher*, 421 F.3d at 1374, 76 USPQ2d at 1232 (“Any EST [expressed sequence tag] transcribed from any gene in the maize genome has the potential to perform any one of the alleged uses.... Nothing about [applicant’s] seven alleged uses set the five claimed ESTs apart from the more than 32,000 ESTs disclosed in the [] application or indeed from any EST derived from any organism. Accordingly, we conclude that [applicant] has only disclosed general uses for its claimed ESTs, not specific ones that satisfy § 101.”).< A general statement of diagnostic utility, such as diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed. Contrast the situation where an applicant discloses a specific biological activity and reasonably correlates that activity to a disease condition. Assertions falling within the latter category are sufficient to identify a specific utility for the invention. Assertions that fall in the former category are insufficient to define a specific utility for the invention, especially if the assertion takes the form of a general statement that makes it clear that a “useful” invention may arise from what has been disclosed by the applicant. *Knapp v. Anderson*, 477 F.2d 588, 177 USPQ 688 (CCPA 1973).

B. Substantial Utility

*> “[A]n application must show that an invention is useful to the public as disclosed in its current form, not that it may prove useful at some future date after further research. Simply put, to satisfy the ‘substantial’ utility requirement, an asserted use must show that the claimed invention has a significant and presently available benefit to the public.” *Fisher*, 421 F.3d at 1371, 76 USPQ2d at 1230. The claims at issue in *Fisher* were directed to expressed sequence tags (ESTs), which are short nucleotide sequences that can be used to discover what genes and downstream proteins are expressed in a cell. The court held that “the claimed ESTs can be used only to gain further information about the underlying genes and the proteins encoded for by those genes. The claimed ESTs themselves are not an end of [applicant’s] research effort,

but only tools to be used along the way in the search for a practical utility.... [Applicant] does not identify the function for the underlying protein-encoding genes. Absent such identification, we hold that the claimed ESTs have not been researched and understood to the point of providing an immediate, well-defined, real world benefit to the public meriting the grant of a patent.” *Id.* at 1376, 76 USPQ2d at 1233-34). Thus a< “substantial utility” defines a “real world” use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a “real world” context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a “substantial utility” define a “real world” context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a “real world” context of use in identifying potential candidates for preventive measures or further monitoring. On the other hand, the following are examples of situations that require or constitute carrying out further research to identify or reasonably confirm a “real world” context of use and, therefore, do not define “substantial utilities”:

(A) Basic research such as studying the properties of the claimed product itself or the mechanisms in which the material is involved;

(B) A method of treating an *unspecified* disease or condition;

(C) A method of assaying for or identifying a material that itself has no specific and/or substantial utility;

(D) A method of making a material that itself has no specific, substantial, and credible utility; and

(E) A claim to an intermediate product for use in making a final product that has no specific, substantial and credible utility.

Office personnel must be careful not to interpret the phrase “immediate benefit to the public” or similar formulations in other cases to mean that products or services based on the claimed invention must be “currently available” to the public in order to satisfy the utility requirement. See, e.g., *Brenner v. Manson*, 383 U.S. 519, 534-35, 148 USPQ 689, 695 (1966). Rather, any reasonable use that an applicant

has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a “substantial” utility.

C. *Research Tools*

Some confusion can result when one attempts to label certain types of inventions as not being capable of having a specific and substantial utility based on the setting in which the invention is to be used. One example is inventions to be used in a research or laboratory setting. Many research tools such as gas chromatographs, screening assays, and nucleotide sequencing techniques have a clear, specific and unquestionable utility (e.g., they are useful in analyzing compounds). An assessment that focuses on whether an invention is useful only in a research setting thus does not address whether the invention is in fact “useful” in a patent sense. Instead, Office personnel must distinguish between inventions that have a specifically identified substantial utility and inventions whose asserted utility requires further research to identify or reasonably confirm. Labels such as “research tool,” “intermediate” or “for research purposes” are not helpful in determining if an applicant has identified a specific and substantial utility for the invention.

II. WHOLLY INOPERATIVE INVENTIONS; “INCREDIBLE” UTILITY

An invention that is “inoperative” (i.e., it does not operate to produce the results claimed by the patent applicant) is not a “useful” invention in the meaning of the patent law. See, e.g., *Newman v. Quigg*, 877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989); *In re Harwood*, 390 F.2d 985, 989, 156 USPQ 673, 676 (CCPA 1968) (“An inoperative invention, of course, does not satisfy the requirement of 35 U.S.C. 101 that an invention be useful.”). However, as the Federal Circuit has stated, “[t]o violate [35 U.S.C.] 101 the claimed device must be totally incapable of achieving a useful result.” *Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571, 24 USPQ2d 1401, 1412 (Fed. Cir. 1992) (emphasis added). See also *E.I. du Pont De Nemours and Co. v. Berkley and Co.*, 620 F.2d 1247, 1260 n.17, 205 USPQ 1, 10 n.17 (8th Cir. 1980) (“A small degree of utility is sufficient . . . The claimed invention must

only be capable of performing some beneficial function An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely A commercially successful product is not required Nor is it essential that the invention accomplish all its intended functions . . . or operate under all conditions . . . partial success being sufficient to demonstrate patentable utility In short, the defense of non-utility cannot be sustained without proof of total incapacity.” If an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate. See *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995); *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA), *reh’g denied*, 480 F.2d 879 (CCPA 1973); *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971).

Situations where an invention is found to be “inoperative” and therefore lacking in utility are rare, and rejections maintained solely on this ground by a Federal court even rarer. In many of these cases, the utility asserted by the applicant was thought to be “incredible in the light of the knowledge of the art, or factually misleading” when initially considered by the Office. *In re Citron*, 325 F.2d 248, 253, 139 USPQ 516, 520 (CCPA 1963). Other cases suggest that on initial evaluation, the Office considered the asserted utility to be inconsistent with known scientific principles or “speculative at best” as to whether attributes of the invention necessary to impart the asserted utility were actually present in the invention. *In re Sichert*, 566 F.2d 1154, 196 USPQ 209 (CCPA 1977). However cast, the underlying finding by the court in these cases was that, based on the factual record of the case, it was clear that the invention could not and did not work as the inventor claimed it did. Indeed, the use of many labels to describe a single problem (e.g., a false assertion regarding utility) has led to some of the confusion that exists today with regard to a rejection based on the “utility” requirement. Examples of such cases include: an invention asserted to change the taste of food using a magnetic field (*Fregeau v. Mossinghoff*, 776 F.2d 1034, 227 USPQ 848 (Fed. Cir. 1985)), a perpetual motion machine (*Newman v. Quigg*, 877 F.2d 1575, 11 USPQ2d 1340 (Fed. Cir. 1989)), a flying machine operating on “flapping or flutter function” (*In re Houghton*, 433 F.2d 820,

167 USPQ 687 (CCPA 1970)), a “cold fusion” process for producing energy (*In re Swartz*, 232 F.3d 862, 56 USPQ2d 1703, (Fed. Cir. 2000)), a method for increasing the energy output of fossil fuels upon combustion through exposure to a magnetic field (*In re Ruskin*, 354 F.2d 395, 148 USPQ 221 (CCPA 1966)), uncharacterized compositions for curing a wide array of cancers (*In re Citron*, 325 F.2d 248, 139 USPQ 516 (CCPA 1963)), and a method of controlling the aging process (*In re Eltgroth*, 419 F.2d 918, 164 USPQ 221 (CCPA 1970)). These examples are fact specific and should not be applied as a *per se* rule. Thus, in view of the rare nature of such cases, Office personnel should not label an asserted utility “incredible,” “speculative” or otherwise unless it is clear that a rejection based on “lack of utility” is proper.

III. THERAPEUTIC OR PHARMACOLOGICAL UTILITY

Inventions asserted to have utility in the treatment of human or animal disorders are subject to the same legal requirements for utility as inventions in any other field of technology. *In re Chilowsky*, 229 F.2d 457, 461-2, 108 USPQ 321, 325 (CCPA 1956) (“There appears to be no basis in the statutes or decisions for requiring any more conclusive evidence of operativeness in one type of case than another. The character and amount of evidence needed may vary, depending on whether the alleged operation described in the application appears to accord with or to contravene established scientific principles or to depend upon principles alleged but not generally recognized, but the degree of certainty as to the ultimate fact of operativeness or inoperativeness should be the same in all cases”); *In re Gazave*, 379 F.2d 973, 978, 154 USPQ 92, 96 (CCPA 1967) (“Thus, in the usual case where the mode of operation alleged can be readily understood and conforms to the known laws of physics and chemistry, operativeness is not questioned, and no further evidence is required.”). As such, pharmacological or therapeutic inventions that provide any “immediate benefit to the public” satisfy 35 U.S.C. 101. The utility being asserted in *Nelson* related to a compound with pharmacological utility. *Nelson v. Bowler*, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980). Office personnel should rely on *Nelson* and other cases as providing general guidance when evaluating the utility of an invention that is

based on any therapeutic, prophylactic, or pharmacological activities of that invention.

Courts have repeatedly found that the mere identification of a pharmacological activity of a compound that is relevant to an asserted pharmacological use provides an “immediate benefit to the public” and thus satisfies the utility requirement. As the Court of Customs and Patent Appeals held in *Nelson v. Bowler*:

Knowledge of the pharmacological activity of any compound is obviously beneficial to the public. It is inherently faster and easier to combat illnesses and alleviate symptoms when the medical profession is armed with an arsenal of chemicals having known pharmacological activities. Since it is crucial to provide researchers with an incentive to disclose pharmacological activities in as many compounds as possible, we conclude that adequate proof of any such activity constitutes a showing of practical utility.

Nelson v. Bowler, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980).

In *Nelson v. Bowler*, the court addressed the practical utility requirement in the context of an interference proceeding. Bowler challenged the patentability of the invention claimed by Nelson on the basis that Nelson had failed to sufficiently and persuasively disclose in his application a practical utility for the invention. Nelson had developed and claimed a class of synthetic prostaglandins modeled on naturally occurring prostaglandins. Naturally occurring prostaglandins are bioactive compounds that, at the time of Nelson’s application, had a recognized value in pharmacology (e.g., the stimulation of uterine smooth muscle which resulted in labor induction or abortion, the ability to raise or lower blood pressure, etc.). To support the utility he identified in his disclosure, Nelson included in his application the results of tests demonstrating the bioactivity of his new substituted prostaglandins relative to the bioactivity of naturally occurring prostaglandins. The court concluded that Nelson had satisfied the practical utility requirement in identifying the synthetic prostaglandins as pharmacologically active compounds. In reaching this conclusion, the court considered and rejected arguments advanced by Bowler that attacked the evidentiary basis for Nelson’s assertions that the compounds were pharmacologically active.

In *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980), an inventor claimed protection for pharmaceuti-

cal compositions for treating leukemia. The active ingredient in the compositions was a structural analog to a known anticancer agent. The applicant provided evidence showing that the claimed analogs had the same general pharmaceutical activity as the known anticancer agents. The court reversed the Board's finding that the asserted pharmaceutical utility was "incredible," pointing to the evidence that showed the relevant pharmacological activity.

In *Cross v. Iizuka*, 753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985), the Federal Circuit affirmed a finding by the Board of Patent Appeals and Interferences that a pharmacological utility had been disclosed in the application of one party to an interference proceeding. The invention that was the subject of the interference count was a chemical compound used for treating blood disorders. Cross had challenged the evidence in Iizuka's specification that supported the claimed utility. However, the Federal Circuit relied extensively on *Nelson v. Bowler* in finding that Iizuka's application had sufficiently disclosed a pharmacological utility for the compounds. It distinguished the case from cases where only a generalized "nebulous" expression, such as "biological properties," had been disclosed in a specification. Such statements, the court held, "convey little explicit indication regarding the utility of a compound." *Cross*, 753 F.2d at 1048, 224 USPQ at 745 (citing *In re Kirk*, 376 F.2d 936, 941, 153 USPQ 48, 52 (CCPA 1967)).

Similarly, courts have found utility for therapeutic inventions despite the fact that an applicant is at a very early stage in the development of a pharmaceutical product or therapeutic regimen based on a claimed pharmacological or bioactive compound or composition. The Federal Circuit, in *Cross v. Iizuka*, 753 F.2d 1040, 1051, 224 USPQ 739, 747-48 (Fed. Cir. 1985), commented on the significance of data from *in vitro* testing that showed pharmacological activity:

We perceive no insurmountable difficulty, under appropriate circumstances, in finding that the first link in the screening chain, *in vitro* testing, may establish a practical utility for the compound in question. Successful *in vitro* testing will marshal resources and direct the expenditure of effort to further *in vivo* testing of the most potent compounds, thereby providing an immediate benefit to the public, analogous to the benefit provided by the showing of an *in vivo* utility.

The Federal Circuit has reiterated that therapeutic utility sufficient under the patent laws is not to be con-

fused with the requirements of the FDA with regard to safety and efficacy of drugs to marketed in the United States.

FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws. *Scott [v. Finney]*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 [(Fed.Cir. 1994)]. Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. Were we to require Phase II testing in order to prove utility, the associated costs would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many crucial areas such as the treatment of cancer.

In re Brana, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). Accordingly, Office personnel should not construe 35 U.S.C. 101, under the logic of "practical" utility or otherwise, to require that an applicant demonstrate that a therapeutic agent based on a claimed invention is a safe or fully effective drug for humans. See, e.g., *In re Sichert*, 566 F.2d 1154, 196 USPQ 209 (CCPA 1977); *In re Hartop*, 311 F.2d 249, 135 USPQ 419 (CCPA 1962); *In re Anthony*, 414 F.2d 1383, 162 USPQ 594 (CCPA 1969); *In re Watson*, 517 F.2d 465, 186 USPQ 11 (CCPA 1975).

These general principles are equally applicable to situations where an applicant has claimed a process for treating a human or animal disorder. In such cases, the asserted utility is usually clear — the invention is asserted to be useful in treating the particular disorder. If the asserted utility is credible, there is no basis to challenge such a claim on the basis that it lacks utility under 35 U.S.C. 101.

See MPEP § 2107.03 for special considerations for asserted therapeutic or pharmacological utilities.

IV. RELATIONSHIP BETWEEN 35 U.S.C. 112, FIRST PARAGRAPH, AND 35 U.S.C. 101

A deficiency under >the utility prong of< 35 U.S.C. 101 also creates a deficiency under 35 U.S.C. 112, first paragraph. See *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995); *In re Jolles*, 628 F.2d 1322, 1326 n.10, 206 USPQ 885, 889 n.11 (CCPA 1980); *In re Fouche*, 439 F.2d 1237, 1243, 169 USPQ 429, 434 (CCPA 1971) ("If such compositions are in

fact useless, appellant's specification cannot have taught how to use them."'). Courts have also cast the 35 U.S.C. 101/35 U.S.C. 112 relationship such that 35 U.S.C. 112 presupposes compliance with 35 U.S.C. 101. See *In re Ziegler*, 992 F.2d 1197, 1200-1201, 26 USPQ2d 1600, 1603 (Fed. Cir. 1993) ("The how to use prong of section 112 incorporates as a matter of law the requirement of 35 U.S.C. 101 that the specification disclose as a matter of fact a practical utility for the invention. ... If the application fails as a matter of fact to satisfy 35 U.S.C. § 101, then the application also fails as a matter of law to enable one of ordinary skill in the art to use the invention under 35 U.S.C. § 112."); *In re Kirk*, 376 F.2d 936, 942, 153 USPQ 48, 53 (CCPA 1967) ("Necessarily, compliance with § 112 requires a description of how to use presently useful inventions, otherwise an applicant would anomalously be required to teach how to use a useless invention."). For example, the Federal Circuit noted, "[o]bviously, if a claimed invention does not have utility, the specification cannot enable one to use it." *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). As such, a rejection properly imposed under 35 U.S.C. 101 >for lack of utility< should be accompanied with a rejection under 35 U.S.C. 112, first paragraph. It is equally clear that a rejection based on "lack of utility," whether grounded upon 35 U.S.C. 101 or 35 U.S.C. 112, first paragraph, rests on the same basis (i.e., the asserted utility is not credible). To avoid confusion, any >lack of utility< rejection that is imposed on the basis of 35 U.S.C. 101 should be accompanied by a rejection based on 35 U.S.C. 112, first paragraph. The 35 U.S.C. 112, first paragraph, rejection should be set out as a separate rejection that incorporates by reference the factual basis and conclusions set forth in the 35 U.S.C. 101 rejection. The 35 U.S.C. 112, first paragraph, rejection should indicate that because the invention as claimed does not have utility, a person skilled in the art would not be able to use the invention as claimed, and as such, the claim is defective under 35 U.S.C. 112, first paragraph. A 35 U.S.C. 112, first paragraph, rejection >based on lack of utility< should not be imposed or maintained unless an appropriate basis exists for imposing a >utility< rejection under 35 U.S.C. 101. In other words, Office personnel should not impose a 35 U.S.C. 112, first paragraph, rejection grounded on a "lack of utility" basis unless a

35 U.S.C. 101 rejection is proper. In particular, the factual showing needed to impose a rejection under 35 U.S.C. 101 must be provided if a rejection under 35 U.S.C. 112, first paragraph, is to be imposed on "lack of utility" grounds.

It is important to recognize that 35 U.S.C. 112, first paragraph, addresses matters other than those related to the question of whether or not an invention lacks utility. These matters include whether the claims are fully supported by the disclosure (*In re Vaeck*, 947 F.2d 488, 495, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991)), whether the applicant has provided an enabling disclosure of the claimed subject matter (*In re Wright*, 999 F.2d 1557, 1561-1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993)), whether the applicant has provided an adequate written description of the invention and whether the applicant has disclosed the best mode of practicing the claimed invention (*Chemcast Corp. v. Arco Indus. Corp.*, 913 F.2d 923, 927-928, 16 USPQ2d 1033, 1036-1037 (Fed. Cir. 1990)). See also *Transco Products Inc. v. Performance Contracting Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994); *Glaxo Inc. v. Novopharm Ltd.* 52 F.3d 1043, 34 USPQ2d 1565 (Fed. Cir. 1995). The fact that an applicant has disclosed a specific utility for an invention and provided a credible basis supporting that specific utility does not provide a basis for concluding that the claims comply with all the requirements of 35 U.S.C. 112, first paragraph. For example, if an applicant has claimed a process of treating a certain disease condition with a certain compound and provided a credible basis for asserting that the compound is useful in that regard, but to actually practice the invention as claimed a person skilled in the relevant art would have to engage in an undue amount of experimentation, the claim may be defective under 35 U.S.C. 112, but not 35 U.S.C. 101. To avoid confusion during examination, any rejection under 35 U.S.C. 112, first paragraph, based on grounds other than "lack of utility" should be imposed separately from any rejection imposed due to "lack of utility" under 35 U.S.C. 101 and 35 U.S.C. 112, first paragraph.

2107.02 Procedural Considerations Related to Rejections for Lack of Utility [R-5]

I. THE CLAIMED INVENTION IS THE FOCUS OF THE UTILITY REQUIREMENT

The claimed invention is the focus of the assessment of whether an applicant has satisfied the utility requirement. Each claim (i.e., each “invention”), therefore, must be evaluated on its own merits for compliance with all statutory requirements. Generally speaking, however, a dependent claim will define an invention that has utility if the independent claim from which the dependent claim depends is drawn to the same statutory class of invention as the dependent claim and the independent claim defines an invention having utility. An exception to this general rule is where the utility specified for the invention defined in a dependent claim differs from that indicated for the invention defined in the independent claim from which the dependent claim depends. Where an applicant has established utility for a species that falls within an identified genus of compounds, and presents a generic claim covering the genus, as a general matter, that claim should be treated as being sufficient under 35 U.S.C. 101. Only where it can be established that other species clearly encompassed by the claim do not have utility should a rejection be imposed on the generic claim. In such cases, the applicant should be encouraged to amend the generic claim so as to exclude the species that lack utility.

It is common and sensible for an applicant to identify several specific utilities for an invention, particularly where the invention is a product (e.g., a machine, an article of manufacture or a composition of matter). However, regardless of the category of invention that is claimed (e.g., product or process), an applicant need only make one credible assertion of specific utility for the claimed invention to satisfy 35 U.S.C. 101 and 35 U.S.C. 112; additional statements of utility, even if not “credible,” do not render the claimed invention lacking in utility. See, e.g., *Raytheon v. Roper*, 724 F.2d 951, 958, 220 USPQ 592, 598 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 (1984) (“When a properly claimed invention meets at least one stated objective, utility under 35 U.S.C. 101 is clearly shown.”); *In re Gottlieb*, 328 F.2d 1016, 1019, 140 USPQ 665, 668 (CCPA 1964) (“Having found that the antibiotic is useful for some purpose, it

becomes unnecessary to decide whether it is in fact useful for the other purposes ‘indicated’ in the specification as possibly useful.”); *In re Malachowski*, 530 F.2d 1402, 189 USPQ 432 (CCPA 1976); *Hoffman v. Klaus*, 9 USPQ2d 1657 (Bd. Pat. App. & Inter. 1988). Thus, if applicant makes one credible assertion of utility, utility for the claimed invention as a whole is established.

Statements made by the applicant in the specification or incident to prosecution of the application before the Office cannot, standing alone, be the basis for a lack of utility rejection under 35 U.S.C. 101 or 35 U.S.C. 112. *Tol-O-Matic, Inc. v. Proma Produkt-Und Mktg. Gesellschaft m.b.h.*, 945 F.2d 1546, 1553, 20 USPQ2d 1332, 1338 (Fed. Cir. 1991) (It is not required that a particular characteristic set forth in the prosecution history be achieved in order to satisfy 35 U.S.C. 101.). An applicant may include statements in the specification whose technical accuracy cannot be easily confirmed if those statements are not necessary to support the patentability of an invention with regard to any statutory basis. Thus, the Office should not require an applicant to strike nonessential statements relating to utility from a patent disclosure, regardless of the technical accuracy of the statement or assertion it presents. Office personnel should also be especially careful not to read into a claim unclaimed results, limitations or embodiments of an invention. See *Carl Zeiss Stiftung v. Renishaw PLC*, 945 F.2d 1173, 20 USPQ2d 1094 (Fed. Cir. 1991); *In re Krimmel*, 292 F.2d 948, 130 USPQ 215 (CCPA 1961). Doing so can inappropriately change the relationship of an asserted utility to the claimed invention and raise issues not relevant to examination of that claim.

II. IS THERE AN ASSERTED OR WELL-ESTABLISHED UTILITY FOR THE CLAIMED INVENTION?

Upon initial examination, the examiner should review the specification to determine if there are any statements asserting that the claimed invention is useful for any particular purpose. A complete disclosure should include a statement which identifies a specific and substantial utility for the invention.

A. *An Asserted Utility Must Be Specific and Substantial*

A statement of specific and substantial utility should fully and clearly explain why the applicant believes the invention is useful. Such statements will usually explain the purpose of or how the invention may be used (e.g., a compound is believed to be useful in the treatment of a particular disorder). Regardless of the form of statement of utility, it must enable one ordinarily skilled in the art to understand why the applicant believes the claimed invention is useful.

Except where an invention has a well-established utility, the failure of an applicant to specifically identify why an invention is believed to be useful renders the claimed invention deficient under 35 U.S.C. 101 and 35 U.S.C. 112, first paragraph. In such cases, the applicant has failed to identify a “specific and substantial utility” for the claimed invention. For example, a statement that a composition has an unspecified “biological activity” or that does not explain why a composition with that activity is believed to be useful fails to set forth a “specific and substantial utility.” *Brenner v. Manson*, 383 US 519, 148 USPQ 689 (1966) (general assertion of similarities to known compounds known to be useful without sufficient corresponding explanation why claimed compounds are believed to be similarly useful insufficient under 35 U.S.C. 101); *In re Ziegler*, 992 F.2d 1197, 1201, 26 USPQ2d 1600, 1604 (Fed. Cir. 1993) (disclosure that composition is “plastic-like” and can form “films” not sufficient to identify specific and substantial utility for invention); *In re Kirk*, 376 F.2d 936, 153 USPQ 48 (CCPA 1967) (indication that compound is “biologically active” or has “biological properties” insufficient standing alone). See also *In re Joly*, 376 F.2d 906, 153 USPQ 45 (CCPA 1967); *Kawai v. Metlesics*, 480 F.2d 880, 890, 178 USPQ 158, 165 (CCPA 1973) (contrasting description of invention as sedative which did suggest specific utility to general suggestion of “pharmacological effects on the central nervous system” which did not). In contrast, a disclosure that identifies a particular biological activity of a compound and explains how that activity can be utilized in a particular therapeutic application of the compound does contain an assertion of specific and substantial utility for the invention.

Situations where an applicant either fails to indicate why an invention is considered useful, or where the applicant inaccurately describes the utility should rarely arise. One reason for this is that applicants are required to disclose the best mode known to them of practicing the invention at the time they file their application. An applicant who omits a description of the specific and substantial utility of the invention, or who incompletely describes that utility, may encounter problems with respect to the best mode requirement of 35 U.S.C. 112, first paragraph.

B. *No Statement of Utility for the Claimed Invention in the Specification Does Not Per Se Negate Utility*

Occasionally, an applicant will not explicitly state in the specification or otherwise assert a specific and substantial utility for the claimed invention. If no statements can be found asserting a specific and substantial utility for the claimed invention in the specification, Office personnel should determine if the claimed invention has a well-established utility. An invention has a well-established utility if (i) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (e.g., properties or applications of a product or process), and (ii) the utility is specific, substantial, and credible. If an invention has a well-established utility, rejections under 35 U.S.C. 101 and 35 U.S.C. 112, first paragraph, based on lack of utility should not be imposed. *In re Folkers*, 344 F.2d 970, 145 USPQ 390 (CCPA 1965). For example, if an application teaches the cloning and characterization of the nucleotide sequence of a well-known protein such as insulin, and those skilled in the art at the time of filing knew that insulin had a well-established use, it would be improper to reject the claimed invention as lacking utility solely because of the omitted statement of specific and substantial utility.

If a person of ordinary skill would not immediately recognize a specific and substantial utility for the claimed invention (i.e., why it would be useful) based on the characteristics of the invention or statements made by the applicant, the examiner should reject the application under 35 U.S.C. 101 and under 35 U.S.C. 112, first paragraph, as failing to identify a specific and substantial utility for the claimed invention. The rejection should clearly indicate that the basis of the

rejection is that the application fails to identify a specific and substantial utility for the invention. The rejection should also specify that the applicant must reply by indicating why the invention is believed useful and where support for any subsequently asserted utility can be found in the specification as filed. See MPEP § 2701.

If the applicant subsequently indicates why the invention is useful, Office personnel should review that assertion according to the standards articulated below for review of the credibility of an asserted utility.

III. EVALUATING THE CREDIBILITY OF AN ASSERTED UTILITY

A. *An Asserted Utility Creates a Presumption of Utility*

In most cases, an applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. 101. See, e.g., *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (CCPA 1965); *In re Langer*, 503 F.2d 1380, 183 USPQ 288 (CCPA 1974); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977). As the Court of Customs and Patent Appeals stated in *In re Langer*:

As a matter of Patent Office practice, a specification which contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented must be taken as sufficient to satisfy the utility requirement of § 101 for the entire claimed subject matter unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope.

In re Langer, 503 F.2d at 1391, 183 USPQ at 297 (emphasis in original). The “Langer” test for utility has been used by both the Federal Circuit and the Court of Customs and Patent Appeals in evaluation of rejections under 35 U.S.C. 112, first paragraph, where the rejection is based on a deficiency under 35 U.S.C. 101. In *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995), the Federal Circuit explicitly adopted the Court of Customs and Patent Appeals formulation of the “Langer” standard for 35 U.S.C. 112, first paragraph rejections, as it was expressed in a slightly reworded format in *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971), namely:

[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. (emphasis added).

Thus, *Langer* and subsequent cases direct the Office to presume that a statement of utility made by an applicant is true. See *In re Langer*, 503 F.2d at 1391, 183 USPQ at 297; *In re Malachowski*, 530 F.2d 1402, 1404, 189 USPQ 432, 435 (CCPA 1976); *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). For obvious reasons of efficiency and in deference to an applicant's understanding of his or her invention, when a statement of utility is evaluated, Office personnel should not begin by questioning the truth of the statement of utility. Instead, any inquiry must start by asking if there is any reason to question the truth of the statement of utility. This can be done by simply evaluating the logic of the statements made, taking into consideration any evidence cited by the applicant. If the asserted utility is credible (i.e., believable based on the record or the nature of the invention), a rejection based on “lack of utility” is not appropriate. Clearly, Office personnel should not begin an evaluation of utility by assuming that an asserted utility is likely to be false, based on the technical field of the invention or for other general reasons.

Compliance with 35 U.S.C. 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) *cert. denied*, 469 U.S. 835 (1984). Thus, to overcome the presumption of truth that an assertion of utility by the applicant enjoys, Office personnel must establish that it is more likely than not that one of ordinary skill in the art would doubt (i.e., “question”) the truth of the statement of utility. The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the totality of the evidence under consideration. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992) (“After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of

argument.”); *In re Corkill*, 771 F.2d 1496, 1500, 226 USPQ 1005, 1008 (Fed. Cir. 1985). A preponderance of the evidence exists when it suggests that it is more likely than not that the assertion in question is true. *Herman v. Huddleston*, 459 U.S. 375, 390 (1983). To do this, Office personnel must provide evidence sufficient to show that the statement of asserted utility would be considered “false” by a person of ordinary skill in the art. Of course, a person of ordinary skill must have the benefit of both facts and reasoning in order to assess the truth of a statement. This means that if the applicant has presented facts that support the reasoning used in asserting a utility, Office personnel must present countervailing facts and reasoning sufficient to establish that a person of ordinary skill would not believe the applicant’s assertion of utility. *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). The initial evidentiary standard used during evaluation of this question is a preponderance of the evidence (i.e., the totality of facts and reasoning suggest that it is more likely than not that the statement of the applicant is false).

B. When Is an Asserted Utility Not Credible?

Where an applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed by Office personnel as being “wrong,” even when there may be reason to believe that the assertion is not entirely accurate. Rather, Office personnel must determine if the assertion of utility is credible (i.e., whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided). An assertion is credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Credibility as used in this context refers to the reliability of the statement based on the logic and facts that are offered by the applicant to support the assertion of utility.

One situation where an assertion of utility would not be considered credible is where a person of ordinary skill would consider the assertion to be “incredible in view of contemporary knowledge” and where nothing offered by the applicant would counter what contemporary knowledge might otherwise suggest. Office personnel should be careful, however, not to label certain types of inventions as “incredible” or

“speculative” as such labels do not provide the correct focus for the evaluation of an assertion of utility. “Incredible utility” is a conclusion, not a starting point for analysis under 35 U.S.C. 101. A conclusion that an asserted utility is incredible can be reached only after the Office has evaluated both the assertion of the applicant regarding utility and any evidentiary basis of that assertion. The Office should be particularly careful not to start with a presumption that an asserted utility is, *per se*, “incredible” and then proceed to base a rejection under 35 U.S.C. 101 on that presumption.

Rejections under 35 U.S.C. 101 >based on a lack of credible utility< have been * sustained by federal courts **>when, for example,< the applicant failed to disclose any utility for the invention or asserted a utility that could only be true if it violated a scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art. *In re Gazave*, 379 F.2d 973, 978, 154 USPQ 92, 96 (CCPA 1967). Special care * should be taken when assessing the credibility of an asserted therapeutic utility for a claimed invention. In such cases, a previous lack of success in treating a disease or condition, or the absence of a proven animal model for testing the effectiveness of drugs for treating a disorder in humans, should not, standing alone, serve as a basis for challenging the asserted utility under 35 U.S.C. 101. >See MPEP § 2107.03 for additional guidance with regard to therapeutic or pharmacological utilities.<

IV. INITIAL BURDEN IS ON THE OFFICE TO ESTABLISH A *PRIMA FACIE* CASE AND PROVIDE EVIDENTIARY SUPPORT THEREOF

To properly reject a claimed invention under 35 U.S.C. 101, the Office must (A) make a *prima facie* showing that the claimed invention lacks utility, and (B) provide a sufficient evidentiary basis for factual assumptions relied upon in establishing the *prima facie* showing. *In re Gaubert*, 524 F.2d 1222, 1224, 187 USPQ 664, 666 (CCPA 1975) (“Accordingly, the PTO must do more than merely question operability - it must set forth factual reasons which would lead one skilled in the art to question the objective truth of the statement of operability”). If the Office cannot develop a proper *prima facie* case and provide evidentiary support for a rejection under 35 U.S.C.

101, a rejection on this ground should not be imposed. See, e.g., *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992) (“[T]he examiner bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability. If that burden is met, the burden of coming forward with evidence or argument shifts to the applicant.... If examination at the initial stage does not produce a *prima facie* case of unpatentability, then without more the applicant is entitled to grant of the patent.”). See also *Fregeau v. Mossinghoff*, 776 F.2d 1034, 227 USPQ 848 (Fed. Cir. 1985) (applying *prima facie* case law to 35 U.S.C. 101); *In re Piasecki*, 745 F.2d 1468, 223 USPQ 785 (Fed. Cir. 1984).

The *prima facie* showing must be set forth in a well-reasoned statement. Any rejection based on lack of utility should include a detailed explanation why the claimed invention has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence regardless of publication date (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the *prima facie* showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial. The *prima facie* showing must contain the following elements:

(A) An explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is neither both specific and substantial nor well-established;

(B) Support for factual findings relied upon in reaching this conclusion; and

(C) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

Where the asserted specific and substantial utility is not credible, a *prima facie* showing of no specific and substantial credible utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention. The *prima facie* showing must contain the following elements:

(A) An explanation that clearly sets forth the reasoning used in concluding that the asserted specific and substantial utility is not credible;

(B) Support for factual findings relied upon in reaching this conclusion; and

(C) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

Where no specific and substantial utility is disclosed or is well-established, a *prima facie* showing of no specific and substantial utility need only establish that applicant has not asserted a utility and that, on the record before the examiner, there is no known well-established utility.

It is imperative that Office personnel use specificity in setting forth and initial rejection under 35 U.S.C. 101 and support any factual conclusions made in the *prima facie* showing.

By using specificity, the applicant will be able to identify the assumptions made by the Office in setting forth the rejection and will be able to address those assumptions properly.

V. EVIDENTIARY REQUESTS BY AN EXAMINER TO SUPPORT AN ASSERTED UTILITY

In appropriate situations the Office may require an applicant to substantiate an asserted utility for a claimed invention. See *In re Pottier*, 376 F.2d 328, 330, 153 USPQ 407, 408 (CCPA 1967) (“When the operativeness of any process would be deemed unlikely by one of ordinary skill in the art, it is not improper for the examiner to call for evidence of operativeness.”). See also *In re Jolles*, 628 F.2d 1322, 1327, 206 USPQ 885, 890 (CCPA 1980); *In re Citron*, 325 F.2d 248, 139 USPQ 516 (CCPA 1963); *In re Novak*, 306 F.2d 924, 928, 134 USPQ 335, 337 (CCPA 1962). In *In re Citron*, the court held that when an “alleged utility appears to be incredible in the light

of the knowledge of the art, or factually misleading, applicant must establish the asserted utility by acceptable proof.” 325 F.2d at 253, 139 USPQ at 520. The court approved of the board’s decision which affirmed the rejection under 35 U.S.C. 101 “in view of the art knowledge of the lack of a cure for cancer and the absence of any clinical data to substantiate the allegation.” 325 F.2d at 252, 139 USPQ at 519 (emphasis in original). The court thus established a higher burden on the applicant where the statement of use is incredible or misleading. In such a case, the examiner should challenge the use and require sufficient evidence of operativeness. The purpose of this authority is to enable an applicant to cure an otherwise defective factual basis for the operability of an invention. Because this is a curative authority (e.g., evidence is requested to enable an applicant to support an assertion that is inconsistent with the facts of record in the application), Office personnel should indicate not only why the factual record is defective in relation to the assertions of the applicant, but also, where appropriate, what type of evidentiary showing can be provided by the applicant to remedy the problem.

Requests for additional evidence should be imposed rarely, and only if necessary to support the scientific credibility of the asserted utility (e.g., if the asserted utility is not consistent with the evidence of record and current scientific knowledge). As the Federal Circuit recently noted, “[o]nly after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention’s asserted utility.” *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995) (citing *In re Bundy*, 642 F.2d 430, 433, 209 USPQ 48, 51 (CCPA 1981)). In *Brana*, the court pointed out that the purpose of treating cancer with chemical compounds does not suggest, *per se*, an incredible utility. Where the prior art disclosed “structurally similar compounds to those claimed by applicants which have been proven *in vivo* to be effective as chemotherapeutic agents against various tumor models . . . , one skilled in the art would be without basis to reasonably doubt applicants’ asserted utility on its face.” 51 F.3d at 1566, 34 USPQ2d at 1441. As courts have stated, “it is clearly improper for the examiner to make a demand for further test data, which as evidence would

be essentially redundant and would seem to serve for nothing except perhaps to unduly burden the applicant.” *In re Isaacs*, 347 F.2d 887, 890, 146 USPQ 193, 196 (CCPA 1965).

VI. CONSIDERATION OF A REPLY TO A *PRIMA FACIE* REJECTION FOR LACK OF UTILITY

If a rejection under 35 U.S.C. 101 has been properly imposed, along with a corresponding rejection under 35 U.S.C. 112, first paragraph, the burden shifts to the applicant to rebut the *prima facie* showing. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992) (“The examiner bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability. If that burden is met, the burden of coming forward with evidence or argument shifts to the applicant. . . . After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of argument.”). An applicant can do this using any combination of the following: amendments to the claims, arguments or reasoning, or new evidence submitted in an affidavit or declaration under 37 CFR 1.132, or in a printed publication. New evidence provided by an applicant must be relevant to the issues raised in the rejection. For example, declarations in which conclusions are set forth without establishing a nexus between those conclusions and the supporting evidence, or which merely express opinions, may be of limited probative value with regard to rebutting a *prima facie* case. *In re Grunwell*, 609 F.2d 486, 203 USPQ 1055 (CCPA 1979); *In re Buchner*, 929 F.2d 660, 18 USPQ2d 1331 (Fed. Cir. 1991). See MPEP § 716.01(a) through § 716.01(c).

If the applicant responds to the *prima facie* rejection, Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific,

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substantial, and credible should a rejection based on lack of utility be maintained. If the record as a whole would make it more likely than not that the asserted utility for the claimed invention would be considered credible by a person of ordinary skill in the art, the Office cannot maintain the rejection. *In re Rinehart*, 531 F.2d 1048, 1052, 189 USPQ 143, 147 (CCPA 1976).

VII. EVALUATION OF EVIDENCE RELATED TO UTILITY

There is no predetermined amount or character of evidence that must be provided by an applicant to support an asserted utility, therapeutic or otherwise. Rather, the character and amount of evidence needed to support an asserted utility will vary depending on what is claimed (*Ex parte Ferguson*, 117 USPQ 229 (Bd. App. 1957)), and whether the asserted utility appears to contravene established scientific principles and beliefs. *In re Gazave*, 379 F.2d 973, 978, 154 USPQ 92, 96 (CCPA 1967); *In re Chilowsky*, 229 F.2d 457, 462, 108 USPQ 321, 325 (CCPA 1956). Furthermore, the applicant does not have to provide evidence sufficient to establish that an asserted utility is true “beyond a reasonable doubt.” *In re Irons*, 340 F.2d 974, 978, 144 USPQ 351, 354 (CCPA 1965). Nor must an applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty. *Nelson v. Bowler*, 626 F.2d 853, 856-57, 206 USPQ 881, 883-84 (CCPA 1980) (reversing the Board and rejecting Bowler’s arguments that the evidence of utility was statistically insignificant. The court pointed out that a rigorous correlation is not necessary when the test is reasonably predictive of the response). See also *Rey-Bellet v. Englehardt*, 493 F.2d 1380, 181 USPQ 453 (CCPA 1974) (data from animal testing is relevant to asserted human therapeutic utility if there is a “satisfactory correlation between the effect on the animal and that ultimately observed in human beings”). Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true.

2107.03 Special Considerations for Asserted Therapeutic or Pharmacological Utilities

The Federal courts have consistently reversed rejections by the Office asserting a lack of utility for inventions claiming a pharmacological or therapeutic utility where an applicant has provided evidence that reasonably supports such a utility. In view of this, Office personnel should be particularly careful in their review of evidence provided in support of an asserted therapeutic or pharmacological utility.

I. A REASONABLE CORRELATION BETWEEN THE EVIDENCE AND THE ASSERTED UTILITY IS SUFFICIENT

As a general matter, evidence of pharmacological or other biological activity of a compound will be relevant to an asserted therapeutic use if there is a reasonable correlation between the activity in question and the asserted utility. *Cross v. Iizuka*, 753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985); *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *Nelson v. Bowler*, 626 F.2d 853, 206 USPQ 881 (CCPA 1980). An applicant can establish this reasonable correlation by relying on statistically relevant data documenting the activity of a compound or composition, arguments or reasoning, documentary evidence (e.g., articles in scientific journals), or any combination thereof. The applicant does not have to prove that a correlation exists between a particular activity and an asserted therapeutic use of a compound as a matter of statistical certainty, nor does he or she have to provide actual evidence of success in treating humans where such a utility is asserted. Instead, as the courts have repeatedly held, all that is required is a reasonable correlation between the activity and the asserted use. *Nelson v. Bowler*, 626 F.2d 853, 857, 206 USPQ 881, 884 (CCPA 1980).

II. STRUCTURAL SIMILARITY TO COMPOUNDS WITH ESTABLISHED UTILITY

Courts have routinely found evidence of structural similarity to a compound known to have a particular therapeutic or pharmacological utility as being supportive of an assertion of therapeutic utility for a new compound. In *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980), the claimed compounds were found to have utility based on a finding of a close structural relationship to daunorubicin and doxorubicin and shared pharmacological activity with those compounds, both of which were known to be

useful in cancer chemotherapy. The evidence of close structural similarity with the known compounds was presented in conjunction with evidence demonstrating substantial activity of the claimed compounds in animals customarily employed for screening anticancer agents. Such evidence should be given appropriate weight in determining whether one skilled in the art would find the asserted utility credible. Office personnel should evaluate not only the existence of the structural relationship, but also the reasoning used by the applicant or a declarant to explain why that structural similarity is believed to be relevant to the applicant's assertion of utility.

III. DATA FROM *IN VITRO* OR ANIMAL TESTING IS GENERALLY SUFFICIENT TO SUPPORT THERAPEUTIC UTILITY

If reasonably correlated to the particular therapeutic or pharmacological utility, data generated using *in vitro* assays, or from testing in an animal model or a combination thereof almost invariably will be sufficient to establish therapeutic or pharmacological utility for a compound, composition or process. A cursory review of cases involving therapeutic inventions where 35 U.S.C. 101 was the dispositive issue illustrates the fact that the Federal courts are not particularly receptive to rejections under 35 U.S.C. 101 based on inoperability. Most striking is the fact that in those cases where an applicant supplied a reasonable evidentiary showing supporting an asserted therapeutic utility, almost uniformly the 35 U.S.C. 101-based rejection was reversed. See, e.g., *In re Brana*, 51 F.3d 1560, 34 USPQ 1436 (Fed. Cir. 1995); *Cross v. Iizuka*, 753 F.2d 1040, 224 USPQ 739 (Fed. Cir. 1985); *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *Nelson v. Bowler*, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980); *In re Malachowski*, 530 F.2d 1402, 189 USPQ 432 (CCPA 1976); *In re Gaubert*, 530 F.2d 1402, 189 USPQ 432 (CCPA 1975); *In re Gazave*, 379 F.2d 973, 154 USPQ 92 (CCPA 1967); *In re Hartop*, 311 F.2d 249, 135 USPQ 419 (CCPA 1962); *In re Krimmel*, 292 F.2d 948, 130 USPQ 215 (CCPA 1961). Only in those cases where the applicant was unable to come forward with any relevant evidence to rebut a finding by the Office that the claimed invention was inoperative was a 35 U.S.C. 101 rejection affirmed by the court. *In re Citron*, 325 F.2d 248, 253, 139 USPQ 516, 520 (CCPA 1963) (therapeutic utility for an

uncharacterized biological extract not supported or scientifically credible); *In re Buting*, 418 F.2d 540, 543, 163 USPQ 689, 690 (CCPA 1969) (record did not establish a credible basis for the assertion that the single class of compounds in question would be useful in treating disparate types of cancers); *In re Novak*, 306 F.2d 924, 134 USPQ 335 (CCPA 1962) (claimed compounds did not have capacity to effect physiological activity upon which utility claim based). Contrast, however, *In re Buting* to *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973), *reh'g denied*, 480 F.2d 879 (CCPA 1973), in which the court held that utility for a genus was found to be supported through a showing of utility for one species. In no case has a Federal court required an applicant to support an asserted utility with data from human clinical trials.

If an applicant provides data, whether from *in vitro* assays or animal tests or both, to support an asserted utility, and an explanation of why that data supports the asserted utility, the Office will determine if the data and the explanation would be viewed by one skilled in the art as being reasonably predictive of the asserted utility. See, e.g., *Ex parte Maas*, 9 USPQ2d 1746 (Bd. Pat. App. & Inter. 1987); *Ex parte Balzarini*, 21 USPQ2d 1892 (Bd. Pat. App. & Inter. 1991). Office personnel must be careful to evaluate all factors that might influence the conclusions of a person of ordinary skill in the art as to this question, including the test parameters, choice of animal, relationship of the activity to the particular disorder to be treated, characteristics of the compound or composition, relative significance of the data provided and, most importantly, the explanation offered by the applicant as to why the information provided is believed to support the asserted utility. If the data supplied is consistent with the asserted utility, the Office cannot maintain a rejection under 35 U.S.C. 101.

Evidence does not have to be in the form of data from an art-recognized animal model for the particular disease or disease condition to which the asserted utility relates. Data from any test that the applicant reasonably correlates to the asserted utility should be evaluated substantively. Thus, an applicant may provide data generated using a particular animal model with an appropriate explanation as to why that data supports the asserted utility. The absence of a

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MANUAL OF PATENT EXAMINING PROCEDURE

certification that the test in question is an industry-accepted model is not dispositive of whether data from an animal model is in fact relevant to the asserted utility. Thus, if one skilled in the art would accept the animal tests as being reasonably predictive of utility in humans, evidence from those tests should be considered sufficient to support the credibility of the asserted utility. *In re Hartop*, 311 F.2d 249, 135 USPQ 419 (CCPA 1962); *In re Krimmel*, 292 F.2d 948, 953, 130 USPQ 215, 219 (CCPA 1961); *Ex parte Krepelka*, 231 USPQ 746 (Bd. Pat. App. & Inter. 1986). Office personnel should be careful not to find evidence unpersuasive simply because no animal model for the human disease condition had been established prior to the filing of the application. See *In re Chilowsky*, 229 F.2d 457, 461, 108 USPQ 321, 325 (CCPA 1956) (“The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it.”); *In re Woody*, 331 F.2d 636, 639, 141 USPQ 518, 520 (CCPA 1964) (“It appears that no one on earth is certain as of the present whether the process claimed will operate in the manner claimed. Yet absolute certainty is not required by the law. The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it.”).

IV. HUMAN CLINICAL DATA

Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an invention related to treatment of human disorders (see *In re Isaacs*, 347 F.2d 889, 146 USPQ 193 (CCPA 1963); *In re Langer*, 503 F.2d 1380, 183 USPQ 288 (CCPA 1974)), even with respect to situations where no art-recognized animal models existed for the human disease encompassed by the claims. *Ex parte Balzarini*, 21 USPQ2d 1892 (Bd. Pat. App. & Inter. 1991) (human clinical data is not required to demonstrate the utility of the claimed invention, even though those skilled in the art might not accept other evidence to establish the efficacy of the claimed therapeutic compositions and the operativeness of the claimed methods of treating humans). Before a drug can enter

human clinical trials, the sponsor, often the applicant, must provide a convincing rationale to those especially skilled in the art (e.g., the Food and Drug Administration) that the investigation may be successful. Such a rationale would provide a basis for the sponsor’s expectation that the investigation may be successful. In order to determine a protocol for phase I testing, the first phase of clinical investigation, some credible rationale of how the drug might be effective or could be effective would be necessary. Thus, as a general rule, if an applicant has initiated human clinical trials for a therapeutic product or process, Office personnel should presume that the applicant has established that the subject matter of that trial is reasonably predictive of having the asserted therapeutic utility.

V. SAFETY AND EFFICACY CONSIDERATIONS

The Office must confine its review of patent applications to the statutory requirements of the patent law. Other agencies of the government have been assigned the responsibility of ensuring conformance to standards established by statute for the advertisement, use, sale or distribution of drugs. The FDA pursues a two-prong test to provide approval for testing. Under that test, a sponsor must show that the investigation does not pose an unreasonable and significant risk of illness or injury and that there is an acceptable rationale for the study. As a review matter, there must be a rationale for believing that the compound could be effective. If the use reviewed by the FDA is not set forth in the specification, FDA review may not satisfy 35 U.S.C. 101. However, if the reviewed use is one set forth in the specification, Office personnel must be extremely hesitant to challenge utility. In such a situation, experts at the FDA have assessed the rationale for the drug or research study upon which an asserted utility is based and found it satisfactory. Thus, in challenging utility, Office personnel must be able to carry their burden that there is no sound rationale for the asserted utility even though experts designated by Congress to decide the issue have come to an opposite conclusion. “FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws.” *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995) (citing *Scott*

v. *Finney*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed. Cir. 1994)).

Thus, while an applicant may on occasion need to provide evidence to show that an invention will work as claimed, it is improper for Office personnel to request evidence of safety in the treatment of humans, or regarding the degree of effectiveness. See *In re Sichert*, 566 F.2d 1154, 196 USPQ 209 (CCPA 1977); *In re Hartop*, 311 F.2d 249, 135 USPQ 419 (CCPA 1962); *In re Anthony*, 414 F.2d 1383, 162 USPQ 594 (CCPA 1969); *In re Watson*, 517 F.2d 465, 186 USPQ 11 (CCPA 1975); *In re Krimmel*, 292 F.2d 948, 130 USPQ 215 (CCPA 1961); *Ex parte Jovanovics*, 211 USPQ 907 (Bd. Pat. App. & Inter. 1981).

VI. TREATMENT OF SPECIFIC DISEASE CONDITIONS

Claims directed to a method of treating or curing a disease for which there have been no previously successful treatments or cures warrant careful review for compliance with 35 U.S.C. 101. The credibility of an asserted utility for treating a human disorder may be more difficult to establish where current scientific understanding suggests that such a task would be impossible. Such a determination has always required a good understanding of the state of the art as of the time that the invention was made. For example, prior to the 1980's, there were a number of cases where an asserted use in treating cancer in humans was viewed as "incredible." *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Buting*, 418 F.2d 540, 163 USPQ 689 (CCPA 1969); *Ex parte Stevens*, 16 USPQ2d 1379 (Bd. Pat. App. & Inter. 1990); *Ex parte Busse*, 1 USPQ2d 1908 (Bd. Pat. App. & Inter. 1986); *Ex parte Krepelka*, 231 USPQ 746 (Bd. Pat. App. & Inter. 1986); *Ex parte Jovanovics*, 211 USPQ 907 (Bd. Pat. App. & Inter. 1981). The fact that there is no known cure for a disease, however, cannot serve as the basis for a conclusion that such an invention lacks utility. Rather, Office personnel must determine if the asserted utility for the invention is credible based on the information disclosed in the application. Only those claims for which an asserted utility is not credible should be rejected. In such cases, the Office should carefully review what is being claimed by the applicant. An assertion that the claimed invention is useful in treating a symptom of an incurable disease may be considered credible by a person of ordinary

skill in the art on the basis of a fairly modest amount of evidence or support. In contrast, an assertion that the claimed invention will be useful in "curing" the disease may require a significantly greater amount of evidentiary support to be considered credible by a person of ordinary skill in the art. *In re Sichert*, 566 F.2d 1154, 196 USPQ 209 (CCPA 1977); *In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980). See also *Ex parte Ferguson*, 117 USPQ 229 (Bd. Pat. App. & Inter. 1957).

In these cases, it is important to note that the Food and Drug Administration has promulgated regulations that enable a party to conduct clinical trials for drugs used to treat life threatening and severely-debilitating illnesses, even where no alternative therapy exists. See 21 CFR 312.80-88 (1994). Implicit in these regulations is the recognition that experts qualified to evaluate the effectiveness of therapeutics can and often do find a sufficient basis to conduct clinical trials of drugs for incurable or previously untreatable illnesses. Thus, affidavit evidence from experts in the art indicating that there is a reasonable expectation of success, supported by sound reasoning, usually should be sufficient to establish that such a utility is credible.

2111 Claim Interpretation; Broadest Reasonable Interpretation [R-5]

CLAIMS MUST BE GIVEN THEIR BROADEST REASONABLE INTERPRETATION

During patent examination, the pending claims must be "given their broadest reasonable interpretation consistent with the specification." >The Federal Circuit's *en banc* decision in *Phillips v. AWH Corp.*, 415 F.3d 1303, 75 USPQ2d 1321 (Fed. Cir. 2005) expressly recognized that the USPTO employs the "broadest reasonable interpretation" standard:

The Patent and Trademark Office ("PTO") determines the scope of claims in patent applications not solely on the basis of the claim language, but upon giving claims their broadest reasonable construction "in light of the specification as it would be interpreted by one of ordinary skill in the art." *In re Am. Acad. of Sci. Tech. Ctr.*, 367 F.3d 1359, 1364[, 70 USPQ2d 1827] (Fed. Cir. 2004). Indeed, the rules of the PTO require that application claims must "conform to the invention as set forth in the remainder of the specification and the terms and phrases used in the claims must find clear support or antecedent basis in the description so that the meaning of the terms in the claims may be ascertainable by reference to the description." 37 CFR 1.75(d)(1).

EXHIBIT 7

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POLICY

The editors have asked selected members of the scientific community to respond to the Policy commentary by J. Doll and the [Review](#) by M. Heller and R. Eisenberg. Their remarks are available at www.sciencemag.org/feature/data/980465.shl

BIOTECHNOLOGY: The Patenting of DNA

[John J. Doll](#)

In the past two decades, there has been an explosion of innovative growth in the field of biotechnology. This growth has resulted in many new products and methodologies that are useful in agriculture, environmental biotechnology, food technology, and the diagnostics and pharmaceutical industries. Other results are new areas of research and development in genomics and bioinformatics. The Human Genome Project is a global coordinated effort to characterize human genetic material and provide a complete human DNA sequence library by 2005. Even though the project is not yet complete, a vast amount of useful DNA sequence information has already been gathered, including sequences of genes and their regulatory regions and genome markers such as expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs) (1).

The United States Patent and Trademark Office (USPTO) recognizes that many people in the biotechnology community are concerned with the possible impact of patents granted for DNA-related inventions (2) on research and innovation in biomedical research and technology. Some in the biotechnology community are concerned that patents on ESTs or SNPs may impede cooperation among laboratories and limit the ready availability of data and materials to researchers. Public access to such sequence data has been the subject of much debate. Several bioinformatics companies are building proprietary sequence databases. On the other hand, some pharmaceutical companies, as well as the National Institutes of Health (NIH), are creating public databases of sequence information to ensure public access to such information. Some critics have even suggested that

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patents should not be granted for these new discoveries and that a new form of intellectual property protection is needed.

However, in the USPTO's view, new areas of technology do not create the need for a whole new specialized patent law. In many ways, the arguments currently being used for DNA sequence technology resemble those voiced 30 to 40 years ago when polymer chemistry was an emerging technology. At that time, people argued that if broad generic claims were granted on the building blocks of basic polymers, it would devastate the industry. In fact, no such disaster occurred. For example, the issuing in 1965 of a basic patent broadly claiming a vulcanizable copolymer of aliphatic mono-olefins and unsaturated bridged-ring hydrocarbons (3) did not preclude the later issuing of patents to different inventors for several copolymers of this type (4). These patents represent early examples of ethylene-propylene-diene monomer (EPDM) rubbers, which are highly weather- and ozone-resistant, stable to thermal aging, and have good electrical insulating properties. These EPDM rubbers have been commercially important as components in tires, weather stripping, radiator hoses, wire insulation, impact modifiers, and roofing.

EPDM copolymers were assembled from three basic building blocks that could be combined in many different ways and, as such, generic and specific claims to these copolymers are analogous to claims that may be issued to DNA inventions. Just as the issuing of broad product claims at the early stages of this technology did not deter development of other new vulcanizable copolymers, the issuing of relatively broad claims in genomic technology should not deter inventions in genomics. Two relevant examples of this in the field of biotechnology are the polymerase chain reaction (PCR) and the human immunodeficiency virus (HIV) protease, which were patented and then widely licensed to permit the biotech industry to continue to grow and benefit from these inventions.

The same patentability analysis is conducted for every patent application, regardless of whether the application is for a computer chip, a mechanical apparatus, a pharmaceutical, or a piece of DNA. In every field of technology--whether emerging, complex, or competitive--all the conditions for patentability (such as statutory subject matter utility, enablement, written description, novelty, and non-obviousness) must be met before a claim is allowed (5).

In applying existing patent law to DNA sequence inventions, a first area of concern is whether such inventions constitute patentable subject matter. As DNA sequences are typically isolated and purified manufactures or compositions of matter under U.S. law; in other words, products of human ingenuity "having a distinctive name, character, [and] use" (6) (see Figure), they are patentable subject matter in the United States. In order for DNA sequences to be distinguished from their naturally occurring counterparts, which cannot be patented, the patent application must state that the invention has been purified or isolated or is part of a recombinant molecule or is now part of a vector.

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Product of Human Ingenuity. This patent was issued to Chakrabarty following the Supreme Court decision, *Diamond v. Chakrabarty* (6), in which the patentability of a living bacterium, genetically engineered to break down crude oil, was affirmed.

Although some SNPs and ESTs may not directly identify genes, they may still be extremely useful and thus satisfy the utility requirement. SNPs and ESTs may have specific utilities that are separate and distinct from the genes to which they correspond. For example, SNPs can be used to trace ancestry or parentage. ESTs can be used for chromosome identification and gene mapping. Both can be used to identify genes that contribute to predisposition to disease.

Claims to DNA elements useful for forensic identification, the identification of tissue type or origin, chromosome mapping, chromosome identification, or tagging of a gene of known and useful function must fulfill the enablement requirement. For any invention, enablement is satisfied when, by reading the patent application, an individual who has skill in the technology would have been able to make and use the invention as intended without undue experimentation.

In fact, it is common for the patentability of DNA elements to hinge on whether sufficient information has been given to enable at least one credible or specific utility. Examples of potentially non-enabled utilities for a DNA sequence fragment include its use to locate disease-associated genes when the disease has no known genetic origin; as an antisense reagent when the corresponding protein to be suppressed is unknown; as a triplex probe to inhibit expression of a protein when the protein and its function are unknown; and to locate and identify genes of unknown utility.

An area of patent law that is still developing relates to the kind of information that must be included in the patent application of a biotechnology-related invention in order to sufficiently identify and distinguish its characteristics from other subject matter (in other words, satisfaction of the written description requirement). In the case of the *Regents of the University of California v. Eli Lilly* (7), the court held that in order to claim a specific DNA sequence, such as the human DNA encoding insulin, more is required than a mere statement that it is part of the invention, plus a fragment of the claimed nucleic acid, plus a reference to a potential method of isolating the entire sequence. As a result of the Lilly case and several earlier cases (8), the USPTO is preparing interim examination guidelines for determining compliance with the written description requirement that should be made available for public comment within the next 3 months.

There has been considerable debate and discussion over how the issuance of a patent on DNA fragments of a gene will affect the patenting of full-length genes. The USPTO views this situation as analogous to having a patent on a picture tube. The picture tube patent does not preclude someone else from obtaining a patent on a television set. However, the holder of the picture tube patent could sue the television set makers for patent infringement if they use the patented picture tube without obtaining a license.

In a second example, a patent might be granted for compound X, which is disclosed to have a specific use (such as a headache remedy). If other investigators find that X has a new and unexpected use, perhaps in combination with compound Y, for treatment of heart arrhythmias, they may have to obtain a license from the individual who first patented compound X

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in order to sell XY.

In summary, once a product is patented, that patent extends to any use, even those that have not been disclosed in the patent. A future nonobvious method of using that product may be patentable, but the first patent would have been dominant.

For DNA to be patentable, it must be novel and nonobvious in light of structurally related DNA or RNA information taught in nonpatent literature or suggested by prior patents. To be considered nonobvious, the invention must have been compared to what was known previously and be judged not to be obvious to someone of ordinary skill working in the field. Because of this, patent claims limited in scope to a specific novel and nonobvious SNP or EST (for example, for forensic identification) would not necessarily preclude the future patenting of the corresponding full-length gene of known function discovered later. The granting of comprehensive claims to downstream DNA products such as full-length genes or to ultimate proteins is unlikely in the absence of a significant amount of information about the gene and protein being disclosed in the patent application.

Two specific examples may be helpful. A patent is granted to a large fragment of DNA, within which exists a gene of great medical interest, even though the location of the open reading frame with the fragment has not been determined. The person who actually discovers and isolates the gene may also be able to receive a patent. Alternatively, many patented DNA fragments such as ESTs or SNPs may be isolated that turn out to be part of the same gene. In both cases, the second patent holder may have to obtain licenses from or pay fees to the primary patent holder but is not prevented from obtaining the second patent.

If the invention has been described in a patent or printed publication anywhere in the world, or if it has been in public use or on sale in the United States for more than 1 year before the date on which an application for patent is filed in the United States, a patent cannot be obtained. Thus, any SNPs or ESTs that have been available in a public database for more than 1 year prior to the filing date of the application cannot be patented. If an SNP is published less than a year before the patent application is filed and the inventor (who was not one of the authors) can show that he or she invented the SNP before the publication date, the SNP may still be patentable.

Without the incentive of patents, there would be less investment in DNA research, and scientists might not disclose their new DNA products to the public. Issuance of patents to such products not only results in the dissemination of technological information to the scientific community for use as a basis for further research but also stimulates investment in the research, development, and commercialization of new biologics. It is only with the patenting of DNA technology that some companies, particularly small ones, can raise sufficient venture capital to bring beneficial products to the marketplace or fund further research. A strong U.S. patent system is critical for the continued development and dissemination to the public of information on DNA sequence elements.

REFERENCES AND NOTES

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3. U.S. Patent No. 3,211,709, filed 14 July 1958 by Adamek *et al.* and

assigned to Hercules Powder Company.

4. U.S. Patent No. 3,527,739, issued 8 September 1970 to Valvassori *et al.* and assigned to Montecatini Edison; U.S. Patent No. 3,531,447, issued 29 September 1970 to Gumboldt *et al.* and assigned to Farbwerke Hoechst Aktiengesellschaft.
5. Title 35 of the United States Code, sections 101, 102, 103, and 112.
6. *Diamond v. Chakrabarty*, 447 U.S. 303, 310, 206 USPQ 193, 197 (1980).
7. *Regents of the University of California v. Eli Lilly and Co.*, 119 F.3 1559, 43 USPQ2d 1398 (Fed. Cir. 1997).
8. *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed. Cir. 1993); *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991).
9. The author acknowledges the contributions of S. G. Kunin, N. J. Linck, R. A. Schwartz, M. M. Parr, R. J. Hill, and S. A. Chambers.

The author is director, Biotechnology Examination, Technology Center 1600, U.S. Patent and Trademark Office, Washington, DC 20231, USA.

THIS ARTICLE HAS BEEN CITED BY OTHER ARTICLES:

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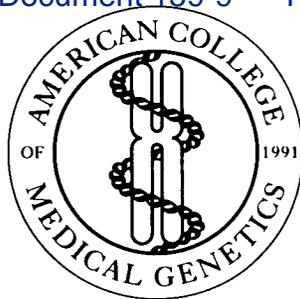
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RE: * Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 "Written Description" Requirement (64 FR 71427)

*Revised Interim Utility Examination Guidelines (64 FR 71440)

I am writing as the President of the American College of Medical Genetics (ACMG), representing over 1,100 clinical and laboratory geneticists in the U.S. These include doctoral trained clinical geneticists, as well as molecular, biochemical and cytogenetics laboratory directors certified by the only specialty level board of the American Board of Medical Specialties (ABMS) in this field, the American Board of Medical Genetics (ABMG). Our members are involved in clinical genetics and genetic research and the translation of genomic knowledge into clinical services, as well as providing genetics laboratory services considered standard of care in the field. I am writing in response to the above guidelines that appeared in the Federal Register on December 21, 1999.

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The members of the ACMG are deeply concerned about the patenting of human genes and fragments, and the enforcement strategies of sequence patent holders and licensees, which are limiting public access

Commissioner of Patents and Trademarks

Attn: Mark Nagumo

March 20, 2000

Page Two.

to important clinical services. Indeed, we have difficulty understanding of how or why patent law has been interpreted to permit naturally occurring sequences, which are uniquely fundamental information to be patented. Such a view has led to patenting of discoveries rather than inventions. However, our comments today are directed at the "Revised Interim Utility Examination Guidelines" and the "Written Description Requirement".

Initially, we commend the Patent and Trademark Office (PTO) for recognizing the need to raise considerably the utility requirements by which determinations of patentability are made. We strongly oppose patents for gene fragments such as expressed sequence tags (ESTs), sequence tagged sites (STSs) and, more recently, single nucleotide polymorphisms (SNPs). These fragments are of use only in the search for complete genes and, as such, are research tools which should be widely available and accessible and not encumbered by patents. We also appreciate the proposal to enhance the requirements for specific, substantial and credible utility claims.

In order to more fully understand our concerns, it is important to recognize the rapid pace at which this field is moving and the speed at which new knowledge renders previous knowledge obsolete. Our members have been at the forefront of the development of gene mapping and genetic technology for the past 40 years thus, we have a unique investment in this issue. Our specific comments on the utility and written description proposal follow.

Revised Utility Guidelines: ACMG believes that there must be specific, substantial, and credible claims for utility. We do not believe that presumed theoretical function based on homology searches is sufficient to claim specific and substantial utility. Frequently, genes have numerous functional domains and knowledge of one or a few can be misleading or incorrect as to the actual role of the gene in question. As such, gene sequences are merely research tools, which ultimately allow for determinations of actual function and, thereby, the invention, to be made; however, the tools are not, themselves, inventions. Unfortunately, thousands of patents are pending for sequences whose presumed function is based on little more than computer searches of regions of homology and we do not believe that such blind searches, lacking both creativity and scientific knowledge, should lead to patentable material. It is noteworthy that such "research" is no longer considered worthy of doctoral level degree awards since there is virtually no novelty involved in such projects.

The PTO must decide the criteria to be used to determine whether or not a DNA sequence meets the standard for specific, substantial and credible utility. The ACMG

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Page Three.

agrees with the NIH (as expressed in a letter of December 21, 1999 from Drs. Varmus and Collins to Commissioner Dickenson) that the revised standard should not be interpreted to embrace claims of a "predicted" function for a gene or its encoded protein, based only on sequence homology with other proteins and genes.

Among those practicing in this area, views of substantial, specific and credible utility change on a weekly basis. This is not surprising given the enormous international investment in genomics. It will be important that the PTO remain apace with the sense of those skilled in this art to avoid a morass of competing claims and the cross-licensing nightmare of our time.

Written Description Guidelines: Our concerns with the descriptions of the patented entity are focused on claims language, which generalizes the claims in order to broaden their scope. These claims should be dealt with in a manner similar to that for chemicals. As such, the descriptions should include complete and unaltered sequence information and claims should not extend beyond that described. Recognition of overbroad claims for gene or genomic fragments would lead to yet another morass of competing claims against those who perform the actual science required to state a complete gene sequence. Those with claims on partial sequence information as is the case for ESTs and other fragments should not be able to exercise superiority over the full gene sequence patent holder.

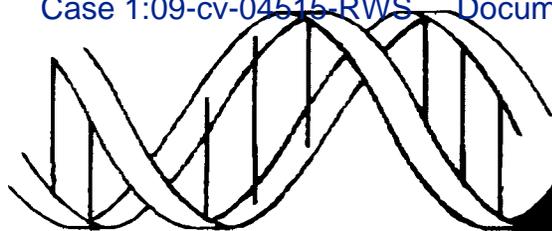
We appreciate the opportunity to comment on the proposed changes to the review of gene patent claims and agree that such claims should be based on thorough understanding of the structure and function of the claimed sequence. We recognize the great responsibility that the Patent and Trademark Office has in ensuring intellectual property protection for inventions involving gene-based discoveries. We are pleased that the PTO is considering changes to its guidelines and we hope that the feedback from our group, and others in the scientific community, will be helpful in developing new guidelines. Please let us know if our group can be of further assistance in this important project.

Sincerely,

A handwritten signature in black ink, appearing to read "R. Rodney Howell". The signature is fluid and cursive, written over a white background.

R. Rodney Howell, M.D.
President

EXHIBIT 9



AMP

ASSOCIATION FOR MOLECULAR PATHOLOGY

9650 Rockville Pike, Bethesda, Maryland 20814-3993 • Telephone (301) 571-1880 Fax (301) 571-1879

Commissioner of Patents and Trademarks
Box 8
Washington, DC 20231

Attn: Mark Nagumo
Fax: (703) 305-9373

March 17, 2000

Dear Commissioner of Patents and Trademarks,

The Association for Molecular Pathology would like to provide brief comments on the Patent and Trademark Office's (PTO) Revised Interim Utility Examination Guidelines that affect the patentability of genetic sequence data. The Association for Molecular Pathology (AMP) is a society of medical professionals engaged in the practice of laboratory-based human molecular diagnostics and translational research in molecular pathology, molecular medicine, and molecular genetics. Our more than 500 members are primarily M.D. and/or Ph.D. diagnostic laboratory directors or are doing translational research or developmental diagnostics in this field. Therefore, the patenting of genetic sequences is of great concern and interest to our members.

The increased stringency of the new utility requirement, to require documentation by the applicant of specific and substantial utility that is credible, is an improvement from the previous guidelines. The new guidelines will prevent the patenting of sequences, such as expressed sequence tags (ESTs), without knowledge of the function of the encoded protein. This will prevent reach through rights to future patents that do demonstrate function and utility of the same sequence, and potentially limit the stacking of patent licenses required for individuals seeking to use the information for research or medical applications. Therefore, we are pleased with the new guidelines.

However, the new guidelines will not solve the current problem in genetic diagnostics that gene sequence patents are creating, since the molecular diagnostic community uses and will continue to use the gene sequences with clearly demonstrated medical utility. The limitation that genetic sequence patents are placing on the clinical application of this human genome information is not in the public's best interest. While AMP understands that the PTO does not set the laws which govern its practice, we feel strongly that the PTO should understand the restraints that gene patents are placing on the clinical practice of molecular diagnostics.

Thank you for providing us the opportunity to share with you our concerns on this matter.

Sincerely,

Debra G.B. Leonard, M.D., Ph.D.
President

<i>President</i> Debra G.B. Leonard	<i>President-elect</i> Karl V. Voelkerding	<i>Past President</i> Mark E. Sobel	<i>Secretary-Treasurer</i> Cathie Leindecker-Foster
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<i>Training & Education Committee Chair</i> Anthony A. Killeen		<i>Executive Officer</i> Frances A. Pitlick	

EXHIBIT 10

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ACLM/"isolated DNA": 2645 patents.

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Refine Search

PAT. NO.	Title
1	7,632,935 T DNA encoding a mast cell-derived membrane protein
2	7,622,631 T Methods for enhancing plant resistance to pathogens
3	7,622,565 T Nucleic acids encoding B7-H4, a T cell immunoregulatory molecule
4	7,619,135 T Seed-specific promoter from the rice glutelin GluB-4 gene and uses thereof
5	7,618,773 T Headloop DNA amplification
6	7,615,678 T Methods for using artificial polynucleotides and compositions thereof to reduce transgene silencing
7	7,611,837 T Kit for detecting non-pathogenic or pathogenic influenza a subtype h5 virus
8	7,605,303 T Stress-responsive root-specific genes
9	7,605,248 T Recombinant constructs of Borrelia burgdorferi
10	7,598,368 T COX5c-1 gene intron for increasing expression level in cassettes, plant cells and transgenic plants
11	7,598,366 T TaBV transcriptional control elements, chimeric constructs and uses therefor
12	7,598,365 T Targeted DNA insertion in plants
13	7,598,066 T Gene encoding vitamin b.sub.6 phosphate phosphatase and use thereof
14	7,595,384 T Seed-specific gene promoter from the rice 10 KDa prolaminin gene and uses thereof
15	7,595,383 T Secreted proteins of Mycobacterium tuberculosis and their use as vaccines and diagnostic reagents
16	7,592,509 T Isolated DNA sequences and polypeptides inducing multiple resistance of plants to phytopathogens and pests
17	7,592,438 T Human gene

- 18 [7,589,186](#) **T** [Isolated hOAT polynucleotide](#)
 - 19 [7,586,023](#) **T** [Methods of conferring ppo-inhibiting herbicide resistance to plants by gene manipulation](#)
 - 20 [7,585,969](#) **T** [MicroRNA and methods for inhibiting same](#)
 - 21 [7,585,495](#) **T** [Method for identifying shampoo-resistant hair-binding peptides and hair benefit agents therefrom](#)
 - 22 [7,582,809](#) **T** [Sorghum aluminum tolerance gene, SbMATE](#)
 - 23 [7,582,447](#) **T** [DNA encoding a testicular carnitine transporter](#)
 - 24 [7,582,437](#) **T** [Adaptor protein that binds to mammalian toll-like receptor 3, and gene thereof](#)
 - 25 [7,569,351](#) **T** [P53 dependent apoptosis-associated gene and protein](#)
 - 26 [7,563,945](#) **T** [Plant regulatory sequences for selective control of gene expression](#)
 - 27 [7,557,264](#) **T** [Gossypium hirsutum tissue-specific promoters and their use](#)
 - 28 [7,557,197](#) **T** [Human soluble neuropilin-1 primary polyadenylation signal and uses thereof](#)
 - 29 [7,556,921](#) **T** [Methods for mapping signal transduction pathways to gene expression programs](#)
 - 30 [7,553,946](#) **T** [Promoters](#)
 - 31 [7,553,638](#) **T** [Candida utilis containing .gamma.-glutamylcysteine](#)
 - 32 [7,547,528](#) **T** [Fluorescent protein and chromoprotein](#)
 - 33 [7,544,795](#) **T** [Recombinant hexose oxidase, a method of producing same and use of such enzyme](#)
 - 34 [7,544,483](#) **T** [Method for the production of protamine](#)
 - 35 [7,541,491](#) **T** [Gene involved in growth-promoting function of acetic acid bacteria and uses thereof](#)
 - 36 [7,541,451](#) **T** [Fluorescent proteins from Fungia](#)
 - 37 [7,541,449](#) **T** [Human kunitz-type inhibitors and methods relating thereto](#)
 - 38 [7,541,172](#) **T** [Transgenic amorpho-4, 11-diene synthesis](#)
 - 39 [7,534,938](#) **T** [Synthetic nucleic acid molecule for imparting multiple traits](#)
 - 40 [7,527,946](#) **T** [Interferon-beta-1a-immunoglobulin fusion proteins and uses](#)
 - 41 [7,524,669](#) **T** [Transgenic Saccharomyces cerevisiae and method for bioremediation](#)
 - 42 [7,521,222](#) **T** [Farnesyl dibenzodiazepinone and processes for its production](#)
 - 43 [7,521,182](#) **T** [Selection system containing non-antibiotic resistance selection marker](#)
 - 44 [7,517,969](#) **T** [Process for isolating nucleic acid with chaotrope agents and ammonium compounds](#)
 - 45 [7,517,677](#) **T** [Recombinant D-amino acid oxidases](#)
 - 46 [7,514,544](#) **T** [Corn event TC1507 and methods for detection thereof](#)
 - 47 [7,514,211](#) **T** [Genomic DNAs participating in rheumatoid arthritis, method of diagnosing the same, method of judging onset risk and diagnostic kit for detecting the same](#)
 - 48 [7,511,126](#) **T** [Position-independent and tissue specific expression of a transgene in milk of transgenic animals](#)
 - 49 [7,504,491](#) **T** [Pigment protein from Cnidopus japonicus](#)
 - 50 [7,501,559](#) **T** [Prevention of Bt resistance development](#)
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ACLM/"isolated DNA": 2645 patents.

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Refine Search

PAT. NO.	Title
51	7,501,555 T Method for obtaining a plant with a lasting resistance to a pathogen
52	7,501,262 T Promoters and gene expression method by using the promoters
53	7,501,123 T Human G-protein chemokine receptor (CCR5) HDGNR10
54	7,498,429 T AHAS small subunit promoter
55	7,498,150 T Materials and methods to increase peptide chain expression
56	7,498,149 T Proteins having effects of controlling cell migration and cell death
57	7,485,714 T Transcription factor having zinc finger domain
58	7,485,463 T Nucleic acids encoding mirafiori lettuce virus proteins and utilization thereof
59	7,485,456 T Modulators of TNF receptor associated factor (TRAF), their preparation and use
60	7,482,509 T Transgenic plants carrying neoxanthin cleavage enzyme gene
61	7,482,157 T Monacolin K biosynthesis genes
62	7,479,386 T HXHV virus, nucleic material, peptide material and uses
63	7,476,730 T Sulfotransferase and DNA encoding the enzyme
64	7,476,539 T DNA containing variant FRT sequences
65	7,470,528 T DNA encoding hydroxylase
66	7,465,792 T Fungi non-LTR retrotransposons and methods related thereto
67	7,465,541 T Vitamin D.sub.3-responsive sequences located 5'-upstream of p27/.sup.kip 1 gene and methods of screening for pharmaceutical agents using the sequences

- 68 [7,462,466](#) **T** [Porcine CD59 nucleic acids and cells containing the same](#)
 - 69 [7,459,308](#) **T** [Nucleic acid molecule encoding a CLASP-2 transmembrane protein](#)
 - 70 [7,459,153](#) **T** [Viral vectors for gene therapy](#)
 - 71 [7,456,271](#) **T** [Glutamate 2,3-aminomutases and methods of use thereof](#)
 - 72 [7,449,568](#) **T** [Alga-origin promoter, intron and terminator](#)
 - 73 [7,446,192](#) **T** [Gene participating in acetic acid tolerance, acetic acid bacteria bred using the gene, and process for producing vinegar with the use of the acetic acid bacteria](#)
 - 74 [7,446,187](#) **T** [Plasmids and utilization thereof](#)
 - 75 [7,446,099](#) **T** [Compositions and methods for biodegradable polymer-peptide mediated transfection](#)
 - 76 [7,445,915](#) **T** [Mutant isopropylmalate isomerase](#)
 - 77 [7,442,530](#) **T** [Process for the production of L-amino acids using strains of the Enterobacteriaceae family which contain an enhanced fadR or iclR gene](#)
 - 78 [7,442,510](#) **T** [Method of identifying hairpin DNA probes by partial fold analysis](#)
 - 79 [7,439,038](#) **T** [Method for producing L-amino acid using methylotroph](#)
 - 80 [7,435,807](#) **T** [Corn event TC1507 and methods for detection thereof](#)
 - 81 [7,435,584](#) **T** [L-lysine-producing corynebacteria and process for the preparation of L-lysine](#)
 - 82 [7,435,571](#) **T** [Microbial production of COQ10](#)
 - 83 [7,432,365](#) **T** [DNA molecules encoding beta clamp proteins of gram positive bacteria](#)
 - 84 [7,432,089](#) **T** [DNA encoding favin-adenine-dinucleotide-dependent-D-erythronate-4-phosphate-de-hydroge- nase, pdxR, and microbial production of vitamin B.sub.6](#)
 - 85 [7,432,078](#) **T** [Estrogen receptor genes and utilization thereof](#)
 - 86 [7,429,657](#) **T** [DNA molecules encoding enzymes involved in starch synthesis, vectors, bacteria, transgenic plant cells and plants containing these molecules](#)
 - 87 [7,429,648](#) **T** [IREN protein, its preparation and use](#)
 - 88 [7,427,677](#) **T** [Expression of zebrafish bone morphogenetic protein 4](#)
 - 89 [7,427,604](#) **T** [DNA encoding an antigenic protein of Eimeria apical membrane antigen 1 and use thereof](#)
 - 90 [7,420,101](#) **T** [Nucleic acid sequences to proteins involved in tocopherol synthesis](#)
 - 91 [7,419,800](#) **T** [Estrogen receptor genes](#)
 - 92 [7,417,180](#) **T** [Genes for increasing grain yield and uses thereof](#)
 - 93 [7,417,132](#) **T** [Corn event TC1507 and methods for detection thereof](#)
 - 94 [7,416,877](#) **T** [Receptor that causes cell death and recombinant production thereof](#)
 - 95 [7,416,871](#) **T** [Thermo-stable lactate oxidase](#)
 - 96 [7,413,895](#) **T** [DNA encoding neutral amino acid transporter](#)
 - 97 [7,413,887](#) **T** [Trichoderma reesei glucoamylase and homologs thereof](#)
 - 98 [7,413,873](#) **T** [Method of detection and treatment of colon cancer](#)
 - 99 [7,410,776](#) **T** [Polypeptides, their production and use](#)
 - 100 [7,408,055](#) **T** [Promoter molecules for use in plants](#)
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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
101	7,407,804 T Human genes relating to respiratory diseases and obesity
102	7,405,345 T Plant seed specific promoters
103	7,405,287 T Method of treating a cancer
104	7,402,412 T Mutated D-aminotransferase and method for producing optically active glutamic acid derivatives using the same
105	7,402,391 T Method for detecting target plant genus
106	7,396,977 T Method of elevating photosynthesis speed of plant by improving pyruvate phosphate dikinase
107	7,396,915 T Monoclonal antibody and gene encoding the same, hybridoma, pharmaceutical composition, and diagnostic reagent
108	7,390,655 T Promoter molecules for use in plants
109	7,388,127 T Multiple stress-inducible peroxidase promoter derived from Ipomoea batatas
110	7,385,048 T Promoters and utilization thereof
111	7,375,204 T Fungal cell wall synthesis gene
112	7,375,201 T Fluorescent protein
113	7,375,199 T Cancer-associated genes
114	7,374,918 T Modified sarcosine oxidases, genes and recombinant DNAs thereof, and methods for preparing the same
115	7,374,911 T Interleukin-1 related gene and protein

- 116 [7,374,910](#) [DNA encoding galanin receptor activating peptide](#)
- 117 [7,374,909](#) [T Modulators of intracellular inflammation, cell death and cell survival pathways](#)
- 118 [7,371,835](#) [T p53-dependent apoptosis-inducing protein and method of screening for apoptosis regulator](#)
- 119 [7,371,548](#) [T Avermectin aglycon synthase genes](#)
- 120 [7,371,546](#) [T Glucoamylase variants](#)
- 121 [7,368,556](#) [T DNA and proteins or peptides specific of bacteria of the Neisseria meningitidis species, methods for obtaining them and biological applications thereof](#)
- 122 [7,361,808](#) [T DNA encoding insecticidal Cry9Fa Bacillus thuringiensis proteins and recombinant hosts expressing same](#)
- 123 [7,361,805](#) [T Ehd1 gene promoting plant flowering, and utilization thereof](#)
- 124 [7,361,751](#) [T Bacillus thuringiensis strains and their insecticidal proteins](#)
- 125 [7,361,748](#) [T von Willebrand factor \(vWF\)--cleaving protease](#)
- 126 [7,361,486](#) [T Polynucleotide, vector, host cell and method for producing human hepatoma-derived growth factor 5 polypeptide](#)
- 127 [7,358,355](#) [T Antibodies against human parathyroid hormone related protein](#)
- 128 [7,358,348](#) [T .beta.-catenin nuclear localized protein](#)
- 129 [7,355,025](#) [T Marker molecules associated with lung tumors](#)
- 130 [7,354,751](#) [T Alcohol dehydrogenase gene of acetic acid bacterium](#)
- 131 [7,354,736](#) [T Caspase-8 binding protein, its preparation and use](#)
- 132 [7,354,390](#) [T Seed coat specific nucleotide sequence encoding peroxidase](#)
- 133 [7,351,815](#) [T Canine pre-proGHRH and mature GHRH genes](#)
- 134 [7,348,171](#) [T N-acetylglucosaminyltransferase Vb coding sequences, recombinant cells and methods](#)
- 135 [7,348,170](#) [T Fungus-origin lysyl oxidases](#)
- 136 [7,348,145](#) [T Clinical assays for the detection and typing of human herpesviruses](#)
- 137 [7,345,157](#) [T Fluorescent protein and chromoprotein](#)
- 138 [7,342,106](#) [T Human antihuman MCP-1 antibody and antibody fragment thereof](#)
- 139 [7,339,047](#) [T Caspase-8 interacting proteins](#)
- 140 [7,335,812](#) [T Method of increasing plant organ and seed size in a plant](#)
- 141 [7,335,757](#) [T Carbonyl reductase, gene encoding the same, and process for producing optically active alcohols using the same](#)
- 142 [7,335,756](#) [T Retinoic acid metabolizing cytochrome P450](#)
- 143 [7,332,595](#) [T DNA sequences encoding peptide sequences specific for the hepatic stages of P. falciparum bearing epitopes capable of stimulating the T lymphocytes](#)
- 144 [7,332,310](#) [T Mutant of homoserine dehydrogenase from Corynebacterium and DNA encoding thereof](#)
- 145 [7,332,301](#) [T Insulin-like growth factor binding protein](#)
- 146 [7,332,171](#) [T Ehrlichia chaffeensis 28 kDa outer membrane protein multigene family](#)
- 147 [7,329,544](#) [T Plant lesion formation suppressing gene, Sp17 and use thereof](#)
- 148 [7,329,510](#) [T Full length human HCN11.sub.h channel subunit and variants](#)

149 [7,326,827](#)  [Sodium/proton antiporter gene](#)

150 [7,323,616](#)  [Genetically altered mice deficient in functional caspase-9](#)



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PAT. NO.	Title
151 7,323,556	Corn event DAS-59122-7 and methods for detection thereof
152 7,320,883	Process for producing coenzyme Q.sub.10
153 7,320,880	Polypeptide having an activity to support proliferation or survival of hematopoietic stem cell and hematopoietic progenitor cell, and DNA coding for the same
154 7,319,019	Glial mitogenic factors lacking an N-terminal signal sequence
155 7,316,916	DNA for encoding D-hydantoin hydrolases, DNA for encoding N-carbamyl-D-amino acid hydrolases, recombinant DNA containing the genes, cells transformed with the recombinant DNA, methods for producing proteins utilizing the transformed cells and methods for producing D-amino acids
156 7,314,864	Humanin, a polypeptide suppressing neuronal death
157 7,314,738	DNA for encoding D-hydantoin hydrolases, DNA for encoding N-carbamyl-D-amino acid hydrolases, recombinant DNA containing the genes, cells transformed with the recombinant DNA, methods for producing proteins utilizing the transformed cells and methods for producing D-amino acids
158 7,309,595	Protein hydrolysates enriched in peptides having a carboxy terminal proline residue
159 7,307,160	OtsA gene encoding trehalose-6-phosphate synthase from a coryneform bacterium
160 7,307,145	Sodium-independent transporter transporting small-sized neutral amino acid, gene thereof and method of analyzing transporter function by constructing

- [fused proteins enabling the specification of the function](#)
- 161 [7,307,142](#) **T** [Leptin antagonists](#)
 - 162 [7,306,909](#) **T** [Canola event pv-bngt04\(rt73\) and compositions and methods for detection thereof](#)
 - 163 [7,303,918](#) **T** [Alteration of flowering time in plants](#)
 - 164 [7,300,791](#) **T** [Production of vascular endothelial cell growth factor and DNA encoding same](#)
 - 165 [7,297,774](#) **T** [Use of inhibitors for the treatment of RTK-hyperfunction-induced disorders, particularly cancer](#)
 - 166 [7,297,773](#) **T** [Presynaptic protein cast](#)
 - 167 [7,297,513](#) **T** [Gene encoding glutathione synthetase from *Candida utilis*](#)
 - 168 [7,291,491](#) **T** [Polypeptides](#)
 - 169 [7,288,643](#) **T** [Corn event TC1507 and methods for detection thereof](#)
 - 170 [7,288,389](#) **T** [Peptide-forming enzyme gene](#)
 - 171 [7,288,388](#) **T** [Peptide-forming enzyme gene, peptide-forming enzyme, and peptide producing method](#)
 - 172 [7,285,656](#) **T** [Root-specific conifer gene promoter and its use](#)
 - 173 [7,285,654](#) **T** [Stimulus-inducible protein kinase complex and methods of use therefor](#)
 - 174 [7,282,622](#) **T** [Flower morphology of plants by targeting mads-box gene](#)
 - 175 [7,282,580](#) **T** [Protein molecule useful for inhibition of anthrax toxin](#)
 - 176 [7,279,335](#) **T** [Nucleic acids encoding lettuce big-vein viral proteins and utilization thereof](#)
 - 177 [7,279,293](#) **T** [Constitutively active histamine H3 receptor mutants and uses thereof](#)
 - 178 [7,271,257](#) **T** [Glutamate receptors and utilization thereof](#)
 - 179 [7,271,003](#) **T** [Rice-derived high expression polypeptide chain elongation factor promoter and method of using the same](#)
 - 180 [7,268,225](#) **T** [Selected nucleotide sequences isolated from pathogenic strains of *Haemophilus influenzae*](#)
 - 181 [7,265,280](#) **T** [Polynucleotides encoding carnation senescence-induced DHS](#)
 - 182 [7,265,269](#) **T** [Nucleic acids encoding a novel Cry2Ae bacillus thuringiensis insecticidal protein](#)
 - 183 [7,265,268](#) **T** [Insecticidal proteins derived from *Bacillus thuringiensis*](#)
 - 184 [7,265,216](#) **T** [Transmembrane serine protease overexpressed in ovarian carcinoma and uses thereof](#)
 - 185 [7,256,278](#) **T** [Sialyltransferase and DNA encoding the same](#)
 - 186 [7,256,276](#) **T** [AtRSp gene promoters](#)
 - 187 [7,256,265](#) **T** [Streptococcal C5a peptidase vaccine](#)
 - 188 [7,253,339](#) **T** [Plant photoperiod sensitivity gene Hd1 and use of the same](#)
 - 189 [7,253,268](#) **T** [Light-driven energy generation using proteorhodopsin](#)
 - 190 [7,252,968](#) **T** [Chromatin regulator genes](#)
 - 191 [7,250,272](#) **T** [G protein-coupled receptor protein and DNA thereof](#)
 - 192 [7,250,161](#) **T** [Equine GM-CSF](#)
 - 193 [7,247,466](#) **T** [Amino acid racemase having low substrate specificity and process for producing racemic amino acid](#)
 - 194 [7,247,449](#) **T** [Fluorescent protein](#)

- 195 [7,244,880](#)  [Nucleic acid molecules encoding novel Bacillus thuringiensis Cry2Ae insecticidal proteins, plant cells, plant or seeds comprising the nucleic acid molecules and methods of using same](#)
- 196 [7,244,435](#)  [DNA vaccine expressing HA1 of equine-2 influenza virus](#)
- 197 [7,241,605](#)  [Polypeptide](#)
- 198 [7,232,674](#)  [Process for producing .alpha.1,4-galactosyltransferase and galactose-containing complex sugar](#)
- 199 [7,229,817](#)  [Recombinant porcine liver esterases, their use and a method for the production thereof](#)
- 200 [7,229,755](#)  [Method for detection of alterations in the DNA mismatch repair pathway](#)
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PAT. NO.	Title
201 RE39,685	T Method for shortening internode of inflorescence by introducing gene for petunia transcription factor petSPL2
202 7,226,993	T Fluorescent protein
203 7,226,784	T Isolated nucleotides encoding arabidopsis senescence-induced eIF-5A
204 7,223,852	T Nucleic acids encoding TRAIN-R: a cysteine rich member of the TNF-receptor family
205 7,223,581	T F.sub.0F.sub.1-ATPase and DNA encoding the same
206 7,223,572	T Methylophilus methylotrophus having enhanced dihydrodipicolinate synthase and/or aspartokinase activity for L-amino acid production
207 7,220,558	T Cartilage-derived morphogenetic proteins
208 7,220,556	T Preparation of factor XIIIa by gene manipulation
209 7,217,866	T DNA encoding a plant deoxyhypusine synthase, a plant eukaryotic initiation factor 5A, transgenic plants and a method for controlling senescence programmed and cell death in plants
210 7,217,865	T Transgenic high tryptophan plants
211 7,217,686	T DNA sequence encoding oncofetal ferritin protein
212 7,217,559	T Gene encoding cyclododecanone monooxygenase
213 7,214,859	T Brassica pyruvate dehydrogenase kinase gene
214 7,214,522	T Thermophilic DNA polymerases from Thermoactinomyces vulgaris
215 7,214,520	T Cloning, sequencing and expression of a comamonas cyclopentanone 1,2-

- [monooxygenase-encoding gene in Escherichia coli](#)
- 216 [7,214,517](#) **T** [.alpha.1,2-fucosyltransferase and DNA encoding the same](#)
- 217 [7,211,422](#) **T** [Polypeptide having .alpha.-isomaltosylglucosaccharide synthase activity](#)
- 218 [7,211,421](#) **T** [Gene encoding dihydrodipicolinate reductase from Bacillus methanolicus](#)
- 219 [7,211,259](#) **T** [4-1BB polypeptides and DNA encoding 4-1BB polypeptides](#)
- 220 [7,208,654](#) **T** [Plant regulatory sequences for selective control of gene expression](#)
- 221 [7,208,273](#) **T** [Common polymorphism in scn5a implicated in drug-induced cardiac arrhythmia](#)
- 222 [7,205,149](#) **T** [Gene of enzyme reactivating DNA damaged by ultraviolet light using visible light](#)
- 223 [7,205,137](#) **T** [.beta.1,2-xylosyltransferase-gene from arabidopsis](#)
- 224 [7,199,282](#) **T** [Floral induction gene](#)
- 225 [7,199,235](#) **T** [Plant promoters](#)
- 226 [7,198,932](#) **T** [Gdp-4-keto-6-deoxy-d-mannose-3,5-epimerase-4-reductase gene derived from arabidopsis thaliana](#)
- 227 [7,198,914](#) **T** [Guanosine triphosphate \(GTP\)-binding protein-coupled receptor protein, BG37](#)
- 228 [7,196,246](#) **T** [HD3a Gene inducing flowering of plant and utilization thereof](#)
- 229 [7,193,072](#) **T** [Compositions to identify swine genetically resistant to F18 E. coli associated diseases](#)
- 230 [7,192,774](#) **T** [Seed-specific promoter from the rice glutelin GluB-1 gene and uses thereof](#)
- 231 [7,192,754](#) **T** [Nomuraeae rileyi-origin ecdysteroid 22-oxidase and molt hormone inactivation system with the use of the same](#)
- 232 [7,189,890](#) **T** [Gene related to regeneration ability of plants and uses thereof](#)
- 233 [7,189,840](#) **T** [Oncosuppressive gene](#)
- 234 [7,189,835](#) **T** [Pichia methanolica secretory signal](#)
- 235 [7,189,570](#) **T** [Putrescine-n-methyltransferase promoter](#)
- 236 [7,189,535](#) **T** [Modulators of the function of receptors of the TNF/NGF receptor family and other proteins](#)
- 237 [7,186,821](#) **T** [Rice sucrose transporter gene promoter](#)
- 238 [7,186,820](#) **T** [Production of humanised antibodies to TNF.alpha.](#)
- 239 [7,186,819](#) **T** [Glutamate receptor and utilization thereof](#)
- 240 [7,186,818](#) **T** [DNA encoding soluble variants of human OX2 receptors](#)
- 241 [7,186,815](#) **T** [Method of isolating secretion signals in lactic acid bacteria and novel secretion signals isolated from Lactococcus lactis](#)
- 242 [7,186,538](#) **T** [Type II restriction endonuclease, CstMI, obtainable from Corynebacterium striatum M82B and a process for producing the same](#)
- 243 [7,186,534](#) **T** [Potent inhibitors of human 9-cis retinol dehydrogenase](#)
- 244 [7,186,526](#) **T** [Recombinant fibroblast growth factor analogs](#)
- 245 [7,183,403](#) **T** [Genes for heat resistant enzymes of amino acid biosynthetic pathway derived from thermophilic coryneform bacteria](#)
- 246 [7,183,077](#) **T** [Promoters and gene expression method by using the promoters](#)
- 247 [7,179,902](#) **T** [Use of regulatory sequences in transgenic plants](#)

- 248 [7,179,901](#) **T** [Renal regulatory elements and methods of use thereof](#)
249 [7,176,347](#) **T** [Vegetative growth specific promoter and transgenic plant obtained with the same](#)
250 [7,176,300](#) **T** [Avian lysozyme promoter](#)
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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
251 7,176,294	Transcription factor, BP1
252 7,175,997	BMP-11 compositions
253 7,175,984	Identification and applications of porcine reproductive and respiratory syndrome virus host susceptibility factor(s) for improved swine breeding and development of a non-simian recombinant cell line for propagation of the virus and a target for a novel class of antiviral compounds
254 7,169,971	DNA encoding insecticidal Cry1Bf Bacillus thuringiensis proteins and recombinant hosts expressing same
255 7,166,770	Cestrum yellow leaf curling virus promoters
256 7,163,807	Nucleic acid sequences encoding modified prepro peptides of an alkaline protease
257 7,163,791	.alpha.,2,8-sialyltransferase
258 7,160,995	DNA encoding anti-TNF antibodies and peptides
259 7,160,722	Master bone formation transcription factor: compositions and methods of use
260 7,160,696	Bovine lymphocyte-derived antibacterial protein
261 7,157,265	Ubiquitin-specific protease occurring in the brain and DNA encoding the same
262 7,157,250	Glutamic acid receptor and utilization thereof
263 7,157,249	Delta subunit of human GABAA receptor
264 7,154,028	Gibberellin 2-oxidase gene, functions and uses thereof
265 7,154,023	Transgenic plants with altered levels of phenolic compounds

- 266 [7,151,202](#) **T** [Environmental stress resistance gene](#)
- 267 [7,151,170](#) **T** [Use of the BNM3 transcriptional activator to control plant embryogenesis and regeneration processes](#)
- 268 [7,151,164](#) **T** [Anti-CD20 antibodies and fusion proteins thereof and methods of use](#)
- 269 [7,148,399](#) **T** [Coffee mannanase](#)
- 270 [7,148,339](#) **T** [Temperature sensitive mutant of bacteriophage T4 endonuclease VII](#)
- 271 [7,148,036](#) **T** [DNA molecules encoding cartilage-derived morphogenetic proteins](#)
- 272 [7,144,720](#) **T** [Protein and DNA thereof](#)
- 273 [7,144,702](#) **T** [Method for detecting target plant genus](#)
- 274 [7,141,389](#) **T** [Chromosome DNA coding for human hepatocyte growth factor](#)
- 275 [7,135,625](#) **T** [Gene concerning brassinosteroid-sensitivity of plants and utilization thereof](#)
- 276 [7,135,561](#) **T** [Infectious bovine viral diarrhea virus clone](#)
- 277 [7,132,525](#) **T** [Hypersensitive response elicitor fragments eliciting a hypersensitive response and uses thereof](#)
- 278 [7,132,292](#) **T** [Anther-specific promoter from the rice TUB8 gene and uses thereof](#)
- 279 [7,132,276](#) **T** [Human p51 genes and gene products thereof](#)
- 280 [7,132,260](#) **T** [DNA encoding parathyroid hormone receptor](#)
- 281 [7,132,106](#) **T** [Infectious cDNA clone of North American porcine reproductive and respiratory syndrome \(PRRS\) virus and uses thereof](#)
- 282 [7,129,065](#) **T** [bHLH-PAS proteins, genes thereof and utilization of the same](#)
- 283 [7,129,064](#) **T** [Canine hepatocyte growth factor](#)
- 284 [7,129,063](#) **T** [Exocrine gland tight junction-constituting protein jeep family](#)
- 285 [7,125,977](#) **T** [Genes for heat resistant enzymes of amino acid biosynthetic pathway derived from thermophilic coryneform bacteria](#)
- 286 [7,125,971](#) **T** [Full-length genomic RNA of papaya leaf-distortion mosaic virus](#)
- 287 [7,125,690](#) **T** [Promoters showing stationary phase-specific activity in gram-positive bacteria](#)
- 288 [7,125,688](#) **T** [Feline hepatocyte growth factor](#)
- 289 [7,125,679](#) **T** [Methods to screen peptide libraries using minicell display](#)
- 290 [7,122,727](#) **T** [Nucleic acid molecules from wheat encoding an R1-protein, and transgenic plant cells and plants comprising the nucleic acid molecules](#)
- 291 [7,122,657](#) **T** [UDP-glucose aglycon-glucosyltransferase](#)
- 292 [7,119,253](#) **T** [DNAs coding for flavone synthase, methods of using flavone synthase DNAs, and plants, flowers, and vectors containing flavone synthase DNAs](#)
- 293 [7,119,252](#) **T** [DNAs coding for flavone synthesis, methods of using flavone synthase DNAs, and plants, flowers, and vectors containing flavone synthase DNAs](#)
- 294 [7,115,732](#) **T** [Selected nucleotide sequences isolated from pathogenic strains of Haemophilus influenzae](#)
- 295 [7,115,420](#) **T** [Promoters and utilization thereof](#)
- 296 [7,115,407](#) **T** [Recombinant type II restriction endonucleases, MmeI and related endonucleases and methods for producing the same](#)
- 297 [7,115,367](#) **T** [Method for the specific detection and identification of retroviral nucleic acids/retroviruses in a specimen](#)
- 298 [7,112,666](#) **T** [Von Willebrand Factor \(Vwf\)-cleaving protease](#)

- 299 [7,112,665](#)  [Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants](#)
- 300 [7,112,428](#)  [Chlorohydrin and hydroxycarboxylic ester asymmetric hydrolase gene](#)
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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
301 7,109,397	T Pseudomonas syringae harpins, HopPtoP and HopPmaH.sub.Pto, and their uses
302 7,109,395	T Dehiscence gene and methods for regulating dehiscence
303 7,109,321	T DNA fragment directing gene expression predominant in flower organ
304 7,109,012	T Recombinant lysophosphatidic acid phosphatase
305 7,108,999	T Modulators of the function of FAS/AP01 receptors
306 7,105,650	T T2R taste receptors and genes encoding same
307 7,101,985	T Methods and compositions in checkpoint signaling
308 7,101,556	T Preparation and usage of plasmodium fusion antigen
309 7,098,324	T Chitinase encoding DNA molecules from cotton expressed preferentially in secondary walled cells during secondary wall deposition and a corresponding promoter
310 7,098,026	T Human desert hedgehog protein
311 7,098,020	T DNA encoding hydantoinase, DNA encoding N-carbamyl-L-amino acid hydrolase, recombinant DNA, transformed cell, method of producing protein, and method of producing optically active amino acid
312 7,098,019	T DNA for encoding D-hydantoin hydrolases, DNA for encoding N-carbamyl-D-amino acid hydrolases, recombinant DNA containing the genes, cells transformed with the recombinant DNA, methods for producing proteins utilizing the transformed cells and methods for producing D-amino acids

- 313 [7,098,013](#) **T** [Polypeptide having .alpha.-isomaltosyl-transferase activity](#)
- 314 [RE39,247](#) **T** [Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases](#)
- 315 [7,094,950](#) **T** [Poly ADP-ribose polymerase gene and it uses](#)
- 316 [7,094,890](#) **T** [Arthritis-associated protein](#)
- 317 [7,094,889](#) **T** [AGE-1 polypeptides and related molecules and methods](#)
- 318 [7,091,027](#) **T** [Transgenic amorpho-4,11-diene synthesis](#)
- 319 [7,091,007](#) **T** [DNA molecules encoding BMP receptor proteins](#)
- 320 [7,090,989](#) **T** [Recombinant human SM-11044-binding receptor proteins exhibiting ligand-binding activities, and their uses](#)
- 321 [7,087,406](#) **T** [Human-derived bradeion proteins, DNA coding for the proteins, and uses thereof](#)
- 322 [RE39,219](#) **T** [Growth arrest homebox gene](#)
- 323 [7,083,976](#) **T** [Tyrosine recombinase for genetic engineering](#)
- 324 [7,081,565](#) **T** [Plant seed specific promoters](#)
- 325 [7,078,592](#) **T** [Nucleic acids encoding coleopteran-toxic polypeptides and insect-resistant transgenic plants comprising them](#)
- 326 [7,078,234](#) **T** [Maize embryo-specific promoter compositions and methods for use thereof](#)
- 327 [7,078,206](#) **T** [UDP-N-acetylglucosamine: galactose-.beta.1,3-N-acetylgalactosamine-.alpha.-R/ \(GlcNAc to GalNAc\) .beta.1,6N-acetylglucosaminyltransferase, C2GnT3](#)
- 328 [7,074,611](#) **T** [Method for the stable inversion of DNA sequence by site-specific recombination and DNA vectors and transgenic cells thereof](#)
- 329 [7,071,376](#) **T** [Genes encoding p-coumarate 3-hydroxylase \(C3H\) and methods of use](#)
- 330 [7,071,325](#) **T** [DNA molecule for detecting glyphosate tolerant wheat plant 33391 and progeny thereof](#)
- 331 [7,071,318](#) **T** [Methods and compositions for stabilizing microtubules and intermediate filaments in striated muscle cells](#)
- 332 [7,071,317](#) **T** [Tissue inhibitor of metalloproteinase type three \(TIMP-3\) composition and methods](#)
- 333 [7,071,171](#) **T** [Unique dendritic cell-associated c-type lectins, dectin-1 and dectin-2 compositions and uses thereof](#)
- 334 [7,070,997](#) **T** [Isolated nucleotides encoding tomato senescence-induced eIF-5A](#)
- 335 [7,070,783](#) **T** [Small molecular weight TNF receptor multimeric molecule](#)
- 336 [7,067,647](#) **T** [Nucleic acid sequences to proteins involved in isoprenoid synthesis](#)
- 337 [7,067,630](#) **T** [Transmembrane serine protease overexpressed in ovarian carcinoma and uses thereof](#)
- 338 [7,067,265](#) **T** [Sodium ion-driven chloride/bi-carbonate exchanger](#)
- 339 [7,063,958](#) **T** [Nucleic acids db, the receptor for leptin](#)
- 340 [7,060,499](#) **T** [DNA containing variant FRT sequences](#)
- 341 [7,060,490](#) **T** [DNA encoding novel estrogen receptor](#)
- 342 [7,060,485](#) **T** [DNA for encoding D-hydantoin hydrolases, DNA for encoding N-carbamyl-D-amino acid hydrolases, recombinant DNA containing the genes, cells transformed with the recombinant DNA, methods for producing proteins utilizing the transformed cells and methods for producing D-amino acids](#)

- 343 [7,060,483](#) **T** [DNA encoding endo-.beta.-galactosidase](#)
344 [7,060,266](#) **T** [Human brain carboxypeptidase B](#)
345 [7,057,088](#) **T** [Gibberellin 2.beta.-hydroxylase genes of rice and uses thereof](#)
346 [7,052,876](#) **T** [Combinatorial method for producing nucleic acids](#)
347 [7,049,490](#) **T** [Gibberellin 3.beta.-hydroxylase genes of rice and uses thereof](#)
348 [7,049,487](#) **T** [Transgenic plants carrying neoxanthin cleavage enzyme gene](#)
349 [7,049,429](#) **T** [Mutations of the 5' region of the human 5-HT1A gene](#)
350 [7,049,131](#) **T** [Methods and deoxyribonucleic acid for the preparation of tissue factor protein](#)
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PAT. NO.	Title
351 7,049,123	T Deoxyribonuclease, gene encoding same and use thereof
352 7,049,111	T Process for producing HMG-CoA reductase inhibitor
353 7,045,324	T Tocopherol associated protein and uses thereof
354 7,045,123	T DNA encoding Pseudomonas syringae hypersensitive response elicitor and its use
355 7,038,110	T Photosensitivity gene of plant and utilization thereof
356 7,037,712	T DNA encoding ovine adenovirus (OAV287) and its use as a viral vector
357 7,037,686	T Human cytoplasmic polyadenylation element binding protein and uses thereof
358 7,034,204	T Plant regulatory sequences for selective control of gene expression
359 7,034,142	T Method to improve translation of polypeptides by using untranslated regions from heat-shock proteins
360 7,033,833	T Polynucleotides encoding carnation senescence-induced EIF-5A
361 7,033,808	T Carbonyl reductase, gene thereof and method of using the same
362 7,033,802	T Penicillin binding protein gene and process for producing L-glutamic acid
363 7,029,845	T DNAs and proteins or peptides specific to bacteria of the species Neisseria meningitidis, processes for obtaining them and their biological uses
364 7,029,667	T DNA encoding Erwinia amylovora hypersensitive response elicitor and its use
365 RE39,062	T INGAP protein involved in pancreatic islet neogenesis
366 7,026,148	T DAP-kinase related protein

- 367 [7,025,969](#) **T** [Protein kinase deficient, immunologically active CMVpp65 mutants](#)
- 368 [7,022,896](#) **T** [Methods and materials for making and using transgenic dicamba-degrading organisms](#)
- 369 [7,022,827](#) **T** [Xylanase, microorganisms producing it, DNA molecules, methods for preparing this xylanase and uses of the latter](#)
- 370 [7,022,499](#) **T** [Nucleic acids encoding differentiation inhibitor delta 2](#)
- 371 [7,015,001](#) **T** [DNA encoding SPNF of the spinosyn cluster](#)
- 372 [7,014,993](#) **T** [Extracellular serine protease](#)
- 373 [7,011,966](#) **T** [Method for cloning and expression of AcuI restriction endonuclease and AcuI methylase in E. coli](#)
- 374 [7,005,512](#) **T** [Methods and compositions for stabilizing microtubules and intermediate filaments in striated muscle cells](#)
- 375 [7,005,290](#) **T** [Best's macular dystrophy gene](#)
- 376 [7,005,284](#) **T** [Transaldolase gene](#)
- 377 [7,002,001](#) **T** [Down-regulation resistant C3 convertase](#)
- 378 [7,001,997](#) **T** [Proteins, their production and use](#)
- 379 [7,001,751](#) **T** [PyrF gene and the utilization thereof](#)
- 380 [7,001,734](#) **T** [Process for identifying para cation channel modulators](#)
- 381 [RE38,981](#) **T** [DNA sequence coding for protein C](#)
- 382 [6,998,255](#) **T** [Human G-protein coupled receptor](#)
- 383 [6,995,250](#) **T** [Thermophilic amino acid biosynthesis system enzyme gene of thermotolerant coryneform bacterium](#)
- 384 [6,995,003](#) **T** [Method to localize expandase in the cytosol](#)
- 385 [6,994,999](#) **T** [Isolated DNA molecule comprising the promoter sequence of a bovine myostatin gene](#)
- 386 [RE38,966](#) **T** [Gene for transcription factor capable of altering characters of a plant and use thereof](#)
- 387 [6,987,088](#) **T** [Compounds that bind HER2](#)
- 388 [6,987,021](#) **T** [Methods and compositions for identifying morphogenic protein analogs using morphogenic protein responsive inhibitory elements](#)
- 389 [6,982,364](#) **T** [Manipulation of a MinD gene in plants to alter plastid size, shape and/or number](#)
- 390 [6,979,731](#) **T** [Gene encoding alkaline liquefying .alpha.-amylase](#)
- 391 [6,977,157](#) **T** [Clock gene promoter](#)
- 392 [6,974,687](#) **T** [.beta.1,3-galactosyltransferase and DNA encoding the enzyme](#)
- 393 [6,974,669](#) **T** [Bio-barcode based on oligonucleotide-modified nanoparticles](#)
- 394 [6,969,762](#) **T** [Selected nucleotide sequences isolated from pathogenic strains of Haemophilus influenzae](#)
- 395 [6,969,760](#) **T** [Jak kinases and regulation of cytokine signal transduction](#)
- 396 [6,967,246](#) **T** [Microbial swollenin protein, DNA sequences encoding such swollenins and method of producing such swollenins](#)
- 397 [6,965,024](#) **T** [Process for producing humanized chimera antibody](#)
- 398 [6,962,984](#) **T** [IgA nephropathy-related DNA](#)
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399 [6,960,705](#) [Nucleic acid encoding a hypersensitive response elicitor from Xanthomonas campestris](#)

400 [6,960,458](#) [Erythrose reductase, its cDNA and cell which the cDNA express](#)

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PAT. NO.	Title
401 6,958,392	T Methods for the isolation of nucleic acids and for quantitative DNA extraction and detection for leukocyte evaluation in blood products
402 6,958,230	T Method for cloning and expression of SbfI restriction endonuclease and SbfI methylase in E. coli
403 6,956,112	T Rapidly degrading GFP-fusion proteins and methods of use
404 6,956,110	T Genes involved in the molecular paths of tumor suppression and/or resistance to viruses
405 6,955,910	T Method for large scale production of recombinant DNA-derived TPA or K2S molecules
406 6,955,906	T Biotin biosynthetic genes having biotin synthase activity
407 RE38,825	T Glyphosate tolerant plants
408 6,953,848	T Gene promoter sequences and uses thereof
409 6,953,847	T Fas antigen derivative
410 6,951,929	T Methods and kits for identifying elite event GAT-ZM1 in biological samples
411 6,951,919	T Fas ligand derivative
412 6,951,653	T Streptococcal C5a peptidase vaccine
413 6,949,636	T Intron/exon structure of the human and mouse .beta.3-adrenergic receptor genes
414 6,949,364	T Testis-specific differentiation-regulatory factor
415 6,949,360	T DNA coding for human cell surface antigen

- 416 [6,949,359](#) **T** [Human IL-1 epsilon DNA and polypeptides](#)
- 417 [6,946,264](#) **T** [Metalloproteinase inhibitor](#)
- 418 [6,943,013](#) **T** [Human checkpoint kinase, hCDS1, compositions and methods](#)
- 419 [6,939,692](#) **T** [Nucleotide sequences coding for the pknB gene](#)
- 420 [6,936,748](#) **T** [Polyphenol oxidase genes from potato tuber, grape, apple and broad bean](#)
- 421 [6,936,707](#) **T** [Modified DNA molecule, recombinant containing the same, and uses thereof](#)
- 422 [6,936,458](#) **T** [Isolated DNA comprising one or more genes specific for 5S clavam biosynthesis, vectors comprising such DNA and streptomyces hosts capable of improved clavulanic acid production](#)
- 423 [6,936,439](#) **T** [OB fusion protein compositions and methods](#)
- 424 [6,933,136](#) **T** [Method for making recombinant proteins](#)
- 425 [6,933,132](#) **T** [Regulation of immune responses by attractin](#)
- 426 [6,930,227](#) **T** [CAMELLIA SINENSIS GENE ENCODING A CAFFEINE SYNTHESIS ASSOCIATED N-METHYL TRANSFERASE WITH 7-METHYLXANTHINE N3 METHYL TRANSFERASE, THEOBROMINE N1 METHYL TRANSFERASE, AND PARAXANTHINE N3 METHYL TRANSFERASE ACTIVITIES AND USE THEREOF](#)
- 427 [6,930,182](#) **T** [Composition and methods of using the Mirabilis mosaic caulimovirus sub-genomic transcript \(Sgt\) promoter for plant genetic engineering](#)
- 428 [6,929,931](#) **T** [Expression constructs using Lactobacillus delbrueckii subsp. lactis lac repressor protein and its lac repressor binding site, microorganisms and methods thereof](#)
- 429 [6,924,366](#) **T** [Recombinant hexose oxidase, a method of producing same and use of such enzyme](#)
- 430 [6,924,135](#) **T** [DNA encoding Eimeria glyceroaldehyde-3-phosphate dehydrogenase and uses thereof](#)
- 431 [6,924,131](#) **T** [Xylitol dehydrogenase of acetic acid bacteria and gene thereof](#)
- 432 [6,924,106](#) **T** [Rifamycin biosynthesis gene cluster](#)
- 433 [6,924,097](#) **T** [Plant promoter and method for gene expression using said promoter](#)
- 434 [6,921,649](#) **T** [Polynucleotide encoding adipocyte complement related protein homolog zacrp2](#)
- 435 [6,919,194](#) **T** [Method for cloning and expression of Tth111I restriction endonuclease-methylase in E. coli](#)
- 436 [6,903,247](#) **T** [Constitutive .alpha.-Tubulin promoter from coffee plants and uses thereof](#)
- 437 [6,902,922](#) **T** [Subtilisin variants](#)
- 438 [6,897,062](#) **T** [DNA encoding the prostate-specific membrane antigen-like gene and uses thereof](#)
- 439 [6,897,053](#) **T** [Enzymes derived from thermophilic organisms that function as a chromosomal replicase, preparation and use thereof](#)
- 440 [6,893,852](#) **T** [Dna encoding sucrose pts enzyme II](#)
- 441 [6,893,848](#) **T** [Desensitized aspartokinase](#)
- 442 [6,893,844](#) **T** [DNA encoding a new human hepatoma derived growth factor and producing method thereof](#)
- 443 [6,891,084](#) **T** [DNA encoding raffinose synthase from soybean](#)

- 444 [6,891,030](#) **T** [T-cell immunoregulatory molecule](#)
- 445 [6,888,047](#) **T** [Transgenic animals as urinary bioreactors for the production of polypeptide in the urine, recombinant DNA construct for kidney-specific expression, and method of using same](#)
- 446 [6,887,694](#) **T** [DNA encoding a polypeptide required for biosynthesis of TA antibiotic](#)
- 447 [6,881,566](#) **T** [Carbamoyl-phosphate synthetase gene of coryneform bacteria and method for producing L-arginine](#)
- 448 [6,881,565](#) **T** [Protein having glutaminase activity and gene encoding the same](#)
- 449 [6,878,818](#) **T** [Modified ubiquitin regulatory system](#)
- 450 [6,878,533](#) **T** [Gene encoding dihydrodipicolinate synthase from Bacillus methanolicus and methods of making lysine wing said gene](#)
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PAT. NO.	Title
451	6,878,525 Peptide leukotriene receptor
452	6,875,852 DNA polymerase III holoenzyme
453	6,875,436 Method for producing cell-fusion type morbillivirus mutants
454	6,872,556 Isoprenoid production
455	6,869,933 HIV-specific CTL inducing peptides and medicaments for preventing or treating AIDS comprising the peptides
456	6,869,790 Preparation of DNA encoding factor XIIIa
457	6,869,786 Method for cloning and expression of BsrGI restriction endonuclease and BsrGI methyltransferase in E. coli
458	6,864,074 DNA FRAGMENT CARRYING TOLUENE MONOOXYGENASE GENE, RECOMBINANT PLASMID, TRANSFORMED MICROORGANISM, METHOD FOR DEGRADING CHLORINATED ALIPHATIC HYDROCARBON COMPOUNDS AND AROMATIC COMPOUNDS, AND METHOD FOR ENVIRONMENTAL REMEDIATION
459	6,864,073 Avermectin aglycon synthase genes
460	6,864,068 Antifungal proteins
461	6,861,574 Sodium/proton antiporter gene
462	6,861,246 L-lysine-producing corynebacteria and process for the preparation of lysine
463	6,861,239 Genes and polynucleotides associated with ultraviolet radiation-mediated skin damage and uses thereof

- 464 [6,858,719](#) **T** [Rh\(D\)-binding proteins and magnetically activated cell sorting method for production thereof](#)
- 465 [6,858,417](#) **T** [DNA FRAGMENT CARRYING TOLUENE MONOOXYGENASE, GENE, RECOMBINANT PLASMID, TRANSFORMED MICROORGANISM, METHOD FOR DEGRADING CHLORINATED ALIPHATIC HYDROCARBON COMPOUNDS AND AROMATIC COMPOUNDS, AND METHOD FOR ENVIRONMENTAL REMEDIATION](#)
- 466 [6,855,873](#) **T** [Recombinant plant expressing non-competitively binding Bt insecticidal cryatal proteins](#)
- 467 [6,855,862](#) **T** [Isolated DNA sequences encoding a flavonoid 5-glucosytransferase and methods of use thereof](#)
- 468 [6,855,814](#) **T** [Sequences of E. coli O157](#)
- 469 [6,855,516](#) **T** [Gene conferring lysozyme insensitivity to corynebacterium](#)
- 470 [6,852,498](#) **T** [Oomycete FtsZ-mt as a target for oomycete-specific antimicrobials](#)
- 471 [6,846,658](#) **T** [Method for cloning and producing the MseI restriction endonuclease](#)
- 472 [6,844,432](#) **T** [Polymorphic DNAs and their use for diagnosis of susceptibility to panic disorder](#)
- 473 [6,844,422](#) **T** [Inhibitor protein of the wnt signal pathway](#)
- 474 [6,841,720](#) **T** [Inducible promoters](#)
- 475 [6,841,370](#) **T** [Site-directed mutagenesis of Escherichia coli phytase](#)
- 476 [6,838,262](#) **T** [Isolated DNA molecule encoding rank ligand](#)
- 477 [6,835,823](#) **T** [Anti-TNF antibodies and peptides of human tumor necrosis factor](#)
- 478 [6,833,135](#) **T** [DNA integration into "Mycobacterium spp." genome by trans-complementation using a site-specific integration system](#)
- 479 [6,830,926](#) **T** [Method for evaluating the ability of a compound to inhibit the protoporphyrinogen oxidase activity](#)
- 480 [6,830,919](#) **T** [Ceramidase gene](#)
- 481 [6,830,905](#) **T** [Glutaminase, its gene and a method of producing it](#)
- 482 [6,828,433](#) **T** [DNA encoding mutated collagen X](#)
- 483 [6,828,428](#) **T** [IGA nephropathy-related genes](#)
- 484 [6,825,399](#) **T** [Genes and vectors for conferring herbicide resistance in plants](#)
- 485 [6,825,024](#) **T** [Metalloproteinase and encoding DNA therefor](#)
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- 487 [6,815,581](#) **T** [Plants which synthesize a modified starch, process for the production thereof and modified starch](#)
- 488 [6,815,537](#) **T** [Method for construction of Thermus-E, coli shuttle vectors and identification of two thermus plasmid replication origins](#)
- 489 [6,815,426](#) **T** [Angiogenesis-inhibitory tripeptides, compositions and their methods of use](#)
- 490 [6,815,189](#) **T** [Gene from acremonium chrysogenum encoding a protein with cephalosporin C acetylhydrolase activity and methods of use of such gene](#)
- 491 [6,809,233](#) **T** [DNA sequences for an amino acid transporter plasmids, bacteria, yeasts and plants containing a transporter and their use](#)
- 492 [6,808,913](#) **T** [Useful mutations of bacterial alkaline protease](#)
- 493 [6,806,359](#) **T** [Hormone receptor compositions and methods](#)

- 494 [6,803,501](#)  [Methods for making plants tolerant to glyphosate and compositions thereof using a DNA encoding an EPSPS enzyme from Eleusine indica](#)
- 495 [6,803,192](#)  [B7-H1, a novel immunoregulatory molecule](#)
- 496 [6,800,468](#)  [UDP-galactose: .beta.-N-acetyl-glucosamine .beta.1,3galactosyltransferases, .beta.3Gal-T5](#)
- 497 [6,797,861](#)  [Seed shattering](#)
- 498 [6,797,509](#)  [Nucleotide sequences which code for the tal gene](#)
- 499 [6,797,265](#)  [Deleted adenovirus vectors and methods of making and administering the same](#)
- 500 [6,794,186](#)  [pCAR and its uses](#)
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PAT. NO.	Title
501 6,794,177	T Modified DNA-polymerase from carboxydotherrnus hydrogenoformans and its use for coupled reverse transcription and polymerase chain reaction
502 6,794,172	T Method for cloning and expression of PpuMI restriction endonuclease and PpuMI methylase in E. coli
503 6,794,169	T UDP-N-acetylglucosamine: galactose-.beta.1,3-N-acetylgalactosamine-.alpha.-R / (GlcNAc to GalNAc) .beta.1,6-N-acetylglucosaminyltransferase, C2GnT3
504 6,794,146	T Modifications of the VEGF receptor-2 protein and methods of use
505 6,793,488	T Flavivirus detection and quantification assay
506 6,790,944	T DNA encoding IGA nephropathy indicating protein
507 6,790,442	T Genomic DNA encoding a polypeptide capable of inducing the production of interferon-.gamma.
508 6,787,309	T Alterations in the long QT syndrome genes KVLQT1 and SCN5A and methods for detecting same
509 6,784,285	T Modifications of the VEGF Receptor-2 Protein and methods of use
510 6,783,978	T Isolation and characterization of the genomic DNA clones of ribosomal protein gene L25 in tobacco
511 6,783,966	T Alpha1, 4-galactosyltransferase and DNA encoding thereof
512 6,780,629	T Subtilase enzymes
513 6,780,408	T Genes encoding hybrid bacillus thuringiensis toxins

- 514 [6,777,537](#) **T** [Osteoclast proton pump subunit](#)
- 515 [6,777,229](#) **T** [Plasmids from *Corynebacterium glutamicum* and use thereof](#)
- 516 [6,777,204](#) **T** [Receptor protein and method for diagnosing inflammatory diseases by using the same](#)
- 517 [6,774,284](#) **T** [DNA encoding a plant lipase, transgenic plants and a method for controlling senescence in plants](#)
- 518 [6,773,919](#) **T** [Expression vector for production of recombinant proteins](#)
- 519 [6,773,908](#) **T** [Proteins encoded by polynucleic acids of porcine reproductive and respiratory syndrome virus \(PRRSV\)](#)
- 520 [6,773,907](#) **T** [Subtilase enzymes](#)
- 521 [6,768,001](#) **T** [Xylanase from *trichoderma reesei*, method for production thereof, and methods employing this enzyme](#)
- 522 [6,764,994](#) **T** [Growth/differential factor of the TGF-B family](#)
- 523 [6,764,843](#) **T** [Method of cloning and expression of BsmBI restriction endonuclease and BsmBI methylase in *E. coli* and purification of BsmBI endonuclease](#)
- 524 [6,764,836](#) **T** [Interleukin-15 receptors](#)
- 525 [6,764,824](#) **T** [Primers for screening schizophrenia and a method thereof](#)
- 526 [6,762,293](#) **T** [Diagnostics and therapeutics for autosomal dominant hemochromatosis](#)
- 527 [6,762,052](#) **T** [DNA sequence encoding flavin-containing monooxygenase](#)
- 528 [6,762,046](#) **T** [Polypeptide having .beta.-fructofuranosidase activity](#)
- 529 [6,759,229](#) **T** [Toxin-phage bacteriocide antibiotic and uses thereof](#)
- 530 [6,759,212](#) **T** [Cell cycle-regulating proteins](#)
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- 532 [6,753,166](#) **T** [IL-1 eta DNA and polypeptides](#)
- 533 [6,750,378](#) **T** [Maize H3C4 promoter combined with the first intron of rice actin, chimeric gene comprising it and transformed plant](#)
- 534 [6,746,866](#) **T** [Preparation of factor XIIIa by gene manipulation](#)
- 535 [6,746,860](#) **T** [Paramyxovirus vectors used for transfer of foreign genes](#)
- 536 [6,743,617](#) **T** [Human lysozyme gene, its encoded polypeptide and the method for preparing them](#)
- 537 [6,743,607](#) **T** [Production of complex carbohydrates](#)
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- 540 [6,737,519](#) **T** [Human genes relating to respiratory diseases and obesity](#)
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- 543 [6,733,994](#) **T** [Highly expressible genes](#)
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- 545 [6,727,409](#) **T** [Bacillus thuringiensis strains and their insecticidal proteins](#)
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548 [6,723,546](#) **T** [Method for cloning and expression of BsaI restriction endonuclease and BsaI methylase in E. coli](#)

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550 [6,717,033](#) **T** [Poly ADP-ribose polymerase gene and its uses](#)

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551	6,716,612 T DNA molecules coding for debranching enzymes derived from plants
552	6,713,618 T DNA which encodes trehalase and uses thereof
553	6,713,270 T Method for identifying ligand of estrogen receptor beta
554	6,710,227 T Cyclin-dependent kinase inhibitors and uses thereof
555	6,710,171 T Physiologically active protein originating in mammals
556	6,709,865 T Sunflower LOX polynucleotides and related compositions
557	6,709,859 T Chromosomal DNA Fragments Encoding Enzymes for Encoding B-Lactam Biosynthesis Enzymes, and Vector and Transformants for Their Expression
558	6,709,842 T DNA encoding a growth factor specific for epithelial cells
559	6,706,688 T Methods for regulating bud-hypha transitions and cAMP levels by the adenylate cyclase-associated protein gene, CAPI
560	6,706,493 T DNA encoding a cholecystokinin receptor
561	6,703,541 T Nematode-upregulated peroxidase gene promoter from nematode-resistant maize line Mp307
562	6,703,225 T Interferon-.alpha.
563	6,703,222 T Soluble LDL receptor, its production and use
564	6,699,981 T Method and compositions for improved polynucleotide synthesis
565	RE38,446 T Sucrose phosphate synthase (SPS), its process for preparation its cDNA, and utilization of cDNA to modify the expression of SPS in plant cells
	T

- 566 [6,696,556](#) [DNA molecule encoding a variant .alpha.2B-adrenoceptor protein, and uses thereof](#)
- 567 [6,696,256](#) [T Method, array and kit for detecting activated transcription factors by hybridization array](#)
- 568 [6,693,228](#) [T Alteration of flowering time in plants](#)
- 569 [6,693,185](#) [T Methods and means to modulate programmed cell death in eukaryotic cells](#)
- 570 [6,692,950](#) [T Clavulanic acid dehydrogenase, preparation and use for the production of clavulanic acid](#)
- 571 [6,692,926](#) [T Recombinant human SM-11044-binding receptor proteins exhibiting ligand-binding activities, and their use](#)
- 572 [6,689,880](#) [T DNA molecule for detecting glyphosate tolerant wheat plant 33391 and progeny thereof](#)
- 573 [6,689,876](#) [T Cloning and sequencing of allergens of dermatophagoides](#)
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- 575 [6,689,583](#) [T Chromatin regulator genes](#)
- 576 [6,686,174](#) [T Method and constructs for inhibiting protein expression in bacteria](#)
- 577 [6,686,149](#) [T Methods for obtaining nucleotide sequences coding for polypeptides specifically active for larvae of *S. littoralis*](#)
- 578 [6,683,165](#) [T Human gene relating to respiratory diseases and obesity](#)
- 579 [6,680,424](#) [T Modified proteinase inhibitors](#)
- 580 [6,680,368](#) [T Estrogen receptor beta](#)
- 581 [6,680,060](#) [T Hepatitis A virus vaccines](#)
- 582 [6,677,444](#) [T Melanoma antigens and methods of use](#)
- 583 [6,673,914](#) [T Human tumor-associated gene](#)
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- 586 [6,673,567](#) [T Method of determination of gene function](#)
- 587 [6,670,466](#) [T Human endogenous retrovirus in breast cancer](#)
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- 592 [6,663,872](#) [T Hemorrhagic enteritis virus DNA sequences, proteins encoded thereby and various uses thereof](#)
- 593 [6,660,851](#) [T DNA fragment elevating gene expression dose](#)
- 594 [6,660,848](#) [T Allelic variant of human STAT3](#)
- 595 [6,660,846](#) [T Vesicular amino acid transporter composition and method](#)
- 596 [6,660,512](#) [T Human lysozyme gene, its encoded polypeptide and the method of preparing them](#)
- 597 [6,657,056](#) [T Compounds for immunotherapy of prostate cancer and methods for their use](#)
- 598 [6,656,708](#) [T Human growth differentiation factor encoding sequence and polypeptide](#)

[encoded by such DNA sequence and producing method thereof](#)

599 [6,653,118](#)  [Deoxyribonuclease, gene encoding same and use thereof](#)

600 [6,649,748](#)  [Peroxidase genomic gene derived from ipomoea batatas and a promoter thereof](#)

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601	6,649,387 T Enzymatic oxidative deamination process
602	6,645,765 T Plant regulatory sequences for control of gene expression
603	6,645,740 T Nucleic acids encodings equine GM-CSF
604	6,642,434 T Transgenic plants with .gamma.-tocopherol methyltransferase
605	6,642,028 T Vectors and genes exhibiting increased expression
606	6,638,748 T Gene encoding alkaline liquifying alpha-amylase
607	6,638,746 T Phytase from bacillus subtilis, gene encoding said phytase, method for its production and use
608	6,638,741 T Differentiation-suppressive polypeptide serrate-2
609	6,635,477 T Human cytomegalovirus DNA sequences
610	6,635,445 T Nucleic acid molecules encoding human luteinizing hormone-human chorionic gonadotropin receptor protein and transformants thereof
611	6,632,981 T DNA sequences encoding polypeptides having beta-1,3-glucanase activity
612	6,632,645 T Thermophilic DNA polymerases from Thermoactinomyces vulgaris
613	6,630,616 T Arabidopsis MPC1 gene and methods for controlling flowering time
614	6,630,345 T Nucleic acids encoding a calcium independent receptor of .alpha.-latrotoxin, characterization and uses thereof
615	6,630,331 T Herbicides test method
616	6,627,793 T Low temperature-inducible wheat WCS120 gene promoter

- 617 [6,620,924](#) **T** [Thermostable glucoamylase](#)
- 618 [6,620,594](#) **T** [Uncoupling protein homologue: UCP 3](#)
- 619 [6,613,960](#) **T** [Phloem-loading-specific promoter](#)
- 620 [6,613,890](#) **T** [Protease inhibitor peptides](#)
- 621 [6,613,547](#) **T** [Pichia methanolica glyceraldehyde-3-phosphate dehydrogenase 1 promoter and terminator](#)
- 622 [RE38,240](#) **T** [DNA encoding human endothelial cell growth factors and plasmids comprising said DNA](#)
- 623 [6,610,908](#) **T** [Manipulation of lignin composition in plants using a tissue-specific promoter](#)
- 624 [6,610,520](#) **T** [Gene encoding human manganese superoxide dismutase and recombinant polypeptide encoded thereby](#)
- 625 [6,610,513](#) **T** [Receptor proteins](#)
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- 628 [6,599,738](#) **T** [Reporter gene system for use in cell-based assessment of inhibitors of the hepatitis C virus protease](#)
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- 630 [6,599,730](#) **T** [Subtilisin 309 variants having decreased adsorption and increased hydrolysis](#)
- 631 [6,596,853](#) **T** [DNA encoding peptides of IL-2](#)
- 632 [6,596,524](#) **T** [Method for cloning and expression of BsmAi restriction endonuclease and BsmAI methylase in E. coli](#)
- 633 [6,596,513](#) **T** [Kluyveromyces lactis maltase/maltose permease bi-directional promoter and use thereof](#)
- 634 [6,596,512](#) **T** [Nucleic acid encoding Nematode dopamine transporter and the protein encoded thereby](#)
- 635 [6,596,509](#) **T** [Recombinant constructs and systems for secretion of proteins via type III secretion systems](#)
- 636 [6,593,462](#) **T** [Purified .beta.1,2-xylosyltransferase and uses thereof](#)
- 637 [6,593,305](#) **T** [Antitumor antisense sequences directed against R1 and R2 components of ribonucleotide reductase](#)
- 638 [6,593,304](#) **T** [RECOMBINANT DNA COMPRISING DNA CODING FOR MYOSIN HEAVY CHAIN SM1 ISO-FORM PROTEIN INSERTED INTO VECTOR DNA MICROORGANISM CARRYING THE RECOMBINANT DNA, AND AN AGENT FOR TREATMENT OF ARTERIOSCLEROSIS COMPRISING THE RECOMBINANT DNA](#)
- 639 [6,593,122](#) **T** [Method for cloning and expression of BseRI restriction endonuclease and BseRI methylase in E. coli](#)
- 640 [6,593,105](#) **T** [Prion propagation inhibition by dominant-negative prion protein mutants](#)
- 641 [6,590,090](#) **T** [Fas ligand-like protein, its production and use](#)
- 642 [6,590,086](#) **T** [Mutants of the Rb and p53 genes and uses thereof](#)
- 643 [6,589,775](#) **T** [DNA sequence encoding enzymes of clavulanic acid biosynthesis](#)
- 644 [6,589,769](#) **T** [Method for cloning and expression of TspRI restriction endonuclease and TspRI methylase in E. coli](#)
- 645 [6,589,752](#) **T** [Recombinant antigen of Taenia solium metacestodes](#)

- 646 [6,586,661](#) **T** [Regulation of quinolate phosphoribosyl transferase expression by transformation with a tobacco quinolate phosphoribosyl transferase nucleic acid](#)
- 647 [6,586,580](#) **T** [Protein rib, a cell surface protein that confers immunity to many strains of the group B Streptococcus: process for purification of the protein, reagent kit and pharmaceutical composition](#)
- 648 [6,586,389](#) **T** [Cubilin protein, DNA sequences encoding cubilin and uses thereof](#)
- 649 [6,586,220](#) **T** [Method for cloning and expression of BsaWI restriction endonuclease and BsaWI methylase in E. coli](#)
- 650 [6,586,202](#) **T** [Isoprenoid production](#)
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PAT. NO.	Title
651 6,583,340	DNA sequence encoding plant 2-acyltransferase
652 6,582,956	Tumor suppressor gene
653 6,579,716	Better emergence characteristics and improved seedling growth under low-light environments
654 6,579,697	Modulator of TNF/NGF superfamily receptors and soluble oligomeric TNF/NGF superfamily receptors
655 6,573,371	Cell growth inhibiting and cell differentiation specific SYG972 gene, genomic DNA and promoter thereof
656 6,570,002	Inhibitor of programmed cell death
657 6,569,665	Calpaines, production and use thereof
658 6,569,650	Process for the fermentative preparation of metabolic products and for the nucleotide sequences encoding for the sod gene
659 6,569,643	Mammalian artificial chromosomes
660 6,569,642	Receptor that binds trail
661 6,569,430	Antibodies to the antigen Campath-1
662 6,566,586	Cotton expansin promoter sequence
663 6,566,511	MAP kinase phosphatase mutant
664 6,563,025	Nucleotide sequences encoding anthranilate synthase
665 6,562,957	Genomic sequence encoding endoglin and fragments thereof
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- 666 [6,562,589](#) [AIB1, a novel steroid receptor co-activator](#)
- 667 [6,559,298](#) [T Polypeptides that bind interleukin-18 \(IL-18\)](#)
- 668 [6,558,953](#) [T Grapevine leafroll virus proteins and their uses](#)
- 669 [6,558,938](#) [T Protease variants and compositions](#)
- 670 [6,558,921](#) [T Promoters of the genes glutamate deshydrogenase, .beta.-N-acetylhexosaminidase and .gamma.-actin and their use in filamentous fungi expression, secretion and antisense systems](#)
- 671 [6,558,728](#) [T .alpha.-glucuronidases of aspergillus, production thereof and their uses](#)
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- 673 [6,555,371](#) [T Sialyltransferase and DNA encoding the same](#)
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- 675 [6,555,355](#) [T Protease variants and compositions](#)
- 676 [6,552,179](#) [T Mutant luciferases](#)
- 677 [6,548,655](#) [T Interleukin-4 receptors](#)
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- 679 [6,541,610](#) [T Fusion proteins comprising tumor necrosis factor receptor](#)
- 680 [6,541,459](#) [T Soluble herpesvirus glycoprotein complex vaccine](#)
- 681 [6,541,238](#) [T Recombinant cellulose synthase](#)
- 682 [6,538,182](#) [T DNA encoding a plant deoxyhypusine synthase, a plant eukaryotic initiation factor 5A, transgenic plants and a method for controlling senescence programmed and cell death in plants](#)
- 683 [6,537,791](#) [T Mammalian DNA-dependent ATPase a polypeptides and fusions thereof](#)
- 684 [6,534,268](#) [T Human BMP-7 promoter and method for exploring bone-related substance by using the same](#)
- 685 [6,534,061](#) [T Tumor necrosis factor receptor homologs and nucleic acids encoding the same](#)
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- 690 [6,528,296](#) [T Endonuclease](#)
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- 694 [6,524,831](#) [T Synthetic enzymes for the production of coniferyl alcohol, coniferylaldehyde, ferulic acid, vanillin and vanillic acid and their use](#)
- 695 [6,524,817](#) [T DNA encoding a receptor subunit for oncostatin M](#)
- 696 [6,521,816](#) [T Nucleic acid molecules from rice and their use for the production of modified starch](#)
- 697 [6,521,438](#) [T Chemoreceptors in plant parasitic nematodes](#)

698 [6,521,410](#) **T** [Mutant of tissue factor pathway inhibitor, DNA sequence and use for detecting thrombotic disorders](#)

699 [6,521,406](#) **T** [SpnG, a gene for spinosyn insecticide biosynthesis](#)

700 [6,515,205](#) **T** [Genes from chromatium vinosum for the production of polyhydroxyalkanoate](#)

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701 6,514,755	T SCA7 gene and methods of use
702 6,514,737	T Method for cloning and expression of AsiSI restriction endonuclease and AsiSI methylase in E. coli
703 6,514,725	T STAT function-regulatory protein
704 6,509,183	T DNA fragment containing gene for alkaline pullulanase
705 6,509,151	T DNA molecule encoding for cellular uptake of Mycobacterium tuberculosis and uses thereof
706 6,506,595	T DNAs encoding new fusion proteins and processes for preparing useful polypeptides through expression of the DNAs
707 6,506,565	T Plant regulatory sequences for selective control of gene expression
708 RE37,958	T DNA sequence coding for protein C
709 6,504,023	T DNA encoding type II IL-1 receptors
710 6,503,706	T Method for identifying human and animal cells having an unlimited proliferation of tumor-formation potential
711 6,503,502	T Nucleotide sequences, proteins, drugs and diagnostic agents of use in treating cancer
712 6,500,943	T Promoter
713 6,500,635	T Insulin-like growth factor binding protein (IGFBF-5)
714 6,498,239	T Sterol glycosyl transferases
715 6,498,019	T Lecithin-cholesterol acyltransferase protein

- 716 [6,497,883](#) **T** [Porcine circovirus recombinant poxvirus vaccine](#)
- 717 [6,495,739](#) **T** [Plant phosphatidic acid phosphatases](#)
- 718 [6,495,344](#) **T** [Phenylalanine-free protein and DNA coding therefor](#)
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- 720 [6,492,106](#) **T** [Mammalian proteins that bind to FKBP12 in a rapamycin-dependent fashion](#)
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- 730 [6,479,628](#) **T** [Methods and materials relating to IMPDH and GMP production](#)
- 731 [6,476,294](#) **T** [Plant phosphatidic acid phosphatases](#)
- 732 [6,475,983](#) **T** [Water-soluble polypeptides having a high affinity for .alpha. and .beta. interferons](#)
- 733 [6,475,761](#) **T** [Glycosyltransferase and DNA encoding the same](#)
- 734 [6,475,735](#) **T** [Human BMP-2 promoter and method for exploring bone-related substance by using the same](#)
- 735 [6,472,519](#) **T** [Plasmodium falciparum antigens inducing protective antibodies](#)
- 736 [6,472,516](#) **T** [Progesterin-regulated gene](#)
- 737 [6,472,191](#) **T** [DNA FRAGMENT CARRYING TOLUENE MONOOXYGENASE GENE, RECOMBINANT PLASMID, TRANSFORMED MICROORGANISM, METHOD FOR DEGRADING CHLORINATED ALIPHATIC HYDROCARBON COMPOUNDS AND AROMATIC COMPOUNDS, AND METHOD FOR ENVIRONMENTAL REMEDIATION](#)
- 738 [6,472,174](#) **T** [DNA encoding cytokine designated LERK-6](#)
- 739 [6,469,231](#) **T** [Starch branching enzyme II of potato](#)
- 740 [6,469,155](#) **T** [HIgR and related domain which binds glycoprotein D of herpes simplex virus](#)
- 741 [6,469,153](#) **T** [EIP-1 and EIP-3 genes, envelope-interacting proteins, EIP-1 and EIP-3](#)
- 742 [6,468,789](#) **T** [Oxygen sensing and hypoxic selection for tumors](#)
- 743 [6,468,775](#) **T** [Thermostable DNA polymerase from carboxydotherrnus hydrogenoformans](#)
- 744 [6,465,636](#) **T** [Pathogen-inducible promoter](#)
- 745 [6,465,618](#) **T** [Mitogen activated protein kinase \(MAPK\) kinase](#)
- 746 [6,465,254](#) **T** [Mutant loxP site and applications thereof](#)
- 747 [6,462,185](#) **T** [Floral organ-specific gene and its promoter sequence](#)
- 748 [6,458,944](#) **T** [Human BMP-4 promoter and method for exploring bone-related substance by](#)

[using the same](#)

749 [6,458,942](#)  [28-kDa immunoreactive protein gene of Ehrlichia canis and uses thereof](#)

750 [6,458,563](#)  [Modified factor VIII](#)

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PAT. NO.	Title
751 6,458,551	T Estrogen receptor
752 6,455,687	T Human lactoferrin
753 6,455,681	T DNA molecules encoding single strand gap response proteins involved in activation of a DNA repair/cell cycle checkpoint pathway
754 6,455,276	T Human .alpha.4 receptor subunit of the GABA-A receptor
755 6,455,274	T Human DNA Ligase IV
756 6,451,583	T Pharmacological targeting of mRNA cap formation for treatment of parasitic infections
757 6,451,570	T Mutants of monoamine oxidase B
758 6,451,568	T Cloning of human choline ethanolaninephospho transferases synthesis of phosphatidyl choline phosphatidyle thanolamine and platelet activating factor
759 6,451,558	T Genes in the control of hematopoiesis
760 6,451,537	T Gene of rice dihydrodipicolinate synthase and DNA relating to the same
761 6,448,381	T DNA encoding heparin-binding growth factor
762 6,448,072	T Transcription factors related to TFIIA
763 6,448,042	T Human tissue inhibitor of metalloproteinase-4
764 6,448,035	T Family of immunoregulators designated leukocyte immunoglobulin-like receptor (LIR)
765 6,444,877	T Liquidambar styraciflua AGAMOUS (LSAG) gene

- 766 [6,444,876](#) [Acyl CoA: cholesterol acyltransferase related nucleic acid sequences](#)
- 767 [6,444,455](#) **T** [Mitogen-activated protein kinase P38-2 and methods of use therefor](#)
- 768 [6,444,426](#) **T** [Production and use of normalized DNA libraries](#)
- 769 [6,441,273](#) **T** [Constitutive and inducible promoters from coffee plants](#)
- 770 [6,440,714](#) **T** [Tyr393 and Tyr398 mutants of monoamine oxidase B](#)
- 771 [6,440,693](#) **T** [TNF receptors, TNF binding proteins and DNAs coding for them](#)
- 772 [6,437,112](#) **T** [Materials for the production of nanometer structures and use thereof](#)
- 773 [6,437,111](#) **T** [Bone morphogenetic protein-11 \(BMP-11\) compositions](#)
- 774 [6,436,688](#) **T** [Human lysozyme gene, its encoding polypeptide and the method preparing for them](#)
- 775 [6,432,702](#) **T** [Mammalian growth factor nucleic acids, vectors, and host cells](#)
- 776 [6,428,979](#) **T** [Receptor protein for human B cell stimulatory factor-2](#)
- 777 [6,423,837](#) **T** [Cloning and sequencing of allergens of dermatophagoides house dust mite](#)
- 778 [6,423,835](#) **T** [Nucleotide deduced amino acid sequence, isolation and purification of heat-shock chlamydial proteins](#)
- 779 [6,423,525](#) **T** [Isolation and composition of a novel glycosidase from chryseobacterium](#)
- 780 [6,423,523](#) **T** [Xylanase derived from a bacillus species, expression vectors for such xylanase and other proteins, host organisms therefor and use thereof](#)
- 781 [6,423,520](#) **T** [Regulation of quinolate phosphoribosyl transferase expression](#)
- 782 [6,423,496](#) **T** [Compositions and methods for the treatment and diagnosis of breast cancer](#)
- 783 [6,420,628](#) **T** [Seed shattering](#)
- 784 [6,420,138](#) **T** [Expression control sequences](#)
- 785 [6,420,134](#) **T** [Vaccines for nontypable haemophilus influenzae](#)
- 786 [6,413,758](#) **T** [Method for cloning and expression of Bpml restriction endonuclease in E. coli](#)
- 787 [6,413,751](#) **T** [DNA adenine methyltransferases and uses thereof](#)
- 788 [6,410,828](#) **T** [Regulatory sequences useful for gene expression in plant embryo tissue](#)
- 789 [6,410,722](#) **T** [Human and mammalian data replication origin consensus sequences](#)
- 790 [6,410,717](#) **T** [Gene encoding a host factor protein indispensable for multiplication of a plant virus](#)
- 791 [6,410,705](#) **T** [Nucleotide sequences coding for the thrE gene and process for the enzymatic production of L-threonine using coryneform bacteria](#)
- 792 [6,410,293](#) **T** [DNA fragments containing biotin biosynthetase gene and use of the same](#)
- 793 [6,406,886](#) **T** [DNA encoding SPA-1 protein](#)
- 794 [6,403,785](#) **T** [Isolated DNA molecule encoding human TSC403](#)
- 795 [6,403,780](#) **T** [Homologous 28-kilodalton immunodominant protein genes of ehrlichia canis and uses thereof](#)
- 796 [6,403,354](#) **T** [Method for cloning and expression of BstYI restriction endonuclease and BstYI methylase in E. coli and purification of BstYI and M.BstYI enzymes](#)
- 797 [6,403,342](#) **T** [DNA coding for mutant isopropylmalate synthase L-leucine-producing microorganism and method for producing L-leucine](#)
- 798 [6,399,348](#) **T** [DNA sequences for matrix metalloproteases, their production and use](#)
- 799 [6,399,325](#) **T** [DNA encoding a galanin receptor](#)
- 800 [6,399,324](#) **T** [Genes encoding branched-chain alpha-ketoacid dehydrogenase complex from](#)

[Streptomyces avermitilis](#)

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PAT. NO.	Title
801 6,395,531	T Method for cloning and expression of MlyI restriction endonuclease and MlyI methylase and BstNBII methylase in E. coli
802 6,395,528	T Phosphoserine phosphatase gene of coryneform bacteria
803 6,395,526	T DNA polymerase
804 6,395,477	T Human potassium channel polynucleotide and polypeptides and uses thereof
805 6,392,030	T Strawberry promoters and genes
806 6,392,023	T Homologous 28-kilodalton immunodominant protein genes of Ehrlichia canis and uses thereof
807 6,392,022	T Gene originating in human chondrocyte
808 6,391,639	T Plant and viral promoters
809 6,391,631	T Bacterial plasmids
810 6,391,608	T Method for cloning and expression of PleI restriction endonuclease and PleI and BstNBII methylases in E. coli
811 6,391,581	T DNA encoding interleukin-4 receptors
812 6,388,174	T Gene encoding .alpha.-subunit of rice anthranilate synthase and DNA relating thereto
813 6,387,696	T Human calcium channel compositions and methods
814 6,387,688	T DNA fragments having basidiomycete-derived promoter activity and expression of foreign genes under control of the promoter activity
815 6,387,681	T Method for cloning and expression of NHEI restriction endonuclease in E.

- [coli.](#)
- 816 [6,384,207](#) **T** [Regulatory sequences for transgenic plants](#)
- 817 [6,384,191](#) **T** [Receptors for fibroblast growth factors](#)
- 818 [6,379,951](#) **T** [Compounds for immunotherapy of breast cancer and methods for their use](#)
- 819 [6,376,753](#) **T** [PURIFIED CYTOCHROME P450 POLYPEPTIDE CYP76B1 FROM HELIANTHUS TUBEROSUS AND ITS APPLICATIONS AS BIOCATALYST IN PARTICULAR FOR THE DEGRADATION OF ENVIRONMENTAL POLLUTANTS AND FOR ALTERING THE RESISTANCE OF PLANTS SENSITIVE TO PHENYLUREA FAMILY OF HERBICIDES](#)
- 820 [6,376,658](#) **T** [Proteins related to encapsulation and genes encoding the same](#)
- 821 [6,376,239](#) **T** [DNA molecules comprising a promoter capable of conferring expression of a heterologous DNA sequence](#)
- 822 [6,376,222](#) **T** [Riboflavin production](#)
- 823 [6,376,216](#) **T** [Promoter from Ashbya gossypii](#)
- 824 [6,368,845](#) **T** [Polypeptides having L-asparaginase activity](#)
- 825 [6,368,833](#) **T** [Esterases, DNA encoding therefor and vectors and host incorporating same](#)
- 826 [6,368,790](#) **T** [cDNA encoding P2P proteins and use of P2P cDNA derived antibodies and antisense reagents in determining the proliferative potential of normal, abnormal, and cancer cells in animals and humans](#)
- 827 [6,368,601](#) **T** [Porcine circovirus vaccine and diagnostics reagents](#)
- 828 [6,365,723](#) **T** [Sequences of E. coli O157](#)
- 829 [6,365,388](#) **T** [Biotin biosynthetic genes](#)
- 830 [6,362,327](#) **T** [High level expression of human cyclooxygenase-2](#)
- 831 [6,362,325](#) **T** [Murine 4-1BB gene](#)
- 832 [6,361,987](#) **T** [Gene encoding a protein having asymmetric hydrolase activity for 4-substituted 1,4-dihydropyridine derivatives and its expression product](#)
- 833 [6,361,986](#) **T** [Process for the preparation of L-amino acids by fermentation and nucleotide sequences coding for the accDA gene](#)
- 834 [6,361,982](#) **T** [Regulatory gene for clavulanic acid biosynthesis](#)
- 835 [6,358,724](#) **T** [Isolation and composition of novel glycosidases](#)
- 836 [6,358,680](#) **T** [Detection of wheat and barley fungal pathogens using the polymerase chain reaction](#)
- 837 [6,355,777](#) **T** [P43 antigen for the immunodiagnosis of canine ehrlichiosis and uses thereof](#)
- 838 [6,355,480](#) **T** [Methods and compositions for modulating spermatogenesis](#)
- 839 [6,355,255](#) **T** [Streptococcal C5a peptidase vaccine](#)
- 840 [6,352,973](#) **T** [Bone stimulating factor](#)
- 841 [6,350,602](#) **T** [Cloning and expression of phytase from aspergillus](#)
- 842 [6,348,643](#) **T** [DNA sequences encoding the arabidopsis acetoxy-acid synthase small subunit and methods of use](#)
- 843 [6,348,334](#) **T** [DNA encoding Fas ligand](#)
- 844 [6,348,331](#) **T** [Pichia methanolica glyceraldehyde-3-phosphate dehydrogenase 2 promoter](#)
- 845 [6,346,394](#) **T** [Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide](#)

- 846 [RE37,543](#) **T** [DNA sequence useful for the production of polyhydroxyalkanoates](#)
- 847 [6,344,552](#) **T** [Compositions and methods comprising DNA sequences encoding B. burgdorferi polypeptides](#)
- 848 [6,344,550](#) **T** [Compositions and methods for the treatment and diagnosis of breast cancer](#)
- 849 [6,344,341](#) **T** [Increased production of secreted proteins by recombinant yeast cells](#)
- 850 [6,344,337](#) **T** [Antigen test to detect equine protozoal myeloencephalitis in horse serum and cerebrospinal fluid](#)
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PAT. NO.	Title
851 6,342,375	T Modified methylotrophic Pichia pastoris yeast which secretes human growth hormone
852 6,342,357	T Alterations in the long QT syndrome genes KVLQT1 and SCN5A and methods for detecting same
853 6,342,215	T Identification of genes
854 6,338,959	T Gene for enzyme having both alkaline pullulanase and alkaline .alpha.-amylase activities
855 6,337,430	T Plant promoters
856 6,335,190	T Method for cloning and producing the BsmI restriction endonuclease in E. coli
857 6,333,448	T Plant enzyme and use thereof
858 6,333,406	T Gene encoding protein antigens of Plasmodium falciparum and uses therefor
859 6,333,191	T Nucleic acids sequence, stress-induced proteins and uses thereof
860 6,333,158	T DNA polymerase-related factors
861 6,331,664	T Acyl-ACP thioesterase nucleic acids from maize and methods of altering palmitic acid levels in transgenic plants therewith
862 6,331,616	T Nucleic acids obtained from the envelope coding region of feline immunodeficiency virus molecular clone designated JSY3
863 6,331,428	T Hexulose phosphate isomerase gene
864 6,329,574	T High lysine fertile transgenic corn plants
865 6,329,568	T Tospovirus resistance in plants

- 866 [6,329,141](#) **T** [Methods for transforming Phaffia strains, transformed Phaffia strains so obtained and recombinant DNA in said methods](#)
- 867 [6,326,165](#) **T** [Recombinant BHLH-PAS/JHR polypeptide and its use to screen potential insecticides](#)
- 868 [6,323,395](#) **T** [Nucleotide sequences of maize and soybean .beta.-Ketoacyl-Acyl Carrier Protein Synthase II and their use in the regulation of fatty acid content of oil](#)
- 869 [6,323,330](#) **T** [Protein C16 and C16N or genes encoding the same](#)
- 870 [6,323,026](#) **T** [Mutations in the KCNE1 gene encoding human mink which cause arrhythmia susceptibility thereby establishing KCNE1 as an LQT gene](#)
- 871 [6,323,020](#) **T** [Neutralization-sensitive epitopes of Cryptosporidium parvum](#)
- 872 [6,322,998](#) **T** [Recombinant DNA compounds that encode ACV synthetase activity of Cephalosporium acremonium](#)
- 873 [6,320,027](#) **T** [Nucleotide sequence of the nucleocapsid gene of oropouche virus](#)
- 874 [6,320,026](#) **T** [Cell growth inhibitor factor](#)
- 875 [6,316,604](#) **T** [Human C3b/C4b receptor \(CR1\)](#)
- 876 [6,316,238](#) **T** [Process for producing activated human ALT](#)
- 877 [6,316,224](#) **T** [Chlorella virus promoters](#)
- 878 [6,313,281](#) **T** [Nucleic acid encoding OMP26 antigen from Haemophilus influenzae](#)
- 879 [6,313,279](#) **T** [Human glutamate receptor and related DNA compounds](#)
- 880 [6,312,696](#) **T** [Antigenic protein originating in infectious laryngotracheitis virus](#)
- 881 [6,312,688](#) **T** [Tyrosine-phosphatase-related protein](#)
- 882 [6,309,868](#) **T** [Cloning of the prolyl-dipeptidyl-peptidase from Aspergillus oryzae](#)
- 883 [6,307,038](#) **T** [Expression systems utilizing autolyzing fusion proteins and a novel reducing polypeptide](#)
- 884 [6,307,036](#) **T** [Tumour suppressor gene](#)
- 885 [6,306,624](#) **T** [Retinoid metabolizing protein](#)
- 886 [6,306,622](#) **T** [cDNA encoding a BMP type II receptor](#)
- 887 [6,303,769](#) **T** [LERK-5 DNA](#)
- 888 [6,303,365](#) **T** [Method of determining activity of 1-deoxy-D-xylulose-5-phosphate reductoisomerase and 1-deoxy-D-xylulose-5-phosphate synthase](#)
- 889 [6,303,359](#) **T** [DNA molecule encoding new aminopeptidase, and method of producing the aminopeptidase](#)
- 890 [6,303,339](#) **T** [DNA segments encoding autoimmune polypeptide epitopes of Ro/ss-A antigen](#)
- 891 [6,300,114](#) **T** [Sequences of xylanase and xylanase expression vectors](#)
- 892 [6,300,103](#) **T** [Anti-microbial proteins](#)
- 893 [6,300,095](#) **T** [Promoters of the genes glutamate dehydrogenase .beta.-N-acetylhexosaminidase and .gamma.-actin and their use in filamentous fungi expression, secretion and antisense systems](#)
- 894 [6,297,032](#) **T** [Recombinant cephalosporin C amidohydrolase in cephalosporin biosynthesis](#)
- 895 [6,294,663](#) **T** [Transmembrane serine protease overexpressed in ovarian carcinoma and uses thereof](#)
- 896 [6,294,359](#) **T** [Human basic fibroblast growth factor analog](#)
- 897 [6,294,358](#) **T** [Thermus promoters for gene expression](#)

- 898 [6,294,352](#) **T** [TNF receptors, TNF binding proteins and DNAs coding for them](#)
899 [6,294,334](#) **T** [Genetic test for equine severe combined immunodeficiency disease](#)
900 [6,291,663](#) **T** [TADG-12: a novel transmembrane serine protease overexpressed in a ovarian carcinoma](#)
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901 6,291,221	Heat tolerant phytases
902 6,291,210	Differentiation inhibitor
903 6,291,206	BMP receptor proteins
904 6,291,168	Nucleic acid sequences diagnostic for pathogenic E.coli O157:H7, methods of identification and kit therefore
905 6,291,156	Plant pest control
906 6,290,965	DNA encoding human papillomavirus type 6A
907 6,287,866	.beta.-casein expressing constructs
908 6,287,858	DeUBiquitinating enzymes that regulate cell growth
909 6,287,816	BMP-9 compositions
910 6,284,948	Genes and methods for control of nematodes in plants
911 6,284,946	Banana DNA associated with fruit development
912 6,284,516	DNA segments and methods for increasing polysaccharide production
913 6,284,508	Glucuronoxylomannan (GXM)-O-acetylhydrolase of Cryptococcus neoformans and uses thereof
914 6,284,506	3-Hydroxy-3-methylglutaryl-CoA reductase polynucleotides in isoprenoid production
915 6,281,409	Blackcurrant promoters and genes
916 6,281,347	Human origin of replication complex genes and uses thereof

- 917 [6,281,346](#) **T** [Rat ob-receptors and nucleotides encoding them](#)
 - 918 [6,280,999](#) **T** [Sorangium polyketide synthases and encoding DNA therefor](#)
 - 919 [6,280,993](#) **T** [Gene encoding class I collagenase](#)
 - 920 [6,280,974](#) **T** [Recombinant feline coronavirus S proteins](#)
 - 921 [6,277,978](#) **T** [KVLQT1--a long QT syndrome gene](#)
 - 922 [6,277,973](#) **T** [Cloning and expression of a DNA sequence encoding a 41 kDa cryptosporidium parvum oocyst wall protein](#)
 - 923 [6,277,968](#) **T** [Human uroplakin II gene and methods for detecting and classifying bladder cancer via human uroplakin genes](#)
 - 924 [6,277,822](#) **T** [Family of peptides known as xenoxins](#)
 - 925 [6,277,598](#) **T** [DNA molecule encoding interferon-gamma \(IFN-.lambda.\) inducing factor \(IGIF, IL-18\) and DNA fragment thereof](#)
 - 926 [6,277,591](#) **T** [Dopamine receptors and genes](#)
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 - 930 [6,274,340](#) **T** [DNA sequences enhancing promoter activity](#)
 - 931 [6,274,330](#) **T** [L-AP4 sensitive glutamate receptors](#)
 - 932 [6,271,443](#) **T** [Cotton and rice cellulose synthase DNA sequences](#)
 - 933 [6,271,442](#) **T** [Method of producing pathogen-resistant plants](#)
 - 934 [6,271,438](#) **T** [Transgenic pathogen-resistant plant](#)
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 - 936 [6,271,364](#) **T** [Analog of NT-3](#)
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 - 939 [6,270,998](#) **T** [DNA coding for human cell surface antigen](#)
 - 940 [6,270,989](#) **T** [Protein production and delivery](#)
 - 941 [6,268,180](#) **T** [Recombinant human recombinant human interleukin-1.alpha.](#)
 - 942 [6,268,171](#) **T** [Recombinant PilC proteins, methods for producing them and their use](#)
 - 943 [6,265,635](#) **T** [DNA sequences coding for enzymes capable of facilitating the synthesis of linear .alpha.-1,4 glucans in plants, fungi and microorganisms](#)
 - 944 [6,265,634](#) **T** [Polyribozyme capable of conferring on plants resistance to cucumber mosaic virus and resistant plants producing this polyribozyme](#)
 - 945 [6,265,194](#) **T** [Serine-threonine kinase gene](#)
 - 946 [6,265,187](#) **T** [Recombinant endotoxin-neutralizing proteins](#)
 - 947 [6,262,344](#) **T** [Nematode-inducible plant gene promoter](#)
 - 948 [6,262,342](#) **T** [DNA sequences encoding polypeptides having .beta.-1,3-glucanase activity](#)
 - 949 [6,262,338](#) **T** [Resistance genes](#)
 - 950 [6,262,336](#) **T** [Expression of a heterologous protein C in mammary tissue of transgenic animals using a long whey acidic protein promoter](#)
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PAT. NO.	Title
951	6,262,245 T Compounds for immunotherapy of prostate cancer and methods for their use
952	6,262,244 T DNA and amino acid sequence specific for natural killer cells
953	6,262,242 T Tumor suppressor designated TS10Q23.3
954	6,258,942 T TADG7: a novel gene expressed in ovarian tumor and uses thereof
955	6,258,583 T Type II restriction endonuclease, Hpy188I, obtainable from helicobacter pylori J188 and a process for producing the same
956	6,258,557 T Smooth muscle cell LIM promoter
957	6,258,556 T cDNA and genomic clones encoding human .mu. opiate receptor and the purified gene product
958	6,258,555 T DNA encoding ACV synthetase
959	6,255,474 T Promoters for swine complement inhibitors
960	6,255,473 T Presenilin-1 gene promoter
961	6,255,467 T Human blood bacterium
962	6,255,092 T Stereospecific alcohol dehydrogenase isolated from Candida parapsilosis, amino acid and DNA sequences therefor, and method of preparation thereof
963	6,255,078 T Pseudorabies virus protein cross reference to related applications
964	6,255,072 T spsA polynucleotides
965	6,255,070 T Folding protein complexes
966	6,254,868 T Glycosylated humanized B-cell specific antibodies

- 967 [6,252,138](#) **T** [Pathogen-induced plant promoters](#)
- 968 [6,251,655](#) **T** [Process for increasing the production of penicillin G \(benzylpenicillin\) in Penicillium chrysogenum by expression of the PCL gene](#)
- 969 [6,248,937](#) **T** [Transcription factor and method for regulation of seed development, quality and stress-tolerance](#)
- 970 [6,248,874](#) **T** [DNA molecules encoding bacterial lysine 2,3-aminomutase](#)
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- 976 [6,245,562](#) **T** [Identification of genes altered in multiple myeloma](#)
- 977 [6,245,545](#) **T** [Method for cloning and producing the SmaI restriction endonuclease](#)
- 978 [6,245,524](#) **T** [Phenylacetyl-CoA ligase from penicillium chrysogenum](#)
- 979 [RE37,206](#) **T** [Gene encoding glycosyltransferase and its uses](#)
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- 981 [6,242,218](#) **T** [Genomic sequences for protein production and delivery](#)
- 982 [6,242,216](#) **T** [Nucleic acids encoding a functional human purinoreceptor P2X2 and P2X4, and methods of production and use thereof](#)
- 983 [6,242,213](#) **T** [Isolated DNA molecules encoding RANK-L](#)
- 984 [6,239,264](#) **T** [Genomic DNA sequences of ashbya gossypii and uses thereof](#)
- 985 [6,239,100](#) **T** [Synthetic polypeptide having fish growth hormone-like activity, nucleic acid encoding for the polypeptide and method using same](#)
- 986 [6,238,916](#) **T** [DNA encoding turkey hypothalamic vasoactive intestinal peptide](#)
- 987 [6,238,901](#) **T** [Type II restriction endonuclease, HPY 188 III, obtainable from Helicobacter pylori J188 and a process for producing the same](#)
- 988 [6,238,864](#) **T** [Analyte detection assay and methods of use](#)
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- 999 [6,228,643](#) **T** [Promoter](#)
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PAT. NO.	Title
1001	6,228,630 T Enzymes with xylanase activity from aspergillus aculeatus
1002	6,228,628 T Mutant chimeric DNA polymerase
1003	6,225,531 T Glucan elicitor receptor, DNA molecule coding therefor, fungus-resistant plants transformed with the DNA molecule and method for creating the plants
1004	6,225,454 T Sialidase localized in plasma membrane and DNA coding for the same
1005	6,225,112 T Human p27Kip1 gene promoter
1006	6,225,097 T Decaprenyl diphosphate synthetase gene
1007	6,225,081 T Protein, DNA coding for same and method of producing the protein
1008	6,225,075 T DNA encoding sterol methyltransferase
1009	6,225,054 T Compositions and methods for the treatment and diagnosis of breast cancer
1010	6,222,096 T Promoter and construct for plant transformation
1011	6,218,179 T Tissue specific hypoxia regulated constructs
1012	6,218,161 T Sugar-chain synthetase and process for producing the same
1013	6,218,148 T DNS encoding stem cell factor
1014	6,215,048 T Nucleic acid sequences encoding an antifungal polypeptide, aly AFP from alyssum and methods for their use
1015	6,215,043 T Method for shortening internode of inflorescence by introducing gene for petunia transcription factor PetSPL2
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- 1016 [6,215,042](#) [Plasmids containing DNA-sequences that cause changes in the carbohydrate concentration and carbohydrate composition in plants, as well as plant cells and plants containing these plasmids](#)
- 1017 [6,214,981](#) [T Moraxella catarrhalis outer membrane protein-106 polypeptide, gene sequence and uses thereof](#)
- 1018 [6,214,797](#) [T Urocortin peptides, nucleic acid encoding same methods for using same](#)
- 1019 [6,214,603](#) [T Method for producing amide compounds using a nitrile hydratase from a thermophilic bacillus](#)
- 1020 [6,214,599](#) [T Protease](#)
- 1021 [6,214,590](#) [T 2-aminothiazoline-4-carboxylate racemase and gene encoding therefor](#)
- 1022 [6,214,584](#) [T Human Interferon-.gamma.inducing factor](#)
- 1023 [6,214,577](#) [T Yeast vectors conferring antibiotic resistance](#)
- 1024 [6,214,550](#) [T Methods of differentiating metastatic and non-metastatic tumors](#)
- 1025 [6,211,432](#) [T DNA sequences coding for a cinnamoyl COA reductase, and applications thereof in the control of lignin contents in plants](#)
- 1026 [6,211,150](#) [T Analogs of cationic proteins](#)
- 1027 [6,210,951](#) [T GMP synthetase and gene coding for the same](#)
- 1028 [6,210,949](#) [T Mouse MTS2 gene](#)
- 1029 [6,210,945](#) [T Method for cloning and producing the RsaI restriction endonuclease in E. coli and purification of the recombinant RsaI restriction endonuclease](#)
- 1030 [6,210,943](#) [T Sucrose phosphate synthase from citrus and DNA encoding the same](#)
- 1031 [6,210,935](#) [T Staurosporin biosynthesis gene clusters](#)
- 1032 [6,210,923](#) [T Mammalian circadian regulator M-RIGUI2 \(MPER2\)](#)
- 1033 [6,207,881](#) [T Control of fruit ripening through genetic control of ACC synthase synthesis](#)
- 1034 [6,207,813](#) [T BMP-6 proteins](#)
- 1035 [6,207,431](#) [T Glutamine:fructose-6-phosphate amidotransferase, its production and use](#)
- 1036 [6,207,419](#) [T Thrombin inhibitory agents and methods of using same](#)
- 1037 [6,207,417](#) [T DNA encoding stem cell factor](#)
- 1038 [6,207,383](#) [T Mutations in and genomic structure of HERG--a long QT syndrome gene](#)
- 1039 [6,204,035](#) [T Methods and compositions to alter the cell surface expression of phosphatidylserine and other clot-promoting plasma membrane phospholipids](#)
- 1040 [6,204,026](#) [T Detection of M. tuberculosis complex via reverse transcriptase SDA](#)
- 1041 [6,204,018](#) [T 66 kDa antigen from Borrelia](#)
- 1042 [6,204,009](#) [T Nucleic acids encoding mutant recombinant hemoglobins containing heme pocket mutations](#)
- 1043 [6,200,785](#) [T L-lysine-producing corynebacteria and process for the preparation of l-lysine](#)
- 1044 [6,200,576](#) [T Swine vesicular disease virus and mutant strains and preparation process and use thereof](#)
- 1045 [6,197,948](#) [T Grapevine leafroll virus \(type 2\) proteins and their uses](#)
- 1046 [6,197,946](#) [T Peptide production as fusion protein in transgenic mammal milk](#)
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1049 [6,197,500](#) **T** [DNA sequences related to fragile X syndrome](#)

1050 [6,194,637](#) **T** [Maize DNA ligase I orthologue and uses thereof](#)

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1051 6,194,558	DNA encoding human monocyte-macrophage apolipoprotein B receptor gene and protein
1052 6,194,177	DNA encoding a hybrid heterodimeric protein
1053 6,194,172	Cytokine designated lerk-6
1054 6,194,168	Expression control sequences
1055 6,194,166	Gene regulating aureobasidin sensitivity
1056 6,191,268	Compositions and methods relating to DNA mismatch repair genes
1057 6,191,267	Cloning and producing the N.BstNBI nicking endonuclease
1058 6,190,892	Microbial production of indigo
1059 6,190,882	Mammalian circadian rhythm-like gene
1060 6,187,574	Process for producing the enzyme D-amino acid oxidase of Rhodotorula gracilis in host cells
1061 6,187,573	DNA encoding a thermostable DNA polymerase
1062 6,187,311	Engineered acarid allergen and process for producing the same
1063 6,184,449	1-Aminocyclopropane-1-carboxylate synthase genes from rosa to control ethylene levels in roses
1064 6,183,986	OspA DNA and lyme disease vaccine
1065 6,180,776	MTS2 gene
1066 6,180,388	Enzymes and micro organisms with amidase activity which hydrolyze

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- 1067 [6,180,364](#) **T** [Protein called epil/placentin, process for the preparation of this protein and pharmaceutical composition containing such, DNA coding for said protein](#)
- 1068 [6,180,344](#) **T** [5' Upstream region sequences of the MyoD1 gene and uses thereof](#)
- 1069 [6,180,334](#) **T** [Artificial recombinant substrate \(rAGG 1\) and native aggrecan to determine the proteolytic activity of `aggrecanase` in cell culture systems](#)
- 1070 [6,177,616](#) **T** [Genes coding for amino acid deacetylases with specificity for N-acetyl-L-phosphinothricin, their isolation and their use](#)
- 1071 [6,177,614](#) **T** [Control of floral induction in plants and uses therefor](#)
- 1072 [6,177,242](#) **T** [Genomic DNA fragments containing regulatory and coding sequences for the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and transgenic animals made using these fragments or mutated fragments](#)
- 1073 [6,175,060](#) **T** [Phosphate-deficiency inducible promoter](#)
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- 1080 [6,169,173](#) **T** [Cloning and functional expression of cholecystokinin/gastrin receptor-encoding DNA](#)
- 1081 [6,169,171](#) **T** [Hybrid protein between CS from plasmodium and HBSAG](#)
- 1082 [6,168,940](#) **T** [Protein having ethylenediamine-N,N'-disuccinic acid:ethylenediamine lyase activity and gene encoding the same](#)
- 1083 [6,166,294](#) **T** [Cotton fiber tissue-specific genes](#)
- 1084 [6,166,292](#) **T** [Raffinose synthetase gene, method of producing raffinose and transgenic plant](#)
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- 1088 [6,165,738](#) **T** [Expression in yeast of antigenically active, recombinant hybrid glutamic acid decarboxylase](#)
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- 1090 [6,160,106](#) **T** [Tumor suppressor genes, proteins encoded thereby and use of said genes and proteins](#)
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- 1094 [6,156,536](#) **T** [Toxins from the wasp Bracon hebetor](#)
- 1095 [6,156,319](#) **T** [Soluble herpesvirus glycoprotein complex vaccine](#)
- 1096 [6,153,815](#) **T** [DNA sequences from brassicaceae encoding squalene epoxidase and process of raising squalene levels in plants therewith](#)

- 1097 [6,150,586](#)  [Plant gene encoding acetyl coenzyme a carboxylase biotin carboxyl carrier protein](#)
- 1098 [6,150,136](#)  [Nucleotide sequence encoding oligodendrocyte-specific protein](#)
- 1099 [6,150,104](#)  [Homozygous mutation in KVLQT1 which causes Jervell and Lange Nielsen syndrome](#)
- 1100 [6,147,281](#)  [Antipathogenic peptides and compositions containing same](#)
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1101 6,147,201	T Cloning and sequencing of allergens of dermatophagoides (house dust mite)
1102 6,146,871	T Process for modifying the enzyme 7.beta.-(4-carboxybutanamide) cephalosporinacylase and purifying said enzyme in a single chromatographic step
1103 6,146,868	T Glucuronoxylomannan (GXM)-O-acetylhydrolase of cryptococcus neoformans and uses thereof
1104 6,143,878	T Sox-9 gene and protein and use in the regeneration of bone or cartilage
1105 6,143,872	T Borrelia burdorferi Osp A and B proteins and immunogenic peptides
1106 6,143,561	T DNA encoding plastid pyruvate dehydrogenase and branched chain oxoacid dehydrogenase components
1107 6,143,550	T Bacillus thuringiensis strains and their insecticidal proteins
1108 6,143,546	T Enzymes with aminopeptidase activity
1109 6,143,526	T Biosynthetic genes for spinosyn insecticide production
1110 6,143,524	T Peptide and protein fusions to thioredoxin, thioredoxin-like molecules, and modified thioredoxin-like molecules
1111 6,140,488	T Ras-binding protein (PRE1)
1112 6,140,115	T Canine .beta.-galactosidase gene and GM1-gangliosidosis
1113 6,140,101	T Polypeptides having L-asparaginase activity
1114 6,140,096	T Enzyme with endo-1,3(4)-.beta.-glucanase activity
1115 6,140,095	T Alkalitolerant xylanases

- 1116 [6,140,074](#) **T** [SH3 protein, gene, chimeric cells, vectors and expression method for producing the novel protein, and uses](#)
- 1117 [6,136,595](#) **T** [Jak kinases and regulations of cytokine signal transduction](#)
- 1118 [6,136,579](#) **T** [Method of producing .alpha.2,3-sialyltransferase](#)
- 1119 [6,136,572](#) **T** [Recombinant KAT enzyme and process for its preparation](#)
- 1120 [6,136,536](#) **T** [Rapid generation of stable mammalian cell lines producing high levels of recombinant proteins](#)
- 1121 [6,136,309](#) **T** [Antibodies against the interferon \(IFN\) .alpha./.beta. receptor \(IFNAR2\) that preferentially block the activity of IFN-.alpha.](#)
- 1122 [6,133,009](#) **T** [Type II Restriction endonuclease, HpyCH4V, obtainable from helicobacter pylori CH4 and a process for producing the same](#)
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- 1124 [6,132,990](#) **T** [Recombinant methods for production of serine protease inhibitors and DNA sequences useful for same](#)
- 1125 [6,132,983](#) **T** [Luciferases](#)
- 1126 [6,130,367](#) **T** [DNA molecules that code for enzymes involved in starch synthesis, vectors, bacteria, transgenic plant cells and plants containing said molecules](#)
- 1127 [6,130,078](#) **T** [Method for cloning the NSPHI restriction-modification system in E. coli and producing the recombinant NSPHI restriction endonuclease](#)
- 1128 [6,130,070](#) **T** [Induction promoter gene and secretory signal gene usable in Schizosaccharomyces pombe, expression vectors having the same, and use thereof](#)
- 1129 [6,130,068](#) **T** [Viral encoded semaphorin protein receptor DNA and polypeptides](#)
- 1130 [6,130,060](#) **T** [Gene encoding for adseverin](#)
- 1131 [6,127,165](#) **T** [Rat glutathione synthetase gene](#)
- 1132 [6,127,160](#) **T** [Protein having cellulase activities and process for producing the same](#)
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- 1134 [6,127,113](#) **T** [Viral obesity methods and compositions](#)
- 1135 [6,124,524](#) **T** [FAE1 genes and their uses](#)
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- 1137 [6,121,521](#) **T** [Chimeric insecticidal protein and DNA coding therefor](#)
- 1138 [6,121,512](#) **T** [Cytochrome P-450 constructs and method of producing herbicide-resistant transgenic plants](#)
- 1139 [6,121,016](#) **T** [DNA encoding human follicle stimulating hormone receptor, vectors and cells containing such DNA, and methods of use thereof](#)
- 1140 [6,121,000](#) **T** [Antitumor antisense sequences directed against R1 and R2 components of ribonucleotide reductase](#)
- 1141 [6,120,765](#) **T** [Urokinase plasminogen activator fragments](#)
- 1142 [6,117,669](#) **T** [Biotin biosynthetic genes](#)
- 1143 [6,114,601](#) **T** [Plant genes encoding flavonoid-3', 5'-hydroxylase](#)
- 1144 [6,114,514](#) **T** [Mycobacterium tuberculosis specific DNA fragment](#)
- 1145 [6,114,159](#) **T** [DNA sequences for matrix metalloproteases, their production and use](#)

- 1146 [6,114,140](#) **T** [DNA encoding density enhanced protein tyrosine phosphatases](#)
1147 [6,114,139](#) **T** [G-protein coupled receptor protein and a DNA encoding the receptor](#)
1148 [6,111,255](#) **T** [Methods of screening for a tumor or tumor progression to the metastatic state](#)
1149 [6,111,091](#) **T** [Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefore](#)
1150 [6,110,737](#) **T** [Human platelet-derived growth factor receptor, type A](#)
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PAT. NO.	Title
1151 6,110,734	Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera
1152 6,110,701	DNA encoding precursor of interleukin-1.beta. converting enzyme--related cysteine proteinase II (ICE.sub.rel -II)
1153 6,110,700	PRAD1 cyclin and its cDNA
1154 6,110,670	Nucleotide sequences, probes and a process for the in vitro diagnosis of chromosomal anomalies correlated with CMT1A disease
1155 6,107,548	DNA sequences from muskmelon (Cucumis melo) related to fruit ripening
1156 6,107,475	Seven transmembrane receptors
1157 6,107,072	Thermostable geranylgeranyl diphosphate synthase
1158 6,107,063	Production of L-isoleucine by means of recombinant microorganisms with deregulated threonine dehydratase
1159 6,107,023	DNA amplification and subtraction techniques
1160 6,103,887	Nucleotide sequence encoding human cyclin A
1161 6,103,488	Method of forming ubiquinone-10
1162 6,103,466	Double-muscling in mammals
1163 6,100,449	Transgenic tomato plants containing a fusarium resistance gene
1164 6,100,446	Microorganisms and plasmids for 2,4-dichlorophenoxyacetic acid (2,4-D) monooxygenase formation and process for the production of these plasmids and strains

- 1165 [6,100,386](#) **T** [Human gene/protein involved in chronic myelogenous leukemia](#)
 - 1166 [6,100,055](#) **T** [DNA encoding f.alpha.-2m-binding protein and protein encoded thereby](#)
 - 1167 [6,100,025](#) **T** [Cloning by complementation and related processes](#)
 - 1168 [6,096,545](#) **T** [Phosphate starvation-inducible proteins](#)
 - 1169 [6,096,514](#) **T** [Human calcium channel compositions and methods](#)
 - 1170 [6,093,808](#) **T** [I.kappa.BEGFP constructs, cell lines and methods of use](#)
 - 1171 [6,091,004](#) **T** [Gene encoding a protein involved in the signal transduction cascade leading to systemic acquired resistance in plants](#)
 - 1172 [6,090,627](#) **T** [Octopine T-DNA structural genes](#)
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 - 1179 [6,087,163](#) **T** [Mycobacterium tuberculosis specific proteins and genes, mixtures of anitgens and uses thereof](#)
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 - 1182 [6,087,116](#) **T** [Interleukin-18 \(IL-18\) receptor polypeptides and their uses](#)
 - 1183 [6,087,097](#) **T** [PCR detection of Borrelia burgdorferi](#)
 - 1184 [6,084,087](#) **T** [DNA encoding conserved T-cell receptor sequences](#)
 - 1185 [6,083,749](#) **T** [DNA encoding a human serotonin receptor \(5-HT.sub.4B\) and uses thereof](#)
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 - 1189 [6,083,690](#) **T** [Methods and compositions for identifying osteogenic agents](#)
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 - 1191 [RE36,755](#) **T** [DNA encoding tumor necrosis factor-.alpha. and -.beta. receptors](#)
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 - 1193 [6,080,545](#) **T** [Oligonucleotides for the detection of salmonella](#)
 - 1194 [6,077,995](#) **T** [Fungal gene encoding resistance to the phytotoxin cercosporin](#)
 - 1195 [6,077,948](#) **T** [Mediators of chronic allograft rejection \(AIF-1\) and DNA encoding them](#)
 - 1196 [6,077,690](#) **T** [Cloning and expression of a protein antigen of Toxoplasma gondii](#)
 - 1197 [6,074,839](#) **T** [Transforming growth factor .alpha.HI](#)
 - 1198 [6,074,649](#) **T** [Recombinant composition containing feline herpes virus type 1, particularly for treating feline infectious peritonitis](#)
 - 1199 [6,072,103](#) **T** [Pathogen and stress-responsive promoter for gene expression](#)
 - 1200 [6,072,048](#) **T** [DNA molecule encoding for cellular uptake of Mycobacterium tuberculosis and uses thereof](#)
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1201	6,072,047 T Receptor that binds trail
1202	6,071,715 T Nucleic acids encoding novel proteins which bind to retinoblastoma protein
1203	6,069,299 T Fungus and insect control with chitinolytic enzymes
1204	6,069,296 T Regulators of G-protein signalling
1205	6,069,241 T Cytochrome P450 gene
1206	6,069,240 T Cloning by complementation and related processes
1207	6,068,990 T Proteins, their production and use
1208	RE36,713 T APC gene and nucleic acid probes derived therefrom
1209	6,066,782 T Combination of DNA sequences which enable the formation of modified starch in plant cells and plants, processes for the production of these plants and the modified starch obtainable therefrom
1210	6,066,726 T Neuron-specific transcriptional promoter
1211	6,066,502 T Endothelin converting enzyme (ECE)
1212	6,066,487 T Method for cloning and expression of BsrFI restriction endonuclease in E. coli
1213	6,066,482 T Acyltransferase and gene encoding acyltransferase
1214	6,066,468 T Chromosomal DNA fragments encoding enzymes for encoding .beta.-lactam biosynthetic enzymes, and vectors and transformants for their expression
1215	6,066,451 T Neural cell protein marker RR/B and DNA encoding the same
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- 1216 [6,063,623](#) [Transport protein which effects the transport of cationic xenobiotics and/or pharmaceuticals, DNA sequences encoding it and their use](#)
 - 1217 [6,063,608](#) [T Cloned genes encoding reverse transcriptase lacking RNase H activity](#)
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1254	6,040,165 T Mutant prenyl diphosphate synthase
1255	6,040,160 T Method of producing L-lysine by fermentation
1256	6,040,156 T DNA encoding glucuronyltransferase
1257	6,037,462 T MTS1 gene mutations
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1259	6,037,161 T Enzyme with acetyl esterase activity
1260	6,037,156 T DNA molecules and vectors encoding clavulanic acid biosynthesis enzymes
1261	6,037,149 T DNA encoding human asthma associated factor 1
1262	6,034,231 T Human CNP gene and precursor protein
1263	6,034,229 T BMP-15 compositions
1264	6,034,227 T DNA molecule encoding a mast cell function-associated antigen (MAFA)
1265	6,033,889 T Gene sequence of Aquifex pyrophilus superoxide dismutase and protein expressed in Escherichia coli
1266	6,033,872 T Polynucleotides encoding a novel human 11cb splice variant
1267	6,033,871 T DNA molecules encoding imidazoline receptive polypeptides and polypeptides encoded thereby

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- 1269 [6,030,832](#) **T** [Carboxy-terminal BRCA1 interacting protein](#)
- 1270 [6,030,806](#) **T** [Human chromosome 16 genes, compositions, methods of making and using same](#)
- 1271 [6,030,804](#) **T** [Polynucleotides encoding G-protein parathyroid hormone receptor HLTDG74 polypeptides](#)
- 1272 [6,028,250](#) **T** [Plant promoter and method for gene expression using said promoter](#)
- 1273 [6,028,249](#) **T** [DNA sequences which lead to the formation of levans, plasmids containing these sequences as well as a process for preparing transgenic plants](#)
- 1274 [6,027,929](#) **T** [Method for cloning and producing the NspI restriction endonuclease in E. coli and purification of the recombinant NspI restriction endonuclease](#)
- 1275 [6,027,919](#) **T** [BMP-12 and BMP-13 proteins and DNA encoding them](#)
- 1276 [6,027,918](#) **T** [Recombinant thermostable enzyme which releases trehalose from non-reducing saccharide](#)
- 1277 [6,027,917](#) **T** [Bone morphogenetic protein \(BMP\)-17 and BMP-18 compositions](#)
- 1278 [6,025,185](#) **T** [Fungus wherein the areA gene has been modified and an areA gene from Aspergillus oryzae](#)
- 1279 [6,025,183](#) **T** [Transgenic animal assay system for anti-cholinesterase substances](#)
- 1280 [6,025,180](#) **T** [ASP1](#)
- 1281 [6,025,179](#) **T** [Method for cloning and producing the SnaBI restriction endonuclease and purification of the recombinant SnaBI restriction endonuclease](#)
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1300 [6,017,535](#) [cDNA sequence of Dengue virus serotype 1 \(Singapore strain\)](#)



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1301	6,015,940 Virus resistant potato plants
1302	6,015,939 Plant VDE genes and methods related thereto
1303	6,015,705 Method of modifying the DNA of an organism, DNA sequence, method of detection and isolation of a gene capable of modifying the DNA of an organism and organisms comprising modified DNA
1304	6,015,690 DNA sequence encoding a human imidazoline receptor and method for cloning the same
1305	6,015,689 Regulation of aureobasidin sensitivity
1306	6,013,862 Wheat aleurone regulatory elements
1307	6,013,515 Cofactors for HIV-1 protein Tat and methods of use therefor
1308	6,013,500 PAK4, a novel gene encoding a serine/threonine kinase
1309	6,013,476 DNA encoding tumor necrosis related receptor TR7
1310	6,013,474 Calcium channel compositions and methods
1311	6,013,468 RNA component of telomerase
1312	6,013,451 Bacillus stearothermophilus DNA Polymerase I (klenow) clones including those with reduced 3'- to -5' exonuclease activity
1313	6,011,201 .beta.-Ketoacyl ACP reductase genes from Brassica napus
1314	6,011,199 Method for producing fruiting plants with improved fruit flavour
1315	6,011,145 Chain length specific UDP-GLC: fatty acid glucosyltransferases

- 1316 [6,011,144](#) [PHA E and PHA C components of poly\(hydroxy fatty acid\) synthase from thiocapsa pfennigii](#)
- 1317 [6,010,886](#) [T Receptor for oncostatin M](#)
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- 1319 [6,008,436](#) [T Nematode-resistant transgenic plants](#)
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- 1326 [6,004,775](#) [T DNA encoding IGFBP-4](#)
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- 1328 [6,004,754](#) [T DNA sequence, related probes and primers for the detection of Streptococcus agalactiae](#)
- 1329 [6,001,986](#) [T Antiviral proteins, amarandin 1 and 2, from Amaranthus viridis, DNAs encoding therefrom](#)
- 1330 [6,001,628](#) [T Debranching enzymes and DNA sequences coding them, suitable for changing the degree of branching of amylopectin starch in plants](#)
- 1331 [6,001,599](#) [T DNAs encoding mammalian ZPBs](#)
- 1332 [5,998,601](#) [T VR-2332 viral nucleotide sequence and methods of use](#)
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- 1337 [5,994,526](#) [T Gene expression in plants](#)
- 1338 [5,994,521](#) [T Full length transcript \(FLT\) promoter from figwort mosaic caulimovirus \(FMV\) and use to express chimeric genes in plant cells](#)
- 1339 [5,994,100](#) [T HAS2 splicing variant HOEFC11: a target in chronic renal failure, inflammatory diseases and myocardial ischemia](#)
- 1340 [5,994,098](#) [T Human 7-TM receptor similar to murine frizzled-6 gene](#)
- 1341 [5,994,095](#) [T MTS2 gene](#)
- 1342 [5,994,094](#) [T Growth/differentiation factor of the TGF-.beta. family](#)
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- 1346 [5,989,887](#) [T Cloning and expression of DNA molecules incoding arabinan-degrading enzymes of fungal origin](#)
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- 1348 [5,989,882](#) [T Processes for preparing acarviosyl transferase and for using it in the](#)

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1351	5,986,174 Maize promoter sequence for leaf- and stalk-preferred gene expression
1352	5,986,172 Rice NADH-dependent reductase, gene therefor, and use thereof
1353	5,986,082 Altered forms of the NIM1 gene conferring disease resistance in plants
1354	5,986,080 Cloned nucleotide pyrophosphohydrolase and uses thereof
1355	5,985,657 Recombinant DNA which codes for interleukin-1
1356	5,985,637 Gene encoding endo-.beta.-N-acetylglucosaminidase A
1357	5,985,623 DNA segments and methods for increasing polysaccharide production
1358	5,985,605 DNA sequences encoding phytases of ruminal microorganisms
1359	5,985,603 P.sub.2x receptor DNA and protein sequence
1360	5,985,600 Nucleic acid encoding delta opioid receptor
1361	5,985,562 Method of detecting thrombotic disease risk associated with plasma carboxypeptidase B polymorphisms
1362	5,981,841 Early seed 5' regulatory sequence
1363	5,981,838 Genetic manipulation of plants to increase stored carbohydrates
1364	5,981,724 DNA encoding CD40 ligand, a cytokine that binds CD40
1365	5,981,723 Retinoblastoma-associated protein 1 CDNA
1366	5,981,280 Method and constructs for inhibiting protein expression in bacteria
1367	5,981,235 Methods for isolating nucleic acids using alkaline protease
1368	5,981,230 Polynucleotide encoding chemokine .beta.-4

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- 1372 [5,981,184](#) **T** [Screening kit and process for determining action of substances inhibiting the P-type ATPase activity of Helicobacter pylori](#)
- 1373 [5,977,440](#) **T** [DNA molecule encoding a 33kD cysteine proteinase and its use in transforming plants to provide insect resistance](#)
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- 1380 [5,972,902](#) **T** [DNA encoding human IL-6 receptor antagonist and protein encoded thereby](#)
- 1381 [5,972,690](#) **T** [DNA strands useful for the synthesis of xanthophylls and the process for producing the xanthophylls](#)
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- 1387 [5,968,816](#) **T** [Tumor suppressor genes, proteins encoded thereby and use of said genes and proteins](#)
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1399 [5,962,327](#)  [Nucleotide sequence encoding the enzyme I-SceI and the uses thereof](#)
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1402 5,962,266	Protease inhibitor peptides
1403 5,962,260	Recombinant production of human and bovine receptors for modified low-density lipoprotein
1404 5,959,178	Modification of lignin synthesis in plants
1405 5,959,174	Plant glutamate receptors
1406 5,959,091	Truncated gene of Bacillus thuringiensis encoding a polypeptide toxin
1407 5,958,893	Genes and proteins for treating cystic fibrosis
1408 5,958,749	DNA encoding a polypeptide possessing maltotetraose-forming amylase activity
1409 5,958,735	Polynucleotides encoding urogenital sinus derived growth inhibitory factor and vectors
1410 5,958,713	Method of detecting biologically active substances by using green fluorescent protein
1411 5,955,653	Callase-related DNAs and their use in artificial male sterility
1412 5,955,652	Plant genes for sensitivity to ethylene and pathogens
1413 5,955,354	RAS P21-interacting protein (RGL) and its RAS interacting domain (RID)
1414 5,955,347	Methods and products for the synthesis of oligosaccharide structures on glycoproteins, glycolipids, or as free molecules, and for the isolation of

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- 1415 [5,952,548](#) **T** [Soybean glucanases, compounds which encode therefor and related methods](#)
- 1416 [5,952,488](#) **T** [Androgen regulation with DNA sequences of rat probasin gene](#)
- 1417 [5,952,486](#) **T** [Materials and methods for the modification of plant lignin content](#)
- 1418 [5,952,483](#) **T** [Human I.kappa.B-.beta.](#)
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- 1446 [5,939,301](#) **T** [Cloned DNA polymerases from Thermotoga neapolitana and mutants thereof](#)
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1452	5,932,781 T Ectoine synthase gene
1453	5,932,449 T Detection of botulinum toxin
1454	5,932,415 T Processes and agents for detecting listerias
1455	5,932,216 T Antibodies to bone morphogenetic protein-10 (BMP-10)
1456	5,929,224 T Leukocyte cell--derived chemotaxin 2b (LECT2b)
1457	5,929,223 T Cloning and characterizing of genes associated with long-term memory
1458	5,929,221 T Gene derived from coryneform bacteria and use thereof
1459	5,928,941 T Repressor kruppel-like factor
1460	5,928,937 T Structure-based designed herbicide resistant products
1461	5,928,931 T Isolation, purification and cloning of retinol dehydratase
1462	5,928,927 T Enzymatic detoxification of organophosphorus compounds
1463	5,928,926 T Isolation and cloning of the human ARSA-I gene and uses thereof
1464	5,928,925 T Rice ornithine carbamyltransferase gene, and a vector containing said gene and a transformant
1465	5,928,915 T CHO cell sialidase by recombinant DNA technology
1466	5,928,898 T Multiple drug resistance gene atrD of Aspergillus nidulans
1467	5,928,897 T Expression of a gram-positive bacteria replicon
1468	5,925,807 T GA4 DNA, protein and method of use

- 1469 [5,925,804](#) **T** [Production of trehalose in plants](#)
- 1470 [5,925,751](#) **T** [Human cytomegalovirus DNA sequences](#)
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- 1487 [5,917,019](#) **T** [Altered telomere repeat binding factor 2](#)
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- 1489 [5,916,769](#) **T** [Polynucleotides encoding extra cellular/epidermal growth factor HCABA58X polypeptides](#)
- 1490 [5,916,768](#) **T** [Contraceptive vaccine based on alloimmunization with zona pellucida polypeptides](#)
- 1491 [5,916,752](#) **T** [Telomerase screening methods](#)
- 1492 [5,916,744](#) **T** [Testing for infestation of rapeseed and other cruciferae by the fungus Leptosphaeria maculans \(blackleg infestation\)](#)
- 1493 [5,914,258](#) **T** [Human deoxycytidine kinase 2](#)
- 1494 [5,914,257](#) **T** [Structural gene for membrane-bound aldehyde dehydrogenase](#)
- 1495 [5,914,253](#) **T** [Recombinant production of murine interferon-.gamma. \(IFN-.gamma.\) inducing factor \(IGIF, IL-18\)](#)
- 1496 [5,914,252](#) **T** [DNA encoding membrane protein having PRE-B cell growth-supporting ability and protein encoded thereby](#)
- 1497 [5,914,251](#) **T** [Nucleic acid molecules encoding placental-derived growth factors](#)
- 1498 [5,910,581](#) **T** [Polypeptides of glycosaminoglycan sulfotransferase originating from human and DNA coding for the polypeptides](#)
- 1499 [5,910,436](#) **T** [Trehalose phosphorylase, its preparation and uses](#)
- 1500 [5,910,412](#) **T** [Method for identifying the sex of spinach by DNA markers](#)

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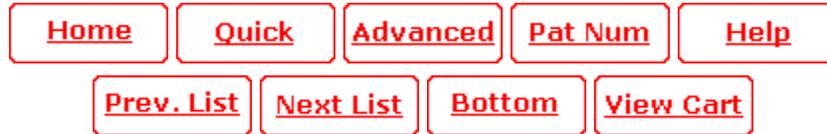
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PAT. NO.	Title
1501 5,908,925	Genetically engineered immunoglobulins with specificity for glycosylated albumin
1502 5,908,827	Protein from urine named component B
1503 5,908,810	Method of improving the growth of crop plants which are resistant to glutamine synthetase inhibitors
1504 5,908,772	Gene encoding lacto-N-biosidase
1505 5,908,761	Galectin-8 and galectin-8-like proteins and DNA molecules coding therefor
1506 5,905,146	DNA binding protein S1-3
1507 5,905,024	Method for performing site-specific affinity fractionation for use in DNA sequencing
1508 5,898,096	Plant promoter
1509 5,892,018	DNA sequences encoding a brain sodium channel protein
1510 5,891,688	DNA encoding lemA-independent GacA and its use in activating gene expression
1511 5,891,665	Untranslated leader sequences from RNA viruses as enhancers of translation
1512 5,889,172	DNA sequences for immunologically active peptides of pertussis toxin
1513 5,888,818	Herbicide resistant plants
1514 5,886,164	DNA encoding enzymes related to ethylene biosynthesis and ripening from

- [banana](#)
- 1515 [5,885,836](#) **T** [FLP-mediated gene modification in mammalian cells, and compositions and cells useful therefor](#)
- 1516 [5,885,819](#) **T** [Enzyme with xylanase activity](#)
- 1517 [5,885,818](#) **T** [Method for cloning and producing ageI restriction endonuclease in E. coli](#)
- 1518 [5,885,571](#) **T** [Bacillus thuringiensis strains and their insecticidal proteins](#)
- 1519 [5,883,244](#) **T** [Lytic .beta.-1,3-glucanase gene](#)
- 1520 [5,883,241](#) **T** [DNA sequences coding for a human metalloproteinase and variants thereof](#)
- 1521 [5,882,911](#) **T** [Enzyme with rhamnogalacturonase activity](#)
- 1522 [5,882,880](#) **T** [Human checkpoint gene and gene for antisense RNA thereof](#)
- 1523 [5,882,879](#) **T** [Method for influencing .beta.-lactam antibiotic production and for isolation of large quantities of ACV synthetase](#)
- 1524 [5,882,869](#) **T** [Plant adenylosuccinate synthetase and DNA coding therefor](#)
- 1525 [5,882,851](#) **T** [Cytochrome P-450 monooxygenases](#)
- 1526 [5,880,332](#) **T** [DNA constructs related to capsanthin capsorubin synthase, cells and plants derived therefrom](#)
- 1527 [5,880,328](#) **T** [DNA encoding plant chitinases](#)
- 1528 [5,879,950](#) **T** [Materials and methods for digestion of DNA or RNA using restriction endonucleases](#)
- 1529 [5,879,909](#) **T** [Human transaldolase: an autoantigen with a function in metabolism](#)
- 1530 [5,879,908](#) **T** [CRFG-1a, a target and marker for chronic renal failure](#)
- 1531 [5,879,883](#) **T** [Method for screening for alzheimer's disease](#)
- 1532 [5,879,879](#) **T** [CIS-acting element in the human LDL receptor promoter and uses thereof](#)
- 1533 [5,877,020](#) **T** [Promoter for the receptor tyrosine kinase, Tie](#)
- 1534 [5,876,999](#) **T** [Preparation of novel streptokinase mutants as improved thrombolytic agents](#)
- 1535 [5,876,991](#) **T** [Polyketide synthase genes](#)
- 1536 [5,876,985](#) **T** [Methods and compositions for the preparation of recombinant Trichomonas vaginalis proteins and peptides](#)
- 1537 [5,876,979](#) **T** [RNA component of mouse, rat, Chinese hamster and bovine telomerase](#)
- 1538 [5,876,974](#) **T** [Method for producing DNA encoding cystic fibrosis transmembrane conductance regulator \(CFTR\) protein in E. coli](#)
- 1539 [5,876,722](#) **T** [DNA encoding Derf II, the major mite allergen, host cells containing such DNA and methods for producing Derf II](#)
- 1540 [5,874,626](#) **T** [Osmotin gene promoter and use thereof](#)
- 1541 [5,874,561](#) **T** [DNA, host cell and vector encoding a protein with cytokine inhibitory activity](#)
- 1542 [5,874,300](#) **T** [Campylobacter jejuni antigens and methods for their production and use](#)
- 1543 [5,874,274](#) **T** [Processing plant material with xylanase](#)
- 1544 [5,874,271](#) **T** [Human glycosyltransferase gene, compounds and method for inhibiting cancerous metastasis](#)
- 1545 [5,874,253](#) **T** [DNA encoding megakaryocyte differentiation factor](#)
- 1546 [5,874,252](#) **T** [Splicing variant of the Epstein-Barr virus-induced G-protein coupled receptor](#)

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PAT. NO.	Title
1551 5,872,225	Method for characterizing the nucleotide sequence of LICAM and the nucleotide sequence characterized thereby
1552 5,872,000	Nitrilase gene
1553 5,871,993	DNA encoding enzyme, recombinant DNA and enzyme, transformant, and their preparations and uses
1554 5,871,990	UDP-N-acetyl-.alpha.-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase, gAlnAc-T3
1555 5,871,917	Identification of differentially methylated and mutated nucleic acids
1556 5,871,742	Recombinant Avipox virus encoding polypeptide of mycoplasma gallisepticum, and utilized a live vaccine
1557 5,869,640	Nucleic acids encoding D-type cyclins and hybridization probes
1558 5,869,638	Bone-related cadherin-like protein and process for its production
1559 5,869,608	Nucleotide and amino acid sequences of the four variable domains of the major outer membrane proteins of Chlamydia trachomatis
1560 5,869,320	Cloning and expression of the gene for bacteriophage T7 RNA polymerase
1561 5,869,318	Gene sequence of the Down syndrome critical region of human chromosome 21, identified by a new "Alu-splicing PCR" technique, coding for a proline-rich protein (DSCR1) highly expressed in foetal brain and in heart and method for characterizing it
1562 5,869,309	Cephalosporin esterase gene from Rhodosporidium toruloides

- 1563 [5,869,304](#) **T** [Technique for specifying the fatty acid at the sn2 position of acylglycerol lipids](#)
- 1564 [5,869,299](#) **T** [Process for producing clavulanic acid](#)
- 1565 [5,869,293](#) **T** [DNA encoding human interferon IFN -.alpha.001](#)
- 1566 [5,869,286](#) **T** [Receptor that binds IL-17](#)
- 1567 [5,869,235](#) **T** [Gene of the hop latent virus and methods for detecting the same](#)
- 1568 [5,869,064](#) **T** [Protein rib, a cell surface protein that confers immunity to many strains of the group B Streptococcus: process for purification of the protein, reagent kit and pharmaceutical composition](#)
- 1569 [5,866,790](#) **T** [DNA sequences and plasmids for the preparation of sugar beet with changed sucrose concentration](#)
- 1570 [5,866,782](#) **T** [Gene which determines cytoplasmic sterility and a method of producing hybrid plants using said gene](#)
- 1571 [5,866,780](#) **T** [Maize chlorotic dwarf virus genome and uses therefor](#)
- 1572 [5,866,779](#) **T** [Recombinant gibberellin DNA and uses thereof](#)
- 1573 [5,866,695](#) **T** [Soybean peroxydase gene family and an assay for detecting soybean peroxidase activity](#)
- 1574 [5,866,422](#) **T** [Method for cloning and producing the Tsp45I restriction endonuclease in E. coli](#)
- 1575 [5,866,398](#) **T** [Method for cloning and producing the BSII restriction endonuclease in E. coli](#)
- 1576 [5,866,395](#) **T** [Purified thermostable pyrococcus furiosus DNA polymerase I](#)
- 1577 [5,866,374](#) **T** [Gene conferring flocculating property on yeast and gene product thereof](#)
- 1578 [5,866,333](#) **T** [Screening methods to detect mRNA targets of editing enzymes](#)
- 1579 [5,863,797](#) **T** [Gene fusion encoding a hypersecretor protein](#)
- 1580 [5,863,783](#) **T** [Cloning and expression of DNA molecules encoding arabinan-degrading enzymes of fungal origin](#)
- 1581 [5,863,758](#) **T** [Nucleic acids encoding osteogenic proteins](#)
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- 1590 [5,859,329](#) **T** [Genetic sequences encoding flavonol synthase enzymes and uses therefor](#)
- 1591 [5,859,328](#) **T** [Isolated DNA elements that direct pistil-specific and anther-specific gene expression and methods of using same](#)
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- 1593 [5,859,183](#) **T** [Altered telomere repeat binding factor](#)
- 1594 [5,858,787](#) **T** [DNA encoding PACAP receptor protein and method for preparing said](#)

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- 1595 [5,858,759](#) **T** [D-N-carbamoyl-amino acid amidohydrolase and hydantoinase](#)
- 1596 [5,858,729](#) **T** [Expression of porcine reproductive respiratory syndrome virus polypeptides in the same cell](#)
- 1597 [5,858,702](#) **T** [Isolation, cloning and expression of transmembrane water channel Aquaporin 5 \(AQP5\)](#)
- 1598 [5,858,353](#) **T** [Insect viruses, sequences, insecticidal compositions and methods](#)
- 1599 [5,856,296](#) **T** [DNA encoding interleukin-4 receptors](#)
- 1600 [5,856,177](#) **T** [Promoters derived from the maize phosphoenolpyruvate carboxylase gene involved in C.sub.4 photosynthesis](#)
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1601 5,856,137	T Nucleic acids encoding and recombinant production of the .beta. subunit of lutenizing hormone
1602 5,856,125	T ETS2 repressor factor (ERF) genetic locus and its products
1603 5,856,124	T DNA encoding high-affinity melatonin receptors
1604 5,856,121	T Growth arrest homebox gene
1605 5,854,420	T Maize acetyl CoA carboxylase encoding DNA clones
1606 5,854,417	T Borna disease virus-specific DNA and proteins
1607 5,854,412	T Chemokine N-terminal deletion mutations
1608 5,854,034	T DNA segments and methods for increasing polysaccharide production
1609 5,854,028	T Compositions comprising IL-11 and methods of making and using IL-11
1610 5,854,021	T Enhance protein production method
1611 5,852,186	T Reactive neutralizing human anti-GP120 recombinant antibody, DNA coding the same and use thereof
1612 5,851,827	T DNA encoding clostridium perfringens alpha-toxin peptides
1613 5,851,824	T Human calcium channel .alpha.-1C/.alpha.-1D, .alpha.-2, .beta.-1, and .gamma.subunits and cells expressing the DNA
1614 5,851,821	T DNA Replication-regulating genes
1615 5,851,805	T Method for producing DNA from mRNA
1616 5,851,794	T Collagen binding protein as well as its preparation

- 1617 [5,850,023](#) **T** [Modified plant viral replicase genes](#)
- 1618 [5,850,020](#) **T** [Materials and method for the modification of plant lignin content](#)
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- 1624 [5,849,895](#) **T** [Human N-methyl-D-aspartate receptor subunits, nucleic acids encoding same and uses therefor](#)
- 1625 [5,849,894](#) **T** [Rhodospirillum rubrum poly-.beta.-hydroxyalkanoate synthase](#)
- 1626 [5,849,558](#) **T** [Discovery of and method for cloning and producing the PspGI restriction endonuclease](#)
- 1627 [5,849,541](#) **T** [DNA encoding triol polyketide synthase](#)
- 1628 [5,849,538](#) **T** [DNA encoding human endothelial cell growth factors and plasmids comprising said DNA](#)
- 1629 [5,849,522](#) **T** [Enhancer for eukaryotic expression systems](#)
- 1630 [5,847,258](#) **T** [DNA encoding .beta.-1,3-glucanases](#)
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- 1636 [5,843,758](#) **T** [Enzyme based bioremediation](#)
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- 1639 [5,843,646](#) **T** [DNA molecules encoding murine son of sevenless \(mSOS\) gene and mSOS polypeptides](#)
- 1640 [5,840,869](#) **T** [DNA encoding interleukin-4 receptors](#)
- 1641 [5,840,865](#) **T** [Eukaryotic transposable element](#)
- 1642 [5,840,558](#) **T** [Soybean peroxidase gene family and an assay for detecting soybean peroxidase activity](#)
- 1643 [5,840,555](#) **T** [Transcriptional regulatory regions derived from the K18 gene](#)
- 1644 [5,840,533](#) **T** [Tissue plasminogen activator](#)
- 1645 [5,840,531](#) **T** [Ingap protein involved in pancreatic islet neogenesis](#)
- 1646 [5,840,530](#) **T** [DNA encoding receptors for the beta-2 chain of human IL-12](#)
- 1647 [5,840,522](#) **T** [Recombinant lectins](#)
- 1648 [5,840,518](#) **T** [DNA fragment, vector containing the DNA fragment, transformant transformed with the vector and process for producing protein using the vector](#)

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1649 [5,840,486](#) [Mutant DNA encoding protein phosphatase 1 G-subunit](#)

1650 [5,840,300](#)  [Methods and compositions comprising single chain recombinant antibodies](#)



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1651	5,837,876 Root cortex specific gene promoter
1652	5,837,850 Regulatory element conferring tapetum specificity
1653	5,837,849 OCS element
1654	5,837,848 Root-specific promoter
1655	5,837,845 Human monoclonal antibody specifically binding to surface antigen of cancer cell membrane
1656	5,837,842 Vascular anticoagulant proteins DNA which codes them, processer for preparing them and their use
1657	5,837,839 DNA coding sequences for mevalonate Pyrophosphate decarboxylase
1658	5,837,545 Genes, polypeptides, and compositions for cold tolerance in plants
1659	5,837,536 Expression of human multidrug resistance genes and improved selection of cells transduced with such genes
1660	5,837,534 Smooth muscle 22.alpha. promoter, gene transfer vectors containing the same, and method of use of the same to target gene expression in arterial smooth muscle cells
1661	5,837,509 Recombinant lactic acid bacterium containing an inserted promoter and method of constructing same
1662	5,837,497 DNAs encoding mammalian ZPC and uses thereof
1663	5,837,495 DNA encoding interleukin-1 antagonist
1664	5,837,492 Chromosome 13-linked breast cancer susceptibility gene

- 1665 [5,837,489](#) **T** [Human neuronal nicotinic acetylcholine receptor and cells transformed with same DNA and mRNA encoding an--subunit of](#)
- 1666 [5,834,284](#) **T** [N-acetylglucosaminyl transferase gene coding therefor and process for production thereof](#)
- 1667 [5,834,265](#) **T** [Multifunctional RNA having self-processing activity, the preparation thereof and the use thereof](#)
- 1668 [5,834,253](#) **T** [Bacillus stearothermophilus DNA polymerase with proof-reading 3'-5' exonuclease activity](#)
- 1669 [5,834,245](#) **T** [PRLTS proteins and DNA's encoding the same](#)
- 1670 [5,834,244](#) **T** [Factor VIIa inhibitors from Kunitz domain proteins](#)
- 1671 [5,831,141](#) **T** [Expression of a heterologous polypeptide in mammary tissue of transgenic non-human mammals using a long whey acidic protein promoter](#)
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- 1673 [5,831,057](#) **T** [Associative learning and the linotte gene](#)
- 1674 [5,831,053](#) **T** [Genes which influence pichia proteolytic activity, and uses therefor](#)
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- 1676 [5,830,759](#) **T** [Unique associated Kaposi's sarcoma virus sequences and uses thereof](#)
- 1677 [5,830,756](#) **T** [DNA and expression vector encoding I.kappa.B Protein](#)
- 1678 [5,830,734](#) **T** [Enzyme with acetyl esterase activity](#)
- 1679 [5,830,714](#) **T** [Biologically active fragment of bacillus stearothermophilus DNA polymerase](#)
- 1680 [5,830,703](#) **T** [DNA encoding adrenomedullin](#)
- 1681 [5,830,700](#) **T** [Hybrid proteins having cross-linking and tissue-binding activities](#)
- 1682 [5,830,688](#) **T** [DNA sequences, vectors, recombinant viruses and method which employs recombinant vaccinia viruses capable of multiplying in CHO cells](#)
- 1683 [5,827,739](#) **T** [Recombinant DNA sequences, vectors containing them and method for the use thereof](#)
- 1684 [5,827,731](#) **T** [Thrombin-inhibitory protein from ticks](#)
- 1685 [5,827,730](#) **T** [Mutant DNA encoding insulin receptor substrate 1](#)
- 1686 [5,827,726](#) **T** [DNA coding protein kinase](#)
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- 1688 [5,827,719](#) **T** [Enzyme with lipolytic activity](#)
- 1689 [5,827,706](#) **T** [Cyclosporin synthetase](#)
- 1690 [5,827,687](#) **T** [Promoter and method of gene expression using the same](#)
- 1691 [5,824,875](#) **T** [1-aminocyclopropane-1-carboxylate synthase genes from pelargonium](#)
- 1692 [5,824,870](#) **T** [Commercial production of aprotinin in plants](#)
- 1693 [5,824,867](#) **T** [Plant glutamate receptors](#)
- 1694 [5,824,864](#) **T** [Maize gene and protein for insect control](#)
- 1695 [5,824,863](#) **T** [Seed coat-specific cryptic promoter in tobacco](#)
- 1696 [5,824,862](#) **T** [DNA encoding ATP-dependent fructose 6-phosphate 1-phosphotransferase originating from plant, recombinant vector containing the same and method for changing sugar content in plant cells under low temperature](#)
- 1697 [5,824,795](#) **T** [Oligonucleotides for the detection of salmonella](#)
- 1698 [5,824,529](#) **T** [Method for cloning and producing the PshAI restriction endonuclease](#)

1699 [5,824,523](#)  [Isolated DNA encoding enzyme for phage resistance](#)

1700 [5,824,509](#)  [Recombinant lymphotoxin cDNA and variants](#)

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1701 5,824,508	T Non-splicing variants of gp350/220
1702 5,824,503	T Gene encoding endoglycoceramidase activator
1703 5,824,302	T Method of controlling insect larvae comprising feeding an insecticidal amount of a transgenic maize plant expressing a polypeptide having Bt-crystal protein toxic properties
1704 5,821,398	T DNA molecules encoding inducible plant promoters and tomato ADH2 enzyme
1705 5,821,104	T Tripeptidyl aminopeptidase
1706 5,821,103	T Deoxyribonuclease
1707 5,821,094	T S-locus receptor kinase gene in a self-incompatible brassica napus line
1708 5,821,090	T Riboflavin-biosynthesis in fungi
1709 5,821,078	T Nucleic acid encoding interferon-.alpha./beta. binding protein
1710 5,821,077	T Process for activating gene expression in bacteria
1711 5,817,794	T Isolated DNA sequence comprising DNA encoding human cytoplasmic Cu-Zn superoxide dismutase
1712 5,817,790	T Reshaped human antibody to human interleukin-6 receptor
1713 5,817,502	T Genes for the synthesis of pyrrolnitrin
1714 5,817,501	T DNA encoding suppressin protein and uses thereof
1715 5,817,499	T DNA encoding an enzyme with endoglucanase activity from Trichoderma harzianum

- 1716 [5,817,496](#) **T** [Recombinant kat enzyme from rat](#)
- 1717 [5,814,509](#) **T** [Prostacyclin synthase derived from human](#)
- 1718 [5,814,506](#) **T** [Over-expression and purification of a truncated thermostable DNA polymerase by protein fusion](#)
- 1719 [5,814,499](#) **T** [DNA encoding phage abortive infection protein from lactococcus lactis and method of use thereof](#)
- 1720 [5,814,456](#) **T** [Sperm antigen corresponding to a sperm zone binding protein autoantigenic epitope](#)
- 1721 [5,811,291](#) **T** [Enzyme with rhamnogalacturonase activity](#)
- 1722 [5,811,285](#) **T** [DSZD utilization in desulfurization of DBT by rhodococcus sp. IGTS8](#)
- 1723 [5,811,233](#) **T** [Compositions and uses thereof in the diagnosis of psoriasis](#)
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- 1727 [5,807,711](#) **T** [Parenchymal hepatocyte growth factors](#)
- 1728 [5,804,558](#) **T** [Protegrins](#)
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- 1734 [5,801,233](#) **T** [Nucleic acid compositions encoding acetyl-coa carboxylase and uses therefor](#)
- 1735 [5,801,043](#) **T** [Amylase variants](#)
- 1736 [5,798,259](#) **T** [Gene coding for eicosapentaenoic acid synthesizing enzymes and process for production of eicosapentaenoic acid](#)
- 1737 [5,798,105](#) **T** [DNA encoding a plasmodium 16kD protein](#)
- 1738 [5,798,099](#) **T** [DNA encoding Derf II, the major mite allergen, host cells containing such DNA and method for producing Derf II](#)
- 1739 [5,795,767](#) **T** [Epimerase](#)
- 1740 [5,795,766](#) **T** [Protein having .alpha.-glucosidase activity, DNA having genetic information thereof, and production of .alpha.-glucosidase](#)
- 1741 [5,795,765](#) **T** [Gene encoding endoglycoceramidase](#)
- 1742 [5,792,933](#) **T** [Fiber-specific protein expression in the cotton plant](#)
- 1743 [5,792,926](#) **T** [Virus/herbicide resistance genes, processes for producing same and their use](#)
- 1744 [5,792,923](#) **T** [DNA sequences which lead to the formation of levans plasmids containing these sequences as well as a process for preparing transgenic plants](#)
- 1745 [5,792,846](#) **T** [Human calcium channel compositions and methods](#)
- 1746 [5,792,638](#) **T** [Human ras-related oncogenes unmasked by expression cDNA cloning](#)
- 1747 [5,792,629](#) **T** [Isolated DNA encoding novel protease inhibitory polypeptide](#)
- 1748 [5,792,612](#) **T** [Use of lipids to improve the polymerase chain reaction](#)
- 1749 [5,789,566](#) **T** [DNA sequence imparting cytoplasmic male sterility, mitochondrial genome, nuclear genome, mitochondria and plant containing said sequence and process for the preparation of hybrids](#)

1750 [5,789,559](#) **T** [DNA sequences of enterically transmitted non-A/non-B hepatitis viral agent](#)



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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
1751 5,789,233	T DNA encoding an Eimekia 50 KD antigen
1752 5,789,211	T Gene encoding a polypeptide having nitrile hydratase activity, a transformant containing the gene and a process for the production of amides using the transformant
1753 5,789,193	T Increased production of secreted proteins by recombinant eukaryotic cells
1754 5,789,170	T Specific co-activator for human androgen receptor
1755 5,786,466	T Method for screening an expression cDNA clone bank for the detection of polynucleotides
1756 5,786,463	T Multiple drug resistance gene of Cryptococcus neoformans
1757 5,786,206	T DNA encoding recombinant lipoprotein antigens
1758 5,786,195	T Method for cloning and producing the bssHII restriction endonuclease in E. coli
1759 5,786,192	T Farnesyl pyrophosphate synthetase and DNA sequence encoding the same
1760 5,786,189	T Vaccine
1761 5,786,140	T DNA's encoding sucrose isomerase and palatinase
1762 5,783,686	T Method for purifying nucleic acids from heterogenous mixtures
1763 5,783,681	T Androgen regulation with DNA sequences of rat probasin gene
1764 5,783,442	T Cloning vector plasmid, vector-primer derived therefrom and preparation method of CDNA bank using the same
1765 5,783,417	T Human-derived tumor cell growth inhibitors

- 1766 [5,783,398](#) **T** [High throughput assay using fusion proteins](#)
- 1767 [5,783,394](#) **T** [Raspberry promoters for expression of transgenes in plants](#)
- 1768 [5,783,385](#) **T** [Method for homologous-recombination screening of recombinant-DNA clones in yeast host libraries](#)
- 1769 [5,780,708](#) **T** [Fertile transgenic corn plants](#)
- 1770 [5,780,271](#) **T** [PCR assays for phytophthora species](#)
- 1771 [5,780,223](#) **T** [Molecular diagnosis of autosomal dominant Charcot-Marie-Tooth disease](#)
- 1772 [5,777,095](#) **T** [Osp A and B Sequence of Borrelia burgdonferi strains ACA1 and IP90](#)
- 1773 [5,776,771](#) **T** [Kanamycin resistance gene derived from microorganisms of the genus rhodococcus](#)
- 1774 [5,776,731](#) **T** [DNA encoding type-I interleukin-I receptor-like protein designated 2F1](#)
- 1775 [5,773,695](#) **T** [Plant nuclear scaffold attachment region and method for increasing gene expression in transgenic cells](#)
- 1776 [5,773,290](#) **T** [Mammary gland-specific promoters](#)
- 1777 [5,773,288](#) **T** [Plant genes affecting gibberellic acid biosynthesis](#)
- 1778 [5,773,274](#) **T** [Gene encoding sulfotransferase](#)
- 1779 [5,773,273](#) **T** [Geranylgeranyl diphosphate synthase and DNA coding therefor](#)
- 1780 [5,773,265](#) **T** [DNA encoding heptaprenyl diphosphate synthetase](#)
- 1781 [5,773,251](#) **T** [DNA clone of human tissue factor inhibitor](#)
- 1782 [5,770,402](#) **T** [DNA encoding macrophage inflammatory protein-1.gamma.](#)
- 1783 [5,770,398](#) **T** [Vector for integration site independent gene expression in mammalian host cells](#)
- 1784 [5,770,372](#) **T** [Detection of mutations in the human ATM gene](#)
- 1785 [5,767,375](#) **T** [Plant genes affecting gibberellic acid biosynthesis](#)
- 1786 [5,767,373](#) **T** [Manipulation of protoporphyrinogen oxidase enzyme activity in eukaryotic organisms](#)
- 1787 [5,767,371](#) **T** [Deacetylase genes for the production of phosphinothricin or phosphinothricyl-alanyl-alanine, process for their isolation, and their use](#)
- 1788 [5,767,370](#) **T** [Deacetylase genes for the production of phosphinothricin or phosphinothricyl-alanyl-alanine, processes for their isolation, and their use](#)
- 1789 [5,767,369](#) **T** [DNA sequences encoding SAR8.2 proteins and uses thereof](#)
- 1790 [5,767,262](#) **T** [Smooth muscle cell LIM protein](#)
- 1791 [5,766,923](#) **T** [Isolated nucleic acid encoding ligands for FGFR](#)
- 1792 [5,766,913](#) **T** [Cloning, expression and nucleotide sequence of an alkaline lipase gene from pseudomonas pseudoalcaligenes F-111](#)
- 1793 [5,766,608](#) **T** [DNA molecules which encode the fimbrin protein of Haemophilus influenzae](#)
- 1794 [5,766,586](#) **T** [Gram-positive bacteria replicon](#)
- 1795 [5,763,590](#) **T** [Isolation of an M.sub.r 52,000 FK506 binding protein and molecular cloning of a corresponding human cDNA](#)
- 1796 [5,763,244](#) **T** [Method for cloning and expression of phosphorylation-dependent protein kinase](#)
- 1797 [5,763,228](#) **T** [Recombinant enzyme for converting maltose into trehalose from pimelobacter sp.](#)

1798 [5,763,223](#) **T** [DNA encoding a cytokine that induces apoptosis](#)

1799 [5,763,216](#) **T** [Gene encoding a human reduced folate carrier \(RFC\)](#)

1800 [5,763,210](#) **T** [Recombinant production of interferon-gamma binding proteins](#)

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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
1801 5,763,183	Allelic variation of the serotonin 5HT7 receptor
1802 5,762,924	Recombinant entomopoxvirus
1803 5,760,204	DNA encoding murine interleukin-5 receptor
1804 5,760,203	Gap gene sequences
1805 5,760,180	DNA encoding precursor of interleukin-1.beta. converting enzyme-related cysteine proteinase III (ICE.sub.rel -III)
1806 5,759,839	Cloned SstI/SacI restriction-modification system
1807 5,759,831	DNA molecules and vectors regarding clavulanic acid biosynthesis enzymes
1808 5,759,824	Genes for butyrobetaine/crotonobetaine-l-carnitine metabolism and their use for the microbiological production of l-carnitine
1809 5,759,807	Process for producing relaxin
1810 5,759,804	Isolated nucleic acid encoding seven transmembrane receptors
1811 5,756,708	DNA sequences of banana bunchy top virus
1812 5,756,348	DNA encoding a glycine transporter and uses thereof
1813 5,756,334	Thermostable DNA polymerase from 9.degree.N-7 and methods for producing the same
1814 5,756,328	Acyltransferase and cDNA encoding acyltransferase
1815 5,756,105	Opacity associated proteins, DNA encoding the same, and methods of use thereof
	T

- 1816 [5,753,507](#) [Plant geraniol/nerol 10-hydroxylase and DNA coding therefor](#)
- 1817 [5,753,502](#) [Neuron-specific ICAM-4 promoter](#)
- 1818 [5,753,464](#) [DNA encoding a polypeptide having pre-B cell growth-supporting ability](#)
- 1819 [5,753,435](#) [Oxido reductase enzyme system obtainable from *P. chrysogenum*, the set of genes encoding the same and the use of oxido reductase enzyme systems or genes encoding the same for increasing antibiotic production](#)
- 1820 [5,750,876](#) [Isoamylase gene, compositions containing it, and methods of using isoamylases](#)
- 1821 [5,750,848](#) [DNA sequence useful for the production of polyhydroxyalkanoates](#)
- 1822 [5,750,399](#) [Isoflavone reductase promoter](#)
- 1823 [5,750,365](#) [Isolated nucleic acid encoding a newt acidic fibroblast growth factor \(AFGF\)](#)
- 1824 [5,747,336](#) [Cloned human genes for muscarinic acetylcholine receptors and cells lines expressing same](#)
- 1825 [5,747,323](#) [Retroviral vectors comprising a VL30-derived psi region](#)
- 1826 [5,747,285](#) [DNA comprising regulatory regions from gene *y* of *penicillium chrysogenum*](#)
- 1827 [5,747,282](#) [17Q-linked breast and ovarian cancer susceptibility gene](#)
- 1828 [5,744,692](#) [Nucleotide sequences coding an endopolygalacturonase inhibitor](#)
- 1829 [5,744,345](#) [Hyperthermostable .beta.-galactosidase gene, enzyme encoded thereby, and process for production](#)
- 1830 [5,744,341](#) [Genes of carotenoid biosynthesis and metabolism and a system for screening for such genes](#)
- 1831 [5,741,704](#) [Hexokinase promoter and assay method](#)
- 1832 [5,741,697](#) [Bacteriophage of *chlamydia psittaci*](#)
- 1833 [5,741,671](#) [Isolation cloning and expression of transmembrane water channel aquaporin 1\(AQP1\)](#)
- 1834 [5,741,645](#) [Gene sequence for spinocerebellar ataxia type 1 and method for diagnosis](#)
- 1835 [5,739,082](#) [Method of improving the yield of herbicide-resistant crop plants](#)
- 1836 [5,739,027](#) [MTS1E1.beta. gene](#)
- 1837 [5,739,008](#) [DNA encoding a protein comprising calmodulin-and actin-binding human caldesmon peptide fragment](#)
- 1838 [5,738,854](#) [Pseudorabies virus vaccine](#)
- 1839 [5,736,375](#) [Expression system for novel pullulanase](#)
- 1840 [5,736,373](#) [Thermostable DNA polymerase from *Bacillus pallidus*](#)
- 1841 [5,736,364](#) [Factor *viia* inhibitors](#)
- 1842 [5,736,359](#) [Isolated DNA molecules for integration site independent gene expression in mammalian host cells](#)
- 1843 [5,736,358](#) [Dictyostelid expression vector and method for expressing a desired protein](#)
- 1844 [5,736,131](#) [Hybrid toxin](#)
- 1845 [5,734,086](#) [Cytochrome P450.sub.lpr gene and its uses](#)
- 1846 [5,734,035](#) [Nematode vaccine](#)
- 1847 [5,733,771](#) [cDNAs encoding minor ampullate spider silk proteins](#)
- 1848 [5,731,412](#) [Protein, DNA coding for same and method of producing the protein](#)
- 1849 [5,731,185](#) [Isolated DNA encoding the hphi restriction endonuclease and related](#)

[methods for producing the same](#)

1850 [5,731,176](#)  [DNA fragment encoding a polypeptide having nitrile hydratase activity, a transformant containing the gene and a process for the production of amides using the transformant](#)

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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
1851 5,731,170	DNA encoding a growth factor specific for epithelial cells
1852 5,731,169	cDNA fragment coding the alpha interferon receptor gene and process for the preparation of a corresponding protein
1853 5,731,167	Autotaxin: motility stimulating protein useful in cancer diagnosis and therapy
1854 5,731,166	Recombinant production of chemotactic CP-10 polypeptides and therapeutic methods using them
1855 5,731,150	IS6110 based molecular detection of mycobacterium tuberculosis
1856 5,728,579	DNA encoding Mat-8
1857 5,728,567	Tissue plasminogen activator having zymogenic or fibrin specific properties
1858 5,726,299	Promoter DNA fragment from coryneform bacteria
1859 5,726,298	Epimorphin and its encoding nucleic acids
1860 5,726,035	Recombinant production of mammalian calcium channel gamma subunits
1861 5,723,759	Pyrrolnitrin biosynthesis genes
1862 5,723,756	Bacillus thuringiensis strains and their genes encoding insecticidal toxins
1863 5,723,754	Tapetum-specific promoters from Brassicaceae spp
1864 5,723,596	European corn borer resistance genetic markers
1865 5,723,332	Translational enhancer DNA
1866 5,723,328	Enzyme with endoglucanase activity

- 1867 [5,723,318](#) **T** [DNA coding for megakaryocyte potentiator](#)
- 1868 [5,723,314](#) **T** [Recombinant antigen for diagnosing rheumatoid arthritis](#)
- 1869 [5,723,293](#) **T** [Diagnostic method and kit for determining Rh blood group genotype](#)
- 1870 [5,721,354](#) **T** [Human cytomegalovirus DNA sequences](#)
- 1871 [5,721,348](#) **T** [DNA encoding PH-20 proteins](#)
- 1872 [5,721,127](#) **T** [Pullulanase](#)
- 1873 [5,721,126](#) **T** [Method for cloning and producing the ScaI restriction endonuclease in E. coli](#)
- 1874 [5,721,115](#) **T** [DNA encoding a novel Haemophilus influenzae protein](#)
- 1875 [5,719,043](#) **T** [DNA sequences for an amino acid transporter, plasmids, bacteria, yeasts and plants containing a transporter and their use](#)
- 1876 [5,719,042](#) **T** [Nucleic acids encoding transcription factor APRF \(acute phase response factor\)](#)
- 1877 [5,719,041](#) **T** [DNA encoding ecotin homologs](#)
- 1878 [5,717,069](#) **T** [DNA sequence coding for enhancin polypeptide which enhances virus infection of host insects](#)
- 1879 [5,716,849](#) **T** [Genes for the biosynthesis of soraphen](#)
- 1880 [5,716,835](#) **T** [Nucleic acid encoding a novel human EP prostaglandin receptor](#)
- 1881 [5,716,834](#) **T** [Cloned factor C cDNA of the Singapore horseshoe crab, Carinoscorpis rotundicauda and purification of factor C proenzyme](#)
- 1882 [5,716,823](#) **T** [Human respiratory virus preparahons and processess](#)
- 1883 [5,714,382](#) **T** [Synthetic plasmids and transformants comprising a feline interferon cDNA](#)
- 1884 [5,714,377](#) **T** [Modified fungal cells and method for producing recombinant products](#)
- 1885 [5,712,382](#) **T** [Plant adenylosuccinate lyase and DNA coding therefor](#)
- 1886 [5,712,155](#) **T** [DNA encoding tumor necrosis factor-.alpha. and -.beta. receptors](#)
- 1887 [5,712,147](#) **T** [DNA encoding Bacillus licheniformis PWD-1 keratinase](#)
- 1888 [5,712,139](#) **T** [Pyranose oxidase, pyranose oxidase gene, novel recombinant DNA and process for producing pyranose oxidase](#)
- 1889 [5,712,121](#) **T** [Chimeric interleukin 5-receptor/immunoglobulin polypeptides](#)
- 1890 [5,712,092](#) **T** [Papillomavirus probe and process for in vitro diagnosis of papillomavirus infections](#)
- 1891 [5,712,091](#) **T** [Method of selecting genetically superior shrimp](#)
- 1892 [5,712,090](#) **T** [PCR-based assay for Mycoplasma hyopneumoniae](#)
- 1893 [5,710,018](#) **T** [Mammalian influx peptide transporter](#)
- 1894 [5,708,156](#) **T** [Epidermal growth factor receptor-like gene product and its uses](#)
- 1895 [5,707,863](#) **T** [Tumor suppressor gene merlin](#)
- 1896 [5,707,840](#) **T** [Multifunctional RNA having self-processing activity, the preparation thereof and the use thereof](#)
- 1897 [5,707,803](#) **T** [DNA regulatory elements responsive to cytokines and methods for their use](#)
- 1898 [5,702,920](#) **T** [DNAS encoding human macrophage migration inhibition factor related peptides](#)
- 1899 [5,702,919](#) **T** [DNA encoding canine granulocyte macrophage colony stimulating factor](#)
- 1900 [5,702,895](#) **T** [Method and kit for detecting methicillin-resistant Staphylococcus aureus](#)

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PAT. NO.	Title
1901	5,700,678 T Protein disulfide-isomerase and production thereof
1902	5,700,667 T Strategy for the production of RNA from immobilized templates
1903	5,700,664 T Mammalian cytokine, IL-11
1904	5,700,658 T ICAM-4 materials and methods
1905	5,698,204 T Recombinant allergenic proteins from ragweed pollen
1906	5,696,250 T DNA encoding megakaryocyte growth and development factor analogs
1907	5,695,966 T DNA encoding daunorubicin 14-hydroxylase and method for preparing doxorubicin
1908	5,695,965 T Neurospora expression system
1909	5,695,957 T Polypeptides and DNA encoding same, associated with human malaria parasites
1910	5,695,956 T Clostridium perfringens type a enterotoxin toxoid and methods of preparation and use as a vaccine and therapeutic agent
1911	5,695,939 T Plant defense genes and plant defense regulatory elements
1912	5,693,781 T Promoter DNA fragment from coryneform bacteria
1913	5,693,774 T DNA sequences involved in soraphen biosynthesis by myxobacteria
1914	5,693,518 T Enzymes with xylanase activity from Aspergillus aculeatus
1915	5,693,506 T Process for protein production in plants
1916	5,693,501 T Compounds and methods to determine presence of Histoplasma capsulatum

- 1917 [5,693,500](#) **T** [Diagnosis of Mycobacterium bovis infection](#)
- 1918 [5,693,498](#) **T** [DNA encoding a plerocerciod growth factor](#)
- 1919 [5,693,487](#) **T** [Nucleic acids encoding max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc and Mad](#)
- 1920 [5,693,486](#) **T** [DNA sequences encoding protegrins and protegrin analogs and their use in recombinant methods of producing protegrins](#)
- 1921 [5,693,473](#) **T** [Linked breast and ovarian cancer susceptibility gene](#)
- 1922 [5,693,472](#) **T** [Detection of cryptosporidium parvum](#)
- 1923 [5,691,199](#) **T** [DNA encoding biocidal proteins](#)
- 1924 [5,691,197](#) **T** [Isolated DNA sequence for a novel macrophage receptor with a collagenous domain](#)
- 1925 [5,691,181](#) **T** [DNA encoding lipase from human gastric mucosal tissue](#)
- 1926 [5,691,168](#) **T** [DNA sequences encoding AlaGlu-IGF-1 and vectors and microorganisms comprising said sequences](#)
- 1927 [5,691,156](#) **T** [Method of inhibiting cell growth with the P.sub.2U receptor](#)
- 1928 [5,691,155](#) **T** [Nucleotide sequences encoding the murine .beta.3-adrenergic receptor and their applications](#)
- 1929 [5,689,051](#) **T** [Transgenic plants and DNA comprising anther specific promoter 5126 and gene to achieve male sterility](#)
- 1930 [5,689,050](#) **T** [Production of gamma linolenic acid by a .DELTA.6-desaturase](#)
- 1931 [5,689,045](#) **T** [Transgenic pathogen-resistant plant](#)
- 1932 [5,689,044](#) **T** [Chemically inducible promoter of a plant PR-1 gene](#)
- 1933 [5,688,939](#) **T** [Plant adenylosuccinate synthetase and DNA coding therefor](#)
- 1934 [5,688,936](#) **T** [Vesicle membrane transport proteins](#)
- 1935 [5,688,691](#) **T** [Insect retinoid-like receptor compositions and methods](#)
- 1936 [5,688,678](#) **T** [DNA encoding and methods for producing BMP-8 proteins](#)
- 1937 [5,686,575](#) **T** [Toxoplasma gondii P28 polypeptides](#)
- 1938 [5,686,294](#) **T** [Protein having heat-resistant malate dehydrogenase activity](#)
- 1939 [5,686,285](#) **T** [Norbornane type ester hydrolase](#)
- 1940 [5,686,283](#) **T** [Genomic DNA encoding the pseudomonas fluorescens alternative sigma factor, RpoS, capable of activating gene expression](#)
- 1941 [5,686,268](#) **T** [Fused proteins](#)
- 1942 [5,684,241](#) **T** [Purified tobacco protein involved in nicotine synthesis, DNA encoding, and use of sense and antisense DNAs corresponding thereto to affect nicotine content in tobacco plants](#)
- 1943 [5,684,238](#) **T** [Biosynthesis of zeaxanthin and glycosylated zeaxanthin in genetically engineered hosts](#)
- 1944 [5,684,146](#) **T** [DNA coding for variable region to human influenza A type virus](#)
- 1945 [5,683,904](#) **T** [Ubiquitin-specific proteases](#)
- 1946 [5,683,898](#) **T** [Gene coding for eicosapentaenoic acid synthesizing enzymes and process for production of eicosapentaenoic acid](#)
- 1947 [5,681,942](#) **T** [Fanconi Anemia Type C gene](#)
- 1948 [5,681,737](#) **T** [Detection system for mutagens that also identifies mutagenic changes](#)

1949 [5,681,735](#) [Transcription control element for increasing gene expression in myoblasts](#)
1950 [5,681,717](#) [DNA encoding novel cell surface protein](#)

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PAT. NO.	Title
1951 5,679,783	DNA encoding a tissue differentiation affecting factor
1952 5,679,635	Aspartoacylase gene, protein, and methods of screening for mutaton associated with canavan disease
1953 5,679,540	Cloning and identification of a two component signal transducing regulatory system from bacteroides fragilis
1954 5,679,511	CDNA clones for a regulatory protein in the melanin-production pathway
1955 5,677,172	Method for production of proteins in yeast
1956 5,674,735	DNA encoding the EHV-4 gH or gC glycoprotein
1957 5,674,734	Cell death protein
1958 5,674,733	Method and materials for introducing DNA into Prevotella ruminicola
1959 5,674,728	Aspergillus niger vacuolar aspartyl protease
1960 5,674,709	Pseudorabies virus protein
1961 5,674,704	Cytokine designated 4-IBB ligand
1962 5,672,509	hPDE IV-C: a human phosphodiesterase IV isozyme
1963 5,670,635	Seed storage protein with nutritionally balanced amino acid composition
1964 5,670,360	Mammalian receptors for glucagon-like-peptide-1 (GLP-1), corresponding DNA and recombinant expression systems, and screening assays for GLP-1 agonists and enhancers
1965 5,670,350	Genomic DNA encoding a pseudomonas global transcriptional activation element and its use in activating gene expression

- 1966 [5,670,338](#) **T** [DNA encoding bone morphogenetic proteins, host cells transformed there by, and uses thereof](#)
- 1967 [5,668,295](#) **T** [Protein involved in nicotine synthesis, DNA encoding, and use of sense and antisense DNAs corresponding thereto to affect nicotine content in transgenic tobacco cells and plants](#)
- 1968 [5,668,263](#) **T** [Conserved yeast nucleic acid sequences](#)
- 1969 [5,668,012](#) **T** [Platelet endothelial cell adhesion molecule-1 promoters and uses thereof](#)
- 1970 [5,668,005](#) **T** [Cloned genes encoding reverse transcriptase lacking RNASE H activity](#)
- 1971 [5,668,004](#) **T** [DNA polymerase III holoenzyme from Escherichia coli](#)
- 1972 [5,665,892](#) **T** [Sucrose phosphate synthase \(SPS\), its process for preparation its cDNA, and utilization of cDNA to modify the expression of SPS in plant cells](#)
- 1973 [5,665,592](#) **T** [Feline immunodeficiency virus isolate NCSU.sub.1](#)
- 1974 [5,665,579](#) **T** [Invertase genes and uses thereof](#)
- 1975 [5,665,566](#) **T** [Cloning of enterokinase and method of use](#)
- 1976 [5,665,564](#) **T** [Isolation and characterisation of genes resistant to anthracycline antibiotics](#)
- 1977 [5,665,551](#) **T** [Purified nucleic acid encoding a thermostable pyrophosphatase](#)
- 1978 [5,665,542](#) **T** [Toxoplasma gondii P28 gene and methods for its use](#)
- 1979 [5,665,349](#) **T** [Recombinant baculovirus with insecticidal activity](#)
- 1980 [5,663,315](#) **T** [Isolated DNA encoding human GP2](#)
- 1981 [5,663,067](#) **T** [Method for cloning and producing the SapI restriction endonuclease in E. coli](#)
- 1982 [5,663,065](#) **T** [DNA encoding infectious Rubella virus](#)
- 1983 [5,663,048](#) **T** [Y-chromosome specific polynucleotide probes for prenatal sexing](#)
- 1984 [5,663,047](#) **T** [HLA-DR antigen gene and its nucleotide sequence and its use](#)
- 1985 [5,661,026](#) **T** [Gene encoding bacterial beta-ketothiolase](#)
- 1986 [5,661,011](#) **T** [Sexing method of bovine embryos](#)
- 1987 [5,661,007](#) **T** [Bone morphogenetic protein-9 compositions](#)
- 1988 [5,661,004](#) **T** [Lymphotoxin-.beta., lymphotoxin-.beta. complexes, pharmaceutical preparations and therapeutic uses thereof](#)
- 1989 [5,661,003](#) **T** [Water channel](#)
- 1990 [5,660,983](#) **T** [Maize cytoplasmic male sterility type T \(cms-T\) mitochondria DNA](#)
- 1991 [5,659,026](#) **T** [ALS3 promoter](#)
- 1992 [5,658,792](#) **T** [Antiproliferative protein](#)
- 1993 [5,658,733](#) **T** [Detection of isoniazid resistant strains of M. tuberculosis](#)
- 1994 [5,656,472](#) **T** [Beta-carotene biosynthesis in genetically engineered hosts](#)
- 1995 [5,656,457](#) **T** [DNA sequence for the unique sequence herpes simplex virus type 2-glycoprotein G protein and method of expressing said unique sequence of HSV-2gG](#)
- 1996 [5,656,452](#) **T** [NF-AT.sub.p, ' a T lymphocyte DNA-binding protein](#)
- 1997 [5,656,451](#) **T** [OspE, OspF, and S1 polypeptides in borrelia burgdorferi](#)
- 1998 [5,654,414](#) **T** [Chemically inducible promoter of a cucumber chitinase/lysozyme gene](#)
- 1999 [5,654,180](#) **T** [Hybrid plasmid vectors, recombinant plasmids containing genes encoding nitrile degrading enzymes, transformants containing the recombinant](#)

[plasmids and methods of producing amides and acids using the transformants](#)
2000 [5,654,139](#)  [Allelic variation of the serotonin 5HT.sub.2c receptor](#)

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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
2001 5,652,357	Nucleic acids for the detection of the Bak polymorphism in human platelet membrane glycoprotein IIb
2002 5,652,353	DNAs encoding tumor necrosis factor-.alpha. muteins
2003 5,652,132	Oxido reductase enzyme system obtainable from P. chrysogenum, the set of genes encoding the same and the use of oxido reductase enzyme systems or genes encoding the same for increasing antibiotic production
2004 5,652,125	Process for preparing daunorubicin
2005 5,650,505	Chemically regulatable and anti-pathogenic DNA sequences and uses thereof
2006 5,650,504	Nucleic acids encoding eck receptor ligands
2007 5,650,320	Lanthionine antibiotic compositions and methods
2008 5,650,314	Recombinant agents affection thrombosis
2009 5,650,298	Tight control of gene expression in eucaryotic cells by tetracycline-responsive promoters
2010 5,648,478	Tissue-specific enhancer active in prostate
2011 5,648,260	DNA encoding antibodies with altered effector functions
2012 5,648,256	Gene encoding a polypeptide having nitrile hydratase activity, a transformant containing the gene and a process for the production of amides using the transformant
2013 5,648,250	Tissue plasminogen activator
2014 5,646,026	Ribosome-inactivating proteins, inactive precursor forms thereof, a process

- for making and a method of using
- 2015 [5,646,016](#) **T** [Peptide and protein fusions to thioredoxin, thioredoxin-like molecules, and modified thioredoxin-like molecules](#)
- 2016 [5,644,045](#) **T** [X-linked adrenoleukodystrophy gene and corresponding protein](#)
- 2017 [5,643,791](#) **T** [Characterization and structure of an endoglucanase gene of Cellulomonas fimi](#)
- 2018 [5,643,749](#) **T** [Soluble interferon .alpha.-receptor, its preparation and use](#)
- 2019 [5,643,747](#) **T** [Genes for the export of pertussis holotoxin](#)
- 2020 [5,643,746](#) **T** [Human epidermal gene promoter](#)
- 2021 [5,643,725](#) **T** [Sequence and analysis of LKP pilin structural genes and the LKP pili operon of nontypable haemophilus influenzae](#)
- 2022 [5,641,874](#) **T** [DNA encoding bactericidal/permeability increasing proteins](#)
- 2023 [5,641,660](#) **T** [Glutamicum threonine biosynthetic pathway](#)
- 2024 [5,641,654](#) **T** [Non-A non-B hepatitis specific antigen and its use in hepatitis](#)
- 2025 [5,641,653](#) **T** [DNA encoding Actinobacillus pleuropneumoniae hemolysin](#)
- 2026 [5,639,949](#) **T** [Genes for the synthesis of antipathogenic substances](#)
- 2027 [5,639,863](#) **T** [Human monoclonal antibodies specific to cell cycle independent glioma surface antigen](#)
- 2028 [5,639,652](#) **T** [DNA encoding a human 5-HT.sub.1F receptor and uses thereof](#)
- 2029 [5,639,640](#) **T** [DNA encoding the beta subunit of human follide stimulating hormone and expression vectors and cells containing same](#)
- 2030 [5,639,638](#) **T** [DNA molecules encoding bone morpogenetic protein-11](#)
- 2031 [5,639,616](#) **T** [Isolated nucleic acid encoding a ubiquitous nuclear receptor](#)
- 2032 [5,637,480](#) **T** [DNA molecules encoding bone morphogenetic protein-10](#)
- 2033 [5,637,476](#) **T** [Method for cloning and producing the SFII restriction endonuclease and methylase](#)
- 2034 [5,635,374](#) **T** [Bone calcification factor and recombinant production of the factor nucleic acid encoding](#)
- 2035 [5,635,373](#) **T** [Bone morphogenic protein-5\(BMP-5\) and DNA encoding same](#)
- 2036 [5,635,372](#) **T** [BMP-15 compositions](#)
- 2037 [5,635,370](#) **T** [DNA encoding BEHAB, a brain hyaluronan-binding protein, and recombinant expression systems for production of BEHAB polypeptides](#)
- 2038 [5,633,449](#) **T** [Induction of resistance to viral diseases in plants](#)
- 2039 [5,633,438](#) **T** [Microspore-specific regulatory element](#)
- 2040 [5,633,435](#) **T** [Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases](#)
- 2041 [5,633,434](#) **T** [Transgenic plants displaying virus and phosphinothricin resistance](#)
- 2042 [5,633,363](#) **T** [Root preferential promoter](#)
- 2043 [5,633,158](#) **T** [Bacterial nitroreductase for the reduction of CB 1954 and analogues thereof to a cytotoxic form](#)
- 2044 [5,633,150](#) **T** [Preparation of functional human factor VIII](#)
- 2045 [5,633,139](#) **T** [Toxoplasma gondii P28 gene and methods for its use](#)
- 2046 [5,633,137](#) **T** [Method for measuring specific gene expression: transcriptional activity per gene dose](#)

- 2047 [5,633,129](#)  [Electrophoretic detection and separation of mutant DNA using replaceable polymer matrices](#)
- 2048 [5,631,358](#)  [Enod2 gene regulatory region](#)
- 2049 [5,631,144](#)  [Application of novel DNA fragments as a coding sequence for a signal peptide for the secretion of mature proteins by recombinant yeast, expression cassettes, transformed yeast and corresponding process for the preparation of proteins](#)
- 2050 [5,629,414](#)  [Diagnostic genes for toxoplasmosis](#)
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PAT. NO.	Title
2051	5,629,204 Peptide related to human programmed cell death and DNA encoding it
2052	5,629,196 DNA encoding peptide hormone that inhibits digestion in insects
2053	5,629,182 DNA fragments coding for a bacteriophage-resistant mechanism
2054	5,627,065 DNA fragment containing a gene encoding creatinine amidohydrolase
2055	5,627,049 K. lactis RP28 ribosomal protein gene promoter and use thereof
2056	5,624,835 Endo-.beta.-1,4-glucanase and a DNA sequence
2057	5,624,819 Germline mutations in the MTS gene
2058	5,624,817 Mutations in the gene encoding the alpha chain of platelet glycoprotein Ib
2059	5,624,816 Transmembrane glycoprotein ASGP-2: nucleotide sequences and recombinant production of proteins
2060	5,623,067 Seed-specific promoter region
2061	5,622,866 Expression cassettes useful in construction of integrative and replicative expression vectors for Streptomyces
2062	5,622,851 Human deoxycytidylate deaminase gene
2063	5,620,867 Bone morphogenetic protein expression and DNA
2064	5,618,722 Photuris firefly luciferase gene
2065	5,618,712 Human lysozyme
2066	5,618,698 Production of erythropoietin
2067	5,618,692 Zwittermicin resistance gene and biocontrol bacteria with the gene

- 2068 [5,616,699](#) **T** [Coding, promoter and regulator sequences of IRF-1](#)
- 2069 [5,616,486](#) **T** [Tissue plasminogen activator having zymogenic or fibrin specific properties](#)
- 2070 [5,616,484](#) **T** [Cloning and expression of the ApaLI restriction endonuclease](#)
- 2071 [5,616,473](#) **T** [Cloning and expression of ligninases](#)
- 2072 [5,616,322](#) **T** [Sperm antigen corresponding to a sperm zona binding protein autoantigenic epitope](#)
- 2073 [5,614,400](#) **T** [Methods and compositions relating to plant palmitoyl-acyl carrier protein desaturase](#)
- 2074 [5,614,190](#) **T** [Tissue plasminogen activator having zymogenic or fibrin specific properties](#)
- 2075 [5,612,191](#) **T** [Plant genes affecting gibberellic acid biosynthesis](#)
- 2076 [5,612,190](#) **T** [DNA molecule encoding bovine group I phospholipase A.sub.2 receptor](#)
- 2077 [5,610,282](#) **T** [cDNA encoding a rat D.sub.1 dopamine receptor linked to adenylyl cyclase activation and expression of the receptor protein in plasmid-transfected cell lines](#)
- 2078 [5,610,053](#) **T** [DNA sequence which acts as a chromatin insulator element to protect expressed genes from cis-acting regulatory sequences in mammalian cells](#)
- 2079 [5,610,048](#) **T** [Xylanase, DNA sequences, coding for the xylanases and methods of use thereof](#)
- 2080 [5,610,010](#) **T** [Process and apparatus for fragmenting biomaterials](#)
- 2081 [5,608,151](#) **T** [Anti-microbial proteins](#)
- 2082 [5,608,150](#) **T** [Fruit specific promoters](#)
- 2083 [5,608,146](#) **T** [DNA sequences with oligosaccharide transporter, plasmids, bacteria and plants containing a transporter as well as a process for the preparation and transformation of yeast strains for identification of the transporter](#)
- 2084 [5,607,844](#) **T** [Mammalian augments of liver regeneration and variants thereof](#)
- 2085 [5,607,836](#) **T** [Methods of detecting compounds which bind to the P.sub.2U receptor](#)
- 2086 [5,605,815](#) **T** [Nucleic acids encoding and expression of parathyroid hormone-like peptide](#)
- 2087 [5,604,123](#) **T** [Luciferase, gene encoding the same and production process of the same](#)
- 2088 [5,604,115](#) **T** [Liver enriched transcription factor](#)
- 2089 [5,602,300](#) **T** [Process for detecting mutations, transgenic mammal transgenic mammalian cell, and process for testing agents or conditioning for mutagenic properties](#)
- 2090 [5,602,031](#) **T** [DNA encoding molecules containing at least one peptide sequence carrying one or several epitopes characteristic of a liver stage antigen produced by p. falciparum in hepatocytes and compositions containing them](#)
- 2091 [5,602,024](#) **T** [DNA encoding a hypothalamic atypical neuropeptide Y/peptide YY receptor \(Y5\) and uses thereof](#)
- 2092 [5,602,016](#) **T** [D-amino acid oxidase from F. solani and DNA therefor](#)
- 2093 [5,602,010](#) **T** [DNA encoding equine-gamma interferon and recombinant production of equine IFN-.gamma. polypeptides](#)
- 2094 [5,602,007](#) **T** [Recombinant DNA molecules](#)
- 2095 [5,602,003](#) **T** [N-acetylglucosaminyltransferase V gene](#)
- 2096 [5,599,709](#) **T** [Recombinant DNA encoding neuronal .alpha.-bungarotoxin-binding proteins](#)
- 2097 [5,599,692](#) **T** [Antigenic polypeptides of Taenia ovis](#)
- 2098 [5,597,911](#) **T** [Mycobacterial nucleic acid hybridization probes and methods of use](#)

- 2099 [5,596,132](#)  [Induction of resistance to virus diseases by transformation of plants with a portion of a plant virus genome involving a read-through replicase gene](#)
- 2100 [5,596,088](#)  [DNA Encoding the human P.sub.2U receptor and null cells expressing P.sub.2U receptors](#)
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PAT. NO.	Title
2101 5,595,911	T Isolation of a cDNA encoding a protein tyrosine phosphatase which localizes to focal adhesions
2102 5,595,902	T DNA encoding human protein kinase C (iota)
2103 5,595,871	T Detection and prevention of mycoplasma hominis infection
2104 5,595,733	T Methods for protecting ZEA mays plants against pest damage
2105 5,593,882	T Selectin variants
2106 5,593,881	T Bacillus thuringiensis delta-endotoxin
2107 5,593,862	T Nucleic acids encoding drosophila tipE cation channel proteins and recombinant expression of the same
2108 5,593,861	T Dog-mouse heterohybridoma and gene fragment coding for constant region of canine immunoglobulins
2109 5,593,837	T Clinical disorders associated with carboxypeptidase E mutation
2110 5,591,574	T Probes, diagnostic method and immunization method based on papillomavirus types HPV 49 and HPV 50
2111 5,591,434	T DNA sequence encoding surface protein of cryptosporidium parvum
2112 5,589,584	T Angiotensinogen gene variants and predisposition to hypertension
2113 5,589,583	T Plant promoter
2114 5,589,380	T Isolated DNA molecule encoding SHET1 of Shigella flexneri 2a and mutant Shigella flexneri 2a
2115 5,589,373	T Thermostable alkaline metalloprotease produced by a hyphomonas and

- [preparation thereof](#)
- 2116 [5,589,372](#) **T** [Squalene synthetase](#)
- 2117 [5,589,360](#) **T** [Polypeptide, DNA fragment encoding the same, drug composition containing the same and process for producing the same](#)
- 2118 [5,589,358](#) **T** [Ileal bile acid transporter compositions and methods](#)
- 2119 [5,589,355](#) **T** [Process for producing riboflavin](#)
- 2120 [5,589,338](#) **T** [Nucleic acids encoding a mutant form of human serum albumin involved in familial dysalbuminemic hyperthyroxinemia](#)
- 2121 [5,587,359](#) **T** [Human derived glycoprotein, biologically active factor which includes glycoprotein and pharmaceutical product](#)
- 2122 [5,587,310](#) **T** [Chimeric blood coagulation proteins](#)
- 2123 [5,587,307](#) **T** [FemA gene of staphylococcus epidermidis, femA protein, and vectors and microorganisms comprising the femA gene](#)
- 2124 [5,587,306](#) **T** [Phospholipase C homolog](#)
- 2125 [5,587,301](#) **T** [DNA encoding a hyaluronan receptor expressed in human umbilical vein endothelial cells](#)
- 2126 [5,585,542](#) **T** [DNA sequences encoding at least part of the tomato enzyme endopolygalacturonase PG1 .beta.-subunit](#)
- 2127 [5,585,269](#) **T** [Isolated DNA encoding c-mer protooncogene](#)
- 2128 [5,585,268](#) **T** [Malaria-specific DNA sequences, expression products thereof, and the use thereof](#)
- 2129 [5,585,256](#) **T** [Aspergillus aculeatus rhamnogalacturon acetyl esterases, DNA sequences encoding the enzymes and methods of use thereof](#)
- 2130 [5,585,253](#) **T** [Extracellular serine protease and a Bacillus subtilis alkaline neutral an serine protease mutant strain](#)
- 2131 [5,582,990](#) **T** [DNA encoding borrelia burgdorferi OspA and a method for diagnosing borrelia burgdorferi infection](#)
- 2132 [5,580,775](#) **T** [High affinity, brain-specific nucleic acids encoding a L-proline transporter, and vectors, and host cells comprising the same](#)
- 2133 [5,580,753](#) **T** [DNA encoding the human cytokine, interleukin-9](#)
- 2134 [5,580,715](#) **T** [Diagnosis of cancer having clonal macrophage involvement](#)
- 2135 [5,576,428](#) **T** [Invertase gene\(s\) and uses thereof](#)
- 2136 [5,576,195](#) **T** [Vectors with pectate lyase signal sequence](#)
- 2137 [5,576,191](#) **T** [Cytokine that binds ST2](#)
- 2138 [5,574,136](#) **T** [DNA encoding granulocyte colony-stimulating factor receptor and protein thereof](#)
- 2139 [5,573,930](#) **T** [DNA encoding various forms of colony stimulating factor-1](#)
- 2140 [5,573,928](#) **T** [Porcine vasoactive intestinal peptide receptor and DNA](#)
- 2141 [5,573,924](#) **T** [CD27 ligand](#)
- 2142 [5,571,693](#) **T** [DNA sequences and amino acid sequences of class B beta lactamase enzymes from bacteroides fragilis](#)
- 2143 [5,571,674](#) **T** [DNA oligomers for use in detection of campylobacter pylori and methods of using such DNA oligomers](#)
- 2144 [5,569,830](#) **T** [Plant inhibitors of fungal polygalacturonases and their use to control fungal](#)

[disease](#)

- 2145 [5,569,823](#) **T** [DNA comprising plum pox virus and tomato spotted wilt virus cDNAs for disease resistance](#)
- 2146 [5,567,611](#) **T** [Multifunctional M-CSF proteins and genes encoding therefor](#)
- 2147 [5,565,358](#) **T** [Enhancer and silencer sequences isolated from the GPIIB promoter](#)
- 2148 [5,565,334](#) **T** [Enhancer sequence for modulating expression in epithelial cells](#)
- 2149 [5,563,048](#) **T** [Human stromal derived factor 1.alpha. and 1.beta., and DNAs encoding the same](#)
- 2150 [5,559,034](#) **T** [Synergistic antifungal protein and compositions containing same](#)
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PAT. NO.	Title
2151 5,559,023	Tumor suppressor gene
2152 5,559,021	DNA encoding a novel mammalian transporter homologous to neurotransmitter transporters and uses thereof
2153 5,559,016	Process for producing alanine
2154 5,559,009	Voltage-gated potassium channel gene, KV1.7, vectors and host cells comprising the same, and recombinant methods of making potassium channel proteins
2155 5,559,008	Leukotoxin gene from Pasteurella suis
2156 5,556,786	Anhidrotic ectodermal dysplasia gene and method of detecting same
2157 5,554,538	DNA sequences derived from papillomavirus HPV-33 genome
2158 5,554,526	Human parainfluenza virus type 4A fusion protein and gene coding for the same
2159 5,554,512	Ligands for flt3 receptors
2160 5,552,537	IgE isoforms and methods of use
2161 5,552,536	DNA encoding precursor of interleukin-1 beta converting enzyme - related cysteine proteinase III (ice rel-III)
2162 5,552,308	CDNA clone of a rat serotonin transporter and protein encoded thereby
2163 5,552,303	DNA encoding epithelium-derived T-cell factor
2164 5,550,223	Lipoprotein I (ompi) of pseudomonas aeruginosa
2165 5,550,037	Mammalian augmenter of liver regeneration (ALR): human and rat

- 2166 [5,547,933](#) **T** [Production of erythropoietin](#)
 - 2167 [5,547,854](#) **T** [DNA encoding a receptor for Mullerian inhibitory substance, misr1, and corresponding vectors, cells, probes, and recombinant methods](#)
 - 2168 [5,547,853](#) **T** [CD2-binding domain of lymphocyte function associated antigen 3](#)
 - 2169 [5,547,845](#) **T** [Aqueous solution containing D.sub.1 dopamine receptor](#)
 - 2170 [5,545,816](#) **T** [Phytoene biosynthesis in genetically engineered hosts](#)
 - 2171 [5,545,563](#) **T** [Human C/EBP gene and vectors for its expression](#)
 - 2172 [5,545,549](#) **T** [DNA encoding a human neuropeptide Y/peptide YY \(Y2\) receptor and uses thereof](#)
 - 2173 [5,545,546](#) **T** [Pollen-specific promoter from maize](#)
 - 2174 [5,545,545](#) **T** [Lysine-insensitive maize dihydrodipicolinic acid synthase](#)
 - 2175 [5,545,525](#) **T** [Detection of candida albicans](#)
 - 2176 [5,543,323](#) **T** [Plasmodium merozoite rhoptries antigenic polypeptides](#)
 - 2177 [5,543,308](#) **T** [Isolated DNA encoding the FSEI restriction endonuclease and related methods for producing the same](#)
 - 2178 [5,541,112](#) **T** [Genes which influence pichia proteolytic activity, and uses therefor](#)
 - 2179 [5,541,109](#) **T** [Expression cloning of c-src SH3-domain binding proteins](#)
 - 2180 [5,541,095](#) **T** [Glycosaminoglycan specific sulfotransferases](#)
 - 2181 [5,538,892](#) **T** [Nucleic acids encoding a TGF-.beta. type 1 receptor](#)
 - 2182 [5,538,869](#) **T** [In-situ hybridization probes for identification and banding of specific human chromosomes and regions](#)
 - 2183 [5,538,866](#) **T** [Prostate-specific membrane antigen](#)
 - 2184 [5,538,861](#) **T** [DNA encoding a stimulating factor for the axl receptor](#)
 - 2185 [5,536,657](#) **T** [Recombinant DNA encoding human receptor for interleukin-12](#)
 - 2186 [5,534,660](#) **T** [Ph genes and their uses](#)
 - 2187 [5,534,409](#) **T** [Cytokine regulated transcription factor](#)
 - 2188 [5,532,347](#) **T** [DNA encoding .alpha. melanocyte stimulating hormone receptor](#)
 - 2189 [5,532,153](#) **T** [Method for cloning and producing the SacI restriction endonuclease](#)
 - 2190 [5,532,152](#) **T** [Platelet-activating factor acetylhydrolase](#)
 - 2191 [5,532,143](#) **T** [Isolated DNA molecules for intergration site independent gene expression in mammalian host cells](#)
 - 2192 [5,530,195](#) **T** [Bacillus thuringiensis gene encoding a toxin active against insects](#)
 - 2193 [5,530,189](#) **T** [Lycopene biosynthesis in genetically engineered hosts](#)
 - 2194 [5,530,177](#) **T** [Transgenic mice producing bovine .alpha.-lactalbumin in their milk](#)
 - 2195 [5,529,919](#) **T** [Method of making endoglucanase I](#)
 - 2196 [5,527,896](#) **T** [Cloning by complementation and related processes](#)
 - 2197 [5,527,884](#) **T** [Mediators of chronic allograft rejection and DNA molecules encoding them](#)
 - 2198 [5,527,682](#) **T** [DNA sequences encoding proteins used to elicit and detect programmed cell death](#)
 - 2199 [5,527,679](#) **T** [.beta..sub.5 protein and DNA encoding the same](#)
 - 2200 [5,525,712](#) **T** [DNA encoding the human neurokinin-1 receptor](#)
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PAT. NO.	Title
2201	5,525,504 T Cytolysin gene and gene product
2202	5,525,502 T DNA encoding chitin deacetylase
2203	5,523,227 T DNA encoding calcium-signal modulating cyclophilin ligand
2204	5,523,225 T DNA sequence encoding human cystathionine .beta.-synthase
2205	5,521,088 T Alcohol acetyltransferase genes and use thereof
2206	5,521,069 T Genomic DNA exons having exons encoding human pituitary adenylate cyclase activity peptide with 38 amino acids residues(PACAP38) and a promoter thereof
2207	RE35,248 T Method for producing the Hinc II restriction endonuclease and methylase
2208	5,519,125 T Plant adenylosuccinate synthetase and DNA coding therefor
2209	5,518,916 T Cloned Babesia DNA
2210	5,516,685 T Isolation and characterization of a novel protease from Streptomyces lividans
2211	5,516,679 T Penicillin V amidohydrolase gene from Fusarium oxysporum
2212	5,516,678 T Method for producing the SSPI restriction endonuclease and methylase
2213	5,516,658 T DNA encoding cytokines that bind the cell surface receptor hek
2214	5,516,653 T DNA encoding a human neuropeptide Y/peptide YY/pancreatic polypeptide receptor (Y4) and uses thereof
2215	5,516,650 T Production of activated protein C
2216	5,514,787 T DNA sequences encoding human membrane cofactor protein (MCP)

- 2217 [5,514,544](#) **T** [Activator gene for macrolide biosynthesis](#)
- 2218 [5,512,669](#) **T** [Gene encoding bacterial acetoacetyl-COA reductase](#)
- 2219 [5,512,478](#) **T** [Genes and enzymes involved in the microbial degradation of pentachlorophenol](#)
- 2220 [5,512,472](#) **T** [DNA sequence encoding sterol .DELTA.14 reductase](#)
- 2221 [5,512,457](#) **T** [Cytokine designated elk ligand](#)
- 2222 [5,512,456](#) **T** [Method for the improved production and recovery of poly-.beta.-hydroxybutyrate from transformed Escherichia coli](#)
- 2223 [5,512,440](#) **T** [Process for lysing Mycobacteria](#)
- 2224 [5,510,474](#) **T** [Plant ubiquitin promoter system](#)
- 2225 [5,510,473](#) **T** [Cloning of the recA gene from thermus aquaticus YT-1](#)
- 2226 [5,510,472](#) **T** [Production of recombinant human interferon-beta2](#)
- 2227 [5,510,466](#) **T** [Scavenger receptor protein and antibody thereto](#)
- 2228 [5,506,137](#) **T** [Purified thermostable Pyrococcus furiosus DNA ligase](#)
- 2229 [5,506,119](#) **T** [DNA encoding variant CD44 surface proteins associated with metastatic tumors](#)
- 2230 [5,504,200](#) **T** [Plant gene expression](#)
- 2231 [5,504,197](#) **T** [DNA encoding neurotrophic growth factors](#)
- 2232 [5,501,976](#) **T** [Methods and compositions for the control of the flesh fly](#)
- 2233 [5,500,370](#) **T** [Thermostable ribonuclease H and genetic constructs therefore](#)
- 2234 [5,500,363](#) **T** [Recombinant thermostable DNA polymerase from archaeobacteria](#)
- 2235 [5,494,806](#) **T** [DNA and vectors encoding the parathyroid hormone receptor, transformed cells, and recombinant production of PTHR proteins and peptides](#)
- 2236 [5,492,825](#) **T** [Mammalian inward rectifier potassium channel cDNA, IRK1, corresponding vectors, and transformed cells](#)
- 2237 [5,492,823](#) **T** [Method for direct cloning and producing the BsoBI restriction endonuclease in E. coli](#)
- 2238 [5,492,811](#) **T** [Bacterial diagnostic probe](#)
- 2239 [5,492,809](#) **T** [Mutations rendering platelet glycoprotein Ib-.alpha. less reactive](#)
- 2240 [5,489,529](#) **T** [DNA for expression of bovine growth hormone](#)
- 2241 [5,489,430](#) **T** [Poultry mycoplasma antigen, gene thereof and recombinant vectors containing the gene as well as vaccines utilizing the same](#)
- 2242 [5,487,990](#) **T** [Glucose-regulated promoter of yeast acetyl-CoA hydrolase](#)
- 2243 [5,487,976](#) **T** [DNA encoding an insect gamma-aminobutyric acid \(GABA\) receptor subunit cells expressing it, and pesticide screening methods using such cells](#)
- 2244 [5,484,905](#) **T** [Receptor protein kinase gene encoded at the self-incompatibility locus](#)
- 2245 [5,484,728](#) **T** [Parathion hydrolase analogs and methods for production and purification](#)
- 2246 [5,484,724](#) **T** [DNA encoding GLSI](#)
- 2247 [5,480,981](#) **T** [CD30 ligand](#)
- 2248 [5,480,805](#) **T** [Composition for modulating sterols in yeast](#)
- 2249 [5,480,799](#) **T** [Sperm antigen corresponding to a sperm zona binding protein autoantigenic epitope](#)
- 2250 [5,480,797](#) **T** [Augmenter of liver regeneration \(ALR\)](#)

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ACLM/"isolated DNA": 2645 patents.

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PAT. NO.	Title
2251 5,478,369	T Nucleotide sequences mediating male fertility and method of using same
2252 5,476,785	T Recombinant DNA clone containing a genomic fragment of PfHRP-II gene from Plasmodium falciparum
2253 5,476,779	T DNA encoding insulin-like growth factor II isolated from rainbow trout
2254 5,475,101	T DNA sequence encoding endoglucanase III cellulase
2255 5,475,100	T Artificial antibody
2256 5,475,098	T Distinctive DNA sequence of E. coli 0157:H7 and its use for the rapid, sensitive and specific detection of 0157:H7 and other enterohemorrhagic E. coli
2257 5,472,872	T Recombinant CviJI restriction endonuclease
2258 5,472,871	T Isolation and characterization of the nematode HER-1 Gene and protein product
2259 5,472,857	T DNA encoding canine granulocyte colony stimulating factor (G-CSF)
2260 5,470,970	T Maspin, a serpin with tumor suppressing activity
2261 5,470,740	T Cloned NsiI restriction-modification system
2262 5,470,706	T Process for the rescue of DNA and for detecting mutations in marker genes
2263 5,470,359	T Regulatory element conferring tapetum specificity
2264 5,468,639	T Isolated DNA molecule encoding ShET2 of Shigella flexneri 2a
2265 5,468,634	T Axl oncogene
	T

- 2266 [5,468,632](#) [Recombinant DNA compounds and expression vectors encoding para-nitrobenzyl esterase activity from bacillus](#)
- 2267 [5,468,630](#) **T** [cDNA clone for human inducible nitric oxide synthase and process for preparing same](#)
- 2268 [5,466,598](#) **T** [Deacetylcephalosporin C acetyltransferase from Acremonium chrysogenum](#)
- 2269 [5,466,597](#) **T** [Bacillus thuringiensis strains and their genes encoding insecticidal toxins](#)
- 2270 [5,466,584](#) **T** [Oxytocin receptor and DNA coding therefor](#)
- 2271 [5,464,774](#) **T** [Bovine basic fibroblast growth factor](#)
- 2272 [5,464,770](#) **T** [DNA encoding \(ASP 113\) and \(LYS 46, ASP 113\) thaumatin I](#)
- 2273 [5,461,145](#) **T** [Sexing method of bovine embryos](#)
- 2274 [5,460,965](#) **T** [DNA and RNA encoding proteins useful in the regulation of KB-containing genes, and cells containing same](#)
- 2275 [5,459,252](#) **T** [Root specific gene promoter](#)
- 2276 [5,459,250](#) **T** [Truncated mammalian growth factor DNA sequence](#)
- 2277 [5,459,064](#) **T** [Protease](#)
- 2278 [5,459,048](#) **T** [DNA encoding 85kd polypeptide useful in diagnosis of Mycoplasma infections in animals](#)
- 2279 [5,459,038](#) **T** [Determination of genetic sex in ruminants using Y-chromosome specific polynucleotides](#)
- 2280 [5,457,049](#) **T** [Tumor suppressor protein pRb2, related gene products, and DNA encoding therefor](#)
- 2281 [5,457,035](#) **T** [Cytokine which is a ligand for OX40](#)
- 2282 [5,455,338](#) **T** [DNA encoding novel human kunitz-type inhibitors and methods relating thereto](#)
- 2283 [5,455,337](#) **T** [DNA encoding chimeric polypeptides comprising the interleukin-5 receptor .alpha.-chain fused to immunoglobulin heavy chain constant regions](#)
- 2284 [5,453,361](#) **T** [Method for producing biologically active human brain derived neurotrophic factor](#)
- 2285 [5,451,516](#) **T** [Bifunctional protein from carrots \(Daucus carota\) with aspartokinase and homoserine dehydrogenase activities](#)
- 2286 [5,449,764](#) **T** [Isolated DNA derived from peach which codes for an ethylene-forming enzyme](#)
- 2287 [5,449,605](#) **T** [Method of detecting a predisposition to cancer by detecting a deletion polymorphism in the gene for human poly \(ADP-ribose\) polymerase](#)
- 2288 [5,447,851](#) **T** [DNA encoding a chimeric polypeptide comprising the extracellular domain of TNF receptor fused to IgG, vectors, and host cells](#)
- 2289 [5,445,956](#) **T** [Recombinant soluble epoxide hydrolase](#)
- 2290 [5,444,167](#) **T** [Variant luteinizing hormone encoding DNA](#)
- 2291 [5,442,050](#) **T** [Molecular cloning of antigens shared by rat- and human-derived Pneumocystis carinii](#)
- 2292 [5,441,881](#) **T** [NSP7524V restriction-modification genes](#)
- 2293 [5,441,868](#) **T** [Production of recombinant erythropoietin](#)
- 2294 [5,439,824](#) **T** [Increased expression of .alpha.-1-antitrypsin in expression vectors through the inclusion of intron II](#)

- 2295 [5,439,822](#) **T** [Gene expression regulatory DNA](#)
2296 [5,439,821](#) **T** [DNA encoding peptide hormone that inhibits digestion in insects](#)
2297 [5,439,814](#) **T** [DNA encoding infectious rubella virus](#)
2298 [5,436,157](#) **T** [Human intra-acrosomal sperm antigen](#)
2299 [5,436,156](#) **T** [Cloning and expression of phytase from aspergillus](#)
2300 [5,436,155](#) **T** [Isolated DNA encoding a somatostatin receptor](#)
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PAT. NO.	Title
2301 5,436,153	Human amyloid protein precursor homolog and Kunitz-type inhibitor
2302 5,434,068	Method for cloning and producing the BglII restriction endonuclease and modification methylase
2303 5,430,134	Method for production of petroselinic acid and OMEGA12 hexadecanoic acid in transgenic plants
2304 5,428,146	Wound-stimulated DNA-sequence from solanum tuberosum and its use
2305 5,427,936	Alkaline bacillus lipases, coding DNA sequences therefor and bacilli, which produce these lipases
2306 5,427,934	Genetic engineering process for the production of S-(+)-2,2-dimethylcyclopropanecarboxamide by microorganisms
2307 5,427,922	DNA encoding a new angiotensin II type 1 receptor subtype and its expression
2308 5,426,181	DNA encoding cytokine-induced protein, TSG-14
2309 5,426,052	Bacillus MGA3 diaminopimelate decarboxylase gene
2310 5,426,049	PS176 gene encoding nematode-active toxin cloned from a bacillus thuringiensis isolate
2311 5,426,048	DNA encoding a fusion receptor for oncostatin M and leukemia inhibitory factor
2312 5,424,410	Bacillus thuringiensis isolates for controlling acarides
2313 5,422,263	DNA encoding the Trichinella spiralis 53kD excretory/secretory antigen for

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- 2314 [5,422,248](#) **T** [DNA sequences encoding granulocyte-colony stimulating factor receptors](#)
- 2315 [5,420,030](#) **T** [Molecular clones of HIV-1 viral strains MN-ST1 and BA-L and uses thereof](#)
- 2316 [5,418,162](#) **T** [Serotonin transporter CDNA](#)
- 2317 [5,418,155](#) **T** [Isolated Renilla luciferase and method of use thereof](#)
- 2318 [5,418,133](#) **T** [Sex determination in cattle, sheep and goats using y-chromosome polynucleotides](#)
- 2319 [5,416,013](#) **T** [Interleukin 1.beta. protease and interleukin 1.beta. protease inhibitors](#)
- 2320 [5,414,076](#) **T** [DNA encoding gibbon ape leukemia virus receptor](#)
- 2321 [5,413,907](#) **T** [Diagnosis for malignant hyperthermia](#)
- 2322 [5,411,857](#) **T** [Probes for papillomaviruses and an in vitro diagnostic procedure for papilloma infections](#)
- 2323 [5,409,815](#) **T** [DNA's encoding signal peptides](#)
- 2324 [5,407,820](#) **T** [Calcium channel .alpha.-2 subunit DNAs and cells expressing them](#)
- 2325 [5,407,819](#) **T** [Human plasminogen activator variants having amino acids 37-42 substituted and a method for their manufacture](#)
- 2326 [5,405,943](#) **T** [Tourette syndrom, autism and associated behaviors](#)
- 2327 [5,405,776](#) **T** [Cloned genes encoding reverse transcriptase lacking RNase H activity](#)
- 2328 [5,405,768](#) **T** [Method for cloning and producing the AATII and ALUI restriction endonuclease and methylase and related method for overexpressing restriction endonucleases](#)
- 2329 [5,405,760](#) **T** [Process for producing recombinant McrBC endonuclease and cleavage of methylated DNA](#)
- 2330 [5,405,758](#) **T** [DNA encoding the recombinant 40 kDA Dermatophagoides farinae allergen](#)
- 2331 [5,403,926](#) **T** [Hepatocellular carcinoma oncogene](#)
- 2332 [5,403,925](#) **T** [Nucleic acids encoding mammalian H-2RIIBP or RXR.sub..beta. and uses thereof](#)
- 2333 [5,401,651](#) **T** [DNA encoding ENA-78, a neutrophil activating factor](#)
- 2334 [5,399,680](#) **T** [Rice chitinase promoter](#)
- 2335 [5,397,702](#) **T** [Assay for and treatment of autoimmune diseases](#)
- 2336 [5,395,760](#) **T** [DNA encoding tumor necrosis factor-.alpha. and -.beta. receptors](#)
- 2337 [5,391,485](#) **T** [DNAs encoding analog GM-CSF molecules displaying resistance to proteases which cleave at adjacent dibasic residues](#)
- 2338 [5,389,543](#) **T** [Cloned genes encoding the D.sub.1 dopamine receptor](#)
- 2339 [5,389,528](#) **T** [Hepatitis .delta. diagnostics and vaccines](#)
- 2340 [5,389,525](#) **T** [DNA-molecules coding for FMDH control regions and structural gene for a protein having FMDH-activity and their use thereof](#)
- 2341 [5,386,025](#) **T** [Calcium channel compositions and methods](#)
- 2342 [5,384,259](#) **T** [Construct and method for expression of tetracycline resistance genes in E. Coli](#)
- 2343 [5,382,519](#) **T** [Periplasmic 3':5'-cyclic nucleotide phosphodiesterase and related methods for producing and using the same](#)
- 2344 [5,380,836](#) **T** [Nucleic acid encoding sodium channel protein](#)
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- 2345 [5,378,820](#) [Gene encoding cytoadhesin protein of mycoplasma gallisepticum and its use](#)
2346 [5,378,620](#) [Streptolysin O derivatives](#)
2347 [5,378,619](#) [Promoter for transgenic plants](#)
2348 [5,376,527](#) [Process for lysing mycobacteria](#)
2349 [5,374,558](#) [Fowlpox virus promoter](#)
2350 [5,374,543](#) [Enhanced indole biosynthesis](#)
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PAT. NO.	Title
2351 5,371,206	DNA encoding chimeric fibroblast growth factors
2352 5,371,205	PDGF .alpha. receptor
2353 5,371,009	Enhancers
2354 5,371,006	Isolated DNA encoding the NotI restriction endonuclease and related methods for producing the same
2355 5,370,995	L-phenylalanyl-tRNA synthetase mutants, a process for the preparation thereof and the use thereof for the in vivo incorporation of non-proteinogenous amino acids into peptides or proteins
2356 5,366,889	DNA encoding a protein-coupled receptor kinase
2357 5,366,882	Method for producing the BGLI restriction endonuclease and methylase
2358 5,364,787	Genes and enzymes involved in the microbial degradation of pentachlorophenol
2359 5,364,779	Process for transforming cells
2360 5,364,772	DNA molecule encoding the .beta..sub.3 -adrenergic receptor
2361 5,362,640	Ornithine carbamoyl transferase gene and utilization of the DNA
2362 5,360,894	C/EBP2 gene and recombinant C/EBP2
2363 5,360,714	Hepadnavirus polymerase gene product having RNA-dependent DNA priming and reverse transcriptase activities and methods of measuring the activities thereof
2364 5,359,047	Nucleic acids encoding DNA structure-specific recognition protein and uses

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- 2365 [5,358,867](#) **T** [DNA cloning and expression of an L7 protein which modulates the 2'-5' oligosynthetase and H2-K*K response to type-I interferon](#)
- 2366 [5,356,802](#) **T** [Functional domains in flavobacterium okeanoikoites \(FokI\) restriction endonuclease](#)
- 2367 [5,356,797](#) **T** [Membrane expression of heterologous genes](#)
- 2368 [5,354,680](#) **T** [Method for producing the DdeI restriction endonuclease and methylase](#)
- 2369 [5,354,670](#) **T** [Site-directed mutagenesis of DNA](#)
- 2370 [5,352,778](#) **T** [Recombinant thermostable DNA polymerase from archaeobacteria](#)
- 2371 [5,352,775](#) **T** [APC gene and nucleic acid probes derived therefrom](#)
- 2372 [5,352,595](#) **T** [Myod regulatory region](#)
- 2373 [5,352,588](#) **T** [Streptococcal immunoglobulin a binding protein encoded by emmL2.2](#)
- 2374 [5,350,840](#) **T** [Localization and characterization of the Wilms' tumor gene](#)
- 2375 [5,350,683](#) **T** [DNA encoding type II interleukin-1 receptors](#)
- 2376 [5,350,673](#) **T** [Detection of a unique Chlamydia strain associated with acute respiratory disease](#)
- 2377 [5,348,874](#) **T** [Eukaryotic transposable element](#)
- 2378 [5,346,823](#) **T** [Subtilisin modifications to enhance oxidative stability](#)
- 2379 [5,346,815](#) **T** [Sodium ion binding proteins](#)
- 2380 [5,344,923](#) **T** [Nucleotide sequence encoding for bifunctional enzyme for proline production](#)
- 2381 [5,344,776](#) **T** [DNA encoding an insect octopamine receptor](#)
- 2382 [5,342,764](#) **T** [Recombinant expression system for human anti-inflammatory phospholipase inhibitor protein](#)
- 2383 [5,340,935](#) **T** [DNAS encoding proteins active in lymphocyte-mediated cytotoxicity](#)
- 2384 [5,340,739](#) **T** [Hematopoietic cell specific transcriptional regulatory elements of serglycin and uses thereof](#)
- 2385 [5,340,733](#) **T** [MboI restriction-modification genes](#)
- 2386 [5,338,841](#) **T** [DNA segments controlling production of xanthan gum](#)
- 2387 [5,338,840](#) **T** [DNA encoding glioma-derived growth factor having vascular endothelial cell growth promoting activity](#)
- 2388 [5,338,839](#) **T** [DNA encoding nestin protein](#)
- 2389 [5,334,525](#) **T** [Hepadnavirus polymerase gene product having RNA-dependent DNA priming and reverse transcriptase activities and methods of measuring the activities thereof](#)
- 2390 [5,332,808](#) **T** [DNA encoding a ribosome inactivating protein](#)
- 2391 [5,332,676](#) **T** [Avipox virus promoter](#)
- 2392 [5,332,671](#) **T** [Production of vascular endothelial cell growth factor and DNA encoding same](#)
- 2393 [5,328,996](#) **T** [Bacterial plasmin receptors as fibrinolytic agents](#)
- 2394 [5,328,987](#) **T** [IgA Fc receptors](#)
- 2395 [5,326,857](#) **T** [ABO genotyping](#)
- 2396 [5,324,830](#) **T** [Chimeric protein that has a human RHo Motif and deoxyribonuclease](#)

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- 2397 [5,324,663](#)  [Methods and products for the synthesis of oligosaccharide structures on glycoproteins, glycolipids, or as free molecules, and for the isolation of cloned genetic sequences that determine these structures](#)
- 2398 [5,324,653](#)  [Recombinant genetic means for the production of serine protease muteins](#)
- 2399 [5,324,651](#)  [DNA encoding rat and human protein kinase C](#)
- 2400 [5,324,638](#)  [Brain transcription factor, nucleic acids encoding same and uses thereof](#)
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PAT. NO.	Title
2401	5,322,937 T Genes encoding a 3-acylation enzyme for macrolide antibiotics
2402	5,322,785 T Purified thermostable DNA polymerase obtainable from thermococcus litoralis
2403	5,320,962 T DNA encoding the human A1 adenosine receptor
2404	5,317,096 T Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants
2405	5,317,094 T Protein PP 15 prepared by genetic manipulation
2406	5,316,935 T Subtilisin variants suitable for hydrolysis and synthesis in organic media
2407	5,316,923 T Synthetic yeast leader peptides
2408	5,315,001 T Acyl carrier protein - DNA sequence and synthesis
2409	5,315,000 T Gene encoding for a L5/3 growth factor and its CDNA
2410	5,308,770 T Cloning and overexpression of glucose-6-phosphate dehydrogenase from Leuconostoc dextranicus
2411	5,308,760 T Crystal proteins of Bacillus thuringiensis, genes encoding them, and host expressing them
2412	5,306,863 T Transformed plant which expresses an insecticidally effective amount of a Bowman-Birk trypsin inhibitor from Vigna unguiculata in leaves, stems or roots, and a method for the production thereof
2413	5,306,616 T Molecular diagnosis of autosomal dominant charcot-marie-tooth disease
2414	5,302,698 T DNA coding for protein binds to enhancer of .alpha.-fetoprotein gene

- 2415 [5,302,519](#) **T** [Method of producing a Mad polypeptide](#)
- 2416 [5,298,407](#) **T** [DNA encoding a protein active in lymphocyte-mediated cytotoxicity](#)
- 2417 [5,298,404](#) **T** [Method for producing the Hpa I restriction endonuclease and methylase](#)
- 2418 [5,296,371](#) **T** [DNA encoding spiroplasma sp. dna methylase](#)
- 2419 [5,294,543](#) **T** [Inhibitor of platelet aggregation](#)
- 2420 [5,292,658](#) **T** [Cloning and expressions of Renilla luciferase](#)
- 2421 [5,292,651](#) **T** [Method for cloning and producing the NaeI restriction endonuclease and methylase](#)
- 2422 [5,290,926](#) **T** [Isolated DNA Encoding plant histidinol dehydrogenase](#)
- 2423 [5,290,694](#) **T** [Recombinant DNA, bacterium of the genus Pseudomonas containing it, and process for preparing lipase by using it](#)
- 2424 [5,288,846](#) **T** [Cell specific gene regulators](#)
- 2425 [5,288,696](#) **T** [Method for producing and cloning SacII restriction endonuclease and methylase](#)
- 2426 [5,288,639](#) **T** [Fungal stress proteins](#)
- 2427 [5,288,622](#) **T** [Human nerve growth factor by recombinant technology](#)
- 2428 [5,284,999](#) **T** [DNA encoding a pituitary-specific thyroid hormone receptor](#)
- 2429 [5,284,755](#) **T** [DNA encoding leukemia inhibitory factor receptors](#)
- 2430 [5,281,525](#) **T** [Cephalosporin acetylhydrolase gene from Bacillus subtilis](#)
- 2431 [5,281,520](#) **T** [Method for producing acyloxyacyl hydrolase](#)
- 2432 [5,281,518](#) **T** [Detection of a unique chlamydia strain associated with acute respiratory disease](#)
- 2433 [5,280,112](#) **T** [DNA sequence encoding bovine and human adrenocorticotrophic hormone receptors](#)
- 2434 [5,279,938](#) **T** [Sensitive diagnostic test for lyme disease](#)
- 2435 [5,278,065](#) **T** [Recombinant DNA encoding an erythropoietin receptor](#)
- 2436 [5,278,060](#) **T** [Method for producing the Nla III restriction endonuclease and methylase](#)
- 2437 [5,278,049](#) **T** [Recombinant molecule encoding human protease nexin](#)
- 2438 [5,273,884](#) **T** [Polypeptides, antigens or vaccines protective against babesiosis](#)
- 2439 [5,272,263](#) **T** [DNA sequences encoding vascular cell adhesion molecules \(VCAMS\)](#)
- 2440 [5,272,078](#) **T** [CDNA encoding the type I iodothyronine 5'deiodinase](#)
- 2441 [5,270,204](#) **T** [Covalent angiogenin/RNase hybrids](#)
- 2442 [5,268,290](#) **T** [Process for producing neuraminidase](#)
- 2443 [5,268,275](#) **T** [Vitamin K-dependent carboxylase](#)
- 2444 [5,268,273](#) **T** [Pichia pastoris acid phosphatase gene, gene regions, signal sequence and expression vectors comprising same](#)
- 2445 [5,268,270](#) **T** [Process for producing proteins using gram negative host cells](#)
- 2446 [5,264,416](#) **T** [Interleukin-7 receptors](#)
- 2447 [5,264,350](#) **T** [DNA sequence performing a function which is expressed in an over-production of extracellular proteins by various strains of bacillus, and vectors containing this sequence](#)
- 2448 [5,262,528](#) **T** [cDNA probe differentiating normal and cancer tissues](#)
- 2449 [5,262,318](#) **T** [Isolated DNA encoding the SPHI restriction endonuclease and related](#)

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2450 [5,260,208](#)  [Enantioselective amidases, DNA sequences encoding them, method of preparation and utilization](#)

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PAT. NO.	Title
2451 5,258,502	Immobilization and purification of fusion proteins using chitin-binding ability
2452 5,258,287	DNA encoding and methods of production of insulin-like growth factor binding protein BP53
2453 5,254,671	Extracellular segments of human e immunoglobulin anchoring peptides and antibodies specific therefor
2454 5,250,425	Process for producing ascorbic acid-2-phosphate
2455 5,248,599	Achromobacter protease I gene and gene product thereof
2456 5,246,852	Bacillus thuringiensis isolate active against lepidopteran pests, and genes encoding novel lepidopteran-active toxins
2457 5,246,845	Heterospecific modification as a means to clone restriction genes
2458 5,246,838	Processing of proteins
2459 5,245,023	Method for producing novel polyester biopolymers
2460 5,244,796	Cloned Leuconostoc mesenteroides glucose-6-phosphate dehydrogenase genes and method of making glucose-6-phosphate dehydrogenase
2461 5,244,793	TGF-.beta.1/.beta.2: a novel chimeric transforming growth factor-beta
2462 5,243,039	Bacillus MGA3 aspartokinase II gene
2463 5,240,849	DNA coding for enzyme capable of acylating the 4"-position of macrolide antibiotic
2464 5,240,831	Methods and compositions for the expression of biologically active

- [eukaryotic cytochrome P450S in bacteria](#)
- 2465 [5,238,839](#) **T** [Nucleic Acids Encoding proteins which induce immunological effector cell activation and chemattraction, vectors, and recombinant cells](#)
- 2466 [5,238,836](#) **T** [Plasmodium falciparum merozoite antigen peptides](#)
- 2467 [5,237,056](#) **T** [DNA encoding a protein which copurifies with acetylcholine receptor inducing activity and uses therefor](#)
- 2468 [5,236,843](#) **T** [Gene encoding a nematode-active toxin cloned from a Bacillus thuringiensis isolate](#)
- 2469 [5,236,834](#) **T** [Allergenic molecules from lepidoglyphus destructor](#)
- 2470 [5,235,049](#) **T** [Nucleic acid sequences encoding a soluble molecule \(SICAM-1\) related to but distinct from ICAM-1](#)
- 2471 [5,227,292](#) **T** [Neurofibromatosis type 1 gene](#)
- 2472 [5,227,289](#) **T** [Method for identifying mutagenic agents which induce large, multilocus deletions in DNA](#)
- 2473 [5,221,624](#) **T** [DNA encoding \(Lys.sup.46, Asp.sup.97, Asp.sup.113\) and \(Lys.sup.46, Asp.sup. .sup.137\) thaumatin I polypeptides](#)
- 2474 [5,219,739](#) **T** [DNA sequences encoding bVEGF120 and hVEGF121 and methods for the production of bovine and human vascular endothelial cell growth factors, bVEGF.sub.120 and hVEGF.sub.121](#)
- 2475 [5,217,902](#) **T** [Method of introducing spectinomycin resistance into plants](#)
- 2476 [5,215,917](#) **T** [Nucleotide sequence encoding the Toxoplasma gondii P22 gene](#)
- 2477 [5,215,915](#) **T** [Cloned gene encoding rat D.sub.1B dopamine receptor](#)
- 2478 [5,215,906](#) **T** [Cloning the HinFI restriction and modification genes](#)
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- 2480 [5,215,892](#) **T** [C/EBP2 gene and recombinant C/EBP2](#)
- 2481 [5,213,969](#) **T** [Cloned N-methylhydantoinase](#)
- 2482 [5,212,296](#) **T** [Expression of herbicide metabolizing cytochromes](#)
- 2483 [5,212,058](#) **T** [Nucleic acid encoding ubiquitin-specific proteases](#)
- 2484 [5,210,189](#) **T** [DNA sequence encoding glycerol 3-phosphate acyltransferase](#)
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- 2486 [5,206,354](#) **T** [DNA sequence encoding active fragment of fibroblast growth factor, HBF-2](#)
- 2487 [5,206,166](#) **T** [Genes encoding lepidopteran-active toxins and transformed hosts](#)
- 2488 [5,206,152](#) **T** [Cloning and expression of early growth regulatory protein genes](#)
- 2489 [5,202,430](#) **T** [Transmissible gastroenteritis virus genes](#)
- 2490 [5,202,248](#) **T** [Method for cloning and producing the NCO I restriction endonuclease and methylase](#)
- 2491 [5,200,337](#) **T** [Type II restriction endonuclease, APO I, obtainable from arthrobacter protophormiae and a process for producing the same](#)
- 2492 [5,200,333](#) **T** [Cloning restriction and modification genes](#)
- 2493 [5,198,542](#) **T** [DNA encoding a pitvitary adenylate cyclase activating protein and use thereof](#)
- 2494 [5,198,541](#) **T** [DNA encoding bactericidal/permeability-increasing proteins](#)
- 2495 [5,198,354](#) **T** [Cloning the Ban I restriction and modification genes](#)
- T**

- 2496 [5,198,347](#) [DNA encoding Plasmodium vivax and Plasmodium knowlesi Duffy receptor](#)
2497 [5,198,342](#) [DNA encoding IgA Fc receptors](#)
2498 [5,196,332](#) [Cloning the Hae II restriction and modification genes](#)
2499 [5,196,331](#) [Cloning the MspI restriction and modification genes](#)
2500 [5,196,329](#) [780 T-DNA gene transcription activator](#)
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PAT. NO.	Title
2501	5,196,319 T Primary biliary cirrhosis autoantigen
2502	5,194,600 T Genes which participate in .beta.-glucan assembly and use thereof
2503	5,194,375 T DNA encoding interleukin-7 receptors and methods of use
2504	5,188,960 T Bacillus thuringiensis isolate active against lepidopteran pests, and genes encoding novel lepidopteran-active toxins
2505	5,188,933 T Protein PP15 prepared by genetic manipulation
2506	5,187,078 T Plasma-type glutathione peroxidase gene and application of the same
2507	5,187,076 T DNA sequences encoding BMP-6 proteins
2508	5,185,441 T DNA sequences, recombinant DNA molecules and processes for producing PI-linked lymphocyte function associated antigen-3
2509	5,185,242 T Method for lysing Mycobacteria using achromopeptidase
2510	5,182,210 T Fowlpox virus promoters
2511	5,180,819 T Purified myeloblastin, nucleic acid molecule encoding same, and uses thereof
2512	5,180,673 T Cloning restriction and modification genes
2513	5,176,997 T DNA probe for male infertility
2514	5,173,403 T Thermostable acid protease from sulfolobus acidocaldarius and gene
2515	5,171,850 T Intestinal oncofetal gene
2516	5,171,685 T Cloning of the Babesia bovis 60 KD antigen
2517	5,171,675 T Macrophage colony stimulating factor-.gamma.

- 2518 [5,171,674](#) **T** [Polynucleotides that encode the human proteoglycan peptide core of the effector cells of the immune response](#)
 - 2519 [5,169,762](#) **T** [Human nerve growth factor by recombinant technology](#)
 - 2520 [5,168,062](#) **T** [Transfer vectors and microorganisms containing human cytomegalovirus immediate-early promoter-regulatory DNA sequence](#)
 - 2521 [5,168,049](#) **T** [Production of streptavidin-like polypeptides](#)
 - 2522 [5,166,329](#) **T** [DNA encoding the alcohol oxidase 2 gene of yeast of the genus Pichia](#)
 - 2523 [5,164,490](#) **T** [Pneumocystis carinii dihydrofolate reductase gene and methods for its use](#)
 - 2524 [5,162,516](#) **T** [Cloning and sequencing of the alcohol dehydrogenase II gene from Zymomonas mobilis](#)
 - 2525 [5,162,227](#) **T** [Recombinant DNA vectors capable of expressing apoaequorin in E. coli](#)
 - 2526 [5,159,066](#) **T** [Recombination activating gene \(RAG-1\)](#)
 - 2527 [5,158,891](#) **T** [Plasmid containing a gene for tetracycline resistance and DNA fragments derived therefrom](#)
 - 2528 [5,155,218](#) **T** [DNA encoding human 5-HT.sub.1D receptors](#)
 - 2529 [5,153,138](#) **T** [Pyruvate oxidase mutants, DNA expressing pyruvate oxidase and methods of use thereof](#)
 - 2530 [5,151,511](#) **T** [DNA encoding avian growth hormones](#)
 - 2531 [5,149,638](#) **T** [Tylosin biosynthetic genes tylA, tylB and tylI](#)
 - 2532 [5,147,643](#) **T** [DNA sequences encoding human t-PA substituted at position 275 or at positions 275 and 277 and pharmaceutical compositions](#)
 - 2533 [5,145,777](#) **T** [Plant cells resistant to herbicidal glutamine synthetase inhibitors](#)
 - 2534 [5,139,942](#) **T** [Method for producing the Nde I restriction endonuclease and methylase](#)
 - 2535 [5,137,823](#) **T** [Method for producing the BamHI restriction endonuclease and methylase](#)
 - 2536 [5,137,821](#) **T** [Gene and process of making glucose-6-phosphate dehydrogenase](#)
 - 2537 [5,130,253](#) **T** [DNAs Encoding mosquito oostatic hormones](#)
 - 2538 [5,128,254](#) **T** [cDNA encoding the long isoform of the D.sub.2 dopamine receptor](#)
 - 2539 [5,126,260](#) **T** [Human erythroid-specific transcriptional enhancer](#)
 - 2540 [5,122,459](#) **T** [Gene encoding biologically active human interleukin 1](#)
 - 2541 [5,120,658](#) **T** [Thermostable tryptophan synthetase gene and extremely thermophilic plasmid vector incorporating said gene](#)
 - 2542 [5,116,738](#) **T** [DNA sequences encoding](#)
 - 2543 [5,114,853](#) **T** [Recombinant DNA, transformant containing said DNA, and process for preparing heat-stable glucose dehydrogenase by use of said transformant](#)
 - 2544 [5,110,730](#) **T** [Human tissue factor related DNA segments](#)
 - 2545 [5,108,922](#) **T** [DNA sequences encoding BMP-1 products](#)
 - 2546 [5,106,748](#) **T** [DNA sequences encoding 5 proteins](#)
 - 2547 [5,106,733](#) **T** [Bovine granulocyte-macrophage colony stimulating factor](#)
 - 2548 [5,102,995](#) **T** [DNA encoding modified heparin cofactor II](#)
 - 2549 [5,100,793](#) **T** [Method for producing the AseI restriction endonuclease and methylase](#)
 - 2550 [5,097,025](#) **T** [Plant promoters](#)
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2551	5,086,169 T Isolated pollen-specific promoter of corn
2552	5,075,431 T Chimeric anti-CEA antibody
2553	5,075,232 T Method for producing the NLA VI restriction endonuclease and methylase
2554	5,075,222 T Interleukin-1 inhibitors
2555	5,073,609 T DNA sequence coding for protein C
2556	5,071,972 T DNA sequences encoding novel thrombolytic proteins
2557	5,064,757 T T4 DNA fragment as a stabilizer for proteins expressed by cloned DNA
2558	5,063,154 T Pheromone - inducible yeast promoter
2559	5,059,519 T Oligonucleotide probes for the determination of the proclivity for development of autoimmune diseases
2560	5,053,330 T Method for producing the MwoI restriction endonuclease and methylase
2561	5,049,655 T Melanin-concentrating hormones
2562	5,049,504 T Bioadhesive coding sequences
2563	5,045,450 T Determination of a mutational spectrum
2564	5,043,276 T DNA strand coding for alpha-acetolactate decarboxylase and yeast transformed with the DNA strand
2565	5,037,756 T Recombinant DNA molecules for producing terminal transferase-like polypeptides
2566	5,032,520 T DNA sequences encoding infectious bronchitis virus spike protein

- 2567 [5,032,511](#) **T** [DNA fragments coding for antigens specific to non-A non-B hepatitis, expression vectors containing said DNA fragments, transformants and process for producing said antigens](#)
- 2568 [5,030,569](#) **T** [Method for producing the AFL II restriction endonuclease and methylase](#)
- 2569 [5,026,839](#) **T** [DNA encoding a basic fibroblast growth factor](#)
- 2570 [5,023,328](#) **T** [Lepidopteran AKH signal sequence](#)
- 2571 [5,019,509](#) **T** [Method and compositions for the production of l-alanine and derivatives thereof](#)
- 2572 [5,015,581](#) **T** [Method for producing the Hinc II restriction endonuclease and methylase](#)
- 2573 [5,013,649](#) **T** [DNA sequences encoding osteoinductive products](#)
- 2574 [5,004,691](#) **T** [Method for producing the ACCI restriction endonuclease and methylase](#)
- 2575 [5,002,882](#) **T** [Method for producing the XmaI restriction endonuclease and methylase](#)
- 2576 [5,002,873](#) **T** [DNA sequence encoding a lymphocyte adhesion receptor for high endothelium](#)
- 2577 [4,999,294](#) **T** [Method for producing the FokI restriction endonuclease and methylase](#)
- 2578 [4,999,293](#) **T** [Method for producing the HhaI restriction endonuclease and methylase](#)
- 2579 [4,999,291](#) **T** [Production of human pluripotent granulocyte colony-stimulating factor](#)
- 2580 [4,997,930](#) **T** [Cloning of complementary DNA encoding maize nitrite reductase](#)
- 2581 [4,996,297](#) **T** [Recombinantly expressed rabbit zona pellucida polypeptides](#)
- 2582 [4,996,151](#) **T** [Method for producing the Eag I restriction endonuclease and methylase](#)
- 2583 [4,994,371](#) **T** [DNA preparation of Christmas factor and use of DNA sequences](#)
- 2584 [4,992,378](#) **T** [C-DNA encoding human prolactin receptor](#)
- 2585 [4,988,620](#) **T** [Method for producing the FnuDI restriction endonuclease and methylase](#)
- 2586 [4,987,074](#) **T** [Method for producing the HgiAI restriction endonuclease and methylase](#)
- 2587 [4,987,066](#) **T** [Process for the detection of restriction fragment length polymorphisms in eukaryotic genomes](#)
- 2588 [4,985,352](#) **T** [DNA encoding serotonin 1C \(5HT1c\) receptor, isolated 5HT1c receptor, mammalian cells expressing same and uses thereof](#)
- 2589 [4,983,542](#) **T** [Method for producing the XbaI restriction endonuclease and methylase](#)
- 2590 [4,983,522](#) **T** [Method for producing the HinPI restriction endonuclease and methylase](#)
- 2591 [4,983,044](#) **T** [Quantitative analysis of biological materials and photogrtaphic film and apparatus therefor](#)
- 2592 [4,968,626](#) **T** [DNA sequence coding for protein C](#)
- 2593 [4,968,607](#) **T** [Interleukin-1 receptors](#)
- 2594 [4,966,841](#) **T** [Enhanced vector production and expression of recombinant DNA products](#)
- 2595 [4,965,189](#) **T** [Probes for the determination of the proclivity for development of autoimmune diseases](#)
- 2596 [4,963,488](#) **T** [DNA sequences, recombinant DNA molecules and process for the preparation of the enzyme mutarotase from acinetobacter calcoaceticus](#)
- 2597 [4,963,487](#) **T** [Method for deletion of a gene from a bacteria](#)
- 2598 [4,960,877](#) **T** [DNA having genetic information of L-.alpha.-glycerophosphate oxidase and application thereof](#)
- 2599 [4,956,280](#) **T** [Biphasic shuttle vectors](#)

2600 4,952,502 **T** [Carbomycin biosynthetic gene, designated carG, for use in streptomyces and other organisms](#)

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PAT. NO.	Title
2601 4,950,603	T Recombinant DNA expression vectors and DNA compounds that encode isopenicillin N synthetase from Streptomyces lipmanii
2602 4,945,052	T Production of a Vitamin C precursor using genetically modified organisms
2603 4,945,051	T Production of human lysozyme
2604 4,940,661	T Metallothionein transcription control sequences and use thereof
2605 4,937,189	T Expression and secretion of heterologous proteins by Yarrowia lipolytica transformants
2606 4,935,361	T Cloning and expression of T4 DNA polymerase
2607 4,929,547	T Antitumor protein gene of streptococcus pyogenes Su, plasmids containing the gene, transformant cells harboring the plasmids, and process for producing the antitumor protein
2608 4,912,044	T Preparation of mesophilic microorganisms which contain a D-hydantoinase which is active at elevated temperature
2609 4,908,312	T Process for producing phenylalanine
2610 4,906,742	T Encoding antigens of M. Leprae
2611 4,900,673	T Mutant human angiogenin (angiogenesis factor with superior angiogenin activity) genes therefor and methods of expression
2612 4,895,802	T DNA strand coding for .alpha.-acetolactate decarboxylase and yeast transformed with the DNA strand
2613 4,895,800	T Yeast production of hepatitis B surface antigen
2614 4,894,333	T Bovine interleukin-1.alpha.
2615 4,892,819	T Recombinant DNA expression vectors and DNA compounds that encode

- [isopenicillin N synthetase from penicillium chrysogenum](#)
- 2616 [4,885,252](#) **T** [Recombinant DNA expression vectors and DNA compounds that encode isopenicillin N synthetase from aspergillus nidulans](#)
- 2617 [4,885,251](#) **T** [Recombinant DNA expression vectors and DNA compounds which encode isopenicillin N synthetase](#)
- 2618 [4,885,242](#) **T** [Genes from pichia histidine pathway and uses thereof](#)
- 2619 [4,882,282](#) **T** [DNA sequences encoding bovine interleukin-2](#)
- 2620 [4,879,374](#) **T** [Bovine interleukin-1.beta. DNA sequence](#)
- 2621 [4,877,729](#) **T** [Recombinant DNA encoding novel family of primate hematopoietic growth factors](#)
- 2622 [4,876,186](#) **T** [Detection and differentiation of coxiella burnetii in biological fluids](#)
- 2623 [4,871,670](#) **T** [Molecular cloning and characterization of a gene sequence coding for human relaxin](#)
- 2624 [4,868,113](#) **T** [Recombinant DNA vector encoding human endothelial cell growth factor](#)
- 2625 [4,855,231](#) **T** [Regulatory region for heterologous gene expression in yeast](#)
- 2626 [4,847,201](#) **T** [DNA encoding for CSF-1 and accompanying recombinant systems](#)
- 2627 [4,839,293](#) **T** [DNA encoding streptavidin, streptavidin produced therefrom, fused polypeptides which include amino acid sequences present in streptavidin and uses thereof](#)
- 2628 [4,837,148](#) **T** [Autonomous replication sequences for yeast strains of the genus pichia](#)
- 2629 [4,820,639](#) **T** [Process for enhancing translational efficiency of eukaryotic mRNA](#)
- 2630 [4,810,643](#) **T** [Production of pluripotent granulocyte colony-stimulating factor](#)
- 2631 [4,786,592](#) **T** [Neisseria gonorrhoeae lectin useful as a vaccine and diagnostic marker and means for producing this lectin](#)
- 2632 [4,761,371](#) **T** [Insulin receptor](#)
- 2633 [4,758,516](#) **T** [Molecular cloning and characterization of a further gene sequence coding for human relaxin](#)
- 2634 [4,753,879](#) **T** [Modified tissue plasminogen activators](#)
- 2635 [4,752,574](#) **T** [Chimeric cloning vectors for use in streptomyces and E. Coli](#)
- 2636 [4,732,847](#) **T** [Monoclonal antibodies for DNA-RNA hybrid complexes and their uses](#)
- 2637 [4,721,672](#) **T** [CDNA and gene for human angiogenin \(angiogenesis factor\) and methods of expression](#)
- 2638 [4,717,666](#) **T** [Cloned streptomycete lividans excretable .beta.-galactosidase gene](#)
- 2639 [4,703,008](#) **T** [DNA sequences encoding erythropoietin](#)
- 2640 [4,680,264](#) **T** [Class II mobilizable gram-negative plasmid](#)
- 2641 [4,631,259](#) **T** [Transposon in cloning DNA](#)
- 2642 [4,613,572](#) **T** [Yeast BAR1 gene plasmid](#)
- 2643 [4,594,318](#) **T** [Isolation and localization of DNA segments](#)
- 2644 [4,535,058](#) **T** [Characterization of oncogenes and assays based thereon](#)
- 2645 [4,237,224](#) **T** [Process for producing biologically functional molecular chimeras](#)
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EXHIBIT 11



US005407796A

United States Patent [19]

[11] **Patent Number:** **5,407,796**

Cutting et al.

[45] **Date of Patent:** **Apr. 18, 1995**

[54] **CYSTIC FIBROSIS MUTATION CLUSTER**

[75] **Inventors:** **Garry R. Cutting**, Towson; **Stylios E. Antonarakis**, Lutherville; **Haig H. Kazazian, Jr.**, Baltimore, all of Md.

[73] **Assignee:** **The Johns Hopkins University**, Baltimore, Md.

[21] **Appl. No.:** **637,621**

[22] **Filed:** **Jan. 4, 1991**

[51] **Int. Cl.⁶** **C07H 21/00**; C12N 15/10; C12P 19/34; C12Q 1/68

[52] **U.S. Cl.** **435/6**; 435/91.2; 536/23.2; 536/24.31; 935/77; 935/78

[58] **Field of Search** 435/6, 91, 91.2; 436/94; 536/27, 23.2, 24.31; 935/77, 78

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Primary Examiner—Robert A. Wax

Assistant Examiner—Dian C. Jacobson

Attorney, Agent, or Firm—Banner, Birch, McKie & Beckett

[57] **ABSTRACT**

Four mutations have been found clustered in exon 11 of the CFTR (cystic fibrosis transmembrane conductance regulator) gene. These mutations occur within a set of amino acids highly conserved among ATP-dependent transport proteins. Humans can be tested to determine whether they carry one of these mutations using a number of methods and/or probes taught herein. Specifically the mutations include: Asn₅₄₉, Asp₅₅₁, Stop₅₅₃, and Thr₅₅₉.

33 Claims, 2 Drawing Sheets

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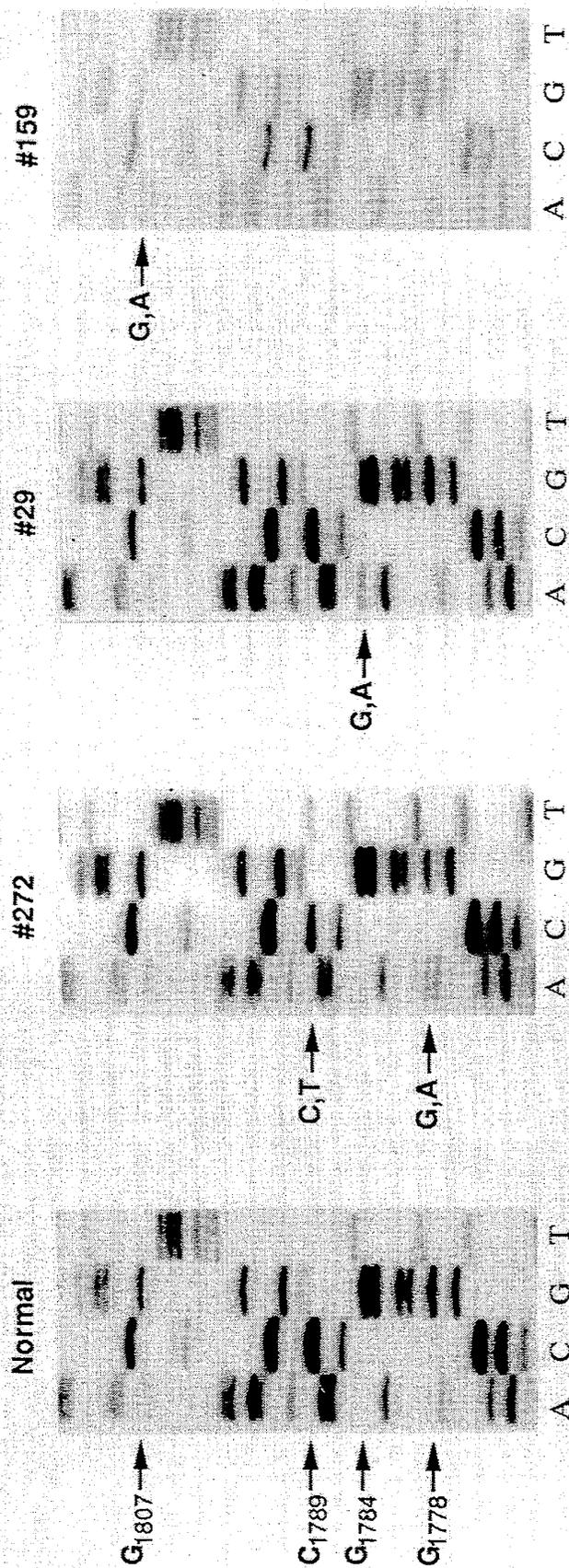
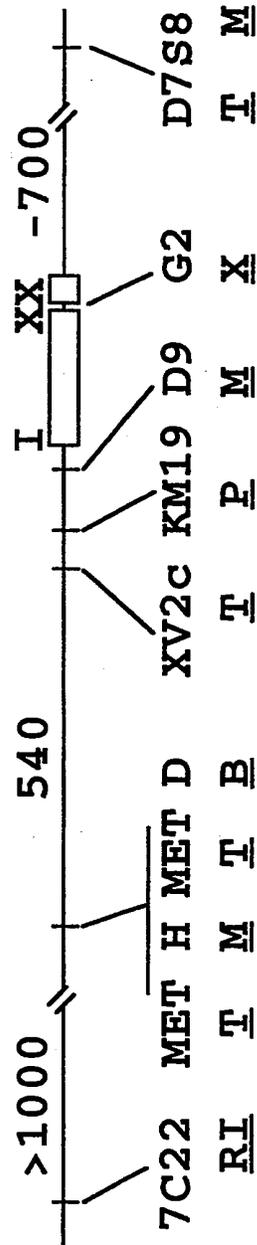


FIG. 1

FIG. 2



CYSTIC FIBROSIS MUTATION CLUSTER

This invention was made using U.S. government funds awarded by the National Institutes of Health as DK 39635 and DK 34944. Therefore the government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Cystic fibrosis (CF), the most common lethal autosomal genetic disorder in the Caucasian population, occurs approximately once in every 2500 live births (Boat, et al., *The Metabolic Basis of Inherited Disease*, eds. Shriver, et al., McGraw-Hill, New York (1989) pp. 2649-2680). A single locus for CF has been mapped to chromosome 7q31 by linkage analysis using DNA marker probes.

Several markers have been shown to have a high degree of linkage disequilibrium with the CF locus in Caucasians suggesting that one mutation of the CF gene predominates in this population (Estivill, et al., *Nature*, (1987), 326:840; Estivill, et al., *Genomics*, (1987), 1:257). DNA polymorphism haplotypes from phenotypically and racially diverse patient populations indicated that several additional mutations of the CF gene may exist in these groups (Ober, et al., *Am. J. Hum. Genet.*, vol. 41, p. 1145, 1987; Estivill, et al., *ibid.*, vol. 43, p. 23 (1988); Fujiwara, et al., *ibid.*, vol. 44, p. 327, (1989); Kerem, et al., *ibid.* p. 827 and Cutting, et al., *ibid.*, p. 307).

The gene responsible for CF has recently been identified (Rommens, et al., *Science*, vol. 245, p. 1059 (1989); Riordan, et al., *ibid.*, p. 1066); it comprises 20 exons and encodes a protein of 1480 amine acids called the CF Transmembrane Conductance Regulator (CFTR). Several regions are postulated to have functional importance in the CFTR protein, including two areas for ATP binding, termed Nucleotide Binding Folds (NBF), a Regulatory (R) region that has multiple potential sites for phosphorylation by protein kinases A and C, and two hydrophobic regions believed to interact with cell membranes.

One mutation has been identified in the CF gene which leads to the omission of phenylalanine residue 508 within the first putative NBF domain, indicating that this region is functionally important. This mutation, termed ΔF_{508} , accounts for about 70% of the CF chromosomes in Caucasian patients and was highly associated with the predominant haplotype found on chromosomes of Caucasian CF patients (Kerem, et al., *Science*, vol. 245, p. 1073 (1989); Lemna, et al., *New Engl. J. Med.*, vol. 322, p. 291 (1990)); the haplotypes associated with Caucasian CF chromosomes without ΔF_{508} are less common, confirming predictions that allelic heterogeneity exists in CF (Ober, et al., *Am. J. Hum. Genet.*, vol. 41, p. 1145, 1987; Estivill, et al., *ibid.*, vol. 43, p. 23 (1988); Fujiwara, et al., *ibid.*, vol. 44, p. 327, (1989); Kerem, et al., *ibid.* p. 827 and Cutting, et al., *ibid.*, p. 307; Kerem, et al., *Science*, vol. 245, p. 1073 (1989)).

There is a need in the art of genetic screening for knowledge of other mutant alleles of CFTR which are present on the other 30% of CF chromosomes in Caucasian CF patients, as well as other alleles found in other racial groups. Knowledge of such alleles can be used to design probes for screening, as well as to devise other screening methods. The more complete the set of probes available for CF mutant alleles, the more accurate diagnoses can be made.

SUMMARY OF THE INVENTION

It is an object of the invention to provide nucleic acid probes for detecting mutant CFTR alleles other than ΔF_{508} .

It is another object of the invention to provide methods of testing a DNA sample of a human for the presence of mutant alleles of the CFTR gene other than ΔF_{508} .

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a nucleic acid probe is provided which is complementary to a mutant allele of the CFTR gene selected from the group consisting of: Asn549, Asp551, Stop553, and Thr559.

In another embodiment of the invention a method is provided for testing a DNA sample of a human to determine if the human is a carrier of Cystic Fibrosis or if the human is affected with Cystic Fibrosis, comprising:

providing a DNA sample from a human;
testing the sample for the presence of a mutation in exon 11 of the CFTR gene of the human, the presence of the mutation indicating that the human is a carrier of Cystic Fibrosis or is affected with Cystic Fibrosis.

These and other embodiments are described with more particularity below. They provide the art with the knowledge of four hitherto unknown mutant alleles which are present in human populations and which can lead to cystic fibrosis if they are not present in a heterozygous configuration with a wild-type allele. In the case of a heterozygote, the individual is a "carrier", but will not be affected himself. If the mutant alleles are present with other mutant alleles, then the individual will be affected with the cystic fibrosis disease. These newly discovered alleles allow for genetic screening to provide more accurate diagnoses. Previously, without knowledge of these alleles, individuals carrying these alleles would have been "false negatives", i.e., they would have appeared to carry a wild-type allele because they did not carry any of the known mutant alleles.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows direct sequence analysis of PCR amplified genomic DNA from exon 11 of CFTR using the primer 11i-5'. The order of nucleotides in each gel panel is A,C,G,T. Patient #272 has exon 11 mutations on each chromosome, A₁₇₇₈ and T₁₇₈₉. Patient #29 has the A₁₇₈₄. Patient #159 has the A₁₈₀₇ mutation.

FIG. 2 shows a map of the relative positions and approximate distances in kb between the markers 7C22, MET, XV2c, KM19, D9, G2 and D7S8 and the CF gene which is shown as a box (Kerem, et al., *Science*, (1989) vol. 245, p. 1073; Estivill, et al., *Am. J. Hum. Genet.* (1989), vol. 44, p. 70 and Ramsay, et al., *Genomics* (1990), vol. 6, p. 39). Roman numerals denote exons 1 and 20 respectively. Enzyme abbreviations are as indicated in legend to Table 3.

DETAILED DESCRIPTION

It is a finding of the present invention that four mutations which cause cystic fibrosis (if present in an individual who lacks a wild-type allele) are clustered in a region of exon 11 of the CFTR gene consisting of nucleotides 1778-1807. (The numbering of nucleotides used herein follows the numbering of Riordan et al., *Science* vol. 245, p.1066, 1989.) See SEQ ID NO: 1. Thus exon

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3

11 comprises a "hotspot" for CF mutations. The corresponding region of the protein is contained within the postulated first (N-terminal) nucleotide binding fold domain, a region which is highly conserved among a large number of homologous proteins. Each of the four mutations is a transversion, three causing amino acid substitutions and one producing a termination codon.

One mutation, G₁₇₈₄ to A, was found in 4% of the Caucasian CF chromosomes studied. (The allele which carries this mutation is termed the Asp551 allele herein.) The stop codon mutation (caused by a thymidine at nucleotide number 1789 leading to a translational stop after 552 amino acid residues,) was found in 5% of the American Black CF chromosomes studied. The other two mutations are rare in the American Black patients. These are both G to A mutations located at nucleotides 1778 and 1807, and lead to an asparagine and threonine residue, respectively. See Table 2.

The possibility that the three missense mutations are normal variants of the CFTR gene was ruled out by sequencing or restriction digestion of non-CF chromosomes with the same haplotype as that associated with each particular mutation. As shown in Table 3, none of the non-CF chromosomes of the same haplotype carried the mutations described herein. Therefore the missense mutations are not normal variants of the gene.

The Asp₅₅₁ allele taught herein is to date the second most common CF mutation in Caucasian chromosomes. The mutation on the allele causes the substitution of glycine, a neutral amino acid, with aspartic acid, a polar amino acid. This charge change makes it unlikely that the allele codes for a normal polymorphic variant of the CFTR protein. In addition, even though the mutation occurs on 4% of Caucasian CF chromosomes, it has not been found on three normal chromosomes with the same ten site haplotype or twenty-four other normal Caucasian chromosomes. In six out of seven Caucasian patients who were found to have this mutation, it was paired with the ΔF₅₀₈ mutation.

The CF gene was identified solely by its location in the human genome (Rommens, et al., *Science*, vol. 245, p. 1059 (1989)). Little is known of the function of its protein product, CFTR, except by analogy to well-characterized proteins that have similar amino acid sequences (Riordan, et al., *Science*, vol. 245, p. 1066 (1989)). The four mutations described here occur within a 13 amino acid segment (codons 548 to 560) (see SEQ ID NO: 2) of the putative first NBF region in the CFTR protein which is highly conserved among similar regions of other membrane-associated transport proteins (Riordan, et al., *Science*, vol. 245, p. 1066 (1989)). Five amino acids in this region are completely conserved in comparable regions from the multiple drug resistance proteins indicating that these positions are probably crucial to protein function (FIG. 2). It appears to be significant that the amino acid substitutions described in this study occur at three of the five completely conserved residues. Moreover, the substitutions occur at the three most conserved residues in that region between CFTR and 14 other membrane associated proteins which bind ATP (Riordan, et al. *Science* (1989), vol. 245, p. 1066). Therefore, the location of these mutations supports the theory that the CFTR protein is a member of the ATP-dependent transport protein superfamily (Riordan, et al., *Science*, vol. 245, p. 1066 (1989); Higgins, *Nature*, vol. 341, p. 103 (1989)).

Nucleic acid probes are provided according to the present invention which comprise either ribonucleic or

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deoxyribonucleic acids. Typically, the size of the probes varies from approximately 18 to 22 nucleotides. Functionally, the probe is long enough to bind specifically to the homologous region of the CFTR gene, but short enough such that a difference of one nucleotide between the probe and the DNA being tested disrupts hybridization. Thus the nucleic acid probes of the present invention are capable of detecting single nucleotide changes in the CFTR gene. The probes of the present invention are complementary to the mutant alleles described here: Asn₅₄₉, Asp₅₅₁, Stop₅₅₃, Thr₅₅₉. The homology of the probes to the mutant alleles is 100%. Probes corresponding to the wild-type sequences in this region of the CFTR gene can also be used. These probes will bind to wild-type sequences but not mutant alleles in the region of nucleotides 1776-1807. Thus, for example, one could determine whether an individual was homozygous or heterozygous for a particular allele using both a wild-type and an allele-specific probe. If no wild-type allele is present in an individual carrying one of these mutant alleles, the individual will be affected by cystic fibrosis.

The DNA samples of humans to be tested according to the present invention include DNA of fetuses, juveniles, and adults. The DNA can be directly analyzed upon removal from the human source, or the DNA may be amplified by the PCR technique (Saiki, et al., *Science*, vol. 230, p. 1350 (1985)). The PCR technique amplifies certain regions of the DNA selectively according to the primers which are used. Alternatively, cells may be isolated from the human source and grown in culture prior to isolation of DNA. Growth in culture may be required where the number of cells available for DNA isolation is limited. Amplification according to the present invention is of exon 11 sequences. All or part of exon 11 may be amplified prior to testing in one of the methods of the present invention. Primers which may be used include the oligonucleotide primers 11i-5' (SEQ ID NO: 3) (5'-CAACTGTGGTTAAAGCAATAGTGT-3') and 11i-3' (SEQ ID NO: 4) (5'-GCACAGATTCTGAGTAACCATAAT-3'). These primer sequences are selected from intron sequences flanking 5' and 3' of exon 11 of the CFTR gene. Other primers may be selected from known CFTR sequences which flank nucleotides 1778 to 1807.

According to one method of the present invention, mutations are detected by sequencing a region of exon 11 of the CFTR gene. The region includes nucleotides 1778 to 1807, which encompasses all four mutational sites taught herein. The sequences can be inspected by eye or by machine to determine if one of the mutations taught herein is present. These include an adenine at nucleotides nos. 1778, 1784, or 1807, or a thymidine residue at nucleotide no. 1789. Sequencing can be accomplished according to any means known in the art. Most simply this region of the genome can be amplified and then the sequence of the amplified region can be determined.

According to other methods of the present invention, the presence of the mutant alleles taught herein can be detected indirectly by testing for the loss or acquisition of specific restriction endonuclease sites. In particular, in the case of the Asn₅₄₉ allele, the mutation leads to a loss of a DdeI site (CTNAG) which can be detected using methods known in the art. Similarly, the Asp₅₅₁ allele carries a mutation which creates an MboI site (GATC) not present on the wild-type allele. Isoschizomers of DdeI and MboI can also be used. Both the

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Asp⁵⁵¹ and the Stop⁵⁵³ alleles carry mutations which destroy a Hinc II site (GTYRN). In addition, any other restriction enzyme having a recognition sequence including one of the nucleotides 1778, 1784, 1807 or 1789 may be used, provided that the mutation either creates or destroys the recognition site. Detection of the new or missing restriction enzyme sites can be accomplished according to any means known in the art. For example, Southern gels of genomic DNA can be used. The genomic DNA is digested with the appropriate restriction endonuclease and separated on an electrophoretic gel matrix such as agarose or acrylamide, as is known in the art. DNA separated on the gel matrix can be then transferred to another solid support on which hybridization can occur. The transfer can be accomplished according to any means known in the art such as wicking or electroblotting. Transferred DNA can be detected by hybridization with a nucleic acid probe which spans nucleotides 1778, 1784, or 1789. The probe should extend far enough beyond nucleotides 1778, 1784, or 1789 such that it is able to hybridize to a piece of DNA which has one end at nucleotide 1778, 1784, or 1789. Further the probe preferably does not span additional sites for the restriction endonuclease being used; this simplifies the analysis but is not necessary. Alternatively, the genomic DNA can be amplified as described above and then tested for the size and number of fragments generated with DdeI, MboI, HincII or other restriction endonuclease which recognize a sequence which includes nucleotides 1778, 1784, or 1789. If enzymes are found which specifically recognize the sequences at nucleotide 1807, they may also be used to detect the Thr⁵⁵⁹ mutant allele.

According to still other methods of the present invention rapid screening techniques are used to determine whether exon 11 of the CFTR gene carries any mutations. Such techniques can be followed by one of the techniques already described above which are specific for a particular allele or mutation. One such rapid screening technique involves the determination of the conformation of single strands of DNA which have been amplified from exon 11 sequences. The single strands are run in non-denaturing electrophoretic gels, such as are typically used for sequencing DNA. The mobility of single stranded DNA on such gels is sensitive to the conformation of the DNA fragments. The conformation of the single stranded DNA is dependent on its base sequence, alterations in even one base affecting the conformation. Thus the presence of one of the CF alleles described herein can be detected by amplifying exon 11 sequences, denaturing the duplex molecules, and separating them on the basis of their conformation on non-denaturing polyacrylamide gels. If mutant alleles are present, they will have a different mobility than wild-type sequences amplified with the same primers. Most conveniently, the amplified sequences will be radiolabeled to facilitate visualization on gels. This can be readily accomplished using labeled primers or a labeled nucleotide. For a general reference on this technique see Orira, et al., *Genomics* vol. 5, pp. 874-879 (1989).

According to another rapid screening technique of the present invention amplified fragments containing mutations are detected using denaturing gradient gel electrophoresis (DGGE). For a general reference on this technique see Sheffield, et al., *Proc. Natl. Acad.*

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Sci. vol. 86, pp. 232-236 (1989). Briefly, double stranded fragments which are generated by amplification (PCR) can be subjected to DGGE. "DGGE is a gel system that separates DNA fragments according to their melting properties. When a DNA fragment is electrophoresed through a linearly increasing gradient of denaturants, the fragment remains double stranded until it reaches the concentration of denaturants equivalent to a melting temperature (t_m) that causes the lower-temperature melting domains of the fragment to melt. At this point, the branching of the molecule caused by partial melting sharply decreases the mobility of the fragment in the gel. The lower-temperature melting domains of DNA fragments differing by as little as a single-base substitution will melt at slightly different denaturant concentrations because of differences in stacking interactions between adjacent bases in each DNA strand. These differences in melting cause two DNA fragments to begin slowing down at different levels in the gel, resulting in their separation from each other." Sheffield, et al., *ibid.* Use of a GC clamp as taught in Myers et al., *Nucleic Acids Res.* vol. 13, pp. 3111-3146 (1985) increases the sensitivity of detection of this method from about 40% to about 100%. If mismatches are present, which would be the case if the DNA sample amplified was heterozygous for an exon 11 CFTR allele, they will be visible on these DGGE gels. Double stranded fragments containing one wild-type strand and one mutant strand will have a different mobility on these gels than will double stranded fragments which contain two wild-type or two mutant strands, due to the different melting temperatures of these species. Thus, the melting temperature of fragments amplified from exon 11 can be determined by DGGE and can indicate whether a mutant allele is present.

The following examples are not intended to limit the scope of the invention, but to illustrate various aspects of the invention.

EXAMPLE 1

This example shows the association of certain haplotypes with the ΔF_{508} and unknown CFTR mutations.

Haplotypes for four DNA markers were determined on 155 Caucasian and 43 Black CF chromosomes using three markers 5' of the CF gene (XV2c, KMI9 and Mp69.9) and one within the gene (G2).

DNA markers XV2e and KM19 and their associated polymorphisms are described for these populations elsewhere (Cutting, et al., *Am. J. Hum. Genet.*, vol. 44, p. 307 (1989)). Probes D9 (Mp6d.9) and G2, which detect MspI and XbaI polymorphism sites respectively, were obtained from Professor Robert Williamson (Estivill, et al., *Am. J. Hum. Genet.*, vol. 44, p. 70 (1989); Ramsay, et al., *Genomics*, vol. 6, p. 39 (1990)). Direct detection of the ΔF_{508} mutation was performed by PCR amplification of genomic DNA using primers C16B and C16D followed by vacuum blotting of amplified DNA to nitrocellulose filters and hybridization with either oligo N (Normal sequence) or oligo F (deletion Phe₅₀₈ sequence) as previously described (Kerem, et al., *Science*, vol. 245, p. 1073 (1989)). The results are shown in Table I below. Parentheses indicate frequency, — indicates that a polymorphism was uninformative or unknown, 1 is the absence and 2 the presence of a restriction site.

TABLE 1

Haplotype	XV2c	KM19	D9	G2	Caucasian		American Black	
	TaqI	PstI	MspI	XbaI	Unk	ΔF ₅₀₈	Unk	ΔF ₅₀₈
A	1	1	1	2	6	0	1	0
B	1	1	2	2	2	0	2	0
C	1	2	1	2	0	0	1	0
D	1	2	2	1	12	5	1	0
E	1	2	2	2	6	92	2	7
F	2	1	1	2	1	0	4	0
G	2	2	1	1	0	0	1	0
H	2	2	2	1	2	1	1	0
I	2	2	2	2	0	6	2	2
	—	—	—	—	6	16	12	7
					35(.23)	120(.77)	27(.63)	16(.37)

The ΔF₅₀₈ mutation was almost exclusively associated with one haplotype (E in Table 1) and accounted for 77% of the mutations on CF chromosomes from our Caucasian patients, similar to other studies of North American Caucasians (Kerem, et al., Science, vol. 245, p. 1073 (1989); Lemna, et al., New. Engl. J. Med., vol. 322, p. 291 (1990)). In contrast, only 16 of 43 (37%) CF chromosomes from American Black patients had the ΔF₅₀₈ mutation, confirming that racial admixture alone does not account for the incidence of CF in this group. Caucasian CF chromosomes without the ΔF₅₀₈ mutation, i.e., unknown, occur on 6 haplotypes with 24 of 29 chromosomes having either an A, D or E haplotype (Table 1). Unknown CF mutations in the American Black patients are associated with a wider distribution of haplotypes than in Caucasians, two of which may be unique to American Black patients.

EXAMPLE 2

This example demonstrates how the four new CFTR mutations were found.

An initial panel of ten Caucasian CF patients having fourteen of the 35 unknown mutations shown in Table 1 representing each haplotype group was selected for nucleotide sequencing (Orkin, et al., Nature, vol. 296, p. 627 (1982).) All of eighteen American Black patients with twenty-seven unknown mutations were examined. Exon 11 was sequenced in these patients as part of a systematic study of regions believed to be functionally important in the CFTR protein.

Four mutations were detected in exon 11 of these patients, three cause amino acid substitutions, while the fourth produces a termination codon (FIG. 1). One mutation (A₁₇₈₄) was found in 4% of our Caucasian CF chromosomes; the stop codon mutation (T₁₇₈₉) occurred in 5% of our American Black CF chromosomes, while the remaining mutations (A₁₈₀₇ and A₁₇₇₈) are rare mutations in American Black patients (Table 2). The presence of each mutation has been confirmed in at least one relative of each patient.

TABLE 2

Nucleotide	Amino Acid	CF Chromosome	
		Racial origin	Haplotypes (# of Chromosomes)*
G ₁₇₇₈ →A	Ser ₅₄₉ →Asn	American Black	A15(1)
G ₁₇₈₄ →A	Gly ₅₅₁ →Asp	Caucasian	D16/18(5) D03(1)
C ₁₇₈₉ →T	Arg ₅₅₃ →Stop	American Black	I12(1) Ii06(1)
G ₁₈₀₇ →A	Ala ₅₅₉ →Thr	American	F(1)

TABLE 2-continued

Nucleotide	Amino Acid	CF Chromosome	
		Racial origin	Haplotypes (# of Chromosomes)*
Black			

*Haplotype codes are created as follows: the first capitalized letter indicates the 4 site (XV2c, KM19, D9 and G2) haplotype shown in Table 1; numbers following the letter indicate the extended haplotype (7C22, MET, D7S8); i indicates an incomplete haplotype that is informative for at least three of the sites in the four site haplotype.

EXAMPLE 3

This example demonstrates how mutations A₁₇₇₈ and A₁₇₈₄ can be detected without sequencing.

PCR amplification of genomic DNA was performed as previously described (Saiki et al. Science, vol. 280, p. 1880 (1985)) using oligonucleotide primers 11i-5' (5'-CAACTGTGGTTAAAGCAATAGTGT-3') and 11i-3' (5'-GCACAGATTCTGAGTAACCATAAT-3') selected from intron sequences flanking 5' and 3' of exon 11 of the CFTR gene. Approximately 500 ng of genomic DNA extracted from peripheral lymphocytes of each subject was amplified using 2 microliters of a 10 micromolar solution of each primer described above in a total volume of 100 microliters containing 1X Taq Polymerase Buffer (50 mM KCl, mM Tris (pH 8.3), 1.5 mM MgCl₂, 0.01% (w/v) gelatin); 0.02 micromoles of each 2' deoxynucleotide 5' triphosphate (Pharmacia) and 2.5 units of Taq Polymerase (Cetus). Amplification was performed by 30 cycles of annealing at 58° for 30 seconds extension at 72° for 1 minute and denaturing at 94° for 30 seconds.

Amplification produced a 425 basepair fragment. Dde I digestion of DNA amplified from normal exon 11 sequence created two fragments of 174 and 251 bp whereas DNA amplified from exon 11 sequence containing the A₁₇₇₈ mutation was not cut with Dde I. DNA amplified from normal exon 11 sequence (425 bp) cannot be cut with MboI whereas digestion of DNA amplified from exon 11 sequence containing the A₁₇₈₄ mutation creates two fragments of 182 and 243 bp.

EXAMPLE 4

This example demonstrates that the three missense mutations are nor normal polymorphic variants of the CFTR gene.

In order to eliminate the possibility that the missense mutations are normal variants, non-CF chromosomes with the same haplotype as that associated with each particular mutation were analyzed by nucleotide sequencing or restriction digestion (Table 3).

TABLE 3

Haplotype*	DNA polymorphism haplotypes associated with each exon 11 mutation										CF Chromosomes**		Normal Chromosomes***		
	RI	T	M	T	B	T	P	M	X	T	M	Caucasian	Black	Caucasian	Black
Mutation G₁₇₇₈→A															
											G ₁₇₇₈ →A	Normal at 1778	G ₁₇₇₈ →A	Normal at 1778	
A15	1	1	1	1	2	1	1	1	2	2	1	0	0	0	0
A	—	—	—	—	—	1	1	1	2	—	—	0	5****	0	0
Ic	—	—	—	—	—	—	1	1	2	—	—	0	1	4	0
c	—	—	—	—	—	—	—	—	1	2	—	0	0	3	0
Other	—	—	—	—	—	—	—	—	—	—	—	0	47	28	0
											1	53	35	0	80
															93
Mutation G₁₇₈₄→A															
											G ₁₇₈₄ →A	Normal at 1784	G ₁₇₈₄ →A	Normal at 1784	
D16/18	1	2	2	1	2	1	2	2	1	1	—	5	0	0	0
D03	1	1	1	1	1	1	2	2	1	1	—	1	5	0	0
D	—	—	—	—	—	1	2	2	1	—	—	0	1	1	0
Other	—	—	—	—	—	—	—	—	—	—	—	0	42	35	0
											6	48	36	0	27
															36
Mutation C₁₇₈₉→A															
											C ₁₇₈₉ →A	Normal at 1789	C ₁₇₈₉ →A	Normal at 1789	
I12	1	1	1	1	2	2	2	2	2	2	1	1	0	0	0
Ii06	1	1	1	1	2	2	2	—	2	—	—	1	0	0	0
d	—	—	—	—	—	—	—	—	2	2	—	0	6	7	0
Other	—	—	—	—	—	—	—	—	—	—	—	0	14	27	0
											2	20	34	0	0
															36
Mutation G₁₈₀₇→A															
											G ₁₈₀₇ →A	Normal at 1807	G ₁₈₀₇ →A	Normal at 1807	
F	—	—	—	—	—	2	1	1	2	—	—	1	1	3	0
Ic	—	—	—	—	—	—	1	1	2	—	—	0	7	4	0
c	—	—	—	—	—	—	—	1	2	—	—	0	0	3	0
Other	—	—	—	—	—	—	—	—	—	—	—	0	12	25	0
											1	20	35	0	0
															36

Table 3 Legends

*Sites 7C22, MET and D7S8 have been previously described (Cutting, et al., Am. J. Hum. Genet. (1989), vol. 44, p. 307); 1 indicates the absence and 2 the presence of a particular site, — indicates that the site is different or uninformative. Enzyme abbreviations — RI(EcoRI), T(TaqI) M(MspI), B(BamI), P(PstI) and X(XbaI). The relative positions of the markers are indicated in FIG. 2.

**CF chromosomes include 21 Caucasian and 9 Black chromosomes with the ΔF₅₀₈ mutation in the G₁₇₇₈→A and G₁₇₈₄→A group and 6 Caucasian and 9 Black chromosomes with the ΔF₅₀₈ mutation in the C₁₇₈₉→T and G₁₈₀₇→A group. Twenty Caucasian CF and 36 Black CF chromosomes were directly sequenced in each case.

***Normal Caucasian chromosomes are from parents and/or siblings of CF patients and are therefore non-CF bearing. Normal Black chromosomes are either non-CF bearing chromosomes from healthy family members or chromosomes from Black patients heterozygous for sickle cell anemia or β-thalassemia (CF carrier frequency in American Blacks is 1 in 65 persons (Cutting, et al., Am. J. Hum. Genet. (1989), vol. 44, p. 307). Normal chromosomes with 4 site haplotypes (XV2c, KM19, Mp6d.9 and G2) identical to the mutation-bearing chromosomes were examined whenever possible. However, two or three site haplotypes, which included the intragenic marker G2 and the closest 5' markers (Mp6d.9± KM19), were also employed. Screening of normal chromosomes and additional Caucasian CF chromosomes for each mutation was as follows: PCR amplification of exon 11 followed by DdeI digestion to detect the G₁₇₇₈→A mutation or MboI digestion to detect the G₁₇₈₄→A mutation or direct sequencing to detect either the C₁₇₈₉→T or G₁₈₀₇→A mutations (Table 2).

****DNA from only five of the six Caucasian patients with an unknown mutation associated with haplotype A was available.

The G₁₇₇₈→A (Ser549→Asn) mutation was identified on one chromosome from a Black CF patient and was inherited from the patient's mother. This mutation causes a conservative substitution between uncharged polar amino acids. To help confirm that this mutation is deleterious, normal chromosomes with the same haplotypes were analyzed. This mutation is associated with an eleven site haplotype (A15) seen only once in 198 (43 American Black, 155 Caucasian) chromosomes. However, the four site haplotype, composed of XV2C, KM19, D9 (Mp6d.9) and G2, designated A, is not rare in either race. This mutation could not be detected on 40 Caucasian or 53 American Black normal chromosomes with at least two sites in common with the haplotype (Table 3).

The G₁₇₈₄→A (Gly551→Asp) mutation was discovered on six Caucasian chromosomes, five of which have the same ten site haplotype D16/18. The sixth occurred on a chromosome which was identical at four sites closest to the gene (D03 haplotype in Table 3) but which differed at the more distant sites. To date, this is the second most common CF mutation in Caucasians. It is unlikely that this mutation is a protein polymorphism since it replaces a neutral with a charged amino acid. Furthermore, the mutation occurs on 4% of Caucasian CF chromosomes in our sample and has not been found on 3 normal chromosomes with the same 10 site haplotype or 24 other normal Caucasian chromosomes. In 6 or 7 Caucasian patients (including two siblings) who were found to have this mutation, it was paired with the

ΔF₅₀₈ mutation. Three of these patients, ages 11 to 13 years, have mild lung disease with normal pulmonary function test results, while the other three patients, ages 15-17 years, have moderate to severe pulmonary disease. The seventh patient with the Gly551→Asp mutation, age 31 years, has an unknown mutation on his other CF chromosome and manifests mild lung disease. All of the patients except one from the sibling pair have exocrine pancreatic insufficiency requiring pancreatic enzyme supplements. The range of illness severity and small number of patients precluded a meaningful assessment of the effect of this mutation on phenotype. All patients are of Northern European ancestry representing different ethnic groups.

The nucleotide substitution C₁₇₈₉ to T (Arg553→Stop) is the first nonsense mutation observed in the CFTR gene. It occurs at a CG dinucleotide, a "hotspot" for mutations, and it conforms to the CG→TG rule (Yousoufian, et al., Nature, vol. 324, p. 380 (1986); Soria, et al., Proc. Natl. Acad. Sci. USA, vol. 86, p. 587 (1989)). This mutation was found on two Black chromosomes having haplotypes identical at 8 informative sites (Table 3) suggesting a common origin of this mutation. It is unknown whether a stable truncated CFTR protein is present in vivo; however, in other disorders, nonsense mutations have been associated with unstable protein products (Adams, et al., Sem. Hematol., in press). Interestingly, one of the two patients with this nonsense mutation is a genetic compound with the G₁₇₇₈→A

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(Ser₅₄₉→Asn) mutation (Patient #272 in FIG. 1). This 13 year old patient has mild disease compared to patients homozygous for the ΔF₅₀₈ mutation.

The fourth mutation, a G₁₈₀₇ to A substitution was found on one chromosome from an American Black patient. This mutation causes a conservative change (Ala₅₅₉→Thr) and since the remainder of the CF gene has not yet been sequenced, we were not convinced that this mutation is associated with disease. Fortunately, this mutation is associated with a relatively common four site haplotype (F) in the Black population. Direct sequencing of twenty-seven chromosomes with at least two sites in common with haplotype F from American Black sickle cell or β thalassemia carriers did not reveal this mutation.

EXAMPLE 5

This example compares the sequence of CFTR in the region of the four disclosed mutations to other known proteins.

The four mutations described here occur within a thirteen amino acid segment (codons 548 to 560) of the first NBF region in the CFTR protein that is highly conserved with similar regions of other membrane-associated transport proteins (Riordan et al. Science vol. 245, pp. 1066-1073 (1989)). Five amino acids in this region are completely conserved in comparable regions from the multiple drug resistance proteins, indicating that these positions are probably crucial to protein function. It appears significant that the amino acid substitutions described in this study occur at three of the five completely conserved residues. Moreover, the substitutions occur at the three most conserved residues in that region between CFTR and fourteen other membrane associated proteins which bind A TP (shown in Riordan). The location of these mutations suggests that the CFTR protein is a member of the ATP-dependent transport protein superfamily (Riordan, supra; and Higgins, Nature, 341:103 (1989)).

We claim:

1. A nucleic acid probe which is complementary to a mutant allele of the CFTR gene said allele being selected from the group consisting of:

Asn₅₄₉, Asp₅₅₁, Stop₅₅₃, and Thr₅₅₉.

2. The probe of claim 1 which is complementary to the Asn₅₄₉ allele.

3. The probe of claim 1 which is complementary to the Thr₅₅₉ allele.

4. The probe of claim 1 which is complementary to the ASP₅₅₁ allele.

5. The probe of claim 1 which is complementary to the Stop₅₅₃ allele.

6. A method of testing a DNA sample of a human to determine if the human is a carrier of Cystic Fibrosis or if the human is affected with Cystic Fibrosis, comprising:

providing a sample of DNA from a human;

testing the sample for the presence of a mutation in exon 11 of the CFTR gene, said mutation comprising a nucleotide selected from the group consisting of: an adenine at nucleotide number 1778, 1784, or 1807, and a thymidine at nucleotide 1789, the presence of the mutation indicating that the human is a carrier of Cystic Fibrosis or is affected with Cystic Fibrosis.

7. The method of claim 6 wherein the step of testing comprises amplifying exon 11 of said gene in a sample of

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DNA from the human to form a population of amplified DNA.

8. The method of claim 7 further comprising the step determining the conformation of single strands of the amplified DNA, a conformation different from that of single strands of amplified exon 11 of wild-type CFTR allele indicating an exon 11 mutation.

9. The method of claim 7 wherein the step of amplifying is performed in the presence of radiolabeled deoxynucleotide triphosphates or radiolabeled primers to form labeled amplified DNA.

10. The method of claim 8 wherein the conformation is determined by electrophoresis on non-denaturing gels.

11. The method of claim 7 further comprising the step of;

determining the melting temperature of double strands of the amplified DNA, the presence of species of amplified DNA in the population of amplified DNA having different melting temperatures from DNA amplified from exon 11 of wild-type CFTR allele indicating a mutation in exon 11 of at least one allele of the CFTR gene in the human.

12. The method of claim 11 wherein the melting temperatures are determined by means of denaturing gradient gel electrophoresis.

13. The method of claim 6 further comprising: contacting the human DNA sample with a nucleic acid probe complementary to a mutant allele of the CFTR gene, said allele being selected from the group consisting of: Asn₅₄₉, Asp₅₅₁, Stop₅₅₃, and Thr₅₅₉, under conditions where totally homologous sequences anneal but sequences differing in one nucleotide do not;

detecting whether the human DNA sample anneals to one of said probes, annealing to one of said probes indicating the presence of a mutant CFTR allele which can cause cystic fibrosis if no wild type allele is present in the human.

14. The method of claim 13 wherein the human DNA sample has been amplified to increase the number of copies of exon 11 of the CFTR gene.

15. The method of claim 6 further comprising: determining the nucleotide sequence of a region of exon 11 of a CFTR allele of a human, said region comprising nucleotides 1778-1807;

inspecting the sequence to determine if there is an adenine at nucleotides number 1778, 1784, or 1807, or a thymidine at nucleotide number 1789, the presence of at least one of said nucleotides indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

16. The method of claim 7 further comprising: digesting an aliquot of the amplified exon 11 DNA with a restriction endonuclease which recognizes a sequence CTNAG which occurs at nucleotide 1778 of the wild-type CFTR allele, to form DNA fragments;

measuring the size of the amplified exon 11 DNA and the DNA fragments, DNA fragments which are the same size as the amplified exon 11 DNA indicating a mutation in a CFTR allele which can cause cystic fibrosis if no wild-type CFTR allele is present in the human.

17. The method of claim 16 wherein the endonuclease is DdeI.

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18. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA
with a restriction endonuclease which recognizes a
sequence GTYRAC which occurs at nucleotide
1784 of the wild-type CFTR allele, to form DNA
fragments;
measuring the size of the amplified exon 11 DNA and
the DNA fragments, DNA fragments which are
the same size as the amplified exon 11 DNA indi-
cating a mutation in a CFTR allele which can cause
cystic fibrosis if no wild-type CFTR allele is pres-
ent in the human.
19. The method of claim 18 wherein the endonuclease
is HincII.
20. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA
with a restriction endonuclease which recognizes a
sequence GTYRAC which occurs at nucleotide
1789 of the wild-type CFTR allele to form DNA
fragments;
measuring the size of the amplified exon 11 DNA and
the DNA fragments, DNA fragments which are
the same size as the amplified exon 11 DNA indi-
cating a mutation in a CFTR allele which can cause
cystic fibrosis if no wild-type CFTR allele is pres-
ent in the human.
21. The method of claim 20 wherein the endonuclease
is HincII.
22. The method of claim 7 further comprising:
digesting an aliquot of the amplified exon 11 DNA
with a restriction endonuclease which recognizes a
sequence GATC which occurs at nucleotide 1784
of a mutant CFTR allele but does not recognize the
sequence in a wild-type CFTR allele, to form
DNA fragments;
measuring the size of the amplified exon 11 DNA and
the DNA fragments, DNA fragments which are
not the same size as the amplified exon 11 DNA
indicating a mutation in a CFTR allele which can
cause cystic fibrosis if no wild-type CFTR allele is
present in the human.
23. The method of claim 22 wherein the endonuclease
is MboI.
24. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endo-
nuclease which recognizes a sequence GATC
which occurs at nucleotide 1784 of a mutant CFTR
allele but not of a wild-type CFTR allele:
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an
exon 11 probe which spans nucleotides 1784 but
does not span any other sequence which the endo-
nuclease recognizes;
detecting the DNA which hybridizes to the probe,
two hybridizing fragments indicating a mutation in
a CFTR allele of the human which can cause cystic

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- fibrosis if no wild-type CFTR allele is present in
the human.
25. The method of claim 24 wherein the endonuclease
is MboI.
26. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endo-
nuclease which recognizes a sequence CTNAG
which occurs at nucleotide 1778 of the wild-type
CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an
exon 11 probe which spans nucleotide 1778 but
does not span any other sequence which the endo-
nuclease recognizes;
detecting the DNA which hybridizes to the probe,
only one hybridizing fragment indicating a muta-
tion in a CFTR gene of the human which can cause
cystic fibrosis if no wild-type CFTR allele is pres-
ent in the human.
27. The method of claim 26 wherein the endonuclease
is DdeI.
28. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endo-
nuclease which recognizes a sequence GTYRAC
which occurs at nucleotide 1784 of the wild-type
CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an
exon 11 probe which spans nucleotide 1784 but
does not span any other sequence which the endo-
nuclease recognizes;
detecting the DNA which hybridizes to the probe,
only one hybridizing fragment indicating a muta-
tion in a CFTR gene of the human which can cause
cystic fibrosis if no wild-type CFTR allele is pres-
ent in the human.
29. The method of claim 28 wherein the endonuclease
is HincII.
30. The method of claim 6 further comprising:
digesting DNA of the human with a restriction endo-
nuclease which recognizes a sequence GTYRAC
which occurs at nucleotide 1789 of the wild-type
CFTR allele;
separating the digested DNA on a gel matrix;
hybridizing the separated, digested DNA with an
exon 11 probe which spans nucleotide 1789 but
does not span any other sequence which the endo-
nuclease recognizes;
detecting the DNA which hybridizes to the probe,
only one hybridizing fragment indicating a muta-
tion in a CFTR gene of the human which can cause
cystic fibrosis if no wild-type CFTR allele is pres-
ent in the human.
31. The method of claim 30 wherein the endonuclease
is HincII.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 4

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6129 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1480 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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			20					25					30		
Glu	Leu	Ser	Asp	Ile	Tyr	Gln	Ile	Pro	Ser	Val	Asp	Ser	Ala	Asp	Asn
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Leu	Ser	Glu	Lys	Leu	Glu	Arg	Glu	Trp	Asp	Arg	Glu	Leu	Ala	Ser	Lys
		50				55					60				

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Phe	Ser	Leu 435	Leu	Gly	Thr	Pro	Val 440	Leu	Lys	Asp	Ile	Asn 445	Phe	Lys	Ile
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His 610	Leu	Lys	Lys	Ala	Asp	Lys 615	Ile	Leu	Ile	Leu	Asn 620	Glu	Gly	Ser	Ser
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Leu	Phe	Asp 835	Asp	Met	Glu	Ser	Ile 840	Pro	Ala	Val	Thr	Thr 845	Trp	Asn	Thr
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		995					1000					1005			
Ala	Val	Leu	Gln	Pro	Tyr	Ile	Phe	Val	Ala	Thr	Val	Pro	Val	Ile	Val
		1010				1015					1020				
Ala	Phe	Ile	Met	Leu	Arg	Ala	Tyr	Phe	Leu	Gln	Thr	Ser	Gln	Gln	Leu
1025					1030					1035					1040
Lys	Gln	Leu	Glu	Ser	Glu	Gly	Arg	Ser	Pro	Ile	Phe	Thr	His	Leu	Val
				1045					1050					1055	
Thr	Ser	Leu	Lys	Gly	Leu	Trp	Thr	Leu	Arg	Ala	Phe	Gly	Arg	Gln	Pro
		1060						1065					1070		
Tyr	Phe	Glu	Thr	Leu	Phe	His	Lys	Ala	Leu	Asn	Leu	His	Thr	Ala	Asn
		1075					1080					1085			
Trp	Phe	Leu	Tyr	Leu	Ser	Thr	Leu	Arg	Trp	Phe	Gln	Met	Arg	Ile	Glu
		1090				1095					1100				
Met	Ile	Phe	Val	Ile	Phe	Phe	Ile	Ala	Val	Thr	Phe	Ile	Ser	Ile	Leu
1105					1110					1115					1120
Thr	Thr	Gly	Glu	Gly	Glu	Gly	Arg	Val	Gly	Ile	Ile	Leu	Thr	Leu	Ala
			1125						1130					1135	
Met	Asn	Ile	Met	Ser	Thr	Leu	Gln	Trp	Ala	Val	Asn	Ser	Ser	Ile	Asp
			1140					1145					1150		
Val	Asp	Ser	Leu	Met	Arg	Ser	Val	Ser	Arg	Val	Phe	Lys	Phe	Ile	Asp
		1155					1160					1165			
Met	Pro	Thr	Glu	Gly	Lys	Pro	Thr	Lys	Ser	Thr	Lys	Pro	Tyr	Lys	Asn
		1170				1175					1180				
Gly	Gln	Leu	Ser	Lys	Val	Met	Ile	Ile	Glu	Asn	Ser	His	Val	Lys	Lys
1185					1190					1195					1200
Asp	Asp	Ile	Trp	Pro	Ser	Gly	Gly	Gln	Met	Thr	Val	Lys	Asp	Leu	Thr
				1205					1210					1215	
Ala	Lys	Tyr	Thr	Glu	Gly	Gly	Asn	Ala	Ile	Leu	Glu	Asn	Ile	Ser	Phe
			1220					1225					1230		
Ser	Ile	Ser	Pro	Gly	Gln	Arg	Val	Gly	Leu	Leu	Gly	Arg	Thr	Gly	Ser
		1235					1240					1245			
Gly	Lys	Ser	Thr	Leu	Leu	Ser	Ala	Phe	Leu	Arg	Leu	Leu	Asn	Thr	Glu
		1250				1255					1260				
Gly	Glu	Ile	Gln	Ile	Asp	Gly	Val	Ser	Trp	Asp	Ser	Ile	Thr	Leu	Gln
1265					1270					1275				1280	
Gln	Trp	Arg	Lys	Ala	Phe	Gly	Val	Ile	Pro	Gln	Lys	Val	Phe	Ile	Phe
			1285						1290					1295	
Ser	Gly	Thr	Phe	Arg	Lys	Asn	Leu	Asp	Pro	Tyr	Glu	Gln	Trp	Ser	Asp
			1300					1305					1310		
Gln	Glu	Ile	Trp	Lys	Val	Ala	Asp	Glu	Val	Gly	Leu	Arg	Ser	Val	Ile
		1315					1320					1325			
Glu	Gln	Phe	Pro	Gly	Lys	Leu	Asp	Phe	Val	Leu	Val	Asp	Gly	Gly	Cys
		1330				1335					1340				
Val	Leu	Ser	His	Gly	His	Lys	Gln	Leu	Met	Cys	Leu	Ala	Arg	Ser	Val
1345					1350					1355					1360
Leu	Ser	Lys	Ala	Lys	Ile	Leu	Leu	Leu	Asp	Glu	Pro	Ser	Ala	His	Leu
				1365					1370					1375	

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5,407,796

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-continued

Asp	Pro	Val	Thr	Tyr	Gln	Ile	Ile	Arg	Arg	Thr	Leu	Lys	Gln	Ala	Phe
			1380					1385					1390		
Ala	Asp	Cys	Thr	Val	Ile	Leu	Cys	Glu	His	Arg	Ile	Glu	Ala	Met	Leu
		1395					1400					1405			
Glu	Cys	Gln	Gln	Phe	Leu	Val	Ile	Glu	Glu	Asn	Lys	Val	Arg	Gln	Tyr
	1410					1415					1420				
Asp	Ser	Ile	Gln	Lys	Leu	Leu	Asn	Glu	Arg	Ser	Leu	Phe	Arg	Gln	Ala
1425					1430				1435						1440
Ile	Ser	Pro	Ser	Asp	Arg	Val	Lys	Leu	Phe	Pro	His	Arg	Asn	Ser	Ser
				1445					1450					1455	
Lys	Cys	Lys	Ser	Lys	Pro	Gln	Ile	Ala	Ala	Leu	Lys	Glu	Glu	Thr	Glu
			1460					1465					1470		
Glu	Glu	Val	Gln	Asp	Thr	Arg	Leu								
		1475					1480								

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAACTGTGGT TAAAGCAATA GTGT

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCACAGATTC TGAGTAACCA TAAT

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32. The probe of claim 1 which comprises 18 to 22 nucleotides.

33. The method of claim 13 wherein said nucleic acid probe comprises 18 to 22 nucleotides.

* * * * *

60

65

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,407,796
DATED : April 18, 1995
INVENTOR(S) : Garry R. Cutting, et. al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In claim 6, column 11, lines 55 and 65 please delete each occurrence of the word "carder" and insert therefor --carrier--.

Please move claims 1-31 which appear at column 11, line 41 to column 14, line 55 and insert them after the sequence listing at column 27.

Signed and Sealed this
Twelfth Day of September, 1995

Attest:



BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

EXHIBIT 12



US006107025A

United States Patent [19][11] **Patent Number:** **6,107,025****Caskey et al.**[45] **Date of Patent:** ***Aug. 22, 2000**[54] **DIAGNOSIS OF THE FRAGILE X SYNDROME**[75] Inventors: **C. Thomas Caskey; David L. Nelson; Maura Pieretti**, all of Houston, Tex.; **Stephen T. Warren**, Clarkston, Ga.; **Ben A. Oostra**, Rotterdam, Netherlands[73] Assignee: **Baylor College of Medicine**, Houston, Tex.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **07/705,490**[22] Filed: **May 24, 1991**[51] **Int. Cl.**⁷ **C07H 21/02**; C07H 21/04; C12Q 1/68; C12P 19/34[52] **U.S. Cl.** **435/6**; 435/91.2; 536/23.1; 536/24.33; 536/24.1[58] **Field of Search** 435/91.2, 6; 536/27, 536/23.1, 24.1, 24.33; 935/77, 78[56] **References Cited****PUBLICATIONS**M. Pieretti, et al., "Absence of Expression of the FMR-1 Gene in Fragile X Syndrome" *Cell* 66:817-822 (1991).J.S. Sutcliffe, et al., "DNA methylation represses FMR-1 transcription in fragile X syndrome" *Human Molecular Genetics* 1:397-400 (1992).D. Devys, et al., "The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation" *Nature Genetics* 4:335-340 (1993).H. Siomi, et al., "The Protein Product of the Fragile X Gene, FMR1, Has Characteristics of an RNA-Binding Protein" *Cell* 74:291-298 (1993).C. Verheij, et al., "Characterization and localization of the FMR-1 gene product associated with fragile X syndrome" *Nature* 363:722-724 (1993).D.P.A. Kuhl, et al., "Fragile X Syndrome Protein FMRP Associates with the Microfilament Fraction of the Cellular Cytoskeleton" Submission to *Cell*.Heitz et al. *Science* vol. 251 pp 1236-1239. Mar. 8, 1991.Suthers, et al., "Physical mapping of new DNA probes near the Fragile X mutation (FRAXA) by using a panel of cell lines" *American Journal of Human Genetics* 47:187-195 (1990). This paper provides order for a number of cloned DNAs using a panel of somatic cell hybrid breakpoints near the Fragile X site. The order of the breakpoints is likewise established by the probes.Warren, et al., "Isolation of the human chromosomal band Xq28 within somatic cell hybrids by Fragile X site breakage" *Proceedings of the National Academy of Sciences* 87:3856-3860 (1990). This paper describes a panel of hybrid cell lines with chromosome breakpoints at or near the Fragile X site, and its use as a mapping reagent.Warren, et al., "The Fragile X site in somatic cell hybrids; An approach for molecular cloning of Fragile sites" *Science* 237:420-423 (1987). This paper describes the first hybrid cell lines with chromosome breakpoints at or near the Fragile X site, and the potential for using these to identify the site.Warren, et al., "Strategy for molecular cloning of the Fragile X site DNA" *American Journal of Medical Genetics* 30:613-623 (1988). This paper describes the strategy of using somatic cell hybrids with Fragile X breakpoints for identifying the Fragile X site.Warren and Davidson, "Expression of Fragile X chromosome in human-rodent somatic cell hybrids" *Somatic Cell and Molecular Genetics* 10:409-413 (1984). This paper demonstrates that the Fragile X site is expressed in rodent cell backgrounds.Warren, "Molecular and somatic cell genetic approaches to the Fragile X syndrome" in: Brosius, J and Freneau, RT (eds.) *Molecular Genetic Approaches to Neuropsychiatric Disease* (Academic Press, San Diego) 1991. A review of state of art.Brown, "Invited editorial: The Fragile X" Progress toward solving the puzzle *American Journal of Human Genetics* 47:175-180 (1990). A review of the status of the physical map as of one year prior to the Fragile X site identification.Nussbaum and Ledbetter, "The Fragile X syndrome" Chapter 8 of *The Metabolic Basis of Inherited Disease*, 6th edition, McGraw-Hill, 1989. A comprehensive review of Fragile X syndrome.Nussbaum and Ledbetter, "Fragile X syndrome: A unique mutation in man" *Annual Review of Genetics* 20:109-145 (1986). Review of the genetic aspects of Fragile X syndrome.Heitz, et al., "Isolation of sequences that span the Fragile X and identification of a Fragile X-related CpG island" *Science* 251:1236-1239 (1991). This paper describes localization of the Fragile X site on a large fragment of DNA and the positions of somatic cell hybrid chromosome breakpoints within the region. It also localizes a CpG island showing Fragile X specific methylation patterns.Craig, "Methylation and the Fragile X" *Nature* 349:742-743 (1991). This news article reviews papers L & M where Fragile X specific methylation was reported.Bell, et al., "Physical mapping across the Fragile X" Hypermethylation and clinical expression of the Fragile X syndrome *Cell* 64:851-866 (1991). This paper describes long-range restriction mapping experiments that determined Fragile X specific hypermethylation of a CpG island in numerous patients.Vincent, et al., "Abnormal pattern detected in Fragile-X patients by pulsed-field gel electrophoresis" *Nature* 349:624-626 (1991). This paper describes long-range restriction mapping experiments that determined Fragile X specific hypermethylation of a CpG island in numerous patients.*Primary Examiner*—Stephanie Zitomer
Attorney, Agent, or Firm—Fulbright & Jaworski LLP[57] **ABSTRACT**

A sequence of the FMR-1 gene is disclosed. This sequence and related probes, cosmids and unique repeats are used to detect X-linked diseases and especially the fragile X syndrome.

4 Claims, 10 Drawing Sheets

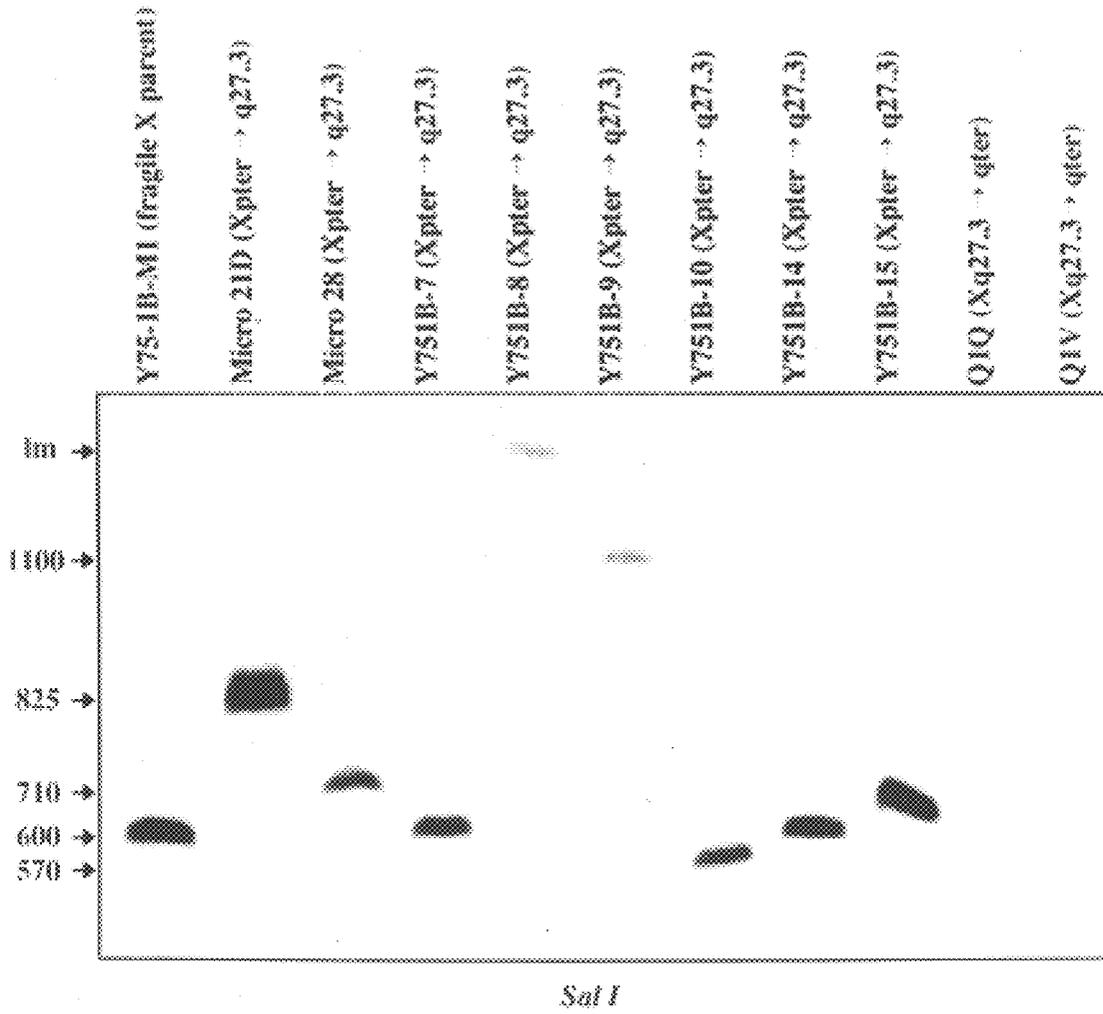


Figure 1

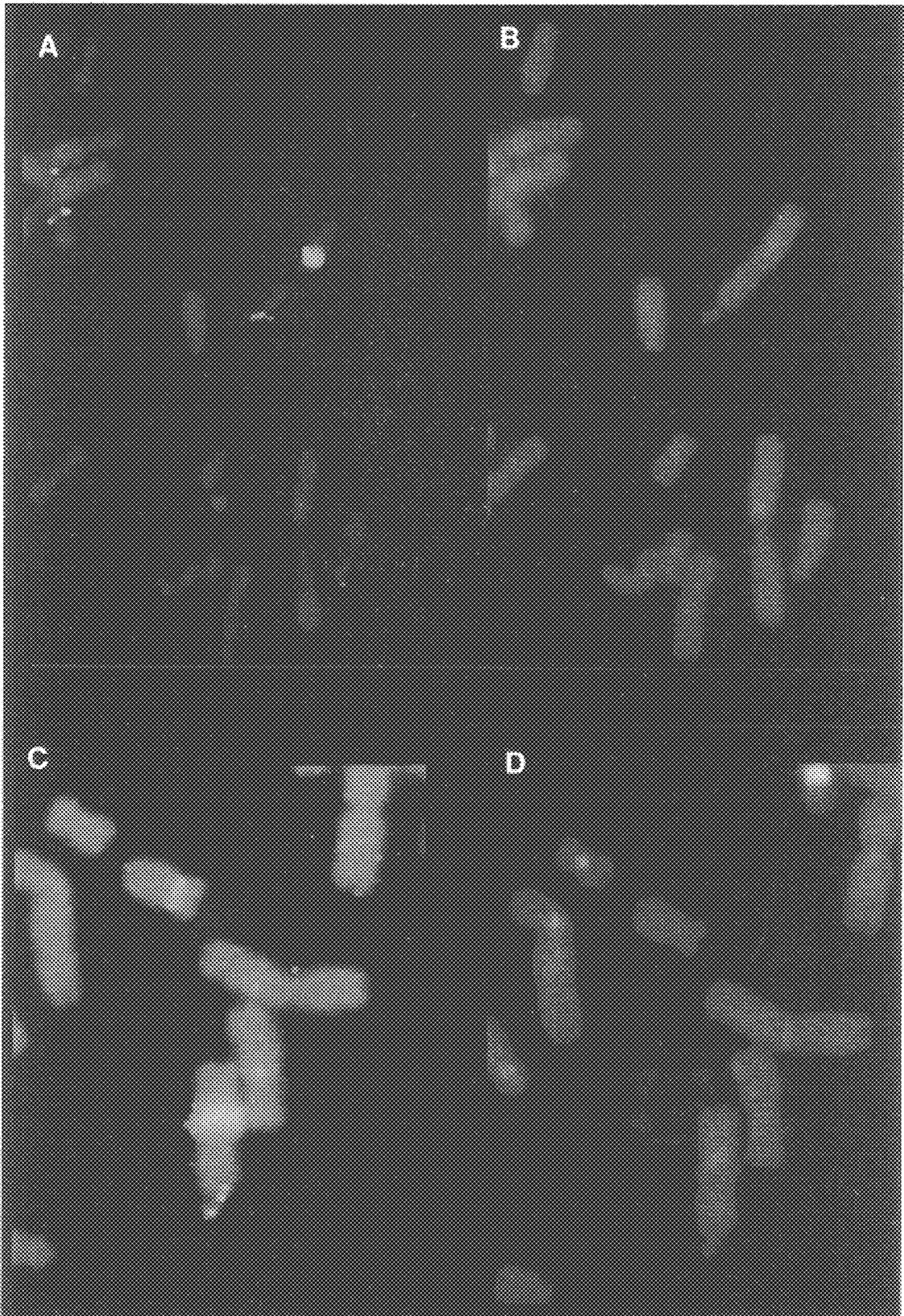


Figure 2

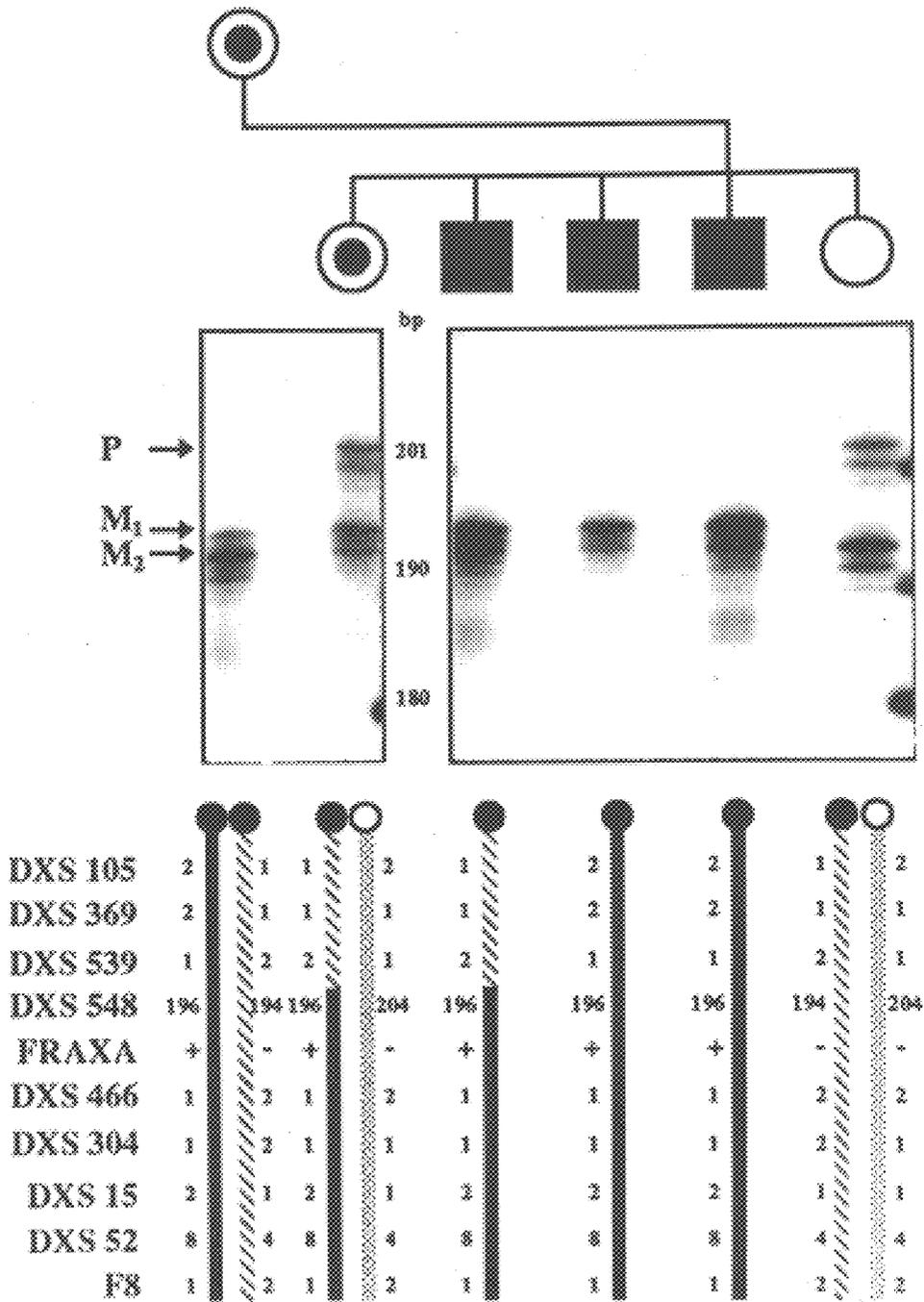


Figure 3

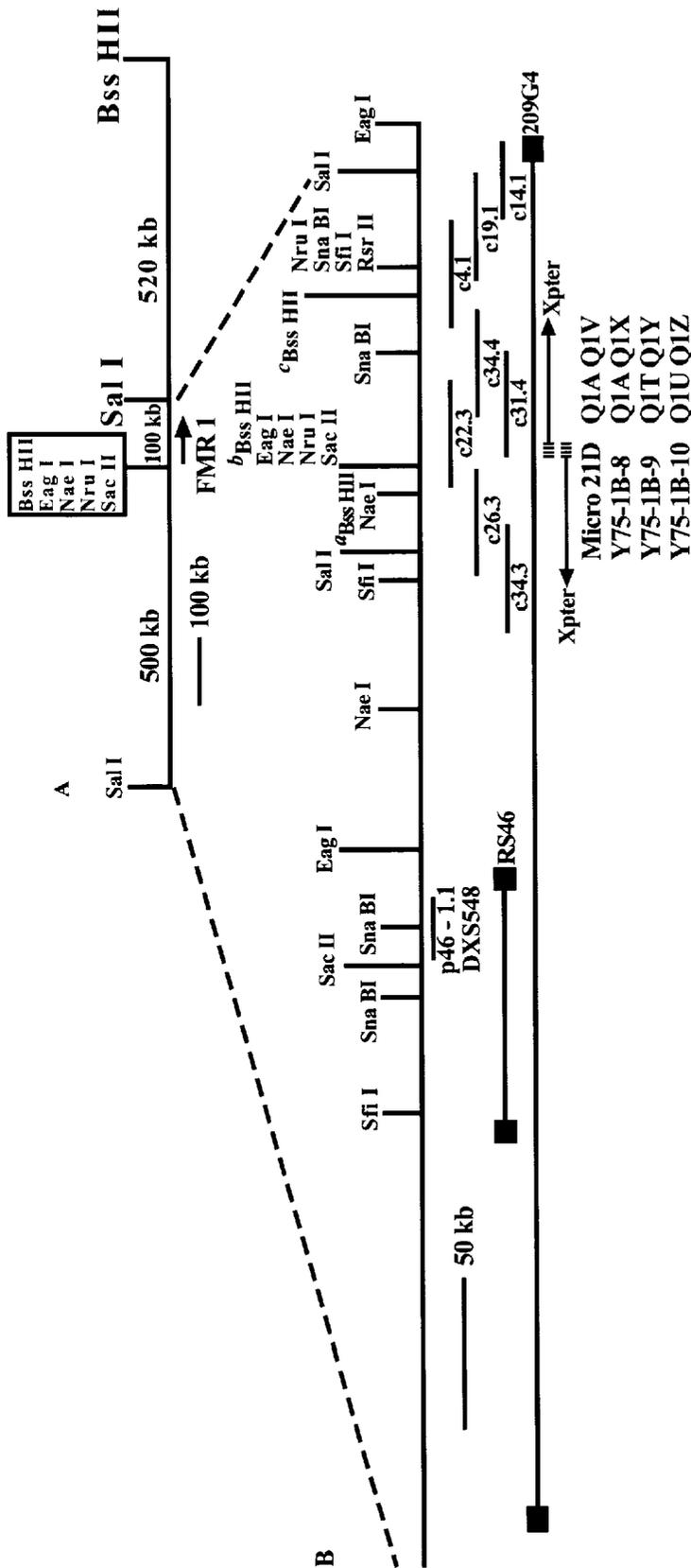


Figure 4

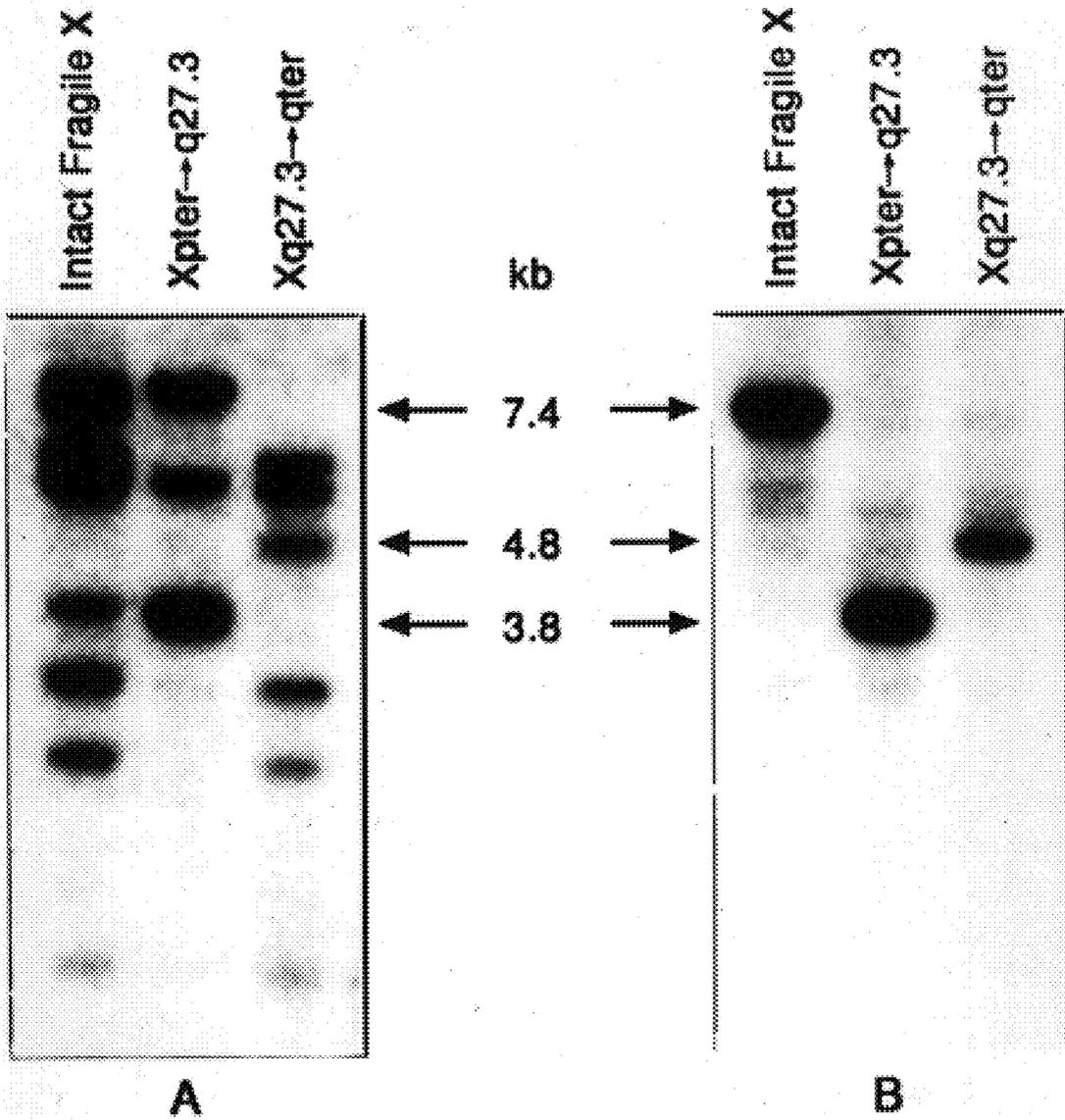


Figure 5

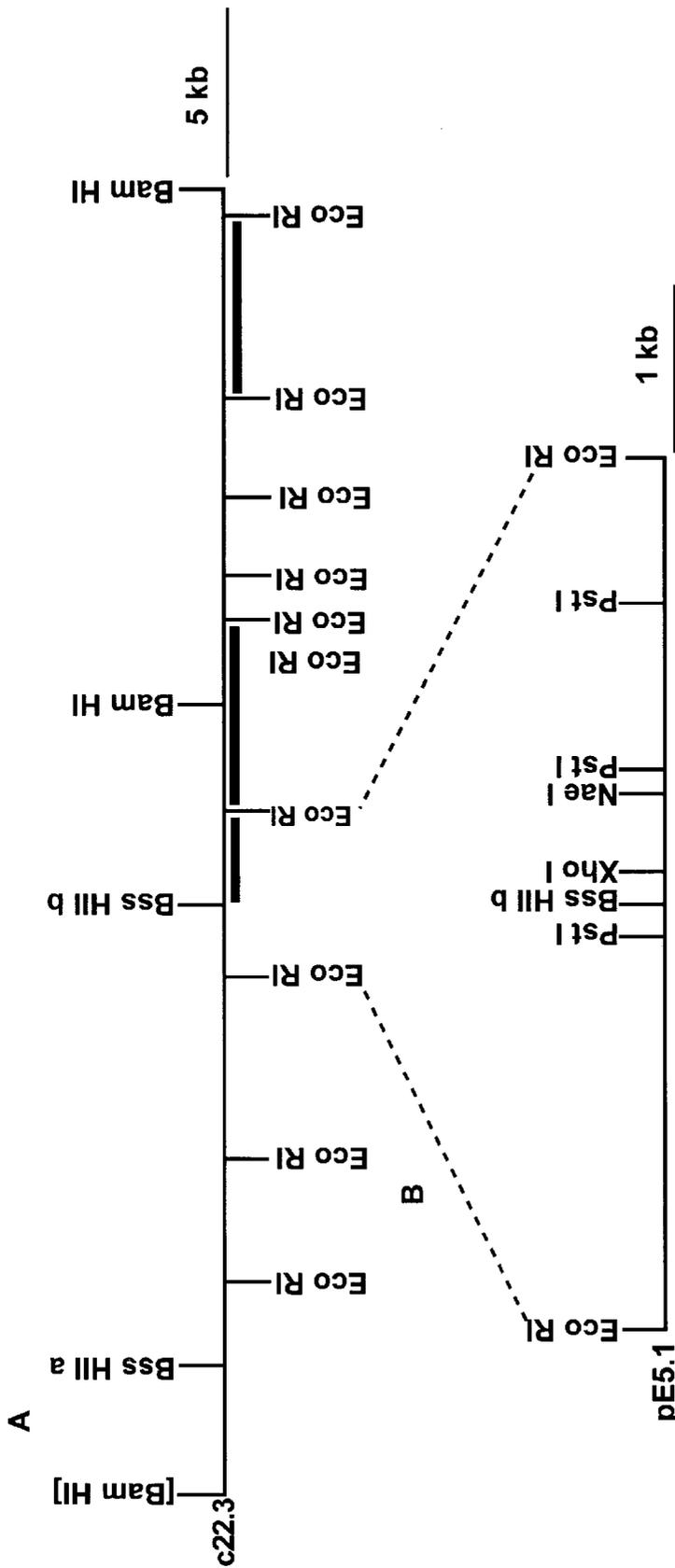


Figure 6

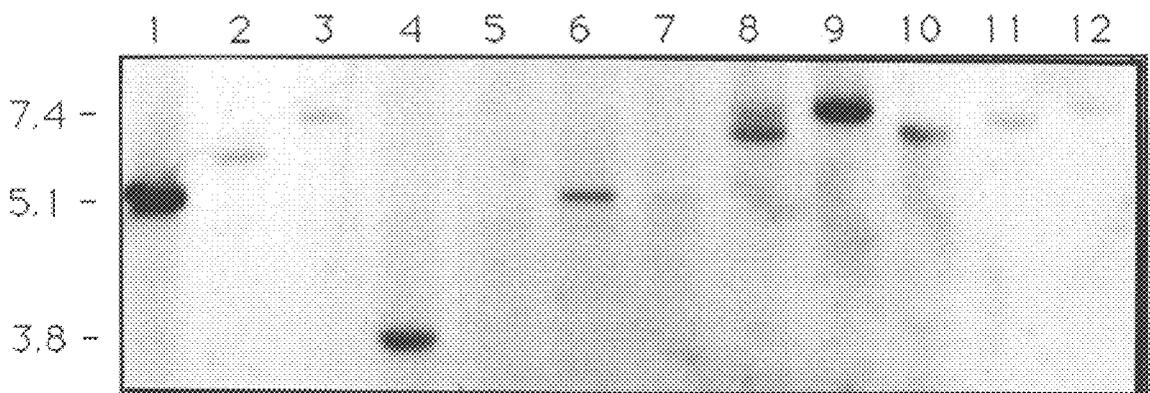


Figure 7

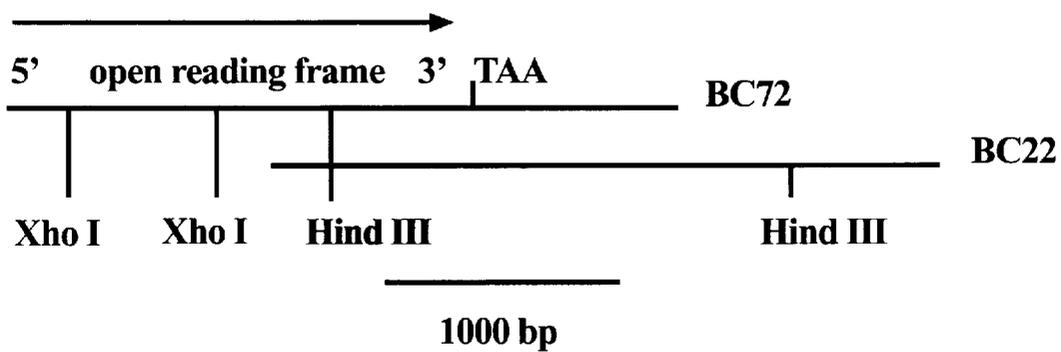


Figure 8

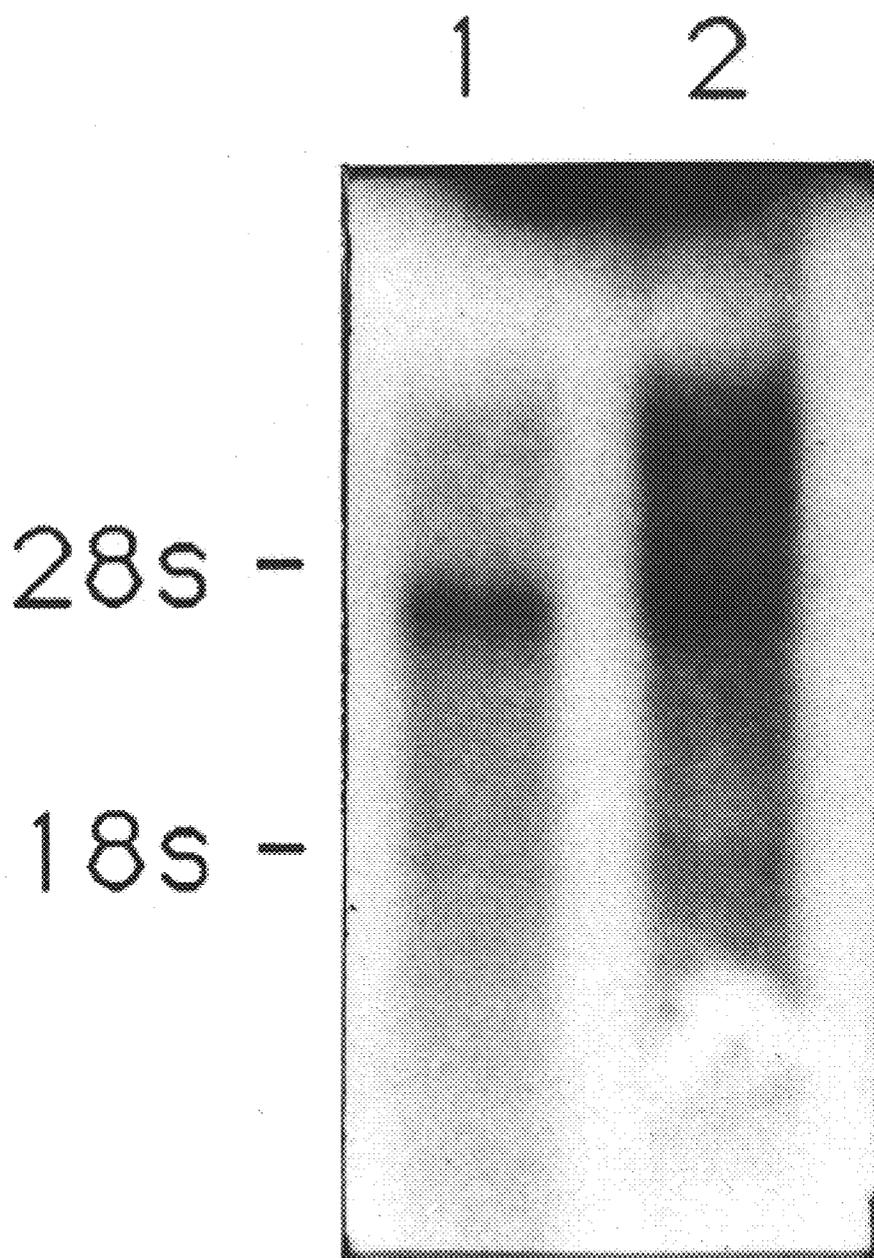


Figure 9

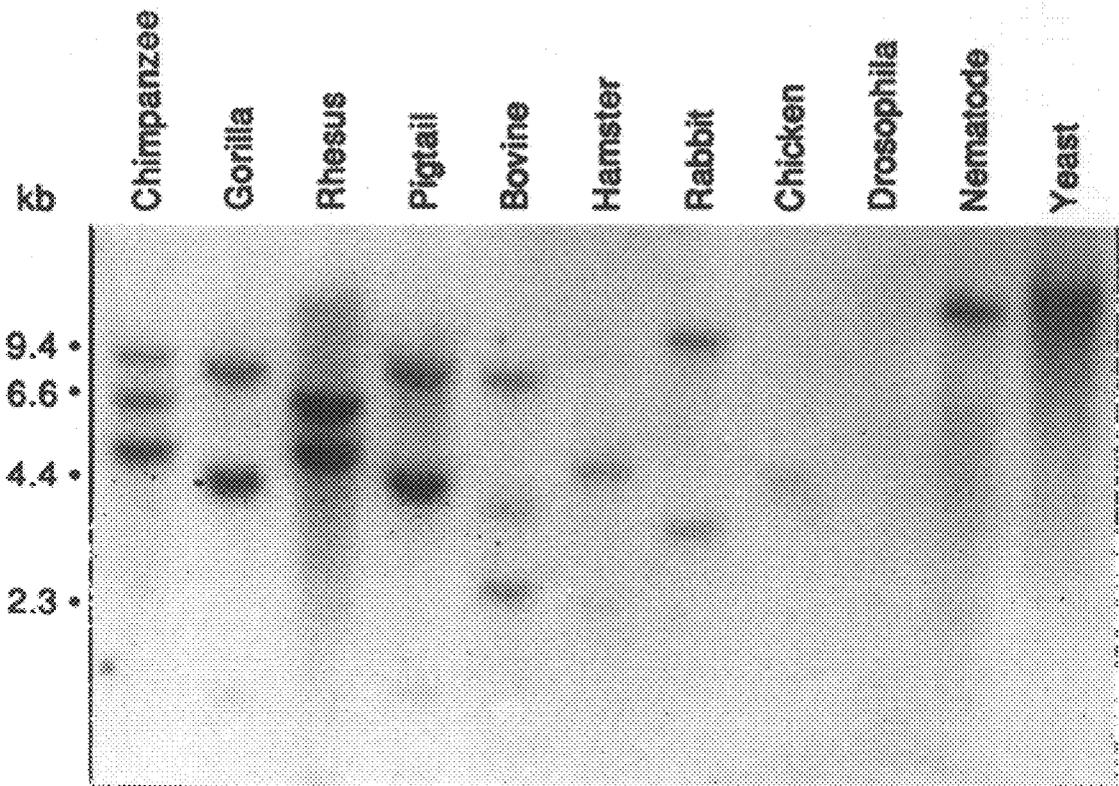


Figure 10

EXHIBIT 12

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DIAGNOSIS OF THE FRAGILE X SYNDROME

This invention was supported by the National Institutes of Health, under grant number LTD 20521. The government may have certain rights under this application. 5

This invention was partially supported by grants from the United States Government, The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates to the field of molecular diagnosis of the fragile X syndrome.

BACKGROUND

The fragile X syndrome is the most frequently encountered form of inherited mental retardation in humans and has a prevalence estimated to be 1/1250 males. The fragile X syndrome segregates as an X-linked dominant disorder with reduced penetrance. Either sex when carrying the fragile X mutation may exhibit mental deficiency. It has been shown that approximately 30% of carrier females are penetrant and that 20% of males carrying the fragile X chromosome are normal but may transmit the disorder and have fully penetrant grandsons. In addition to the mental retardation which is variable in severity, penetrant males exhibit additional phenotypic involvement including macroorchidism and distinctive facies. Since fully penetrant males rarely reproduce, it has been suggested that the frequency of new mutations of the fragile X site may be as high as 1/3000 germ cells to maintain the population frequency. 20

The fragile X syndrome, as implied by its name, is associated with a fragile site expressed as an isochromatid gap in the metaphase chromosome at map position Xq 27.3. The fragile X site is induced by cell culture conditions which perturb deoxy pyrimidine pools and is rarely observed in greater than 50% of the metaphase spreads. Neither the molecular nature of the fragile X site, nor its relationship to the gene responsible for the clinical expression of the syndrome is understood. However, based upon genetic linkage studies, as well as in situ hybridizations, the fragile X site and its associated gene are tightly linked if not coincident. 25

The present application provides a new procedure for detecting the fragile X site at the molecular level. It provides a molecular method for the diagnosis of the fragile X syndrome, describes a unique open reading sequence at the suspected gene locus and provides probes to the fragile X region. 30

SUMMARY OF THE INVENTION

An object of the present invention is a method for diagnosing fragile X syndrome. 35

A further object of the present invention is the provision of a sequence of the FMR-1 gene.

An additional object of the present invention is a method of detecting the fragile X syndrome by measuring the mRNA or protein from the FMR-1 gene. 40

Thus in accomplishing the foregoing objects there is provided in accordance with one aspect of the present invention as a composition of matter, a 3.8 kb cDNA clone containing the FMR-1 gene. A further aspect is a 4242 bp genomic DNA sequence containing at least a fraction of the FMR-1 gene. 45

A further embodiment of the present invention is a group of cosmid probes for the selection of the FMR-1 gene in the fragile X syndrome. 50

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An additional embodiment of the present invention is a method of detecting fragile X syndrome comprising the steps of digesting DNA from an individual to be tested with a restriction endonuclease and detecting the restriction fragment length polymorphism with hybridization to probes within the fragile X locus and southern blot analysis. In a preferred embodiment of the present invention, the probe is pE5.1 and the restriction endonucleases are selected from the group consisting of EcoR I, Pst I, Xho I and BssH II.

Alternate embodiments of the present invention include detecting the fragile X syndrome by measuring the expression of the FMR-1 gene either as the amount of mRNA expressed or as the amount of FMR-1 protein produced. Another embodiment of the present invention includes a method of detecting X-linked disease comprising the steps of detecting variation in the (CGG)_n repeat at the 5' end of the FMR-1 gene by measuring the length of the repeat, wherein n for normal ranges between 16 and 30 and n for X-linked disease is greater than 30. A variety of methods are available to detect the dosage measurements of the repeat. These procedures can be selected from the group consisting of visual examination, densitometry measurement, quantitative radioactivity and quantitative fluorescence as well as pulsed field gel electrophoresis and fluorescence in situ hybridization. 55

Other and further objects, features and advantages will be apparent and eventually more readily understood from a reading of the following specification and by reference to the accompanying drawings forming a part thereof, wherein examples of the presently preferred embodiments of the invention are given for the purpose of the disclosure. 60

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a Southern blot analysis of pulsed field gel resolved Sal I digested DNA of proximal translocation hybrids probed with p46-1.1.

FIGS. 2A-2D show fluorescent in situ hybridization of YAC 209G4 and cosmids to the fragile X site at Xq 27.3 of an affected male patient. 65

FIG. 3 is a PCR analysis of DXS548 alleles in a fragile X family with recombinant individuals.

FIGS. 4A and 4B comprise a physical map of the fragile X region of a genomic and YAC 209G4 DNA.

FIGS. 5A and 5B depict a Southern blot analysis of fragile X associated translocation breakpoints. In (A) the Southern blot is hybridized with cosmid 22.3 and in (B) the same filter is hybridized with pE5.1. 70

FIGS. 6A and 6B comprise a restriction map of cosmid 22.3 and pE5.1. In (A) is cosmid 22.3 showing BssH II sites a and b as well as EcoR I and BamH I sites. The BamH I site in parentheses was destroyed during cloning. The solid lines below the map show fragments which hybridize to cDNAs BC72 and BC22. In (B) is the map of the cloned 5.1 kb EcoR I fragment of cosmid 22.3 (pE5.1). The solid line below the map shows the position of the FMR-1 exonic sequence which contains the Xho I site. 75

FIG. 7 shows length variation of EcoRI fragments from normal and fragile X human chromosomes with probe pE5.1.

FIG. 8 is a map of the FMR-1 cDNA clones.

FIG. 9 is a Northern blot analysis of a poly(A)RNA hybridized with cDNA BC22.

FIG. 10 is a zoo blot analysis of DNA isolated from several species hybridized with cDNA BC22.

The drawings and figures are not necessarily to scale and certain features mentioned may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness. 80

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DETAILED DESCRIPTION OF THE INVENTION

It will be readily apparent to one skilled in the art that variations, substitutions and modifications may be made to the invention disclosed herein without departing from the scope and the spirit of the invention.

Each sample to be tested herein for the fragile X site is derived from genomic DNA, mRNA or protein. The source of the genomic DNA to be tested can be any medical specimen which contains DNA. Some examples of medical specimen include blood, semen, vaginal swabs, buccal mouthwash, tissue, hair and mixture of body fluids. As used herein the term "polymerase chain reaction" or "PCR" refers to the PCR procedure described in the patents to *Mullis*, et al., U.S. Pat. Nos. 4,683,195 and 4,683,202. The procedure basically involves: (1) treating extracted DNA to form single-stranded complementary strands; (2) adding a pair of oligonucleotide primers, wherein one primer of the pair is substantially complementary to part of the sequence in the sense strand and the other primer of each pair is substantially complementary to a different part of the same sequence in the complementary antisense strand; (3) annealing the paired primers to the complementary sequence; (4) simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product complementary to the strands annealed to each primer wherein said extension products after separation from the complement serve as templates for the synthesis of an extension product for the other primer of each pair; (5) separating said extension products from said templates to produce single-stranded molecules; and (6) amplifying said single-stranded molecules by repeating at least once said annealing, extending and separating steps.

As used herein fluorescence in situ hybridization or "FISH" refers to the procedure described in Wotta, et al., *Am. J. of Human Genetics*, 46, 95-106 (1988) and Kievits, et al., *Cytogenet. Cell Genet.*, 53:134-136 (1990). The procedure basically involves the steps of preparing interphase or metaphase spreads from cells of peripheral blood lymphocytes and hybridizing labeled probes to the interphase or metaphase spreads. Using probes with mixed labels allows visualization of space, order and distance between hybridization sites. After hybridization the labels are examined to determine the order and distance between the hybridization sites.

As used herein, the term "pulsed field gel electrophoresis" or "PFGE" refers to a procedure described by Schwartz, et al., *Cold Springs Harbor Symposium, Quantitative Biology*, 47:189-195 (1982). The procedure basically comprises running a standard electrophoresis gel (agarose, polyacrylamide or other gel known to those skilled in the art) under pulsing conditions. One skilled in the art recognizes that the strength of the field as well the direction of the field is pulsed and rotated in order to separate megabase DNA molecules. Current commercial systems are computer controlled and select the strength, direction and time of pulse depending on the molecular weight of DNA to be separated.

One embodiment of the present invention as a composition of matter is a 3.8 kb cDNA clone (SEQ. ID. No. 1) containing the FMR-1 gene.

Another embodiment of the present invention is a 4242 bp genomic DNA (SEQ. ID. No. 2). This DNA is a sequence of pE5.1 from the distal Eco RI site containing the fragile X region. Further, there is a 229 bp genomic DNA (SEQ. ID. No. 3) of pE5.1 from the proximal Eco RI site.

One embodiment of the present invention is a method of detecting Fragile X syndrome comprising the steps of

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digesting DNA from an individual to be tested with a restriction endonuclease and detecting the restriction fragment length to polymorphism (RFLP) with hybridization to probes within the fragile X locus and southern blot analysis. One skilled in the art will readily recognize that a variety of restriction endonucleases can be used. In the preferred embodiment the restriction endonuclease is selected from the group consisting of EcoR I, Pst I, Xho I and BssH II.

In the method of detection, it is found that the probe pE 5.1 is used in the preferred embodiment. Again, one skilled in the art readily recognizes that other probes or fractions of the probe pE5.1 which hybridize to the unique fragment lengths can be used.

An alternative method for detecting the Fragile X syndrome comprises the step of measuring the expression of the FMR-1 gene. The FMR-1 gene can be measured by either measuring the amount of mRNA expressed or by measuring the amount of FMR-1 protein.

When measuring the amount of mRNA expressed, the amount of mRNA is determined by the steps of extracting RNA from any tissue source including fibroblast and lymphoblastoid cell lines of the individuals to be tested. From the RNA of FMR-1, a cDNA is prepared. From RNA of a control gene a cDNA is prepared. Then quantification is achieved by comparing the amount of mRNA from FMR-1 with the mRNA from the controlled gene. In the preferred embodiment, the quantification step includes PCR analysis of the FMR-1 cDNA and PCR analysis of the control gene cDNA. The PCR products are electrophoresed and ethidium bromide stained. The products are then quantified by comparing the FMR-1 product versus the control gene product after the ethidium bromide staining. The oligonucleotide primers for the fragile X site are SEQ. ID. No. 8 and SEQ. ID. No. 9. One example of the control gene is HPRT and the oligonucleotides are SEQ. ID. No. 12 and SEQ. ID. No. 13.

When measuring the amount of FMR-1 protein produced, one can use any of the variety of methods known in the art to detect proteins, including monoclonal antibodies, polyclonal antibodies and protein assays. In the preferred embodiment, the antibodies detect SEQ. ID. No. 14.

The methods described herein can also be used to detect X-linked disease. The method comprises the steps of detecting variation of the (CGG)_n repeat found at the 5' end of the FMR-1 gene by measuring the length of the repeat wherein n (number of repeats) for normal is in the range between 16 and 30 and n for X-linked diseases is in the range of greater than 30. In the case of Fragile X, n is usually at least twice the range of normal. Types of disease which can be detected are X-linked mental retardation both of fragile X and non-fragile X type, X linked manic depressive disease, TKCR syndrome and Martin-Bell syndrome.

The method of dosage compensation by measuring the amount or length of the repeat can be done by using FISH. In the FISH method, the repetitive sequence can be used as a probe to distinguish between normal and fragile X syndrome simply by the presence or absence of a signal to the repetitive sequence. In this case, the application of the repeat sequence provides a sufficiently large target for the hybridization. Thus, it is possible that very sensitive FISH might detect transmitting males (with 50-100 copies of the CGG) even though these would be lost to routine microscopy and detection. Although FISH is usually applied to metaphase nuclei, in the present invention it is applicable to both metaphase and interphase for the detection of X-linked disease.

Alternate methods to measure the dosage measurement of the repeat can include visual examination, densitometry measurement, quantitative radioactivity and quantitative fluorescence.

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In one embodiment the size of the repeat is determined by dosage measurements of Southern blotting analysis of restriction enzyme digests with probes contained within the FMR-1 gene region.

It is also known that the method of PFGE can be used to detect variation at the fragile X locus.

In another embodiment the variation of the (CGG)_n repeat is measured by PCR. In this method the oligonucleotide primers are SEQ. ID. No. 10 and SEQ. ID. No. 11.

Another embodiment of the present invention is the cosmid probes shown in FIG. 4. These cosmid probes can be selected from the group consisting of C 22.3, C 34.4, C 31.4, C 4.1, C 34.3, C 26.3 C 19.1 and C14. 1. These cosmid clones are Sau 3A digests of the YAC 209G4. These digests were cloned into p2CpG. This results in inserts from 35–45 Kb. The ends are defined by their positions on the map of FIG. 4. These cosmid probes overlap the range in which the FMR-1 gene is located.

In detecting the fragile X sites the length of CA polymorphisms at the fragile X site can be measured by performing a PCR assay and measuring the length of the amplified products. In the PCR assay, the oligonucleotide primers are SEQ. ID. No. 6 and SEQ. ID. No. 7.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner. In the examples all percentages are by weight, if for solids and by volumes, if for liquids and all temperatures are in degrees Celsius unless otherwise noted.

EXAMPLE 1

Pulsed Field Gel Electrophoresis

Southern blot analysis of genomic DNA or YAC DNA resolved by PFGE was performed essentially as described (Smith, et al., Pulsed-field gel electrophoresis and the technology of large DNA molecules. In *Genome Analysis: A Practical Approach*; Oxford:IRB Press, pp.41–72, 1988). In this procedure, trypsinized and washed mammalian cells were suspended in molten agarose (final concentration 0.5% wt/vol; Baker) prepared in SE buffer (75 mM NaCl, 25 mM EDTA, pH 8.0) at a final concentration of 1.5×10^7 cells/ml. Chromosomal DNAs were isolated from YAC clones. Yeast cells from a 10 ml saturated culture were harvested, rinsed once in 50 mM EDTA, pH 8.0 and recovered in 0.5 ml SBE-zymolase (1 M sorbitol, 25 mM EDTA pH 8.0, 14 mM 2-mercaptoethanol, 1 mg/ml zymolase [1CN]). 0.5 ml 1% Seaplaque agarose (FMC) in SBE (without zymolase) was added and the suspension transferred to plug molds. Spheroplast generation (for yeast cells) was for 5 hours to overnight in SBE-zymolase. Cell lysis (mammalian or yeast cells) was for 2 days in ESP (0.5 M EDTA, pH 9.5, 1% N-lauroylsarcosine, 1 mg/ml proteinase K) at 50° C. Restriction endonuclease digestion was performed using the manufacturer's recommended buffers and conditions with a 50 μ l plug slice in 250 μ l of buffer containing 50 units of enzyme. For double digests, the plugs were rinsed and equilibrated, following digestion with the first enzyme, with the second buffer several times prior to digestion with the second enzyme. PFGE was carried out on a Bio-Rad Contour-Clamped Homogeneous Electric Field (CHEF) DRII apparatus through 1% agarose (BRL) at 200 V and 14° C. in 0.5 \times TBE buffer (45 mM Tris-borate, 1 mM EDTA). For resolution of fragments of \approx 200–1200 kb, switch time was 60 sec for 17 hrs followed by 90 sec for 10 hrs; for resolution of fragments \approx 10–500 kb, the switch times were ramped from 5 sec to 50 sec over 27 hrs. Southern blotting and

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hybridization were carried out as described in the art with the exception that acid depurination in 0.25 M HCl was allowed to proceed 20 min for pulsed-field gels. Radiolabeled probes were synthesized by random priming from 50 ng gel purified fragments except when intact cosmids were used which were nick translated (Boehringer Mannheim kit; following manufacturer's recommendations). For genomic probes containing repetitive elements, repeat suppression was accomplished by preassociation with 1–3 mg of sonicated human placental DNA in 100–300 μ l of 5 \times SSC (1 \times SSC is 150 mM NaCl, 15 mM NaCitrate, pH 7.0) for 3–10 min at 65° C. prior to the addition to the filter. Washing was carried out to a final stringency wash of 0.2 \times SSC for 15 min at 65° C. prior to autoradiography. *S. cerevisiae* strain YNN295 chromosomes (BioRad), concatamers of phage lambda (BioRad) or high molecular weight markers (BRL) were used as size standards.

EXAMPLE 2

PCR Analysis of DXS548 Alleles

Amplification was carried out on 0.2–0.5 μ g of genomic DNA in a 10 μ l total reaction containing 0.25 mM dNTPs, 40 ng of primers SEQ. ID. NO. 6 and SEQ. ID. No. 7, and 0.25 units of Taq polymerase in a buffer of 10 mM Tris-HCl, 50 mM KCl, 12 mM MgCl and 0.01% gelatin. Twenty three cycles of PCR were carried out in the following fashion; 3 cycles of 1 min each at 97° C., 62° C. annealing and 72° C. extension followed by 20 additional cycles with the annealing temperature lowered to 55° C. The reaction volume was then increased to 50 μ l with the same reaction components and concentrations except that one primer was 5' end-labelled with $Y^{32}P$ -ATP. PCR was continued for 10 cycles of 1 min each at 95° C. denaturation, 62° C. annealing and 72° C. extension. PCR products were analyzed by electrophoresis of 2 μ l of reaction through a 40 cm 6% polyacrylamide denaturing sequencing gel for approximately 2.25 hrs. The gel was dried without fixing and exposed to X-ray film overnight at room temperature.

EXAMPLE 3

Cosmid Library Construction of YAC 209G4

Agarose plugs (0.5% SeaPlaque FMC) containing 5–10 μ g of yeast DNA were prepared. 100 μ l blocks of DNA were equilibrated on ice in 0.5 ml of Mbo I digestion buffer, containing 0.1 mg/ml bovine serum albumin (BSA, MB grade; Boehringer Mannheim). After 2–3 hrs, the buffer was replaced by 150 μ l of fresh buffer to which Mbo I was added (0.0001–0.0007 units). Following overnight incubation on ice, digestion was carried out for 40 min at 37° C. The agarose blocks were melted, the DNA dephosphorylated with 1 unit calf intestinal alkaline phosphatase (Boehringer Mannheim), and treated with 2.5 units of agarase (Calbiochem). The solution was extracted twice with phenol/chloroform, once with chloroform, the DNA precipitated with ethanol and dissolved in 10 mM Tris, 0.1 mM EDTA (pH 7.4) at a concentration of 500 ng/ μ l. 250 ng of DNA was ligated to 500 ng of Bst BI (dephosphorylated) and Bam HI digested vector (p2CpG). Ligation and packaging was carried out according to standard procedures. Cosmids containing human inserts were selected by hybridizing with human specific Alu-repeat probe. These cosmids can be seen in FIG. 4.

EXAMPLE 4

YAC and Cosmid Subcloning

YACs were subcloned following isolation of the intact chromosome by preparative PFGE and EcoR I digestion of

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the DNA in molten agarose (Seaplaque; FMC). Fragments were phenol/chloroform extracted, ethanol precipitated, recovered and ligated into EcoR I cut, dephosphorylated, lambda ZAP II arms according to manufacturer's recommendations (Stratagene). Cosmids were subcloned following an alkaline lysis isolation and EcoR I digestion. Fragments were phenol/chloroform extracted and ethanol precipitated prior to ligation into lambda ZAP II arms as with YAC fragments. In the case of both cosmids and YACs, 75 ng EcoR I fragments were ligated to 1 ug vector arms. Selected phage were converted into pBluescript II SK-clones following in vivo excision of plasmid with insert according to manufacturer's guidelines.

EXAMPLE 5

cDNA Library Screening

A human fetal brain lambda gt11 cDNA library (Clontech, Palo Alto, Calif.) of 1.3×10^6 independent clones with insert lengths of 0.7–4.0 kb was used. The library was plated on 15 cm plates at a density of 50,000 pfu per dish using strain LE392. Filter lifts were prepared according to standard techniques and the library screened with cosmid DNA hexanucleotide labelled with ^{32}P -dATP and ^{32}P -dCTP. The labelled DNA was first prehybridized with 100 μg of total sheared human genomic DNA and 100 μg cosmid vector DNA in $5 \times \text{SSC}$ at 65°C . for 2 hrs. Following hybridization for 16 hrs, the filters were washed to a stringency of $0.1 \times \text{SSC}$. The filters were exposed to Fuji film with intensifying screens for 2 days at -80°C .

EXAMPLE 6

Fluorescent In Situ Hybridization

In situ hybridizations of total YAC-containing yeast DNA and cosmids were performed. Fragile X expression was induced by 96 hr culturing of lymphocytes (PHA stimulated from a male fragile X patient) in medium TC199 (Gibco) supplemented with 10% bovine fetal calf serum and, for the last 24 hrs, 10 $\mu\text{g}/\text{ml}$ methotrexate (Lederle). Chromosomes were prepared on slides using standard techniques.

Slides were washed with PBS and incubated for 1 hr at 37°C . in RNase A (100 $\mu\text{g}/\text{ml}$) in $2 \times \text{SSC}$. The slides were then incubated 10 min with pepsin (Serva; 0.1 mg/ml in 0.01 N HCL), fixed in 1% (vol/vol) in PBS, 50 mM MgCl_2 formaldehyde (Merck) and dehydrated in cold ethanol. Biotinylated total yeast and cosmid DNA were preannealed for 1–4 hrs in the presence of sonicated human genomic DNA and hybridized to the chromosomes overnight using 150 ng (yeast) or 40 ng (cosmid) of probe in 10 μl of 50% formamide, $2 \times \text{SSC}$, 10% dextran sulfate under an 18 mm² coverslip sealed with rubber cement. In some experiments, 2 ng/ μl pBamX5, a human repetitive sequence detecting the pericentromeric region of the human X, was separately denatured and added to the hybridization solution.

The signals were amplified by two layers of avidin-FITC (Vector) and one layer of biotinylated goat anti-avidin (Vector). The slides were then washed with PBS and mounted in antifade medium of 2% DABCO in glycerol containing propidium iodide (0.03 $\mu\text{g}/\text{ml}$). Microscopic analysis was performed with a Leitz Aristoplan microscope with FITC (K3 block) and DAPI (A block) detection. Photographs were made using Ektachrome 400 (Kodak) daylight slide film.

EXAMPLE 7

Northern Blot Analysis

Total RNA was extracted using guanidinium isothiocyanate followed by centrifugation through cesium chloride.

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Poly(A)⁺ RNA was selected by passage through oligo(dT) cellulose. Human brain, liver, and fetal poly(A) RNA was purchased from Clontech Laboratories (Palo Alto, Calif.).

Five μg of poly(A) containing RNA or 25 μg of total RNA were precipitated and dissolved in 20 μl of 50% (vol/vol) formaldehyde and $1 \times \text{MEN}$ (20 mM MOPS, pH 6.8, 5 mM sodium acetate, 1 mM EDTA) and incubated for 10 min at 60°C .; 5 μl of dye marker (50% sucrose, 0.5% bromophenolblue) was added and the samples were loaded on a formaldehyde-agarose gel. Electrophoresis was carried out for 3 hrs. at 100 V and the gel then soaked for 30 min in $20 \times \text{SSC}$ and blotted onto a nitrocellulose or nylon (GeneScreen Plus, Dupont) overnight in $10 \times \text{SSC}$ (Thomas, 1980). The RNA was fixed to the membranes by baking under vacuum for 2 hrs at 80°C . The membranes were prehybridized in 50% formamide, $5 \times \text{Denhart's}$, 50 mM sodium phosphate, pH 6.8, 10% dextran sulfate and 100 μg of denatured salmon sperm DNA at 42°C . for 2–4 hrs. Hybridization with the probe was for 16–20 hrs at 42°C . in the above buffer. Filters were washed with $3 \times \text{SSC}$, 0.1% SDS at 50°C . and then the SSC concentration was lowered according to the level of background, with a final wash in $0.1 \times \text{SSC}$, 0.1% SDS.

EXAMPLE 8

RT-PCR Quantitation of the FMR-1 Transcript

A PCR based test is devised in which the transcription product from the FMR-1 gene is quantitated with respect to an internal control (HPRT gene), in RNA samples from Fragile X and normal cell lines. In this method the total RNA was extracted from lymphoblastoid cell lines derived from Fragile X affected individuals and normal controls. The cDNA synthesis was performed in vitro from 5 μg of total RNA using oligo-dT and random primers via a reverse transcriptase reaction. Then PCR from single stranded cDNA was carried out using primers specific for the HPRT cDNA (SEQ. ID. Nos. 12 and 13) and primers specific for the FMR-1 cDNA (SEQ. ID. Nos. 8 and 9). The PCR conditions were as follows: 94°C . 1 min; 55°C . 1 min; 72°C . 1 min 45 sec; for 28 cycles and 7 min final extension at 72°C . The PCR products were run on an ABI Horizontal Electrophoresis device, by which the ethidium bromide stained products of each gene were exactly quantitated with respect to each other. Quantitative variations in the expression of the FMR-1 gene in Fragile X patients derived cell lines was then monitored.

EXAMPLE 9

Isolation of YACs Spanning the Fragile X Translocation Breakpoints

Through regional mapping of YAC clones containing DNA inserts derived from the distal human Xq, an 80 kb YAC (RS46) was found to map within Xq27.3 proximal to the fragile X-associated hybrid breakpoints. A 4.0 kb subclone (p46-1.1) of RS46 identified a normal 600 kb Sal I fragment on PFGE that was altered in size in 6 of 8 proximal translocation hybrids (FIG. 1). In FIG. 1, Y75-1B-M1 is a somatic cell hybrid containing the intact fragile X chromosome from which all other hybrids were derived. Lanes 2–9 are proximal translocation hybrids containing centric human Xpter→q27.3 translocated to different rodent chromosome arms. Q1Q and Q1V are distal translocation hybrids containing human Xq27.3→qter translocated to different centric rodent chromosome. The distal translocation hybrids have

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lost the human sequence detected by p46-1.1. Hybrids Y751B-7 and Y751B-14 show the same 600 kb Sal I fragment as the parental hybrid, however all other proximal translocation hybrids show variant bands indicating that probe p46-1.1 detects a sequence within 600 kb of these translocation breakpoints.

PFGE analyses of these hybrids, with more distant X-linked probes, showed identical band sizes and therefore similar methylation patterns as might be expected since the hybrids were all derived from the same parental fragile X somatic cell hybrid (Y75-1B-M1). These data suggest that in 75% of the proximal translocation hybrids, the human breakpoint is within the 600 kb Sal I fragment observed in the parental, intact fragile X hybrid. In the translocation hybrids, the distal human Sal I site is lost and replaced by heterologous translocations containing different rodent Sal I sites.

Since YAC RS46 does not hybridize to the DNA of the distal translocation hybrids and therefore does not cross these translocation breakpoints, additional YACs were sought of this region. A YAC library developed at the Human Polymorphism Study Center (CEPH) was screened using RS46 specific oligonucleotide primers SEQ. ID. Nos. 4 and 5 or SEQ. ID. Nos. 6 and 7. A YAC of 475 kb (209G4) was identified which completely overlaps YAC RS46 and includes sequences distal to the proximal translocation breakpoints which are present in 13 or 14 distal translocation breakpoints. YAC 209G4 encompasses 86% (19/22) of both the proximal and distal translocation breakpoints and thus identifies a fragile X-associated breakpoint cluster region. In situ hybridization using YAC 209G4 showed localization to the expressed fragile X site (FIG. 2). In FIG. 2, panel A represents the localization of YAC 209G4 to the expressed fragile X site. The centromeric signal is due to pBamX5, indicating the human X chromosome with slight hybridization to acrocentric chromosomes; Panel B shows a DAPI stained spread of panel A showing the expressed fragile X site; Panel C shows localization of cosmid 7.1 to the fragile X region; and finally, panel D shows localization of cosmid 22.3 to the fragile X region.

The signal includes both flanking boundaries of the isochromatid gap of the fragile site as well as the gap itself, suggesting the presence of uncondensed DNA within the fragile site and indicating that YAC 209G4 includes this region.

The close proximity of these YACs to the fragile X locus was independently supported by genetic linkage studies between a polymorphism identified in YAC RS46 and the fragile X locus. DXS548 is a dinucleotide repeat which reveals 9 alleles of variable length that are informative in >80% of fragile X families. In highly selected families previously shown to have crossovers with tightly linked flanking markers, DXS548 cosegregated, without recombination, with the fragile X locus (lod score of 6.95 at $\Theta=0$). As shown in FIG. 3, a carrier daughter and affected son are recombinant between the fragile X locus (FRAXA) and proximal markers DXS 539 (probe JH89) and DXS 369 (probe RN1) which map approximately 5 cM proximal to FRAXA with lod scores >40. The carrier mother shows two DXS 548 alleles at 196 and 194 bp (M1 and M2, respectively). The paternal 204 allele of the father is seen in the carrier daughter (II-1) who also inherited the maternal 196 bp allele. All three affected males inherited the 196 bp maternal allele (compare with the 194 allele of the normal daughter (II-5)). The carrier daughter (II-1) and affected son (II-2) are both recombinants between proximal markers DXS 150, DXS 369 and DXS 539. However, these indi-

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viduals are non-recombinant with DXS 548, placing this locus to the crossovers closer to the fragile X locus. Therefore, DXS 548 positions YACs RS46 and 209G4 near the mutation responsible for the clinical phenotype of the fragile X syndrome.

EXAMPLE 10

Physical Map of YAC 209 G4

A physical map of YAC 209G4 and of the corresponding genomic region was developed and is shown in FIG. 4. In FIG. 4(A), the physical map of the fragile X chromosome in the vicinity of the Fragile X locus is shown. The Sal I sites which give rise to the 600 kb fragment seen in hybrid Y75-1B-M1 probed with p46-1.1 and the normal 620 kb BssH II fragment observed in normal X chromosomes can be seen. The sites within the box are those previously shown to be methylated on the fragile X chromosome. The position and orientation of FMR-1 is shown.

In FIG. 4(B), a higher resolution physical map derived from both YAC inserts and genomic DNA is shown. Probe p46-1.1 and the DXS 548 loci are shown as are the positions of cDNAs and cosmids. YACs RS46 and 209G4 are shown below in alignment with the map (Hatched boxes indicate YAC vector sequences). The positions of the translocation breakpoints are shown as well as the orientation of the map relative to the X chromosome telomeres.

A CpG-island containing 5 infrequent-cleaving restriction endonuclease sites was identified 150 kb distal to CSX 548. This CpG-island appears hypermethylated on the fragile X chromosome. It is known in the art that there is an absence of a normal 620 kb BssH II fragment (FIG. 4A) in patients and most carriers of the fragile X syndrome. The absence of the fragment appears to be due to the methylation (and therefore resistance to cleavage) of the BssH II site (b in FIG. 4B) leading to a very large band which fails to resolve on PFGE. Since CpG-islands often are found 5' to mammalian genes and since methylation of such islands may influence expression of associated genes, it is possible a gene may reside nearby this fragile X-related CpG-island and its expression (or lack of) may be responsible for at least a portion of the fragile X phenotype.

EXAMPLE 11

Cosmid Contig Surrounding the Fragile X-Related CpG Island and Breakpoint Cluster Region

To characterize the region surrounding the CpG-island, a cosmid library was constructed from the yeast clone harboring YAC 209G4 and cosmids containing human DNA were identified by hybridization to human-specific repetitive elements. In situ hybridization with several human cosmids showed signals in (FIG. 2C) and on the edge (FIG. 2D) of the fragile X gap. A four cosmid contig was identified which spans the fragile X-related CpG island (FIG. 4B) from BssH II site a (cosmid 22.3) through BssH II site c (cosmid 4.1).

Cosmid 22.3 was found to include the breakpoints of 11 of 16 tested translocation hybrids (4/5 proximal translocations and 7/11 distal translocations; all 16 breakpoints map within YAC 209G4). As shown in FIG. 5A, nine bands (including doublet bands at 5.6 and 5.5 kb), surveying approximately 44 kb of genomic DNA, are observed on Southern analysis of EcoR I digested DNA of the intact fragile X hybrid (Y75-1B-M1) following hybridization with radiolabeled and preannealed cosmid 22.3. Of these nine bands, three are present in the distal Q1X (with a novel 4.8

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kb junctional fragment). The 7.4 kb band of the intact X hybrid Y75-1B-M1 is absent in both translocation hybrids indicating that both breakpoints fall within this interval. The other nine hybrids all exhibited patterns similar to either micro21D or Q1X, with distinct junctional fragments allowing identification of a fragile X-associated breakpoint cluster region (FXBCR) with this 7.4 kb fragment.

The 7.4 kb EcoR I fragment observed above on the fragile X chromosome was not observed in restriction digests of the overlapping cosmids 22.3 and 31.4. However, comparison of the cosmid restriction maps with the EcoR I fragments detected by c22.3 show a 5.1 kb fragment in the cosmids that is absent in Y75-1B-M1 and replaced by the 7.4 kb fragment. As shown in FIG. 6A, this 5.1 kb fragment contains the BssH II site b exhibiting fragile X specific hypermethylation. This fragment was subcloned from c31.4 and used to analyze hybrid breakpoints. As shown in FIG. 5B, the 5.1 kb fragment (pE5.1; FIG. 6B) hybridizes specifically to the 7.4 kb EcoR I fragment of the fragile X chromosome and clearly shows the junctional fragments in micro21D and Q1X. Thus a fragment length difference exists between the normal DNA used to construct YAC 209G4 and the fragile X chromosome of hybrid Y75-1B-M1, and this fragment identifies the FXBCR.

EXAMPLE 12**Fragile X Breakpoint Cluster Region Rearranged in Fragile X Patients**

The results of Southern hybridization of EcoR I digested DNA from two normal and seven unrelated fragile X individuals using pE5.1 as probe are shown in FIG. 7. In FIG. 7, Lanes 1, 6 and 7 demonstrate hybridization of the normal 5.1 kb EcoR I fragment in placental DNA (lane 1) and cloned into a cosmid (22.3) or YAC vector (209G4) and seeded into hamster DNA at single-copy level. Somatic cell hybrids containing portions of fragile X chromosomes in hamster backgrounds show bands of altered size from the normal 5.1 kb fragment. Lane 2 contains the hybrid X3000-11.1. Lane 3 contains DNA from micro28D, a proximal hybrid with a breakpoint distal to the fragile site and lane 4 contains DNA from miceo21D, a proximal hybrid with the same chromosome as micro28D, however with a breakpoint detected by pE5.1. Lane 5 contains hamster DNA. Lanes 8-12 contain DNA from 5 unrelated fragile X patients' lymphoblastoid lines. The bands altered from the normal 5.1 kb are seen in each fragile X sample.

The normal samples (two of five normal samples are shown) exhibit the expected 5.1 kb fragment while all seven fragile X patient DNAs exhibited larger EcoR I fragments with variable increases in size, including the 7.4 kb fragment observed from hybrid Y75-1B-M1. These data suggest an insertion or amplification event within the normal 5.1 kb fragment that is specific for the fragile X chromosome and is coincident with the fragile X-associated breakpoint cluster region and the fragile X-related CpG island.

EXAMPLE 13**Identification and Characterization of FMR-1**

In order to search for transcripts associated with the fragile X region, the cosmid subclones of YAC 209G4 were used as hybridization probes to screen a cDNA library derived from normal human fetal brain RNA. Cosmid 4.1, containing BssH II site c (FIG. 4B), identified cDNA clone BC22. A map of FMR-1 cDNA clones is shown in FIG. 8.

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Restriction digestion and sequence analysis revealed an insert in BC22 of 2835 bp at location 934 to 3765 of SEQ. ID. No. 1, with an open reading frame at one end extending 1033 bp to a stop codon. Since the reading frame remains open at the 5' end of the clone, BC22 was used to identify related cDNAs from the same library. Several overlapping clones were isolated, one of which, BC72, was characterized in greater detail. This clone extended the cDNA sequence another 933 bp in the 5' direction, and overlapped BC22 for approximately 2000 bp toward the 3' end. Sequence analysis demonstrated that the same reading frame remained open through the 5' end of BC72, indicating that the 5' end of the mRNA has not yet been reached, and allowing prediction of a portion (657 amino acids) of the encoded protein. It remains unclear if the entire 3' portion also was isolated since no poly(A) tract was found at the end of BC22, however a putative polyadenylation addition signal is observed in position 3741 following numerous in frame stop codons. In SEQ. ID. No. 1, nucleotides 1-1027 derive from BC72 and nucleotides 934-3765 are from BC22.

A repeated DNA sequence is found close to the 5' end of BC72 with 28 CGG triplets interspersed with two AGG triplets. This CGG repeat encoding 30 contiguous arg residues begins with base 37 and extends to base 127. In the predicted open reading frame, this repeat would generate a protein domain composed of 30 contiguous arginine residues. Homology searches with the predicted protein sequence identify significant overlaps with a number of arginine-rich proteins, although none contain a polyarginine stretch of equivalent length. The remainder of the protein shows no significant homology in protein database searches. However, searches against DNA sequence databases identify several related sequences, the strongest of which is with the human androgen receptor (AR). This is an X-linked gene (mapping to Xq12) with an identical, though smaller, CGG repeat in the first exon which encodes a polyglycine stretch.

EXAMPLE 14**Northern Hybridization**

Northern hybridization using the BC22 insert as probe was run. (FIG. 9). Five μ g of poly(A) selected RNA from human brain (lane 1) and normal placenta (lane 2) were electrophoresed, blotted onto a GeneScreen Plus filter and hybridized with radiolabeled BC22 insert. A single hybridizing species of approximately 4.8 kb is observed in each lane. As seen in FIG. 9, this procedure detects a mRNA of approximately 4.8 kb in human brain and placenta. This indicates that the 3.8 kb of cDNA obtained does not contain the entire mRNA of this gene. The probe failed to detect signal in human liver, fetal lung and fetal kidney but did detect message in lymphocytes.

EXAMPLE 15**Zoo Blot Analysis**

Hybridization of BC22 to DNA samples isolated from a number of different organisms was run (FIG. 10). Ten μ g of DNA from each species was cleaved with EcoRI and electrophoresed and blotted onto a nylon membrane. Hybridization was carried out with labelled cDNA overnight using standard conditions and washed to a final stringency of 0.2 \times SSC for 5 min at 65 $^{\circ}$ C. Hybridization signals were observed with all organisms with the exception of *Drosophila melanogaster*. Since this blot was washed under very stringent conditions (final wash in 0.2 \times SSC at 65 $^{\circ}$ C. for 5

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min), cross hybridization may be observed in *Drosophila* under less stringent conditions. However, the high stringency of the final wash does indicate the highly conserved nature of this sequence particularly in *C. elegans*.

EXAMPLE 16**Location of FMR-1 Gene Relative to the Fragile X-Related CpG Island and FXBCR**

BC22 demonstrates hybridization to the 70 kb fragment of YAC 209G4 between BssH II sites b and c as well as to cosmids 4.1, 34.4, 31.4 and 22.3 (FIG. 4), indicating exons spanning over 80 kb of DNA. The proximal/distal orientation of the transcript was determined by hybridizing end fragments of BC22 to the cosmid contig. Since the 3' end of BC22 detected cosmid 4.1 and the 5' end detected cosmid 22.3, the transcriptional orientation was distal from BssH II site b toward the Xq telomere. This suggests the potential involvement of the fragile X-related CpG island in the regulation of this gene. A 1 kb 5' fragment of BC72 (to the Hind III site at position 1026 of SEQ. ID. No. 1) was used to study the location of the exons encoding this portion of the mRNA in the cosmid and YAC clones. In cosmid 22.3, this probe identifies three EcoR I fragments (FIG. 6A) distal to the BssH II site b. One of the fragments contains the BssH II site (b) as well as the breakpoint cluster region and exhibits length variation in fragile X patients. Restriction mapping and direct sequencing of the 5.1 kb EcoR I fragment using a primer derived from BC72 sequence (position 223 to 246) demonstrated an exon immediately distal to the BssH II site b. This exon contains an Xho I site (position 137 in FMR-1 cDNA sequence) that is found 310 nucleotides from the BssHII II site in genomic DNA (FIG. 6B). This exon also contains the block of CGG repeats which are seen in the sequence analysis of the genomic DNA as well. Thus the CGG repeat block is found within the fragile X-related CpG island and constitutes a portion of this CpG-rich region.

EXAMPLE 17**A PCR Assay to Determine Fragile X Disease**

A PCR based test is devised in which the length of genomic DNA at the fragile X site from an individual is determined. In this method the total DNA was extracted from lymphoblastoid cells from fragile X and normal individuals. Oligonucleotide primers (SEQ. ID. No. 10 and SEQ. ID. No. 11) were used in PCR using the following conditions: 94° C. 1 min. 72° 2 min. for 50 cycles and a 7 min final extension at 72° C. The use of 10% dimethylsul-

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foxide in the reaction is important for enhancing the ability to amplify this GC-rich sequence. The PCR products are visualized after size separation by electrophoresis using ethidium bromide staining. Differences in size between PCR products from normal and fragile X samples are observed, and these correspond to variation in the number of CGG repeats present.

EXAMPLE 18**Elucidation of Fragile X Site**

To elucidate the fragile X site at the molecular level, somatic cell hybrids were isolated that contained translocations between rodent chromosomes and the human fragile X chromosome, retaining either human Xpter→q27.3 or human Xq27.3→qter, referred to as proximal or distal translocations, relative to the fragile X site. Since the high frequency and specificity of the chromosome breakage was not observed in normal X hybrids and since the translocation breakpoints map within the same interval defined by polymorphic loci which flank the fragile X locus, these breakpoints are likely to coincide with the fragile X site.

A yeast artificial chromosome (YAC) has been isolated which spans some of these translocation breakpoints and includes polymorphic loci which flank the fragile X locus. Within this region, a fragile X-related CpG island was identified which is aberrantly hypermethylated in patients and most carriers of the fragile X syndrome. Although the significance of this CpG-island hypermethylation remains unclear, these data do imply the presence of a gene, perhaps inactivated by methylation, within a genomic region which includes the fragile X-associated hybrid breakpoints.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual patent or publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and attain the ends and advantages mentioned as well those inherent therein. The sequences, methods, procedures and techniques described herein are presently representative of the preferred embodiments and are intended to be exemplary and not intended as limitations on the scope. Changes therein and other uses which are encompassed within the spirit of the invention or defined by the scope of the appended claims will occur to those skilled in the art.

SEQUENCE LISTING**(1) GENERAL INFORMATION:**

(iii) NUMBER OF SEQUENCES: 14

(2) INFORMATION FOR SEQ ID NO:1:**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 3765 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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-continued

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACGGAGGCG CCCGTGCCAG GGGCGTGC GACGCGCGG GCGGGCGGCG GCGGCGGCGG 60

CGGCGGAGGC GCGGCGGCG GCGGCGGCG CGGCGGAGGC GCGGCGGCG GCGGCGGCGG 120

CGGCGGCTGG GCCTCGAGCG CCCGCAGCCC ACCTCTCGGG GCGGGGCTCC GCGGCTAGC 180

AGGGCTGAAG AGAAGATGGA GGAGCTGGTG GTGGAAGTGC GGGGCTCCAA TGGCGCTTTC 240

TACAAGGCAT TTGTAAAGGA TGTTTCATGAA GATTCAATAA CAGTTGCATT TGAAAAAAC 300

TGGCAGCCTG ATAGGCAGAT TCCATTTTCAT GATGTCAGAT TCCCACCTCC TGTAGGTAT 360

AATAAAGATA TAAATGAAAG TGATGAAGTT GAGGTGTATT CCAGAGCAA TGA AAAAAGAG 420

CCTTGCTGTT GGTGGTTAGC TAAAGTGAGG ATGATAAAGG GTGAGTTTTA TGTGATAGAA 480

TATGCAGCAT GTGATGCAAC TTACAATGAA ATTGTCACAA TTGAACGTCT AAGATCTGTT 540

AATCCCAACA AACCTGCCAC AAAAGATACT TTCCATAAGA TCAAGCTGGA TGTGCCAGAA 600

GACTTACGGC AAATGTGTGC CAAAGAGGCG GCACATAAGG ATTTTAAAAA GGCAGTTGGT 660

GCCTTTTCTG TAACTTATGA TCCAGAAAAT TATCAGCTTG TCATTTTGTG CATCAATGAA 720

GTCACCTCAA AGCGAGCACA TATGCTGATT GACATGCACT TTCGGAGTCT GCGCACTAAG 780

TTGTCTCTGA TAATGAGAAA TGAAGAAGCT AGTAAGCAGC TGGAGAGTTC AAGGCAGCTT 840

GCCTCGAGAT TTCATGAACA GTTTATCGTA AGAGAAGATC TGATGGGTCT AGCTATTGGT 900

ACTCATGGTG CTAATATTC ACAAGCTAGA AAAGTACCTG GGGTCACTGC TATTGATCTA 960

GATGAAGATA CCTGCACATT TCATATTTAT GGAGAGGATC AGGATGCAGT GAAAAAAGCT 1020

AGAAGCTTTC TCGAATTTGC TGAAGATGTA ATACAAGTTC CAAGGAAGCT AGTAGTAATA 1080

GGAAAAAATG GAAAGCTGAT TCAGGAGATT GTGGACAAGT CAGGAGTTGT GAGGGTGAGG 1140

ATTGAGGCTG AAAATGAGAA AAATGTTCCA CAAGAAGAGG AAATTATGCC ACCAAATTC 1200

CTTCTTCCA ATAATTC AAG GTTTGGACCT AATGCCCAG AAGAAAAAA ACATTTAGAT 1260

ATAAAGGAAA ACAGCACCCA TTTTCTCAA CCTAACAGTA CAAAAGTCCA GAGGGTATG 1320

GTACCATTG TTTTGTGGG AACAAAGGAC AGCATCGCTA ATGCCACTGT TCTTTTGGAT 1380

TATCACCTGA ACTATTTAAA GGAAGTAGAC CAGTTGCGTT TGGAGAGATT ACAAATTGAT 1440

GAGCAGTTGC GACAGATTGG AGCTAGTTCT AGACCACCAC CAAATCGTAC AGATAAGGAA 1500

AAAAGCTATG TGAATGATGA TGGTCAAGGA ATGGGTCGAG GTAGTAGACC TTACAGAAAT 1560

AGGGGCGCAG GCAGACGCGG TCCTGGATAT ACTTCAGGAA CTAATTCCTGA AGCATCAAAT 1620

GCTTCTGAAA CAGAATCTGA CCACAGAGAC GAACTCAGTG ATTGGTCATT AGCTCCAACA 1680

GAGGAAGAGA GGGAGAGCTT CTGCGCAGA GGAGACGGAC GCGGCGTGG AGGGGAGGA 1740

AGAGACAAG GAGGAAGAGG ACGTGGAGGA GGC'TTCAAAG GAAACGACGA TCACTCCCGA 1800

ACAGATAATC GTCCACGTAA TCCAAGAGAG GCTAAAGGAA GAACAACAGA TGGATCCCTT 1860

CAGAATACCT CCAGTGAAGG TAGTCGGCTG CGCACGGGTA AAGATCGTAA CCAGAAGAAA 1920

GAGAAGCCAG ACAGCGTGA TGGTCAAGCA CCACTCGTGA ATGGAGTACC CTA AACTGCA 1980

TAATTCGAA GTTATATTTCT TATAACCAT TCCGTAATTC TTATTCATA TTAGAAAACT 2040

TTGTTAGGCC AAAGACAAAT AGTAGCAAG ATGGCACAGG GCATGAAATG AACACAAAT 2100

ATGCTAAGAA TTTTATTTT TTTGGTATTG GCCATAAGCA ACAATTTTCA GATTTGCACA 2160

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AAAAGATACC TTAAAATTTG AAACATTGCT TTTAAAAC TA CTTAGCACTT CAGGGCAGAT 2220
TTTAGTTTTA TTTTCTAAAG TACTGAGCAG TGATATTCTT TGTTAATTTG GACCATTTTC 2280
CTGCATTGGG TGATCATTCA CCAGTACATT CTCAGTTTTT CTTAATATAT AGCATTATAG 2340
GTAATCATAT TAGACTTCTG TTTTCAATCT CGTATAGAAG TCTTCATGAA ATGCTATGTC 2400
ATTTTCATGC CTGTGTCAGT TTATGTTTTG GTCCACTTTT CCAGTATTTT AGTGGACCCT 2460
GAAATGTGTG TGATGTGACA TTTGTCAATTT TCATTAGCAA AAAAAGTTGT ATGATCTGTG 2520
CCTTTTTTAT ATCTTGGCAG GTAGGAATAT TATATTTGGA TGCAGAGTTC AGGGAAGATA 2580
AGTTGGAAC ACTAAATGTT AAAGATGTAG CAAACCCTGT CAAACATTAG TACTTTATAG 2640
AAGAATGCAT GCTTCCATA TTTTTCCTT TACATAAACA TCAGGTTAGG CAGTATAAAG 2700
AATAGGACTT GTTTTTGTTT TTGTTTTGTT GCACTGAAGT TTGATAAATA GTGTTATTGA 2760
GAGAGATGTG TAATTTTTCT GTATAGACAG GAGAAGAAAG AACTATCTTC ATCTGAGAGA 2820
GGCTAAAATG TTTTCAGCTA GGAACAAATC TTCCTGGTCG AAAGTTAGTA GGATATGCCT 2880
GCTCTTTGGC CTGATGACCA ATTTTAACCT AGAGCTTTTT TTTTAAATTT TGTCTGCCCC 2940
AAGTTTTGTG AAATTTTTCA TATTTTAATT TCAAGCTTAT TTTGGAGAGA TAGGAAGGTC 3000
ATTTCCATGT ATGCATAATA ATCCTGCAAA GTACAGGTAC TTTGTCTAAG AAACATTGGA 3060
AGCAGGTTAA ATGTTTTGTA AACTTTGAAA TATATGGTCT AATGTTTAAAG CAGAATTGGA 3120
AAAGACTAAG ATCGGTTAAC AAATAACAAC TTTTTTTTCT TTTTTTCTTT TGTTTTTTGA 3180
AGTGTGGGG TTTGGTTTTG TTTTGTGAGT CTTTTTTTTT TAAGTAAAT TTATTGAGGA 3240
AAAATATGTG AAGGACCTTC ACTCTAAGAT GTTATATTTT TCTTAAAAAG TAACTCCTAG 3300
TAGGGGTACC ACTGAATCTG TACAGAGCCG TAAAACTGA AGTCTGCCT CTGATGTATT 3360
TTGTGAGTTT GTTCTTTTGA ATTTTCATTT TACAGTTACT TTTCTTGC TAACAAACAAG 3420
CATATAAAAT GGCAACAAAC TGCACATGAT TTCACAAATA TTAAAAAGTC TTTTAAAAAG 3480
TATTGCCAAA CATTAATGTT GATTCTAGT TATTTATTCT GGAATGTAT AGTATTTGAA 3540
AACAGAAAT GGTACCTTGC ACACATCATC TGTAAGCTGT TTGGTTTAA AATACTGTAG 3600
ATAATTAACC AAGGTAGAAT GACCTGTAA TGTAAGTCT CTTGGGCAAT ATTCTCTGTA 3660
CATATTAGCG ACAACAGATT GGATTTTATG TTGACATTTG TTTGGTTATA GTGCAATATA 3720
TTTTGTATGC AAGCAGTTTC AATAAAGTTT GATCTTCCTC TGCTA 3765
    
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4242 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

ACTTGGAGGG GTATAATCAT TCTAATCAAT GTGTCCCCTT TTAATAAAT ACATTGGAGT 60
TGCAGCTAAT GCTCTGTCTC CATTACAGCCT ATGATGAGAT TCTCTTTCAG CCCTATTGGG 120
TTCTTGGCCT CATGTGACTA CTCCAAGAC CCTAGTCCAA AAGGTCTTTC CTGTTTGCTA 180
TGGCCTTGAG GAATGTGGCC CTAGATCCAC CGCTTTAAAG CTGGAGTTCC ACCAGCAGCA 240
ACATCCTCTC ATTTCTGGGG ACCTGCCTGG GGCAGGTCAT CCTGCCTCTG CCAACTCAGT 300
    
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GCTATTAGTT	AACTCTCACC	TGCCATATTC	CAGCTGGAAT	CATCTCCCCT	TCTCCACCCC	360
AGACTAGGTC	ATGTTCCGCC	ATCATGGAAG	CGCCTATTCT	TCATACCCCT	TATCACAGCT	420
GCAACTACTC	ATTTACTTGT	CTGACAATTT	GATTIATGTC	CACCTACTTT	GCTAGGTACT	480
AAGTTCAATG	CTGGCAGTCG	TTTCTTCTTT	TTTTTCTTTT	TCTGTTTTCG	TCACCGATTT	540
CTCGTTAGCA	CTTAGCACAG	TGTCTGGCAC	ACGATAGATG	CTCCGTCAAC	TTCTCAGTTG	600
GATACCAGCA	TCCCGAAGGG	ACATGGATTA	AGGCAGCTAT	AAGCACGGTG	TAAAAACAGG	660
AATAAGAAAA	AGTTGAGGTT	TGTTTCACAG	TGGAATGTAA	AGGGTTGCAA	GGAGGTGCAT	720
CGGCCCTGT	GGACAGGACG	CATGACTGCT	ACACACGTGT	TCACCCACC	CTCTGGCACA	780
GGGTGCACAT	ACAGTAGGGG	CAGAAATGAA	CCTCAAGTGC	TTAACACAAT	TTTTAAAAAA	840
TATATAGTCA	AGTGAAAGTA	TGAAAATGAG	TTGAGGAAAG	GCGAGTACGT	GGGTCAAAGC	900
TGGGTCTGAG	GAAAGGCTCA	CATTTTGAGA	TCCCGACTCA	ATCCATGTCC	CTTAAAGGGC	960
ACAGGGTGTG	TCCACAGGGC	CGCCAAAAT	CTGGTGAGAG	AGGGCGTAGA	CGCCTCACCT	1020
TCTGCCTCTA	CGGGTCACAA	AAGCCTGGGT	CACCCTGGTT	GCCACTGTTC	CTAGTTCAAA	1080
GTCTTCTTCT	GTCTAATCCT	TCACCCTAT	TCTCGCCTTC	CACTCCACCT	CCCGCTCAGT	1140
CAGACTGCGC	TACTTTGAAC	CGGACCAAAC	CAAACCAAAC	CAAACCAAAC	CAAACCAGAC	1200
CAGACACCCC	CTCCCGCGGA	ATCCAGAGA	GGCCGAACTG	GGATAACCGG	ATGCATTTGA	1260
TTTCCCACGC	CACTGAGTGC	ACCTCTGCAG	AAATGGGCGT	TCTGGCCCTC	GCGAGGCAGT	1320
GCGACCTGTC	ACCGCCCTTC	AGCCTTCCCG	CCCTCCACCA	AGCCCGCGCA	CGCCCGGCC	1380
GCGCGTCTGT	CTTTCGACCC	GGCACCCCGG	CCGGTTCCCA	GCAGCGCGCA	TGCGCGCGCT	1440
CCCAGGCCAC	TTGAAGAGAG	AGGGCGGGGC	CGAGGGGCTG	AGCCCGCGGG	GGGAGGGAAC	1500
AGCGTTGATC	ACGTGACGTG	GTTTCAGTGT	TTACACCCGC	AGCGGGCCGG	GGGTTCCGGC	1560
TCAGTCAGGC	GCTCAGCTCC	GTTTCGGTTT	CACTTCCGGT	GGAGGGCCGC	CTCTAGCGGG	1620
CGGGGGGCGC	ACGGCGAGCG	CGGGCGGGCG	CGGTGACGGA	GGCGCCGCTG	CCAGGGGGCG	1680
TGCGGCAGCG	CGGCGGCGGC	GGCGGCGGCG	GCGGCGGCGG	CGGCGGCGGC	GGCGGCGGCT	1740
GGGCCTCGAG	CGCCCGCAGC	CCACCTCTTG	GGGGCGGGCT	CCCGGCGCTA	CAGGGCTGAA	1800
GAGAAGATGG	AGGAGCTGGT	GGTGAAGTG	CGGGCTCCAA	TGGCGCTTTC	TACAAGGTAC	1860
TTGGCTCTAG	GGCAGGCCCC	ATCTTCGCCC	TTCCTTCCCT	CCCTTTTTC	TTGGTGTCCG	1920
CGGGAGGCAG	GCCCGGGGCC	CTCTTCCCGA	GCACCGCGCC	TGGGTGCCAG	GGCACGCTCG	1980
GCGGGATGTT	GTTGGGAGGG	AAGGACTGGA	CTTGGGGCCT	GTTGGAAGCC	CCTCTCCGAC	2040
TCCAGAGGCC	CTAGCGCCTA	TCGAAATGAG	AGACCAGCGA	GGAGAGGGTT	CTCTTTCGGC	2100
GCCGAGCCCC	GCCGGGGTGA	GCTGGGGATG	GGCGAGGGCC	GGCGGCAGGT	ACTAGAGCCG	2160
GGCGGGAAGG	GCCGAAATCG	GCGCTAAGTG	ACGGCGATGG	CTTATTCCCC	CTTTCCTAAA	2220
CATCATCTCC	CAGCGGGATC	CGGGCCTGTC	GTGTGGGTAG	TTGTGGAGGA	GCGGGGGGCG	2280
CTTCAGCCGG	GCCGCCCTCT	GCAGCGCCAA	GAGGGCTTCA	GGTCTCCTTT	GGCTTCTCTT	2340
TTCCGGTCTA	GCATTGGGAC	TTCGGAGAGC	TCCACTGTTC	TGGGCGAGGG	CTGTGAAGAA	2400
AGAGTAGTAA	GAAAGCGTAG	TCGGCACCAA	ATCACAAATG	CAACTGATTT	TTAGTGGCTT	2460
CTCTTTGTGG	ATTTTCGAGG	AGATTTTAGA	TCCAAAAGTT	TCAGGAAGAC	CCTAACATGG	2520
CCCAGCAGTG	CATTGAAGAA	GTGTATCATC	GTGAATATTC	GCGTCCCCCT	TTTTGTAAAA	2580
CGGGTAAAT	TCAGGAATGC	ACATGCTTCA	GCGTCTAAAA	CCATTAGCAG	CGTGCTACT	2640

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TAAAAATTGT	GTGTGTGTGT	TTAAGTTTCC	AAAGACCTAA	ATATATGCCA	TGAAACTTCA	2700
GGTAATTAAC	TGAGAGTATA	TTATTACTAG	GGCATTTTTT	TTTAACTGA	GCGAAAATAT	2760
TTTTGTGCC	CTAAGAAGCT	GACCACATTT	CCTTTGAATT	TGTGGTGTG	CAGTGGACTG	2820
AATTGTTGAG	GCTTTATATA	GGCATTTCATG	GGTTTACTGT	GCTTTTTAAA	GTTACACCAT	2880
TGCAGATCAA	CTAACACCTT	TCAGTTTTAA	AAGGAAGATT	TACAAAATTG	ATGTAGCAGT	2940
AGTGCCTTTG	TTGGTATGTA	GGTGTGTAT	AAATTCATCT	ATAAATTCCTC	ATTCCTTTTT	3000
GAATGTCTAT	AACTCTTTTC	AATAATATCC	CACCTTACTA	CAGTATTTTG	GCAATAGAAG	3060
GTGCGTGTGG	AAGGAAGGCT	GGAAAATAGC	TATTAGCAGT	GTCCAACACA	ATTCTTAAAT	3120
GTATTGTAGA	ATGGCTTGAA	TGTTTCAGAC	AGGACACGTT	TGGCTATAGG	AAAATAAACA	3180
ATTGACTTTA	TTCTGTGTTT	ACCAATTTTA	TGAAGACATT	TGGAGATCAG	TATATTTTCT	3240
AAATGAGTAA	AGTATGTAAA	CTGTTCCATA	CTTTGAGCAC	AAAGATAAAG	CCTTTTGCTG	3300
TAAAAGGAGG	CAAAGGTAA	CCCCGCGTTT	ATGTTCTTAA	CAGTCTCATG	AATATGAAAT	3360
TGTTTCAGTT	GACTCTGCAG	TCAAAATTTT	AATTCATTG	ATTTTATGA	TCCATAATTT	3420
CTTCTGGTGA	GTTTGCCTAG	AATCGTTCAC	GGTCCTAGAT	TAGTGGTTTT	GGTCACTAGA	3480
TTTCTGGCAC	TAATAACTAT	AATACATATA	CATATATATG	TGTGAGTAAC	GGCTAATGGT	3540
TAGGCAAGAT	TTTGATTGAC	CTGTGATATA	AACCTAGATT	GGATGCCACT	AAAGTTTGCT	3600
TATCACAGAG	GGCAAGTAGC	ACATTATGGC	CTTGAAGTAC	TTATTGTTCT	CTTCCAGCAA	3660
CTTATGATTT	GCTCCAGTGA	TTTTCTTGCA	CACTGACTGG	AATATAAGAA	ATGCCTTCTA	3720
TTTTTGCTAT	TAATCCCTC	CTTTTTTGTT	TTGTTTTGTA	ACGAAGTTGT	TTAACTTGAA	3780
GGTGAATGAA	GAATAGTTG	GTGCCCCCTT	AGTCCCTGA	GGAGAAATGT	TAATACTTGA	3840
ACAAGTGTGT	GTCAGACAAA	TTGCTGTTAT	GTTTATTTAA	TTAAGTTTGA	TTTCTAAGAA	3900
AATCTCAAAT	GGTCTGCACT	GATGGAAGAA	CAGTTTCTGT	AACAAAAAAG	CTTGAAATTT	3960
TTATATGACT	TATAAATACTG	CTGTGAGTTT	TAAAAGTAAA	GCAAAAGTAA	ACTGAGTTGC	4020
TTGTCCAGTG	GGATGGACAG	GAAAGATGTG	AAATAAAAAC	CAATGAAAAA	TGAAC TGCTG	4080
TGGAGAAGTG	TTACATTTAT	GGAAAAAGAA	ATAGGAACCT	TGTTTCATCAA	ATTGATAGAA	4140
AAGCTTTTAA	AACTAAACAA	ATCAAACAAC	TTGAGTATAA	TGGAATTCAG	GTAAGCTATC	4200
TTGAAAGGGG	AAATATCAAA	AGCTAGAGAT	CAGAGTAAGG	CT		4242

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 229 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCAGGT	AAGCTATCTT	GAAAGGGGAA	ATATCAAAAG	CTAGAGATCA	GAGTAAGGCT	60
GAGACTCAGA	GTCAAGTGGG	GAAGACTAAG	TTGCAGTATG	TACTGGCAGT	GAAGATAAGT	120
ATTTATTCAT	TCATTGAACA	TACCTTGAAA	TCAACCACTT	TTAATGTGCC	AGGGACACAA	180
AGATAGAAAA	GACATTTGCC	CTGTCTGGAA	GGTACTAATA	ATCCAATAA		229

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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTTGCCAACC GTTCAGCCAC

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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATTCCTGGA GCACAGACTG

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(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGAGCTTCAC TATGCAATGG AATC

24

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTACATTAGA GTCACCTGTG GTGC

24

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
TAGCTAACCA CCAACAGCAA GGC 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
AACTGGCAGC CTGATAGGCA GATTC 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GCTCAGCTCC GTTTCGGTTT CACTTCCGGT 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
AGCCCCGCAC TTCCACCACC AGCTCCTCCA 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
CGTGGGGTCC TTTTCACCAG CAAG 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATTATGGAC AGGACTGAAC GTC

23

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 657 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Gly Gly Ala Arg Ala Arg Gly Arg Ala Ala Ala Arg Arg Arg Arg
 1 5 10 15
 Arg
 20 25 30
 Arg Leu Gly Leu Glu Arg Pro
 35 40 45
 Gln Pro Thr Ser Arg Gly Arg Ala Pro Gly Ala Ser Arg Ala Glu Glu
 50 55 60
 Lys Met Glu Glu Leu Val Val Glu Val Arg Gly Ser Asn Gly Ala Phe
 65 70 75 80
 Tyr Lys Ala Phe Val Lys Asp Val His Glu Asp Ser Ile Thr Val Ala
 85 90 95
 Phe Glu Asn Asn Trp Gln Pro Asp Arg Gln Ile Pro Phe His Asp Val
 100 105 110
 Arg Phe Pro Pro Pro Val Gly Tyr Asn Lys Asp Ile Asn Glu Ser Asp
 115 120 125
 Glu Val Glu Val Tyr Ser Arg Ala Asn Glu Lys Glu Pro Cys Cys Trp
 130 135 140
 Trp Leu Ala Lys Val Arg Met Ile Lys Gly Glu Phe Tyr Val Ile Glu
 145 150 155 160
 Tyr Ala Ala Cys Asp Ala Thr Tyr Asn Glu Ile Val Thr Ile Glu Arg
 165 170 175
 Leu Arg Ser Val Asn Pro Asn Lys Pro Ala Thr Lys Asp Thr Phe His
 180 185 190
 Lys Ile Lys Leu Asp Val Pro Glu Asp Leu Arg Gln Met Cys Ala Lys
 195 200 205
 Glu Ala Ala His Lys Asp Phe Lys Lys Ala Val Gly Ala Phe Ser Val
 210 215 220
 Thr Tyr Asp Pro Glu Asn Tyr Gln Leu Val Ile Leu Ser Ile Asn Glu
 225 230 235 240
 Val Thr Ser Lys Arg Ala His Met Leu Ile Asp Met His Phe Arg Ser
 245 250 255
 Leu Arg Thr Lys Leu Ser Leu Ile Met Arg Asn Glu Glu Ala Ser Lys
 260 265 270

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Gln Leu Glu Ser Ser Arg Gln Leu Ala Ser Arg Phe His Glu Gln Phe
 275 280 285

Ile Val Arg Glu Asp Leu Met Gly Leu Ala Ile Gly Thr His Gly Ala
 290 295 300

Asn Ile Gln Gln Ala Arg Lys Val Pro Gly Val Thr Ala Ile Asp Leu
 305 310 315 320

Asp Glu Asp Thr Cys Thr Phe His Ile Tyr Gly Glu Asp Gln Asp Ala
 325 330 335

Val Lys Lys Ala Arg Ser Phe Leu Glu Phe Ala Glu Asp Val Ile Gln
 340 345 350

Val Pro Arg Asn Leu Val Val Ile Gly Lys Asn Gly Lys Leu Ile Gln
 355 360 365

Glu Ile Val Asp Lys Ser Gly Val Val Arg Val Arg Ile Glu Ala Glu
 370 375 380

Asn Glu Lys Asn Val Pro Gln Glu Glu Glu Ile Met Pro Pro Asn Ser
 385 390 395 400

Leu Pro Ser Asn Asn Ser Arg Val Gly Pro Asn Ala Pro Glu Glu Lys
 405 410 415

Lys His Leu Asp Ile Lys Glu Asn Ser Thr His Phe Ser Gln Pro Asn
 420 425 430

Ser Thr Lys Val Gln Arg Gly Met Val Pro Phe Val Phe Val Gly Thr
 435 440 445

Lys Asp Ser Ile Ala Asn Ala Thr Val Leu Leu Asp Tyr His Leu Asn
 450 455 460

Tyr Leu Lys Glu Val Asp Gln Leu Arg Leu Glu Arg Leu Gln Ile Asp
 465 470 475 480

Glu Gln Leu Arg Gln Ile Gly Ala Ser Ser Arg Pro Pro Pro Asn Arg
 485 490 495

Thr Asp Lys Glu Lys Ser Tyr Val Thr Asp Asp Gly Gln Gly Met Gly
 500 505 510

Arg Gly Ser Arg Pro Tyr Arg Asn Arg Gly His Gly Arg Arg Gly Pro
 515 520 525

Gly Tyr Thr Ser Gly Thr Asn Ser Glu Ala Ser Asn Ala Ser Glu Thr
 530 535 540

Glu Ser Asp His Arg Asp Glu Leu Ser Asp Trp Ser Leu Ala Pro Thr
 545 550 555 560

Glu Glu Glu Arg Glu Ser Phe Leu Arg Arg Gly Asp Gly Arg Arg Arg
 565 570 575

Gly Gly Gly Gly Arg Gly Gln Gly Gly Arg Gly Arg Gly Gly Gly Phe
 580 585 590

Lys Gly Asn Asp Asp His Ser Arg Thr Asp Asn Arg Pro Arg Asn Pro
 595 600 605

Arg Glu Ala Lys Gly Arg Thr Thr Asp Gly Ser Leu Gln Asn Thr Ser
 610 615 620

Ser Glu Gly Ser Arg Leu Arg Thr Gly Lys Asp Arg Asn Gln Lys Lys
 625 630 635 640

Glu Lys Pro Asp Ser Val Asp Gly Gln Gln Pro Leu Val Asn Gly Val
 645 650 655

Pro

What is claimed is:

1. A method of detecting Fragile X syndrome comprising the step of measuring the expression of the FMR-1 gene,

wherein the expression is measured by determining the amount of mRNA expressed, the method comprising the steps of:

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extracting RNA from lymphoblastoid cell lines from individuals to be tested;
preparing FMR-1 cDNA and control gene cDNA from said extracted RNA;
quantifying the FMR-1 cDNA by comparing with the control gene cDNA; and
comparing the amount of FMR-1 cDNA with the amount of FMR-1 cDNA in normal individuals.
2. The method of claim **1**, wherein the quantification step includes PCR of the control gene, electrophoresis of the

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PCR products, ethidium bromide staining of the products and quantification of FMR-1 products versus control gene products.
3. The method of claim **2**, wherein the oligonucleotide primers SEQ. ID. No. 8 and SEQ. ID. No. 9 are used to amplify the mRNA from the fragile X site.
4. The method of claim **3**, wherein the control gene is HPRT and the oligonucleotide primers are SEQ. ID. No. 12 and SEQ. ID. No. 13.

* * * * *

EXHIBIT 13



US006180337B1

(12) **United States Patent**
Caskey et al.

(10) **Patent No.:** **US 6,180,337 B1**
(45) **Date of Patent:** ***Jan. 30, 2001**

(54) **DIAGNOSIS OF THE FRAGILE X SYNDROME**

(75) Inventors: **C. Thomas Caskey; David L. Nelson; Maura Pieretti**, all of Houston, TX (US); **Stephen T. Warren**, Clarkston, GA (US); **Ben A. Oostra**, Rotterdam (NL); **Ying-hui Fu**, Houston, TX (US)

(73) Assignee: **Baylor College of Medicine**, Houston, TX (US)

(*) Notice: Under 35 U.S.C. 154(b), the term of this patent shall be extended for 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **07/751,891**

(22) Filed: **Aug. 29, 1991**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/705,490, filed on May 24, 1991.

(51) **Int. Cl.**⁷ **C12Q 1/68**; G01N 33/53; C07H 21/02; C07K 14/435

(52) **U.S. Cl.** **435/6**; 435/7.1; 435/91.2; 536/23.5; 536/24.33; 530/350

(58) **Field of Search** 435/6, 91.2; 536/27, 536/23.1, 23.5, 24.33; 530/350; 935/77, 78

(56) **References Cited**

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Primary Examiner—Stephanie Zitomer

(74) *Attorney, Agent, or Firm*—Fulbright & Jaworski LLP

(57) **ABSTRACT**

A sequence of the FMR-1 gene is disclosed. This sequence and related probes, cosmids and unique repeats are used to detect X-linked diseases and especially the fragile X syndrome. Also, methods using methylation-sensitive restriction endonuclease and PCR primer probes were used to detect X-linked disease.

9 Claims, 15 Drawing Sheets

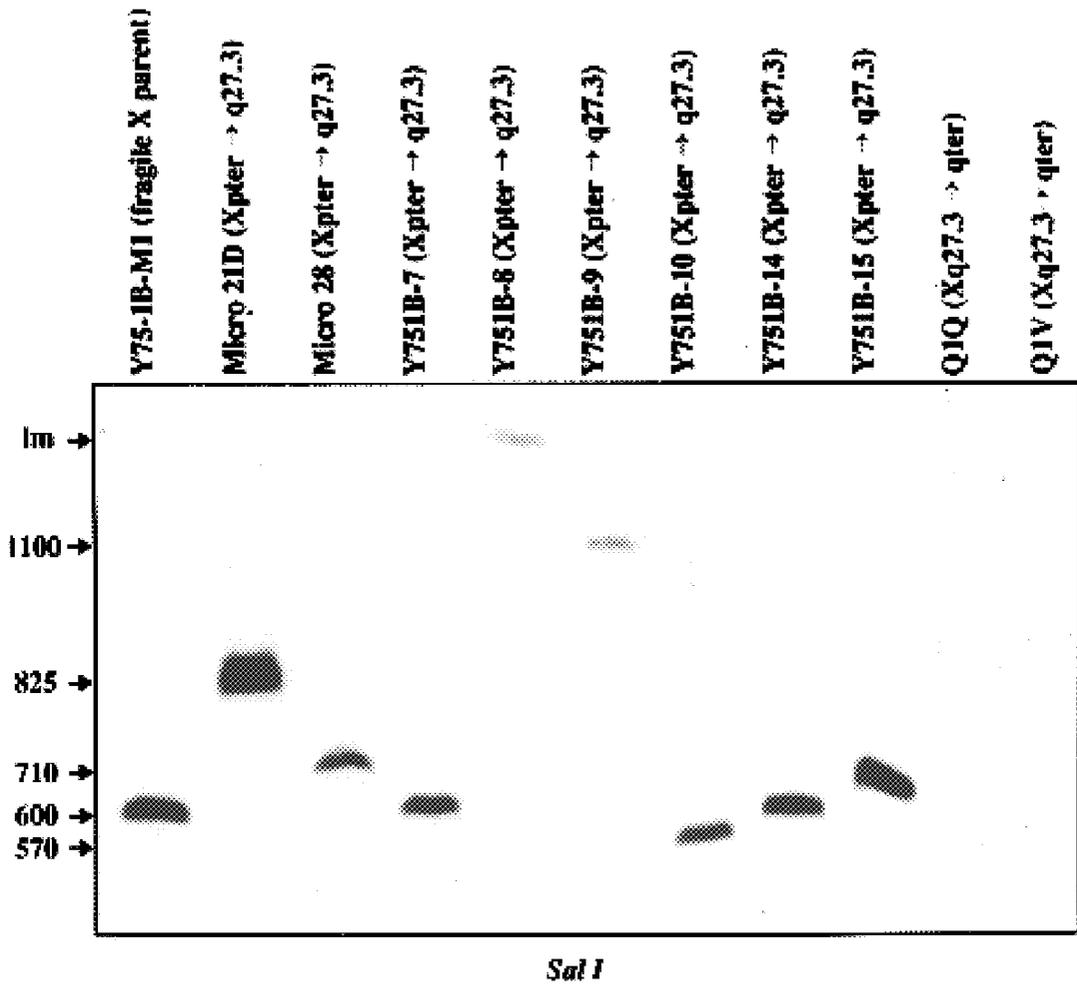


Figure 1

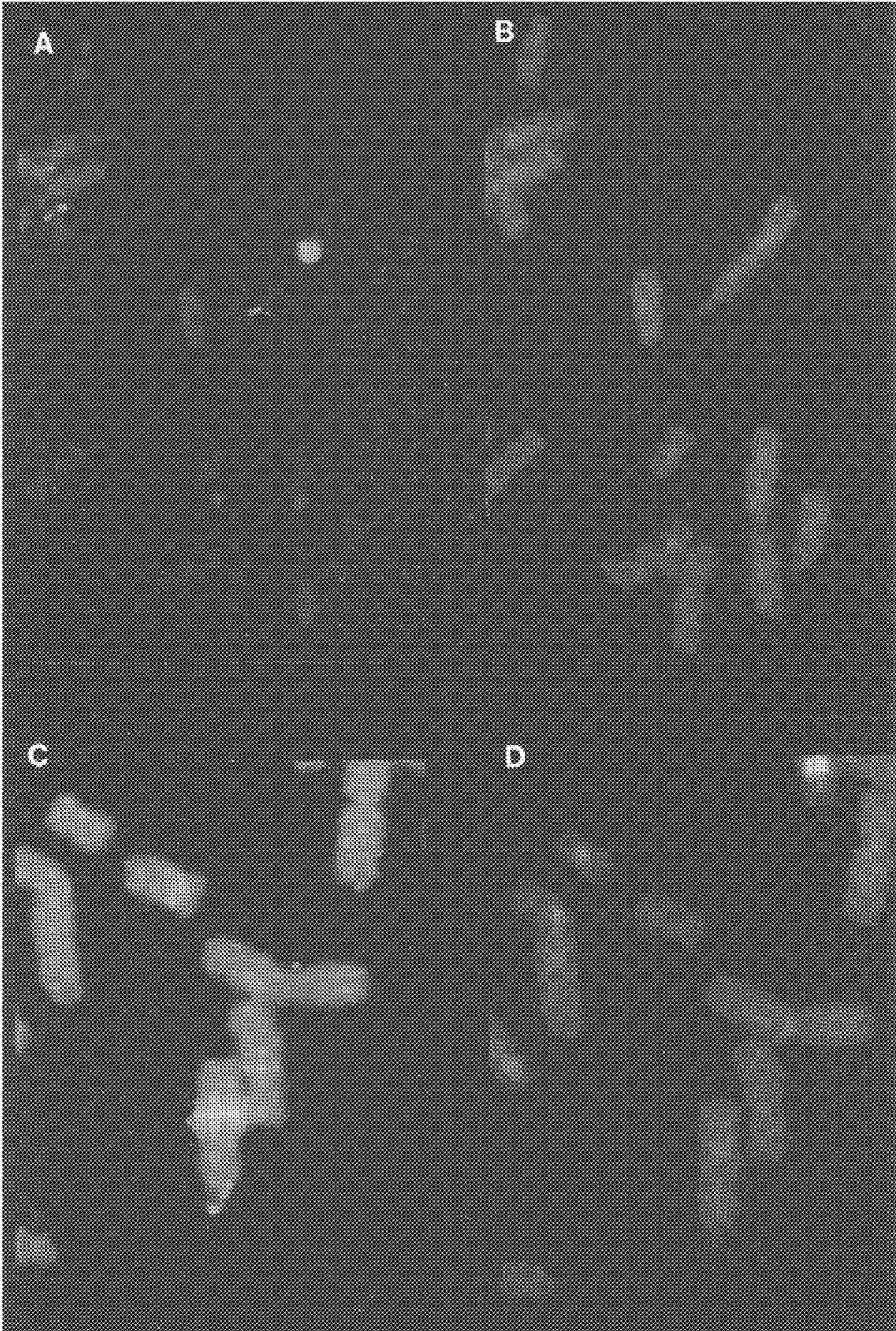


Figure 2

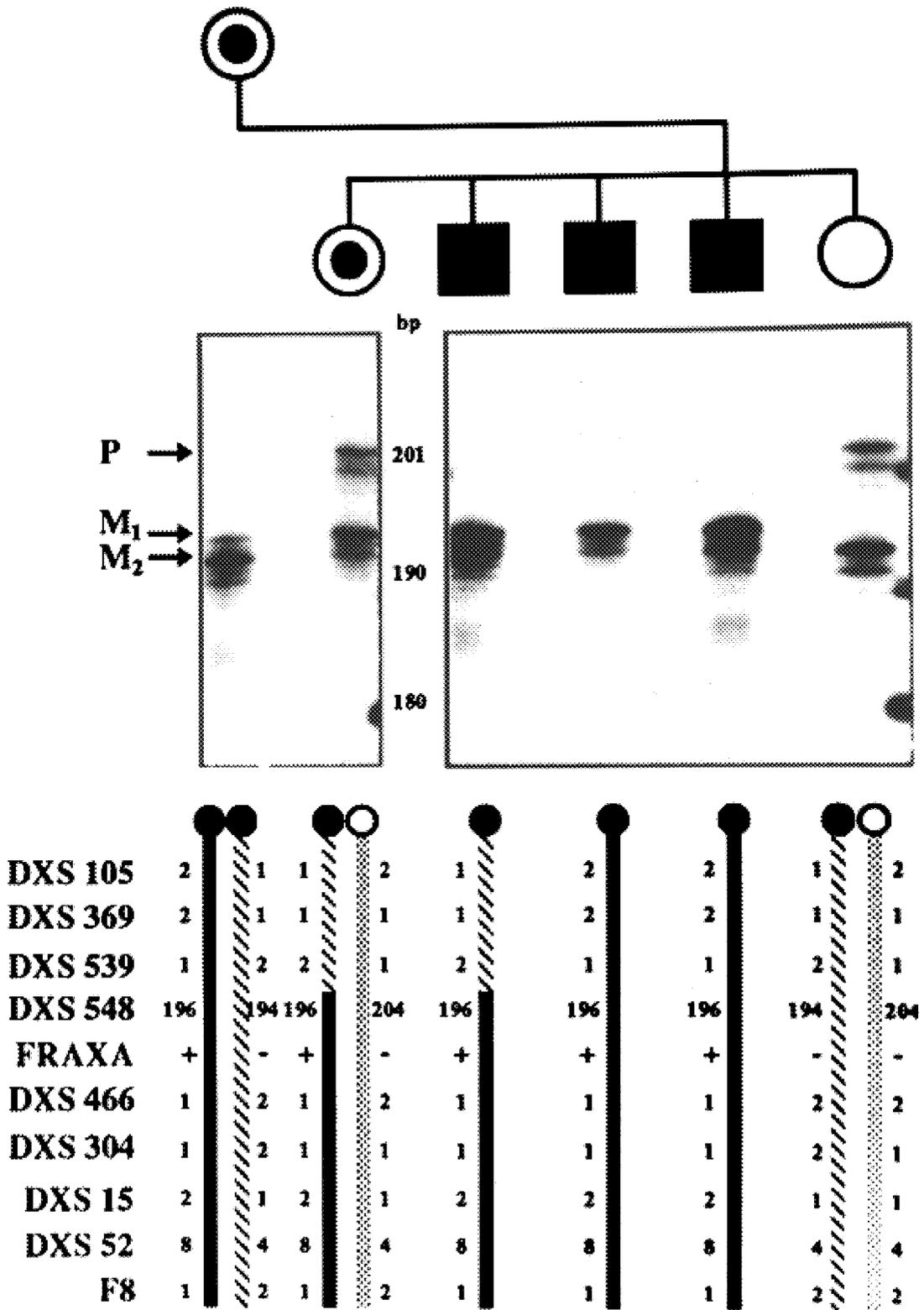


Figure 3

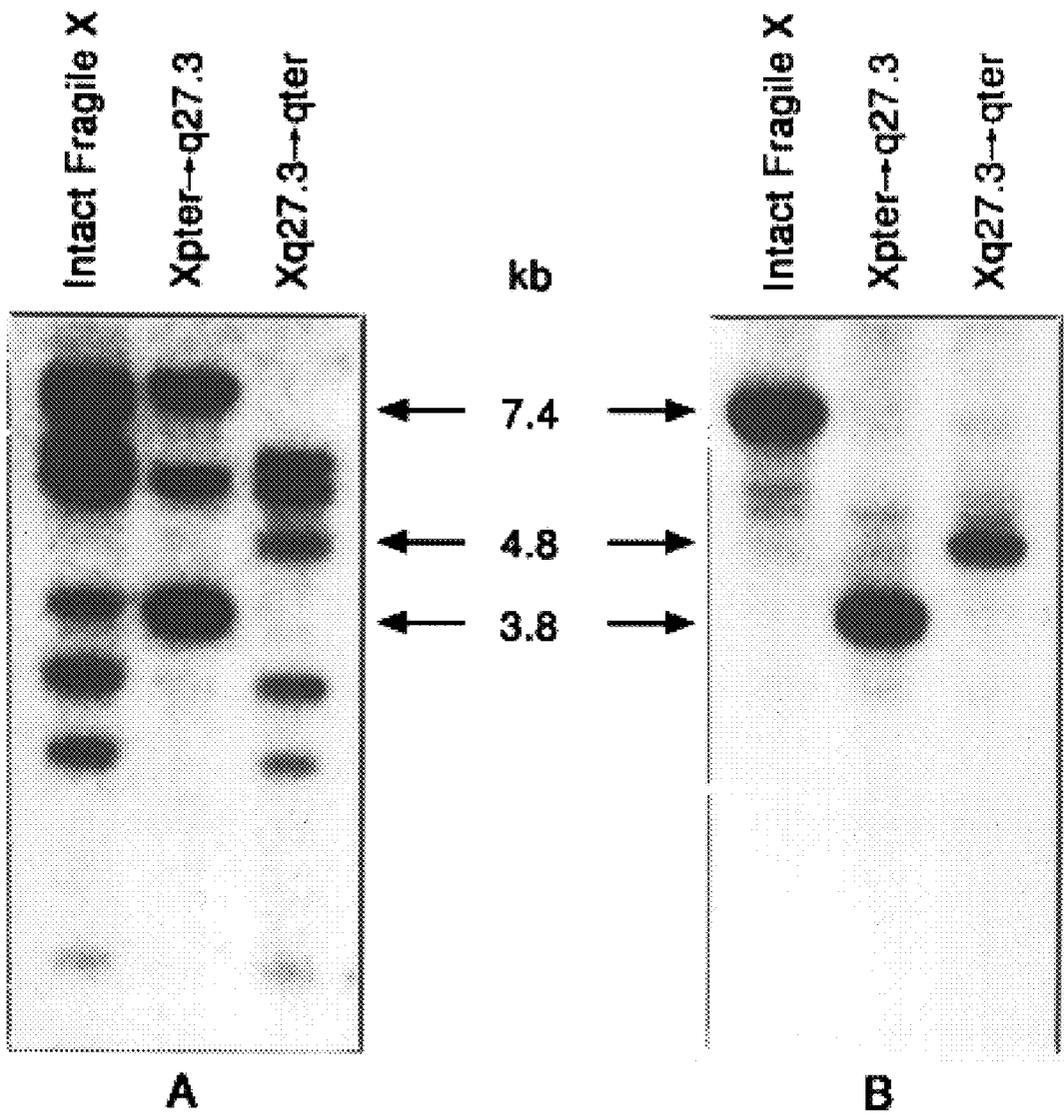


Figure 5

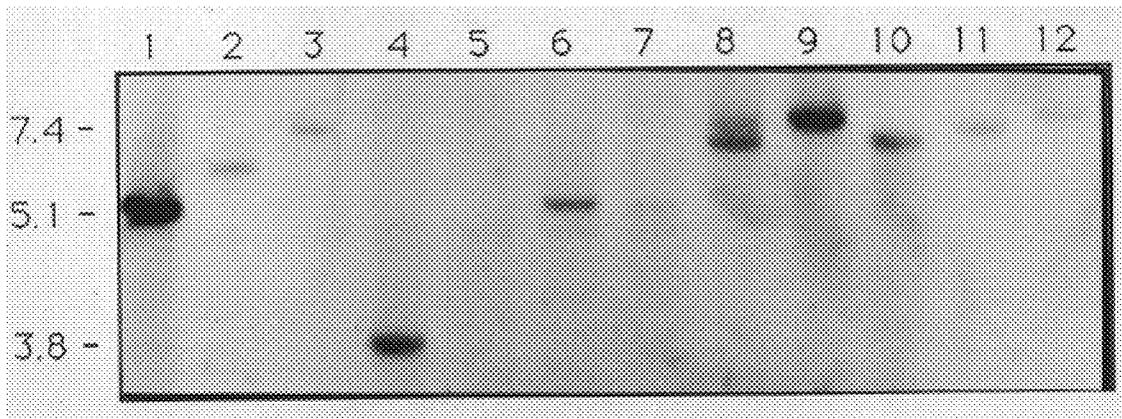


Figure 7

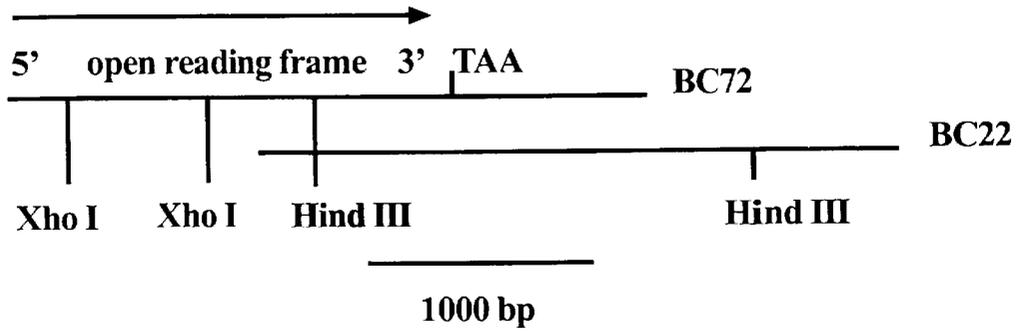


Figure 8

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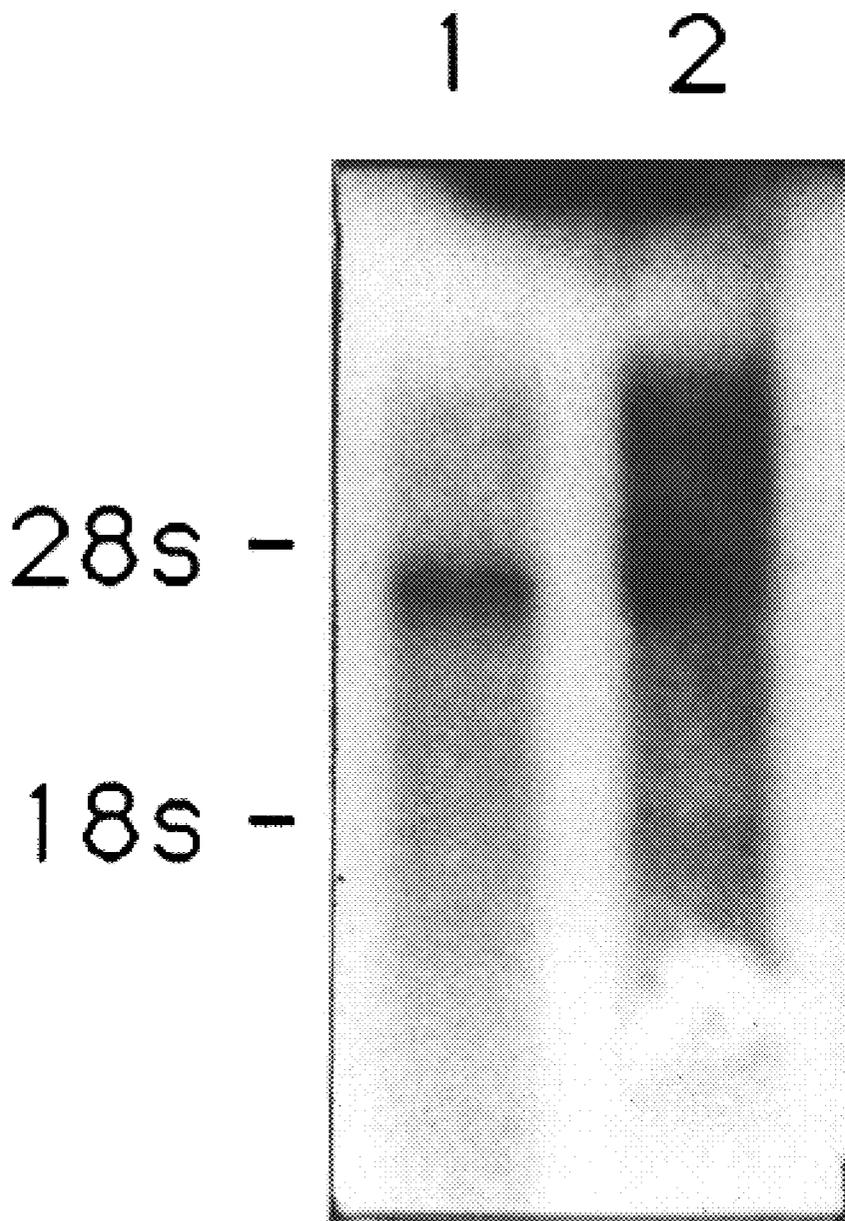


Figure 9

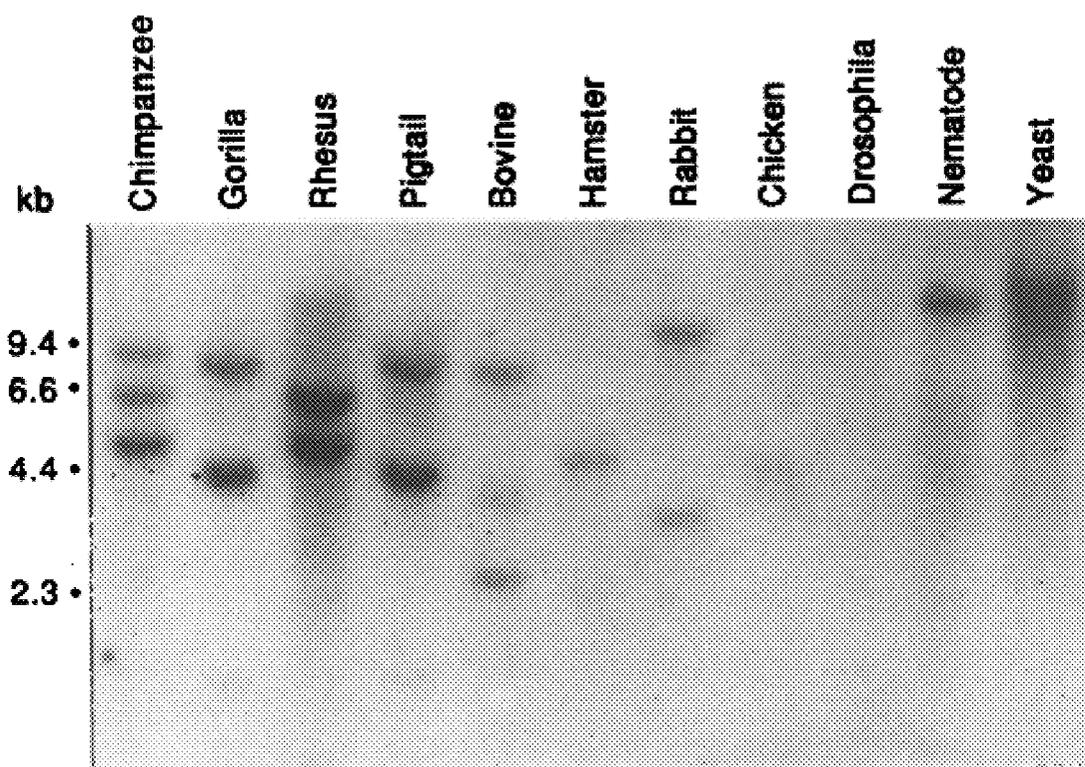


Figure 10

CTGCAGAAATGGGGGTTCTGGCCCTCGCGAGGCAGTGGGACCTGTCACCGCCCTTCAGCC
 primer g ▶

61 TTCCCGCCCTCCACCAAGCCCGCGCACGCCCGGCCCGCGCTGTCTTTTGACCCGGCA
EagI BssHII BssHII

121 CCCC GCCCGCCGGTTCACAGC a GCQCGCATGCg c GCGTCCCAGGCCACTTGAAGAGAGAGGG
SacII Sma3AI

181 CGGGGCCGAGGGGCTGAGc CC GCGGGGGGAGGGAACAGCGTTGATCACGTGACGTGGTTT
 primer a ▶ primer h ◀

241 CAGTGTTTACACCCGACGCGGGCCGGGGGTTCCGGCCCTAGTCAGGCCTCAGCTCCGTTT
 primer c ▶

301 Cg GTTTCACCTCCGGTGGAGGGCCGCCTCTGAGCGGGCGCCGGCCGACGGCGAGCCGGg
 ◀primer b end of FMR-1

361 g c g g c g g c g g t g a c g g a g g e g e e g e t g e e a g g g g g c g t g c g g c a g c g c g G G C G C G G G G C C
 primer d ◀ XhoI

421 GCGCGCGCGGGCGGGCGCGGGCGGGCGGGCGGct g g g c c t c g a g c c C C C G C A G C C C A
NheI primer e ▶

481 CCTCTCGGGGGCGGGCTCCCGGCC TAGCAGGGCTGAAGA GAAGATGGAGGAGCTGGTGG
 exon boundary ◀primer f

541 TGGAAGTGGGGGCTCCAAATGGCGCTTCTACAAGTACTTGGCTCTAGGGCAGGCCCA

601 TCTTCGCCCTTCCTTCCCTCCCTTTCTTCTTGGTGTGCGCGGGAGGCAGGCCCGGGGC

661 CTCTTCCC GAGCACCGCGCCTGGGTGCCAGGGCAOGCTCGGCGGA TGTGTgGGAOGG

721 AAGGACTGGA CTTGGGCTGTGGAAGCCCTCTCCGACTCCGAGAGGCCCTAGCGCCT

781 ATCGAAATGAGAGACCAGCGAGGAGAGGGTTCTCTTTCGGCGCCGAGc CCCGCCGGGTG

841 AGCTGGGGATGGCCGAGGGCCGGCGGCAGTACTAGAGCCGGCCGGGAAGGGCCGAAATC
BamHI

901 GGCCTAAGTGACGGCGATGCC TTATCCCCCTTCTAAACATCATCTCCAgCGGGAT

961 CCGGGCTGTCTGTGGGTAGTGTGGAGGAGCGGGGGCGCTTCAGCCGGGCCgCCTCC

1021 TGCAG

Figure 11

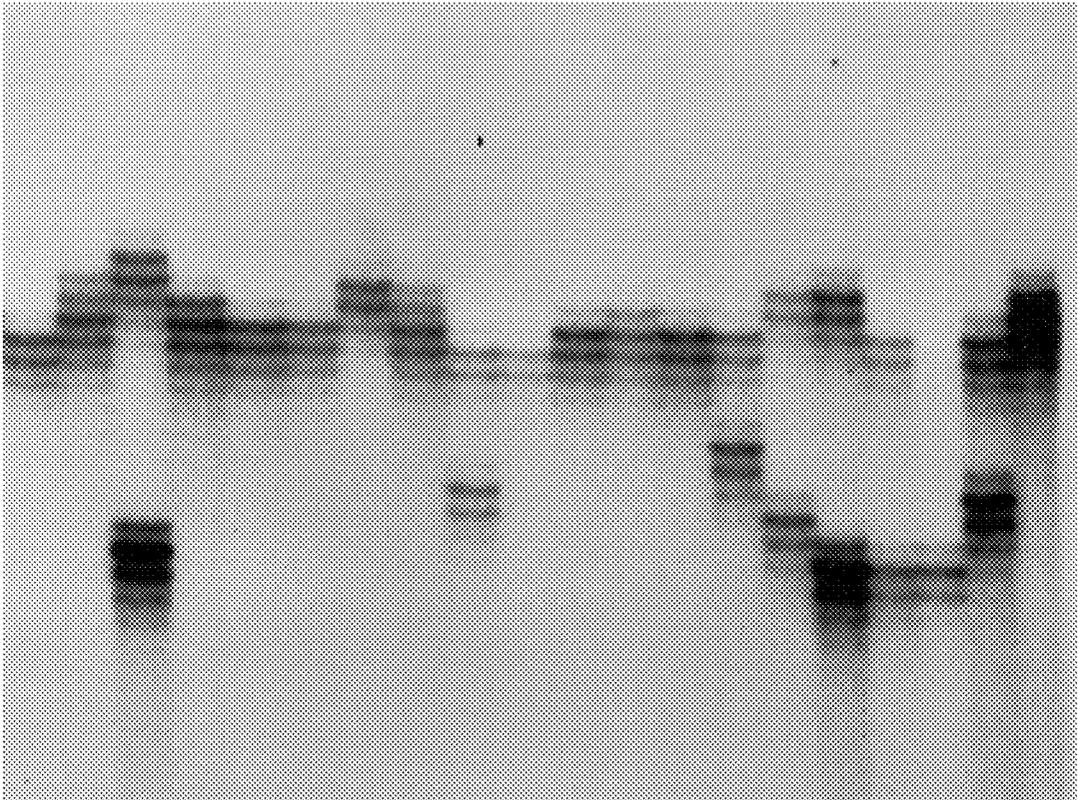


Figure 12

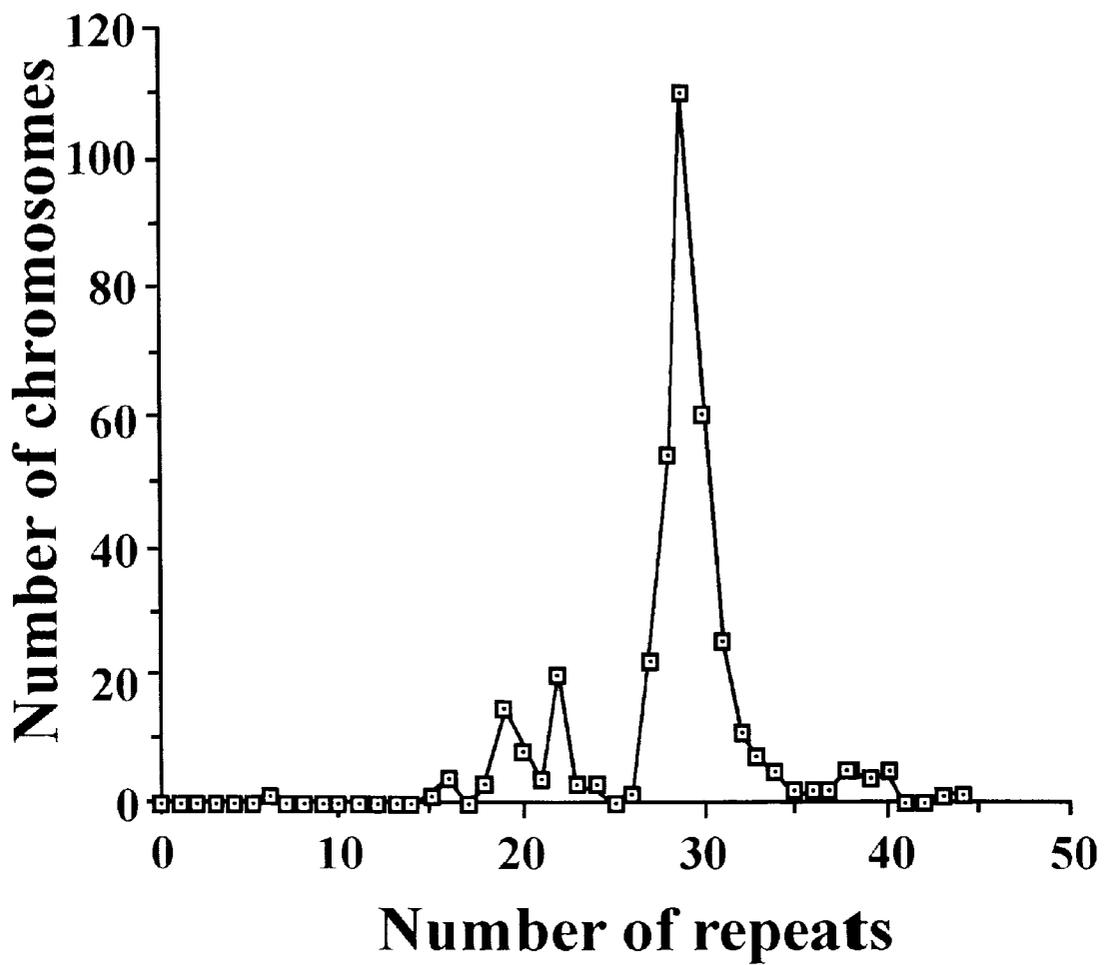


Figure 13

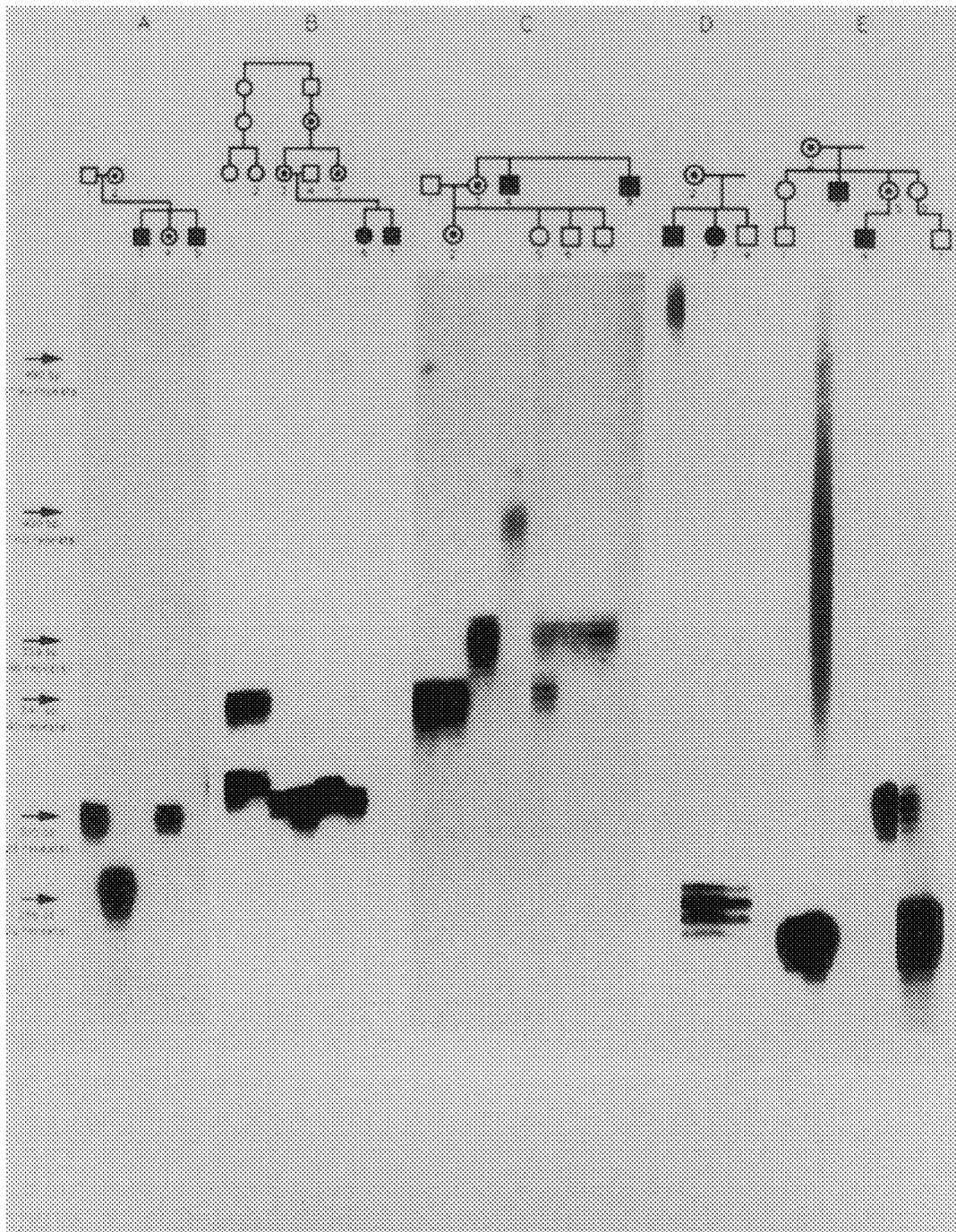


Figure 14

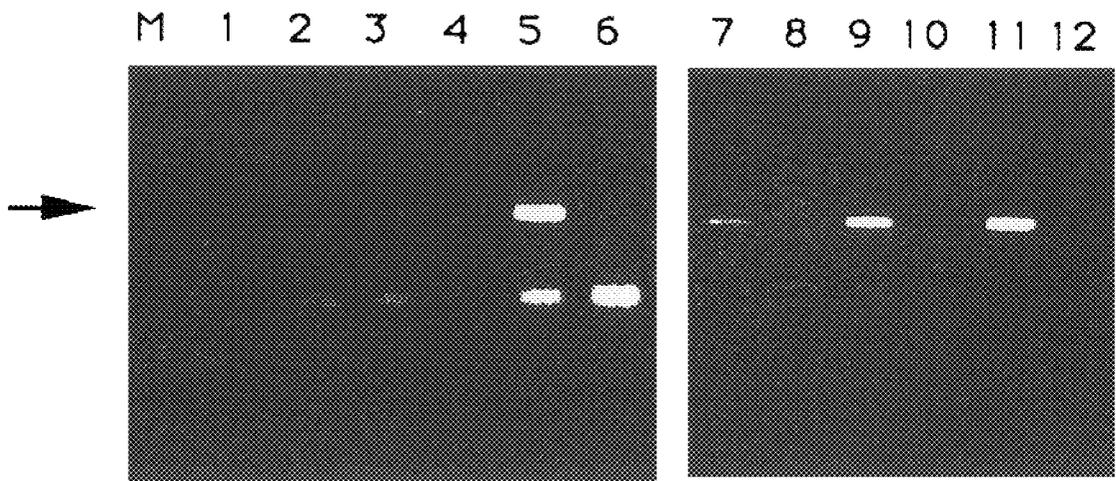


Figure 15

EXHIBIT 13

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DIAGNOSIS OF THE FRAGILE X SYNDROME

This application is a Continuation-In-Part of U.S. Ser. No. 07/705,490 filed May 24, 1991.

This invention was supported by the National Institutes of Health, under grant number LTD 20521. The government may have certain rights under this application.

FIELD OF THE INVENTION

This invention relates to the field of molecular diagnosis of the fragile X syndrome.

BACKGROUND

The fragile X syndrome is the most frequently encountered form of inherited mental retardation in humans and has a prevalence estimated to be 1/1250 males. The fragile X syndrome segregates as an X-linked dominant disorder with reduced penetrance. Either sex when carrying the fragile X mutation may exhibit mental deficiency. It has been shown that approximately 30% of carrier females are penetrant and that 20% of males carrying the fragile X chromosome are normal but may transmit the disorder and have fully penetrant grandsons. In addition to the mental retardation which is variable in severity, penetrant males exhibit additional phenotypic involvement including macroorchidism and distinctive facies. Since fully penetrant males rarely reproduce, it has been suggested that the frequency of new mutations of the fragile X site may be as high as 1/3000 germ cells to maintain the population frequency.

The fragile X syndrome, as implied by its name, is associated with a fragile site expressed as an isochromatid gap in the metaphase chromosome at map position Xq 27.3. The fragile X site is induced by cell culture conditions which perturb deoxypyrimidine pools and is rarely observed in greater than 50% of the metaphase spreads. Neither the molecular nature of the fragile X site, nor its relationship to the gene responsible for the clinical expression of the syndrome is understood. However, based upon genetic linkage studies, as well as in situ hybridizations, the fragile X site and its associated gene are tightly linked if not coincident.

The present application provides a new procedure for detecting the fragile X site at the molecular level. It provides a molecular method for the diagnosis of the fragile X syndrome, describes a unique open reading sequence at the suspected gene locus and provides probes to the fragile X region.

SUMMARY OF THE INVENTION

An object of the present invention is a method for diagnosing fragile X syndrome.

A further object of the present invention is the provision of a sequence of the FMR-1 gene.

An additional object of the present invention is a method of detecting the fragile X syndrome by measuring the mRNA or protein from the FMR-1 gene.

Another object of the present invention is a method of detecting the fragile X syndrome by measuring CGG repeats.

A further object of the present invention is a method of detecting the fragile X syndrome by measuring the methylation associated with a CpG island.

Thus in accomplishing the foregoing objects there is provided in accordance with one aspect of the present

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invention as a composition of matter, a 3.8 kb cDNA clone containing the FMR-1 gene. A further aspect is a 5222 bp genomic DNA sequence containing at least a fraction of the FMR-1 gene.

A further embodiment of the present invention is a group of cosmid probes for the selection of the FMR-1 gene in the fragile X syndrome.

An additional embodiment of the present invention is a method of detecting fragile X syndrome comprising the steps of digesting DNA from an individual to be tested with a restriction endonuclease and detecting the restriction fragment length polymorphism with hybridization to probes within the fragile X locus and southern blot analysis. In a preferred embodiment of the present invention, the probe is pE5.1 and the restriction endonucleases are selected from the group consisting of EcoR I, Pst I, Xho I and BssH II.

Alternate embodiments of the present invention include detecting the fragile X syndrome by measuring the expression of the FMR-1 gene either as the amount of mRNA expressed or as the amount of FMR-1 protein produced. Another embodiment of the present invention includes a method of detecting X-linked disease comprising the steps of detecting variation in the (CGG)_n repeat at the 5' end of the FMR-1 gene by measuring the length of the repeat, wherein n for normal ranges between 16 and 30 and n for X-linked disease is greater than 30. A variety of methods are available to detect the dosage measurements of the repeat. These procedures can be selected from the group consisting of visual examination, densitometry measurement, quantitative radioactivity and quantitative fluorescence as well as pulsed field gel electrophoresis and fluorescence in situ hybridization.

Other and further objects, features and advantages will be apparent and eventually more readily understood from a reading of the following specification and by reference to the accompanying drawings forming a part thereof, wherein examples of the presently preferred embodiments of the invention are given for the purpose of the disclosure.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a Southern blot analysis of pulsed field gel resolved Sal I digested DNA of proximal translocation hybrids probed with p46-1.1.

FIGS. 2A-2D are show fluorescent in situ hybridization of YAC 209G4 and cosmids to the fragile X site at Xq 27.3 of an affected male patient.

FIG. 3 is a PCR analysis of DXS548 alleles in a fragile X family with recombinant individuals.

FIG. 4 is a physical map of the fragile X region of a genomic and YAC 209G4 DNA.

FIGS. 5A and 5B depict a Southern blot analysis of fragile X associated translocation breakpoints. In (A) the Southern blot is hybridized with cosmid 22.3 and in (B) the same filter is hybridized with pE5.1.

FIGS. 6A and 6B depict a restriction map of cosmid 22.3 and pE5.1. In (A) is cosmid 22.3 showing BssH II sites a and b as well as EcoR I and BamH I sites. The BamH I site in parentheses was destroyed during cloning. The solid lines below the map show fragments which hybridize to cDNAs BC72 and BC22. In (B) is the map of the cloned 5.1 kb EcoR I fragment of cosmid 22.3 (pE5.1). The solid line below the map shows the position of the FMR-1 exonic sequence which contains the Xho I site.

FIG. 7 shows length variation of EcoRI fragments from normal and fragile X human chromosomes with probe pE5.1.

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FIG. 8 is a map of the FMR-1 cDNA clones.

FIG. 9 is a Northern blot analysis of a poly(A)RNA hybridized with cDNA BC22.

FIG. 10 is a zoo blot analysis of DNA isolated from several species hybridized with cDNA BC22.

FIG. 11 is a sequence (SEQ ID NO: 24) of the 1 kb PstI DNA fragment containing the CpG island and "CGG" repeat. The differences between this sequence and that reported by Kremer et. al. *Science* 252:1711-1714 (1991) are shown in lower case letters. The name of restriction sites are shown above their recognition sequences. The locations of PCR primers are shown by solid line below. The sequence has been corrected for the FMR-1 at the positions 384-385 (CG vs GC) Verkerk et. al. *Cell* 65:905-914 (1991). Primer a (SEQ ID NO: 15), Primer b (SEQ ID NO: 16), Primer c (SEQ ID NO: 10), Primer d (SEQ ID NO: 17), Primer e (SEQ ID NO: 18), Primer f (SEQ ID NO: 11), Primer g (SEQ ID NO: 19) and Primer h (SEQ ID NO: 20).

FIG. 12 shows the polymorphic nature of the "CGG" locus in normal human genomic DNAs. Genomic DNA was obtained from unrelated volunteer donors at a local blood bank.

FIG. 13 shows the distribution of different fragile X alleles among the normal population. No obvious difference was observed for the pattern of distribution among different races (Caucasian, Black, Hispanic and Asian).

FIGS. 14A-14E represent a PCR study of CGG repeats in fragile X families. Lymphoblastoid cell line DNA was used for these analyses.

FIG. 15 shows the methylation status of normal and affected male DNAs tested by PCR. Lanes 1-6 are patient DNAs and lanes 7-12 are normal DNAs. Genomic DNAs were digested to completion by BssHII. 200 ng of undigested (odd numbered lanes) or digested (even numbered lanes) DNA was used for PCR amplification. The conditions for the PCR reactions were those described in FIG. 1. The PCR products were examined on a 2% agarose gel and stained with ethidium bromide.

The drawings and figures are not necessarily to scale and certain features mentioned may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness.

DETAILED DESCRIPTION OF THE INVENTION

It will be readily apparent to one skilled in the art that variations, substitutions and modifications may be made to the invention disclosed herein without departing from the scope and the spirit of the invention.

Each sample to be tested herein for the fragile X site is derived from genomic DNA, mRNA or protein. The source of the genomic DNA to be tested can be any medical specimen which contains DNA. Some examples of medical specimen include blood, semen, vaginal swabs, buccal mouthwash, tissue, hair and mixture of body fluids. As used herein the term "polymerase chain reaction" or "PCR" refers to the PCR procedure described in the patents to Mullis, et al., U.S. Pat. Nos. 4,683,195 and 4,683,202. The procedure basically involves: (1) treating extracted DNA to form single-stranded complementary strands; (2) adding a pair of oligonucleotide primers, wherein one primer of the pair is substantially complementary to part of the sequence in the sense strand and the other primer of each pair is substantially complementary to a different part of the same sequence in the complementary antisense strand; (3) annealing the paired

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primers to the complementary sequence; (4) simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product complementary to the strands annealed to each primer wherein said extension products after separation from the complement serve as templates for the synthesis of an extension product for the other primer of each pair; (5) separating said extension products from said templates to produce single-stranded molecules; and (6) amplifying said single-stranded molecules by repeating at least once said annealing, extending and separating steps.

As used herein fluorescence in situ hybridization or "FISH" refers to the procedure described in Wotta, et al., *Am. J. of Human Genetics*, 46, 95-106 (1988) and Kievits, et al., *Cytogenet. Cell Genet.*, 53:134-136 (1990). The procedure basically involves the steps of preparing interphase or metaphase spreads from cells of peripheral blood lymphocytes and hybridizing labeled probes to the interphase or metaphase spreads. Using probes with mixed labels allows visualization of space, order and distance between hybridization sites. After hybridization the labels are examined to determine the order and distance between the hybridization sites.

As used herein, the term "pulsed field gel electrophoresis" or "PFGE" refers to a procedure described by Schwartz, et al., *Cold Springs Harbor Symposium, Quantitative Biology*, 47:189-195 (1982). The procedure basically comprises running a standard electrophoresis gel (agarose, polyacrylamide or other gel known to those skilled in the art) under pulsing conditions. One skilled in the art recognizes that the strength of the field as well the direction of the field is pulsed and rotated in order to separate megabase DNA molecules. Current commercial systems are computer controlled and select the strength, direction and time of pulse depending on the molecular weight of DNA to be separated.

One embodiment of the present invention as a composition of matter is a 3.8 kb cDNA clone (SEQ ID NO: 1) containing the FMR-1 gene.

Another embodiment of the present invention is a 5222 bp genomic DNA (SEQ ID NO: 23). This DNA includes a 4188 bp (SEQ ID NO: 2) sequence from the distal Eco RI site containing the fragile X region and a 229 bp genomic DNA (SEQ ID NO: 3) from the proximal Eco RI site.

One embodiment of the present invention is a method of detecting Fragile X syndrome comprising the steps of digesting DNA from an individual to be tested with a restriction endonuclease and detecting the restriction fragment length to polymorphism (RFLP) with hybridization to probes within the fragile X locus and southern blot analysis. One skilled in the art will readily recognize that a variety of restriction endonucleases can be used. In the preferred embodiment the restriction endonuclease is selected from the group consisting of EcoR I, Pst I, Xho I and BssH II.

In the method of detection, it is found that the probe pE 5.1 is used in the preferred embodiment. One skilled in the art readily recognizes that other probes consisting of some sub fraction (i.e., a fragment) of the full probe pE5.1 will hybridize to the unique fragment lengths and thus can be used.

An alternative method for detecting the Fragile X syndrome comprises the step of measuring the expression of the FMR-1 gene. The FMR-1 gene can be measured by either measuring the amount of mRNA expressed or by measuring the amount of FMR-1 protein.

When measuring the amount of mRNA expressed, the amount of mRNA is determined by the steps of extracting

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RNA from any tissue source including fibroblast and lymphoblastoid cell lines of the individuals to be tested. From the RNA of FMR-1, a cDNA is prepared. From RNA of a control gene a cDNA is prepared. Then quantification is achieved by comparing the amount of mRNA from FMR-1 with the mRNA from the controlled gene. In the preferred embodiment, the quantification step includes PCR analysis of the FMR-1 cDNA and PCR analysis of the control gene cDNA. The PCR products are electrophoresed and ethidium bromide stained. The products are then quantified by comparing the FMR-1 product versus the control gene product after the ethidium bromide staining. The oligonucleotide primers for the fragile X site are SEQ ID NO: 8 and SEQ ID NO: 9. One example of the control gene is HPRT and the oligonucleotides are SEQ ID NO: 12 and SEQ ID NO: 13.

When measuring the amount of FMR-1 protein produced, one can use any of the variety of methods known in the art to detect proteins, including monoclonal antibodies, polyclonal antibodies and protein assays. In the preferred embodiment, the antibodies detect SEQ ID NO: 14.

The methods described herein can also be used to detect X-linked disease. The method comprises the steps of detecting variation of the (CGG)_n repeat found at the 5' end of the FMR-1 gene by measuring the length of the repeat wherein n (number of repeats) for normal is in the range between 16 and 30 and n for X-linked diseases is in the range of greater than 30. In the case of Fragile X, n is usually at least twice the range of normal. Types of disease which can be detected are X-linked mental retardation both of fragile X and non-fragile X type, X linked manic depressive disease, TKCR syndrome and Martin-Bell syndrome.

The method of dosage compensation by measuring the amount or length of the repeat can be done by using FISH. In the FISH method, the repetitive sequence can be used as a probe to distinguish between normal and fragile X syndrome simply by the presence or absence of a signal to the repetitive sequence. In this case, the application of the repeat sequence provides a sufficiently large target for the hybridization. Thus, it is possible that very sensitive FISH might detect transmitting males (with 50–100 copies of the CGG) even though these would be lost to routine microscopy and detection. Although FISH is usually applied to metaphase nuclei, in the present invention it is applicable to both metaphase and interphase for the detection of X-linked disease.

Alternate methods to measure the dosage measurement of the repeat can include visual examination, densitometry measurement, quantitative radioactivity and quantitative fluorescence.

In one embodiment the size of the repeat is determined by dosage measurements of Southern blotting analysis of restriction enzyme digests with probes contained within the FMR-1 gene region.

It is also known that the method of PFGE can be used to detect variation at the fragile X locus.

In another embodiment the variation of the (CGG)_n repeat is measured by PCR. A variety of PCR primer pairs can be used including SEQ ID NOS: 19 and 11 or SEQ ID NOS: 15 and 11 or SEQ ID NOS: 10 and 11. In this method the preferred oligonucleotide primer pair is SEQ ID NO: 10 and SEQ ID NO: 11.

Another embodiment of the present invention is the cosmid probes shown in FIG. 4. These cosmid probes can be selected from the group consisting of C 22.3, C 34.4, C 31.4, C 4.1, C 34.3, C 26.3 C 19.1 and C14.1. These cosmid clones are Sau 3A digests of the YAC 209G4. These digests were

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cloned into p2CpG. This results in inserts from 35–45 Kb. The ends are defined by their positions on the map of FIG. 4. These cosmid probes overlap the range in which the FMR-1 gene is located.

In detecting the fragile X sites the length of CA polymorphisms at the fragile X site can be measured by performing a PCR assay and measuring the length of the amplified products. In the PCR assay, the oligonucleotide primers are SEQ ID NO: 6 and SEQ ID NO: 7.

Another method of detecting X linked mental retardation (fragile X syndrome) is to measure the methylation associated with a CpG island in the fragile X area, wherein a methylation-sensitive restriction endonuclease is used to digest the extracted DNA to be tested and then the digested DNA is amplified. If products are amplified in males it indicates the presence of methylation and the fragile X gene defect. In this procedure a variety of restriction endonuclease can be used including BssH II, Eag I, Sac II, Hpa II and Msp I. The oligonucleotide primer pairs are selected from the group consisting of SEQ ID NOS: 19 and 20, SEQ ID NOS: 19 and 11, SEQ ID NOS: 19 and 17 and SEQ ID NOS: 19 and 16. Additionally, restriction endonuclease Nhe I and Xha I can be used with primer pair SEQ ID NOS: 19 and 11 or SEQ ID NOS: 15 and 11 or SEQ ID NOS: 10 and 11. The restriction endonuclease Nhe I can be used with primer pair SEQ ID NOS: 18 and 11. In the preferred embodiment the restriction endonuclease is BssH II and the primer pair is SEQ ID NOS: 19 and 20.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner. In the examples all percentages are by weight, if for solids and by volumes, if for liquids and all temperatures are in degrees Celsius unless otherwise noted.

EXAMPLE 1

Pulsed Field Gel Electrophoresis Southern blot analysis of genomic DNA or YAC DNA resolved by PFGE was performed essentially as described (Smith, et al., Pulsed-field gel electrophoresis and the technology of large DNA molecules. In Genome Analysis: A Practical Approach; Oxford:IRB Press, pp.41–72, 1988). In this procedure, trypsinized and washed mammalian cells were suspended in molten agarose (final concentration 0.5% wt/vol; Baker) prepared in SE buffer (75 mM NaCl, 25 mM EDTA, pH 8.0) at a final concentration of 1.5×10^7 cells/ml. Chromosomal DNAs were isolated from YAC clones. Yeast cells from a 10 ml saturated culture were harvested, rinsed once in 50 mM EDTA, pH 8.0 and recovered in 0.5 ml SBE-zymolase (1 M sorbitol, 25 mM EDTA pH 8.0, 14 mM 2-mercaptoethanol, 1 mg/ml zymolase [ICN]). 0.5 ml 1% Seaplaque agarose (FMC) in SBE (without zymolase) was added and the suspension transferred to plug molds. Spheroplast generation (for yeast cells) was for 5 hours to overnight in SBE-zymolase. Cell lysis (mammalian or yeast cells) was for 2 days in ESP (0.5 M EDTA, pH 9.5, 1% N-lauralosarcosine, 1 mg/ml proteinase K) at 50° C. Restriction endonuclease digestion was performed using the manufacturer's recommended buffers and conditions with a 50 μ l plug slice in 250 μ l of buffer containing 50 units of enzyme. For double digests, the plugs were rinsed and equilibrated, following digestion with the first enzyme, with the second buffer several times prior to digestion with the second enzyme. PFGE was carried out on a Bio-Rad Contour-Clamped Homogeneous Electric Field (CHEF) DRII apparatus through 1% agarose (BRL) at 200 V and 14° C. in 0.5 \times TBE buffer (45 mM Tris-borate, 1 mM EDTA). For resolution of fragments of \approx 200–1200 kb, switch time was 60

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sec for 17 hrs followed by 90 sec for 10 hrs; for resolution of fragments \approx 10–500 kb, the switch times were ramped from 5 sec to 50 sec over 27 hrs. Southern blotting and hybridization were carried out as described in the art with the exception that acid depurination in 0.25 M HCl was allowed to proceed 20 min for pulsed-field gels. Radiolabeled probes were synthesized by random priming from 50 ng gel purified fragments except when intact cosmids were used which were nick translated (Boehringer Mannheim kit; following manufacturer's recommendations). For genomic probes containing repetitive elements, repeat suppression was accomplished by preassociation with 1–3 mg of sonicated human placental DNA in 100–300 μ l of 5 \times SSC (1 \times SSC is 150 mM NaCl, 15 mM NaCitrate, pH 7.0) for 3–10 min at 65 $^{\circ}$ C. prior to the addition to the filter. Washing was carried out to a final stringency wash of 0.2 \times SSC for 15 min at 65 $^{\circ}$ C. prior to autoradiography. *S. cerevisiae* strain YNN295 chromosomes (BioRad), concatamers of phage lambda (BioRad) or high molecular weight markers (BRL) were used as size standards.

EXAMPLE 2

PCR Analysis of DXS548 Alleles

Amplification was carried out on 0.2–0.5 μ g of genomic DNA in a 10 μ l total reaction containing 0.25 mM dNTPs, 40 ng of primers SEQ. ID. NO. 6 and SEQ. ID. No. 7, and 0.25 units of Taq polymerase in a buffer of 10 mM Tris-HCl, 50 mM KCl, 12 mM MgCl and 0.01% gelatin. Twenty three cycles of PCR were carried out in the following fashion; 3 cycles of 1 min each at 97 $^{\circ}$ C., 62 $^{\circ}$ C. annealing and 72 $^{\circ}$ C. extension followed by 20 additional cycles with the annealing temperature lowered to 55 $^{\circ}$ C. The reaction volume was then increased to 50 μ l with the same reaction components and concentrations except that one primer was 5' end-labelled with Y³²P-ATP. PCR was continued for 10 cycles of 1 min each at 95 $^{\circ}$ C. denaturation, 62 $^{\circ}$ C. annealing and 72 $^{\circ}$ C. extension. PCR products were analyzed by electrophoresis of 2 μ l of reaction through a 40 cm 6% polyacrylamide denaturing sequencing gel for approximately 2.25 hrs. The gel was dried without fixing and exposed to X-ray film overnight at room temperature.

EXAMPLE 3

Cosmid Library Construction of YAC 209G4

Agarose plugs (0.5% SeaPlaque FMC) containing 5–10 μ g of yeast DNA were prepared. 100 μ l blocks of DNA were equilibrated on ice in 0.5 ml of Mbo I digestion buffer, containing 0.1 mg/ml bovine serum albumin (BSA, MB grade; Boehringer Mannheim). After 2–3 hrs, the buffer was replaced by 150 μ l of fresh buffer to which Mbo I was added (0.0001–0.0007 units). Following overnight incubation on ice, digestion was carried out for 40 min at 37 $^{\circ}$ C. The agarose blocks were melted, the DNA dephosphorylated with 1 unit calf intestinal alkaline phosphatase (Boehringer Mannheim), and treated with 2.5 units of agarase (Calbiochem). The solution was extracted twice with phenol/chloroform, once with chloroform, the DNA precipitated with ethanol and dissolved in 10 mM Tris, 0.1 mM EDTA (pH 7.4) at a concentration of 500 ng/ μ l. 250 ng of DNA was ligated to 500 ng of Bst BI (dephosphorylated) and Bam HI digested vector (p2CpG). Ligation and packaging was carried out according to standard procedures. Cosmids containing human inserts were selected by hybridizing with human specific Alu-repeat probe. These cosmids can be seen in FIG. 4.

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EXAMPLE 4

YAC and Cosmid Subcloning

YACs were subcloned following isolation of the intact chromosome by preparative PFGE and EcoR I digestion of the DNA in molten agarose (Seaplaque; FMC). Fragments were phenol/chloroform extracted, ethanol precipitated, recovered and ligated into EcoR I cut, dephosphorylated, lambda ZAP II arms according to manufacturer's recommendations (Stratagene). Cosmids were subcloned following an alkaline lysis isolation and EcoR I digestion. Fragments were phenol/chloroform extracted and ethanol precipitated prior to ligation into lambda ZAP II arms as with YAC fragments. In the case of both cosmids and YACs, 75 ng EcoR I fragments were ligated to 1 μ g vector arms. Selected phage were converted into pBluescript II SK-clones following in vivo excision of plasmid with insert according to manufacturer's guidelines.

EXAMPLE 5

cDNA Library Screening

A human fetal brain lambda gt11 cDNA library (Clontech, Palo Alto, Calif.) of 1.3×10^6 independent clones with insert lengths of 0.7–4.0 kb was used. The library was plated on 15 cm plates at a density of 50,000 pfu per dish using strain LE392. Filter lifts were prepared according to standard techniques and the library screened with cosmid DNA hexanucleotide labelled with ³²P-dATP and ³²P-dCTP. The labelled DNA was first prehybridized with 100 μ g of total sheared human genomic DNA and 100 μ g cosmid vector DNA in 5 \times SSC at 65 $^{\circ}$ C. for 2 hrs. Following hybridization for 16 hrs, the filters were washed to a stringency of 0.1 \times SSC. The filters were exposed to Fuji film with intensifying screens for 2 days at –80 $^{\circ}$ C.

EXAMPLE 6

Fluorescent In Situ Hybridization

In situ hybridizations of total YAC-containing yeast DNA and cosmids were performed. Fragile X expression was induced by 96 hr culturing of lymphocytes (PHA stimulated from a male fragile X patient) in medium TC199 (Gibco) supplemented with 10% bovine fetal calf serum and, for the last 24 hrs, 10 μ g/ml methotrexate (Lederle). Chromosomes were prepared on slides using standard techniques.

Slides were washed with PBS and incubated for 1 hr at 37 $^{\circ}$ C. in RNase A (100 μ g/ml) in 2 \times SSC. The slides were then incubated 10 min with pepsin (Serva; 0.1 mg/ml in 0.01 N HCl), fixed in 1% (vol/vol in PBS, 50 mM MgCl₂) formaldehyde (Merck) and dehydrated in cold ethanol. Biotinylated total yeast and cosmid DNA were preannealed for 1–4 hrs in the presence of sonicated human genomic DNA and hybridized to the chromosomes overnight using 150 ng (yeast) or 40 ng (cosmid) of probe in 10 μ l of 50% formamide, 2 \times SSC, 10% dextran sulfate under an 18 mm² coverslip sealed with rubber cement. In some experiments, 2 ng/ μ l pBamX5, a human repetitive sequence detecting the pericentromeric region of the human X, was separately denatured and added to the hybridization solution.

The signals were amplified by two layers of avidin-FITC (Vector) and one layer of biotinylated goat anti-avidin (Vector). The slides were then washed with PBS and mounted in antifade medium of 2% DABCO in glycerol containing propidium iodide (0.03 μ g/ml). Microscopic analysis was performed with a Leitz Aristoplan microscope with FITC (K3 block) and DAPI (A block) detection. Photographs were made using Ektachrome 400 (Kodak) daylight slide film.

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EXAMPLE 7

Northern Blot Analysis

Total RNA was extracted using guanidinium isothiocyanate followed by centrifugation through cesium chloride. Poly(A)⁺ RNA was selected by passage through oligo(dT) cellulose. Human brain, liver, and fetal poly(A) RNA was purchased from Clontech Laboratories (Palo Alto, Calif.).

Five μ g of poly(A) containing RNA or 25 μ g of total RNA were precipitated and dissolved in 20 μ l of 50% (vol/vol) formaldehyde and 1 \times MEN (20 mM MOPS, pH 6.8, 5 mM sodium acetate, 1 mM EDTA) and incubated for 10 min at 60 $^{\circ}$ C.; 5 μ l of dye marker (50% sucrose, 0.5% bromophenolblue) was added and the samples were loaded on a formaldehyde-agarose gel. Electrophoresis was carried out for 3 hrs. at 100 V and the gel then soaked for 30 min in 20 \times SSC and blotted onto a nitrocellulose or nylon (GeneScreen Plus, Dupont) overnight in 10 \times SSC (Thomas, 1980). The RNA was fixed to the membranes by baking under vacuum for 2 hrs at 80 $^{\circ}$ C. The membranes were prehybridized in 50% formamide, 5 \times Denhart's, 50 mM sodium phosphate, pH 6.8, 10% dextran sulfate and 100 μ g of denatured salmon sperm DNA at 42 $^{\circ}$ C. for 2-4 hrs. Hybridization with the probe was for 16-20 hrs at 42 $^{\circ}$ C. in the above buffer. Filters were washed with 3 \times SSC, 0.1% SDS at 50 $^{\circ}$ C. and then the SSC concentration was lowered according to the level of background, with a final wash in 0.1 \times SSC, 0.1% SDS.

EXAMPLE 8

RT-PCR Quantitation of the FMR-1 Transcript

A PCR based test is devised in which the transcription product from the FMR-1 gene is quantitated with respect to an internal control (HPRT gene), in RNA samples from Fragile X and normal cell lines. In this method the total RNA was extracted from lymphoblastoid cell lines derived from Fragile X affected individuals and normal controls. The cDNA synthesis was performed in vitro from 5 μ g of total RNA using oligo-dT and random primers via a reverse transcriptase reaction. Then PCR from single stranded cDNA was carried out using primers specific for the HPRT cDNA (SEQ ID NOS: 12 and 13) and primers specific for the FMR-1 cDNA (SEQ ID NOS: 8 and 9). The PCR conditions were as follows: 94 $^{\circ}$ C., 1 min; 55 $^{\circ}$ C. 1 min; 72 $^{\circ}$ C. 1 min 45 sec; for 28 cycles and 7 min final extension at 72 $^{\circ}$ C. The PCR products were run on an ABI Horizontal Electrophoresis device, by which the ethidium bromide stained products of each gene were exactly quantitated with respect to each other. Quantitative variations in the expression of the FMR-1 gene in Fragile X patients derived cell lines was then monitored.

EXAMPLE 9

Isolation of YACs Spanning the Fragile X Translocation Breakpoints

Through regional mapping of YAC clones containing DNA inserts derived from the distal human Xq, an 80 kb YAC (RS46) was found to map within Xq27.3 proximal to the fragile X-associated hybrid breakpoints. A 4.0 kb subclone (p46-1.1) of RS46 identified a normal 600 kb Sal I fragment on PFGE that was altered in size in 6 of 8 proximal translocation hybrids (FIG. 1). In FIG. 1, Y75-1B-M1 is a somatic cell hybrid containing the intact fragile X chromosome from which all other hybrids were derived. Lanes 2-9 are proximal translocation hybrids containing centric human Xpter-q27.3 translocated to different rodent chromosome arms. Q1Q and Q1V are distal translocation hybrids containing human Xq27.3-pter translocated to different centric rodent chromosome. The distal translocation hybrids have

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lost the human sequence detected by p46-1.1. Hybrids Y751B-7 and Y751B-14 show the same 600 kb Sal I fragment as the parental hybrid, however all other proximal translocation hybrids show variant bands indicating that probe p46-1.1 detects a sequence within 600 kb of these translocation breakpoints.

PFGE analyses of these hybrids, with more distant X-linked probes, showed identical band sizes and therefore similar methylation patterns as might be expected since the hybrids were all derived from the same parental fragile X somatic cell hybrid (Y75-1B-M1). These data suggest that in 75% of the proximal translocation hybrids, the human breakpoint is within the 600 kb Sal I fragment observed in the parental, intact fragile X hybrid. In the translocation hybrids, the distal human Sal I site is lost and replaced by heterologous translocations containing different rodent Sal I sites.

Since YAC RS46 does not hybridize to the DNA of the distal translocation hybrids and therefore does not cross these translocation breakpoints, additional YACs were sought of this region. A YAC library developed at the Human Polymorphism Study Center (CEPH) was screened using RS46 specific oligonucleotide primers SEQ ID NOS: 4 and 5 or SEQ ID NOS: 6 and 7. A YAC of 475 kb (209G4) was identified which completely overlaps YAC RS46 and includes sequences distal to the proximal translocation breakpoints which are present in 13 or 14 distal translocation breakpoints. YAC 209G4 encompasses 86% (19/22) of both the proximal and distal translocation breakpoints and thus identifies a fragile X-associated breakpoint cluster region. In situ hybridization using YAC 209G4 showed localization to the expressed fragile X site (FIG. 2). In FIG. 2, panel A represents the localization of YAC 209G4 to the expressed fragile X site. The centromeric signal is due to pBamX5, indicating the human X chromosome with slight hybridization to acrocentric chromosomes; Panel B shows a DAPI stained spread of panel A showing the expressed fragile X site; Panel C shows localization of cosmid 7.1 to the fragile X region; and finally, panel D shows localization of cosmid 22.3 to the fragile X region.

The signal includes both flanking boundaries of the isochromatid gap of the fragile site as well as the gap itself, suggesting the presence of uncondensed DNA within the fragile site and indicating that YAC 209G4 includes this region.

The close proximity of these YACs to the fragile X locus was independently supported by genetic linkage studies between a polymorphism identified in YAC RS46 and the fragile X locus. DXS548 is a dinucleotide repeat which reveals 9 alleles of variable length that are informative in >80% of fragile X families. In highly selected families previously shown to have crossovers with tightly linked flanking markers, DXS548 cosegregated, without recombination, with the fragile X locus (lod score of 6.95 at $\Theta=0$). As shown in FIG. 3, a carrier daughter and affected son are recombinant between the fragile X locus (FRAXA) and proximal markers DXS 539 (probe JH89) and DXS 369 (probe RN1) which map approximately 5 cM proximal to FRAXA with lod scores >40. The carrier mother shows two DXS 548 alleles at 196 and 194 bp (M1 and M2, respectively). The paternal 204 allele of the father is seen in the carrier daughter (II-1) who also inherited the maternal 196 bp allele. All three affected males inherited the 196 bp maternal allele (compare with the 194 allele of the normal daughter (II-5). The carrier daughter (II-1) and affected son (II-2) are both recombinants between proximal markers DXS 150, DXS 369 and DXS 539. However, these indi-

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viduals are non-recombinant with DXS 548, placing this locus to the crossovers closer to the fragile X locus. Therefore, DXS 548 positions YACs RS46 and 209G4 near the mutation responsible for the clinical phenotype of the fragile X syndrome.

EXAMPLE 10

Physical Map of YAC 209 G4

A physical map of YAC 209G4 and of the corresponding genomic region was developed and is shown in FIG. 4. In FIG. 4(A), the physical map of the fragile X chromosome in the vicinity of the Fragile X locus is shown. The Sal I sites which give rise to the 600 kb fragment seen in hybrid Y75-1B-M1 probed with p46-1.1 and the normal 620 kb BssH II fragment observed in normal X chromosomes can be seen. The sites within the box are those previously shown to be methylated on the fragile X chromosome. The position and orientation of FMR-1 is shown.

In FIG. 4(B), a higher resolution physical map derived from both YAC inserts and genomic DNA is shown. Probe p46-1.1 and the DXS 548 loci are shown as are the positions of cDNAs and cosmids. YACs RS46 and 209G4 are shown below in alignment with the map (Hatched boxes indicate YAC vector sequences). The positions of the translocation breakpoints are shown as well as the orientation of the map relative to the X chromosome telomeres.

A CpG-island containing 5 infrequent-cleaving restriction endonuclease sites was identified 150 kb distal to CSX 548. This CpG-island appears hypermethylated on the fragile X chromosome. It is known in the art that there is an absence of a normal 620 kb BssH II fragment (FIG. 4A) in patients and most carriers of the fragile X syndrome. The absence of the fragment appears to be due to the methylation (and therefore resistance to cleavage) of the BssH II site (b in FIG. 4B) leading to a very large band which fails to resolve on PFGE. Since CpG-islands often are found 5' to mammalian genes and since methylation of such islands may influence expression of associated genes, it is possible a gene may reside nearby this fragile X-related CpG-island and its expression (or lack of) may be responsible for at least a portion of the fragile X phenotype.

EXAMPLE 11

Cosmid Contig Surrounding the Fragile X-Related CpG Island and Breakpoint Cluster Region

To characterize the region surrounding the CpG-island, a cosmid library was constructed from the yeast clone harboring YAC 209G4 and cosmids containing human DNA were identified by hybridization to human-specific repetitive elements. In situ hybridization with several human cosmids showed signals in (FIG. 2C) and on the edge (FIG. 2D) of the fragile X gap. A four cosmid contig was identified which spans the fragile X-related CpG island (FIG. 4B) from BssH II site a (cosmid 22.3) through BssH II site c (cosmid 4.1).

Cosmid 22.3 was found to include the breakpoints of 11 of 16 tested translocation hybrids (4/5 proximal translocations and 7/11 distal translocations; all 16 breakpoints map within YAC 209G4). As shown in FIG. 5A, nine bands (including doublet bands at 5.6 and 5.5 kb), surveying approximately 44 kb of genomic DNA, are observed on Southern analysis of EcoR I digested DNA of the intact fragile X hybrid (Y75-1B-M1) following hybridization with radiolabeled and preannealed cosmid 22.3. Of these nine bands, three are present in the distal Q1X (with a novel 4.8 kb junctional fragment). The 7.4 kb band of the intact X hybrid Y75-1B-M1 is absent in both translocation hybrids indicating that both breakpoints fall within this interval. The other nine hybrids all exhibited patterns similar to either

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micro21D or Q1X, with distinct junctional fragments allowing identification of a fragile X-associated breakpoint cluster region (FXBCR) with this 7.4 kb fragment.

The 7.4 kb EcoR I fragment observed above on the fragile X chromosome was not observed in restriction digests of the overlapping cosmids 22.3 and 31.4. However, comparison of the cosmid restriction maps with the EcoR I fragments detected by c22.3 show a 5.1 kb fragment in the cosmids that is absent in Y75-1B-M1 and replaced by the 7.4 kb fragment. As shown in FIG. 6A, this 5.1 kb fragment contains the BssH II site b exhibiting fragile X specific hypermethylation. This fragment was subcloned from c31.4 and used to analyze hybrid breakpoints. As shown in FIG. 5B, the 5.1 kb fragment (pE5.1; FIG. 6B) hybridizes specifically to the 7.4 kb EcoR I fragment of the fragile X chromosome and clearly shows the junctional fragments in micro21D and Q1X. Thus a fragment length difference exists between the normal DNA used to construct YAC 209G4 and the fragile X chromosome of hybrid Y75-1B-M1, and this fragment identifies the FXBCR.

EXAMPLE 12

Fragile X Breakpoint Cluster Region Rearranged in Fragile X Patients

The results of Southern hybridization of EcoR I digested DNA from two normal and seven unrelated fragile X individuals using pE5.1 as probe are shown in FIG. 7. In FIG. 7, Lanes 1, 6 and 7 demonstrate hybridization of the normal 5.1 kb EcoR I fragment in placental DNA (lane 1) and cloned into a cosmid (22.3) or YAC vector (209G4) and seeded into hamster DNA at single-copy level. Somatic cell hybrids containing portions of fragile X chromosomes in hamster backgrounds show bands of altered size from the normal 5.1 kb fragment. Lane 2 contains the hybrid X3000-11.1. Lane 3 contains DNA from micro28D, a proximal hybrid with a breakpoint distal to the fragile site and lane 4 contains DNA from miceo21D, a proximal hybrid with the same chromosome as micro28D, however with a breakpoint detected by pE5.1. Lane 5 contains hamster DNA. Lanes 8-12 contain DNA from 5 unrelated fragile X patients' lymphoblastoid lines. The bands altered from the normal 5.1 kb are seen in each fragile X sample.

The normal samples (two of five normal samples are shown) exhibit the expected 5.1 kb fragment while all seven fragile X patient DNAs exhibited larger EcoR I fragments with variable increases in size, including the 7.4 kb fragment observed from hybrid Y75-1B-M1. These data suggest an insertion or amplification event within the normal 5.1 kb fragment that is specific for the fragile X chromosome and is coincident with the fragile X-associated breakpoint cluster region and the fragile X-related CpG island.

EXAMPLE 13

Identification and Characterization of FMR-1

In order to search for transcripts associated with the fragile X region, the cosmid subclones of YAC 209G4 were used as hybridization probes to screen a cDNA library derived from normal human fetal brain RNA. Cosmid 4.1, containing BssH II site c (FIG. 4B), identified cDNA clone BC22. A map of FMR-1 cDNA clones is shown in FIG. 8. Restriction digestion and sequence analysis revealed an insert in BC22 of 2835 bp at location 934 to 3765 of SEQ ID NO: 1, with an open reading frame at one end extending 1033 bp to a stop codon. Since the reading frame remains open at the 5' end of the clone, BC22 was used to identify related cDNAs from the same library. Several overlapping clones were isolated, one of which, BC72, was characterized in greater detail. This clone extended the cDNA sequence

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another 933 bp in the 5' direction, and overlapped BC22 for approximately 2000 bp toward the 3' end. Sequence analysis demonstrated that the same reading frame remained open through the 5' end of BC72, indicating that the 5' end of the mRNA has not yet been reached, and allowing prediction of a portion (657 amino acids) of the encoded protein. It remains unclear if the entire 3' portion also was isolated since no poly(A) tract was found at the end of BC22, however a putative polyadenylation addition signal is observed in position 3741 following numerous in frame stop codons. In SEQ ID NO: 1, nucleotides 1–1027 derive from BC72 and nucleotides 934–3765 are from BC22.

A repeated DNA sequence is found close to the 5' end of BC72 with 28 CGG triplets interspersed with two AGG triplets. This CGG repeat encoding 30 contiguous arg residues begins with base 37 and extends to base 127. In the predicted open reading frame, this repeat would generate a protein domain composed of 30 contiguous arginine residues. Homology searches with the predicted protein sequence identify significant overlaps with a number of arginine-rich proteins, although none contain a polyarginine stretch of equivalent length. The remainder of the protein shows no significant homology in protein database searches. However, searches against DNA sequence databases identify several related sequences, the strongest of which is with the human androgen receptor (AR). This is an X-linked gene (mapping to Xq12) with an identical, though smaller, CGG repeat in the first exon which encodes a polyglycine stretch.

EXAMPLE 14

Northern Hybridization

Northern hybridization using the BC22 insert as probe was run. (FIG. 9). Five μ g of poly(A) selected RNA from human brain (lane 1) and normal placenta (lane 2) were electrophoresed, blotted onto a GeneScreen Plus filter and hybridized with radiolabeled BC22 insert. A single hybridizing species of approximately 4.8 kb is observed in each lane. As seen in FIG. 9, this procedure detects a mRNA of approximately 4.8 kb in human brain and placenta. This indicates that the 3.8 kb of cDNA obtained does not contain the entire mRNA of this gene. The probe failed to detect signal in human liver, fetal lung and fetal kidney but did detect message in lymphocytes.

EXAMPLE 15

Zoo Blot Analysis

Hybridization of BC22 to DNA samples isolated from a number of different organisms was run (FIG. 10). Ten μ g of DNA from each species was cleaved with EcoRI and electrophoresed and blotted onto a nylon membrane. Hybridization was carried out with labelled cDNA overnight using standard conditions and washed to a final stringency of 0.2 \times SSC for 5 min at 65 $^{\circ}$ C. Hybridization signals were observed with all organisms with the exception of *Drosophila melanogaster*. Since this blot was washed under very stringent conditions (final wash in 0.2 \times SSC at 65 $^{\circ}$ C. for 5 min), cross hybridization may be observed in *Drosophila* under less stringent conditions. However, the high stringency of the final wash does indicate the highly conserved nature of this sequence particularly in *C. elegans*.

EXAMPLE 16

Location of FMR-1 Gene Relative to the Fragile X-Related CpG Island and FXBCR

BC22 demonstrates hybridization to the 70 kb fragment of YAC 209G4 between BssH II sites b and c as well as to cosmids 4.1, 34.4, 31.4 and 22.3 (FIG. 4), indicating exons spanning over 80 kb of DNA. The proximal/distal orienta-

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tion of the transcript was determined by hybridizing end fragments of BC22 to the cosmid contig. Since the 3' end of BC22 detected cosmid 4.1 and the 5' end detected cosmid 22.3, the transcriptional orientation was distal from BssH II site b toward the Xq telomere. This suggests the potential involvement of the fragile X-related CpG island in the regulation of this gene. A 1 kb 5' fragment of BC72 (to the Hind III site at position 1026 of SEQ ID NO: 1) was used to study the location of the exons encoding this portion of the mRNA in the cosmid and YAC clones. In cosmid 22.3, this probe identifies three EcoR I fragments (FIG. 6A) distal to the BssH II site b. One of the fragments contains the BssH II site (b) as well as the breakpoint cluster region and exhibits length variation in fragile X patients. Restriction mapping and direct sequencing of the 5.1 kb EcoR I fragment using a primer derived from BC72 sequence (position 223 to 246) demonstrated an exon immediately distal to the BssH II site b. This exon contains an Xho I site (position 137 in FMR-1 cDNA sequence) that is found 310 nucleotides from the BssHII II site in genomic DNA (FIG. 6B). This exon also contains the block of CGG repeats which are seen in the sequence analysis of the genomic DNA as well. Thus the CGG repeat block is found within the fragile X-related CpG island and constitutes a portion of this CpG-rich region.

EXAMPLE 17

A PCR Assay to Determine Fragile X Disease

A PCR based test is devised in which the length of genomic DNA at the fragile X site from an individual is determined. In this method the total DNA was extracted from lymphoblastoid cells from fragile X and normal individuals. Oligonucleotide primers (SEQ ID NO: 10 and SEQ. ID. No. 11) were used in PCR using the following conditions: 94 $^{\circ}$ C. 1 min. 72 $^{\circ}$ 2 min. for 50 cycles and a 7 min final extension at 72 $^{\circ}$ C. The use of 10% dimethylsulfoxide in the reaction is important for enhancing the ability to amplify this GC-rich sequence. The PCR products are visualized after size separation by electrophoresis using ethidium bromide staining. Differences in size between PCR products from normal and fragile X samples are observed, and these correspond to variation in the number of CGG repeats present.

Alternative conditions using oligonucleotide primers (SEQ ID NO: 10 and SEQ ID NO: 11) can be used in PCR: 95 $^{\circ}$ C. for 10 min. for initial denaturation, followed by 25 cycles of DNA reannealing (65 $^{\circ}$ C., 1 min.), elongation (72 $^{\circ}$ C., 2 min.), and denaturation (95 $^{\circ}$ C., 1.5 min.). The reaction contains 100 ng of test DNA, 3 pmoles of each primer, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM MgCl₂, 200 uM dATP, 200 uM dCTP, 200 uM dTTP, 50 uM dGTP, 150 uM 7-deaza-dGTP, 10% DMSO, 2–4 uCi of ³²P-dCTP and 0.45 units of Ampli-Taq DNA polymerase in a 15 ul volume. To visualize results of these assays, radioactive PCR products were heated to 95 $^{\circ}$ C. for 2 min., then separated on a denaturing DNA sequencing gel (acrylamide). Alleles are sized relative to a sequencing ladder derived from bacteriophage M13, and the size differences are taken to correspond to the number of CGG repeats present. The range of repeats in the normal population is from 4–46, with a mean number of 29. In some fragile X chromosomes, the number of repeats can be assayed, and is between 50 and about 150. At present when there are greater than about 150 repeats these assay conditions do not amplify the fragile X chromosome. The use of 7-deaza dGTP, DMSO, high annealing and denaturing temperatures and ³²P for detection are all important parameters for the success of these reactions. Lack of amplification in males, or amplification of only one of the two expected alleles in females with this protocol is taken as

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an indication of the presence of the fragile X mutation. A pair of oligonucleotide primers capable of acting as an internal control for amplification under these conditions has been derived from the human androgen receptor gene (Xq11-q12) (SEQ ID NO 21 and SEQ ID NO 22). A product is obtained from these primers in all the negative fragile X patients tested.

Results from five unrelated fragile X families are shown. B6 and D3 are affected females and C2 has been clinically diagnosed as "slow".

Consistent length amplification products were obtained in multiple assays of the same allele in the same sample and in multiple generations in pedigrees (FIG. 14) indicating that this PCR assay is faithful to the genomic organization and that the normal number of CGG repeats appears stable in meiosis.

This PCR assay in fragile X families was capable of identifying all alleles of normal length, as well as some alleles of increased length (FIG. 14). In general, all affected males failed to amplify. This is not surprising given the presence of large (1000–2000 bp) length increases present in these individuals. Two affected males (FIG. 14, C4 and D1) did yield PCR products which are larger than normal (60 and >100 repeats). These individuals are mosaic by Southern hybridization, with EcoRI fragments of near normal length when assayed with pE5.1. Thus, all affected males give abnormal results (no amplification or larger than normal). Flanking region amplification of all affected males indicates that the null result obtained for the CGG assay is not due to technical difficulties or deletion. For some female carriers (A2, A4, C3, D2 and E5), only one normal allele can be detected by PCR while the other allele is too large to amplify. These results were further confirmed by Southern blot analysis. In family A, the daughter A4 was cytogenetically diagnosed as a normal female. However, the PCR assay indicated that she is indeed a carrier, having inherited the maternal fragile X allele. This is an example where the PCR based method can be a powerful diagnostic assay for carriers.

Normal transmitting males (NTM) and their daughters exhibit abnormal sized products when the CGG region is assayed. These products are 69–220 bp larger than the average normal product, suggesting repeats numbering between 52 and 100 CGGs. For female carriers (B3, B5, and E2) who are daughters of NTMs, the normal allele is accompanied by a mutant allele approximately 200 bp larger than the normal. These premutation alleles can be stably inherited (see FIG. 14, family B). In the case of family E, the carrier mother E4 has a normal allele and a 200 bp larger allele. Her daughter E5 received one normal allele presumably from her father and one abnormal allele much larger than her mother's according to Southern blot analysis. Her son (E4) has an even larger allele and is penetrant for fragile X syndrome. This is a case where amplification events occurred through more than one generation before phenotypic expression.

EXAMPLE 18

Elucidation of Fragile X Site

To elucidate the fragile X site at the molecular level, somatic cell hybrids were isolated that contained translocations between rodent chromosomes and the human fragile X chromosome, retaining either human Xpter-q27.3 or human Xq27.3-qter, referred to as proximal or distal translocations, relative to the fragile X site. Since the high frequency and specificity of the chromosome breakage was not observed in normal X hybrids and since the translocation breakpoints map within the same interval defined by polymorphic loci

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which flank the fragile X locus, these breakpoints are likely to coincide with the fragile X site.

A yeast artificial chromosome (YAC) has been isolated which spans some of these translocation breakpoints and includes polymorphic loci which flank the fragile X locus. Within this region, a fragile X-related CpG island was identified which is aberrantly hypermethylated in patients and most carriers of the fragile X syndrome. Although the significance of this CpG-island hypermethylation remains unclear, these data do imply the presence of a gene, perhaps inactivated by methylation, within a genomic region which includes the fragile X-associated hybrid breakpoints.

EXAMPLE 19

PCR-Based Assay for Methylation at the Fragile X-Associated CpG Island

A PCR-based test is devised in which the methylation status of the genomic DNA at the fragile X site from an individual is determined. In this method the total DNA is extracted from lymphoblastoid cells or whole blood from normal and fragile X individuals. The DNA is then subjected to digestion with a methylation-sensitive restriction endonuclease such as BssH II. Both digested and undigested DNAs are then subjected to PCR. Oligonucleotide primers (SEQ ID NO: 19 and SEQ ID NO: 20) were used in PCR under the following conditions: 95° C. for 10 min. for initial denaturation, followed by 35 cycles of DNA reannealing (65° C., 1 min.), elongation (72° C., 2 min.), and denaturation (95° C., 1.5 min.). The reaction contains 100 ng of test DNA, 10 pmoles of each primer, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM MgCl₂, 200 uM dATP, 200 uM dCTP, 200 uM dTTP, 200 uM dGTP, 10% DMSO, and 1.5 units of Ampli-Taq DNA polymerase in a 50 ul volume. Detection of the amplification products is accomplished by agarose gel electrophoresis and staining with ethidium bromide. The presence of a PCR product in digested samples is indicative of methylation at the restriction cleavage site. Amplification of undigested samples serves as a control—the absence of amplification in the digested sample indicates no methylation at the site.

FIG. 15 shows the methylation status of normal and affected male DNAs tested by PCR. Lanes 1–6 are patient DNAs and lanes 7–12 are normal DNAs. Genomic DNAs were digested to completion by BssH II. 200 ng of undigested (odd numbered lanes) or digested (even numbered lanes) DNA was used for PCR amplification. The conditions for the PCR reactions were those described in the example. The PCR products were examined on a 2% agarose gel and stained with ethidium bromide.

PCR products are obtained from male patient DNAs, but not from normal DNAs after digestion with BssH II. Examples of 3 normal and 3 affected males are shown in FIG. 15. While not useful in females due to methylation of this CpG island on the inactive X chromosome, this test in conjunction with the CGG assay represents a rapid and simple screen for fragile X males.

EXAMPLE 20

PCR-Based Assay for the Integrity of the Sequences Surrounding the CGG Repeat

A PCR-based test is devised in which the length of the genomic DNA at the fragile X site from an individual is determined. In this method the total DNA is extracted from lymphoblastoid cells or whole blood from normal and fragile X individuals. Oligonucleotide primers (SEQ ID NO: 15 and SEQ ID NO: 16) or primers (SEQ ID NO: 10 and SEQ ID NO: 17) or primers (SEQ ID NO: 11 and SEQ ID NO: 18) were used in PCR under the following conditions:

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95° C. for 10 min. for initial denaturation, followed by 50 cycles of DNA reannealing (65° C., 1 min.), elongation (72° C., 2 min.), and denaturation (95° C., 1.5 min.). The reactions contains 100 ng of test DNA, 10 pmoles of each primer, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM MgCl₂, 200 uM dATP, 200 uM dCTP, 200 uM dTTP, 200 uM dGTP, 10% DMSO, and 1.5 units of Ampli-Taq DNA polymerase in a 50 ul volume. Detection of the amplification products is accomplished by agarose gel electrophoresis and staining with ethidium bromide. Alternatively, the inclusion of ³²P and detection via autoradiography can be employed. Presence of a product of the expected length is indicative of normal sequence composition between primer binding sites. No alterations have been observed in fragile X individuals. These assays can serve as controls for the CGG alterations inferred from negative PCR results obtained with primers (SEQ ID NO: 10 and SEQ ID NO: 11).

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All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual patent or publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and attain the ends and advantages mentioned as well those inherent therein. The sequences, methods, procedures and techniques described herein are presently representative of the preferred embodiments and are intended to be exemplary and not intended as limitations on the scope. Changes therein and other uses which are encompassed within the spirit of the invention or defined by the scope of the appended claims will occur to those skilled in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 24

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3765 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GACGGAGGCG CCCGTGCCAG GGGGCGTGCG GCAGCGCGGC GCGGCGGCG GCGGCGGCGG      60
CGGCGGAGGC GCGGCGGCG GCGGCGGCG GCGGCGGAGC GCGGCGGCG GCGGCGGCGG      120
CGGCGGCTGG GCCTCGAGCG CCCGCAGCCC ACCTCTCGGG GCGGGGCTCC CGGCGCTAGC      180
AGGGCTGAAG AGAAGATGGA GGAGCTGGTG GTGGAAGTGC GGGGCTCCAA TGGCGCTTTC      240
TACAAGGCAT TTGTAAGAAG TGTTTCATGAA GATTCAATAA CAGTTGCATT TGAACAACAAC      300
TGGCAGCCTG ATAGGCAGAT TCCATTTTCAT GATGTCAGAT TCCCACCTCC TGTTAGGTTAT      360
AATAAAGATA TAAATGAAAG TGATGAAGTT GAGGTGTATT CCAGAGCAAA TGA AAAAAGAG      420
CCTTGCTGTT GGTGTTAGC TAAAGTGAGG ATGATAAAGG GTGAGTTTTA TGTGATAGAA      480
TATGCAGCAT GTGATGCAAC TTACAATGAA ATGTGCACAA TTGAACGCTT AAGATCTGTT      540
AATCCCAACA AACCTGCCAC AAAAGATACT TTCCATAAGA TCAAGCTGGA TGTGCCAGAA      600
GACTTACGGC AAATGTGTGC CAAAGAGGCG GCACATAAGG ATTTTAAAAA GGCAGTTGGT      660
GCCTTTTCTG TAACTTATGA TCCAGAAAAT TATCAGCTTG TCATTTTGTC CATCAATGAA      720
GTCACCTCAA AGCGAGCACA TATGCTGATT GACATGCACT TTCGGAGTCT GCGCACTAAG      780
TTGCTCTGTA TAATGAGAAA TGAAGAAGCT AGTAAGCAGC TGGAGAGTTC AAGGCAGCTT      840
GCCTCGAGAT TTCATGAACA GTTTATCGTA AGAGAAGATC TGATGGGTCT AGCTATTGGT      900
ACTCATGGTG CTAATATTCA GCAAGCTAGA AAAGTACCTG GGGTCACTGC TATTGATCTA      960
GATGAAGATA CCTGCACATT TCATATTTAT GGAGAGGATC AGGATGCAGT GAAAAAAGCT     1020
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AGAAGCTTTC	TCGAATTTGC	TGAAGATGTA	ATACAAGTTC	CAAGGAACTT	AGTAGTAATA	1080
GGAAAAATG	GAAAGCTGAT	TCAGGAGATT	GTGGACAAGT	CAGGAGTTGT	GAGGGTGAGG	1140
ATTGAGGCTG	AAAATGAGAA	AAATGTTCCA	CAAGAAGAGG	AAATTATGCC	ACCAAATTCC	1200
CTTCCTTCCA	ATAATTCAAG	GGTTGGACCT	AATGCCCCAG	AAGAAAAAAA	ACATTTAGAT	1260
ATAAAGGAAA	ACAGCACCCA	TTTTTCTCAA	CCTAACAGTA	CAAAAGTCCA	GAGGGGTATG	1320
GTACCATTTG	TTTTTGTGGG	AACAAAGGAC	AGCATCGCTA	ATGCCACTGT	TCTTTTGGAT	1380
TATCACCTGA	ACTATTTAAA	GGAAGTAGAC	CAGTTGCGTT	TGGAGAGATT	ACAAATTGAT	1440
GAGCAGTTGC	GACAGATTGG	AGCTAGTTCT	AGACCACCAC	CAAATCGTAC	AGATAAGGAA	1500
AAAAGCTATG	TGACTGATGA	TGGTCAAGGA	ATGGGTCGAG	GTAGTAGACC	TTACAGAAAT	1560
AGGGGGCACG	GCAGACGCGG	TCCTGGATAT	ACTTCAGGAA	CTAATTCTGA	AGCATCAAAT	1620
GCTTCTGAAA	CAGAATCTGA	CCACAGAGAC	GAACTCAGTG	ATTGGTCATT	AGCTCCAACA	1680
GAGGAAGAGA	GGGAGAGCTT	CCTGCGCAGA	GGAGACGGAC	GGCGGCGTGG	AGGGGGAGGA	1740
AGAGGACAAG	GAGGAAGAGG	ACGTGGAGGA	GGCTTCAAAG	GAAACGACGA	TCACTCCCGA	1800
ACAGATAATC	GTCCACGTAA	TCCAAGAGAG	GCTAAAGGAA	GAACAACAGA	TGGATCCCTT	1860
CAGAATACCT	CCAGTGAAGG	TAGTCGGCTG	CGCACGGGTA	AAGATCGTAA	CCAGAAGAAA	1920
GAGAAGCCAG	ACAGCGTGGA	TGGTCAGCAA	CCACTCGTGA	ATGGAGTACC	CTAAACTGCA	1980
TAATCTGAAA	GTTATATTTT	CTATACCATT	TCCGTAATTC	TTATTCATA	TTAGAAAAT	2040
TTGTTAGGCC	AAAGACAAAT	AGTAGCAAG	ATGGCACAGG	GCATGAAATG	AACACAAATT	2100
ATGCTAAGAA	TTTTTTATTT	TTTGGTATTG	GCCATAAGCA	ACAAATTTCA	GATTTGCACA	2160
AAAAGATACC	TTAAATTTG	AAACATTGCT	TTTAAACTA	CTTAGCACTT	CAGGGCAGAT	2220
TTTAGTTTTA	TTTTCTAAG	TACTGAGCAG	TGATATTCTT	TGTTAATTTG	GACCATTTTC	2280
CTGCATTGGG	TGATCATTCA	CCAGTACATT	CTCAGTTTTT	CTTAATATAT	AGCATTTATG	2340
GTAATCATAT	TAGACTTCTG	TTTTCAATCT	CGTATAGAAG	TCTTCATGAA	ATGCTATGTC	2400
ATTTTCATGC	CTGTGTCAGT	TTATGTTTTG	GTCCACTTTT	CCAGTATTTT	AGTGGACCCCT	2460
GAAATGTGTG	TGATGTGACA	TTTGTCAATT	TCATTAGCAA	AAAAAGTTGT	ATGATCTGTG	2520
CCTTTTTTAT	ATCTTGGCAG	GTAGGAATAT	TATATTTGGA	TGCAGAGTTC	AGGGAAGATA	2580
AGTTGGAAAC	ACTAAATGTT	AAAGATGTAG	CAAACCCGTG	CAAACATTAG	TACTTTATAG	2640
AAGAATGCAT	GCTTTCATA	TTTTTTTCCT	TACATAAACA	TCAGGTTAGG	CAGTATAAAG	2700
AATAGGACTT	GTTTTTGT	TTGTTTTGTT	GCACTGAAGT	TTGATAAATA	GTGTTATTGA	2760
GAGAGATGTG	TAATTTTTCT	GTATAGACAG	GAGAAGAAAG	AACTATCTTC	ATCTGAGAGA	2820
GGCTAAAATG	TTTTTCAGCTA	GGAACAAATC	TTCCTGGTCG	AAAGTTAGTA	GGATATGCCT	2880
GCTCTTTGGC	CTGATGACCA	ATTTTAACTT	AGAGCTTTTT	TTTTTAATTT	TGTCTGCCCC	2940
AAGTTTTGTG	AAATTTTTCA	TATTTTAATT	TCAAGCTTAT	TTTGGAGAGA	TAGGAAGGTC	3000
ATTTCCATGT	ATGCATAATA	ATCCTGCAAA	GTACAGGTAC	TTTGTCTAAG	AAACATTGGA	3060
AGCAGGTTAA	ATGTTTTGTA	AACTTTGAAA	TATATGGTCT	AATGTTAAG	CAGAATTGGA	3120
AAAGACTAAG	ATCGGTTAAC	AAATAACAAC	TTTTTTTTCT	TTTTTCTTTT	TGTTTTTTGA	3180
AGTGTGGGG	TTTGGTTTTG	TTTTTTGAGT	CTTTTTTTTT	TAAGTAAAT	TTATTGAGGA	3240
AAAAATATGT	AAGGACCTTC	ACTCTAAGAT	GTTATATTTT	TCTTAAAAAG	TAACTCCTAG	3300
TAGGGGTACC	ACTGAATCTG	TACAGAGCCG	TAAAAACTGA	AGTTCTGCCT	CTGATGTATT	3360

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TTGTGAGTTT	GTTTCTTTGA	ATTTTCATTT	TACAGTTACT	TTTCCTTGCA	TACAAACAAG	3420
CATATAAAAT	GGCAACAAAC	TGCACATGAT	TTCACAAATA	TTAAAAAGTC	TTTTAAAAAG	3480
TATTGCCAAA	CATTAATGTT	GATTTCTAGT	TATTTATCT	GGGAATGTAT	AGTATTTGAA	3540
AACAGAAAT	GGTACCTTGC	ACACATCATC	TGTAAGCTGT	TTGGTTTTAA	AATACTGTAG	3600
ATAATTAACC	AAGGTAGAAT	GACCTTGTA	TGTAAGCTGT	CTTGGGCAAT	ATTCTCTGTA	3660
CATATTAGCG	ACAACAGATT	GGATTTTATG	TTGACATTG	TTTGGTTATA	GTGCAATATA	3720
TTTTGTATGC	AAGCAGTTTC	AATAAAGTTT	GATCTTCCTC	TGCTA		3765

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4188 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACTTGGAGGG	GTATAATCAT	TCTAATCAAT	GTGTCCCCTT	TTACTATAAT	ACATTGGAGT	60
TGCAGCTAAT	GCTCTGCTCC	CATTCAGCCT	ATGATGAGAT	TCTCTTTCAG	CCCTATTGGG	120
TTCTTGGCCT	CATGTGACTA	CTCCAAGAC	CCTAGTCCAA	AAGGCTTTTC	CTGTTTGCTA	180
TGGCCTTGAG	GAATGTGGCC	CTAGATCCAC	CGCTTTAAAG	CTGGAGTTCC	ACCAGCAGCA	240
ACATCCTCTC	ATTCTGGGGC	ACCTGCCTGG	GGCAGGTCAT	CCTGCCTCTG	CCAACTCAGT	300
GCTATTAGTT	AACTCTCACC	TGCCATATTC	CAGCTGGAAT	CATCTCCCCT	TCTCCACCCC	360
AGACTAGGTC	ATGTTCCGCC	ATCATGGAAG	CGCCTATCT	TCATACCCCT	TATCACAGCT	420
GCAACTACTC	ATTTACTTGT	CTGACAATTT	GATTTATGTC	CACCTACTTT	GCTAGGTACT	480
AAGTTCAATG	CTGGCAGTCG	TTTCTTCTTT	TTTTTTCITT	TCTGTTTTGC	TCACCGATTT	540
CTCGTTAGCA	CTTAGCACAG	TGTCTGGCAC	ACGATAGATG	CTCCGTCAAC	TTCTCAGTTG	600
GATACCAGCA	TCCCAGAGGG	ACATGGATTA	AGGCAGCTAT	AAGCACGGTG	TAAAAACAGG	660
AATAAGAAAA	AGTTGAGGTT	TGTTTCACAG	TGGAATGTAA	AGGTTGCAA	GGAGGTGCAT	720
CGGCCCTGT	GGACAGGACG	CATGACTGCT	ACACACGTGT	TCACCCACC	CTCTGGCACA	780
GGGTGCACAT	ACAGTAGGGG	CAGAAATGAA	CCTCAAGTGC	TTAACACAAT	TTTTAAAAAA	840
TATATAGTCA	AGTGAAAGTA	TGAAAATGAG	TTGAGGAAAG	GCGAGTACGT	GGTCAAAGC	900
TGGGTCTGAG	GAAAGGCTCA	CATTTTGAGA	TCCCAGTCA	ATCCATGTCC	CTTAAAGGGC	960
ACAGGGTGTG	TCCACAGGGC	CGCCAAAAT	CTGGTGAGAG	AGGGCGTAGA	CGCCTCACCT	1020
TCTGCCTCTA	CGGGTCACAA	AAGCCTGGGT	CACCTGGTT	GCCACTGTTT	CTAGTCAAA	1080
GTCTTCTTCT	GTCTAATCCT	TCACCCCTAT	TCTCGCCTTC	CACTCCACCT	CCCCTCAGT	1140
CAGACTGCGC	TACTTTGAAC	CGGACCAAAC	CAAACCAAAC	CAAACCAAAC	CAAACCAGAC	1200
CAGACACCCC	CTCCCGCGGA	ATCCCAGAGA	GGCCGAAGTG	GGATAACCGG	ATGCATTTGA	1260
TTTCCCACGC	CACTGAGTGC	ACCTCTGCAG	AAATGGGCGT	TCTGGCCCTC	GCGAGGCAGT	1320
GCGACCTGTC	ACCGCCCTTC	AGCCTTCCCG	CCCTCCACCA	AGCCCGCGCA	CGCCCGGCC	1380
GCGCGTCTGT	CTTTCGACCC	GGCACCCCGG	CCGGTTCCCA	GCAGCGCGCA	TGCGCGCGCT	1440
CCCAGGCCAC	TTGAAGAGAG	AGGGCGGGGC	CGAGGGGCTG	AGCCCGCGGG	GGGAGGGAAC	1500

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AGCGTTGATC	ACGTGACGTG	GTTTCAGTGT	TTACACCCGC	AGCGGGCCGG	GGGTTCGGCC	1560
TCAGTCAGGC	GCTCAGCTCC	GTTTCGGTTT	CAC TTCGGT	GGAGGGCCGC	CTCTAGCGGG	1620
CGGCGGGCCG	ACGCGGAGCG	CGGGCGGCGG	CGGTGACGGA	GGCGCCGCTG	CCAGGGGGCG	1680
TGCGGCAGCG	CGGCGGCGCG	GGCGGCGGCG	GCGGCGGCGG	CGGCGGCGGC	GGCGGCGGCT	1740
GGGCCTCGAG	CGCCCGCAGC	CCACCTCTTG	GGGGCGGGCT	CCC GGCGCTA	CAGGGCTGAA	1800
GAGAAGATGG	AGGAGCTGGT	GGTGGAAAGT	CGGGCTCCAA	TGGCGCTTTC	TACAAGGTAC	1860
TTGGCTCTAG	GGCAGGCCCC	ATCTTCGCCC	TTCCTTCCTT	CCCTTTTTTTC	TTGGTGTCCG	1920
CGGGAGGCAG	GCCCGGGGCC	CTCTTCCCGA	GCACCGCGCC	TGGGTGCCAG	GGCAGCTCG	1980
GCGGGATGTT	GTTGGGAGGG	AAGGACTGGA	CTTGGGGCCT	GTTGGAAGCC	CCTCTCCGAC	2040
TCCAGAGGCC	CTAGCGCCTA	TCGAAATGAG	AGACCAGCGA	GGAGAGGGTT	CTCTTTCGGC	2100
GCCGAGCCCC	GCCGGGTGA	GCTGGGGATG	GGCGAGGGCC	GGCGGCAGGT	ACTAGAGCCG	2160
GGCGGGAAGG	GCCGAAATCG	GCGCTAAGTG	ACGGCGATGG	CTTATTCCTC	CTTTCCTAAA	2220
CATCATCTCC	CAGCGGATC	CGGGCTGTG	GTGTGGGTAG	TTGTGGAGGA	GCGGGGGGCG	2280
CTTCAGCCGG	GCCGCTCCTT	GCAGCGCCAA	GAGGGCTTCA	GGTCTCCTTT	GGCTTCTCTT	2340
TTCCGGTCTA	GCATTGGGAC	TTCGGAGAGC	TCCACTGTTC	TGGGCGAGGG	CTGTGAAGAA	2400
AGAGTAGTAA	GAGCGGTAG	TCGGCACCAA	ATCACAATGG	CAACTGATTT	TTAGTGGCTT	2460
CTCTTTGTGG	ATTCGGAGG	AGATTTTAGA	TCCAAAAGTT	TCAGGAAGAC	CCTAACATGG	2520
CCCAGCAGTG	CATTGAAGAA	GTTGATCATC	GTGAATATTC	GCGTCCCCCT	TTTTGTAAAA	2580
CGGGGTAAT	TCAGGAATGC	ACATGCTTCA	GCGTCTAAAA	CCATTAGCAG	CGCTGCTACT	2640
TAAAAATTTG	GTGTGTGTGT	TTAAGTTTCC	AAAGACCTAA	ATATATGCCA	TGAAACTTCA	2700
GGTAATTAAC	TGAGAGTATA	TTATTACTAG	GGCATTTTTTT	TTTTAACTGA	GCGAAAATAT	2760
TTTTGTGCC	CTAAGAACTT	GACCACATTT	CCTTTGAATT	TGTGGTGTG	CAGTGGACTG	2820
AATTGTGTAG	GCTTTATATA	GGCATTCATG	GGTTTACTGT	GCTTTTAAAA	GTTACACCAT	2880
TGCAGATCAA	CTAACACCTT	TCAGTTTTAA	AAGGAAGATT	TACAAATTTG	ATGTAGCAGT	2940
AGTGCCTTTG	TTGGTATGTA	GGTGCCTGAT	AAATTCATCT	ATAAATCTC	ATTTCTTTTT	3000
GAATGTCTAT	AACCTCTTTC	AATAATATCC	CACCTTACTA	CAGTATTTTG	GCAATAGAAG	3060
GTGCGTGTGG	AAGGAAGGCT	GGAAAATAGC	TATTAGCAGT	GTCCAACACA	ATTCTTAAAT	3120
GTATTGTAGA	ATGGCTTGAA	TGTTTCAGAC	AGGACACGTT	TGGCTATAGG	AAAATAAACA	3180
ATTGACTTTA	TTCTGTGTTT	ACCAATTTTA	TGAAGACATT	TGGAGATCAG	TATATTTTAT	3240
AAATGAGTAA	AGTATGTAAA	CTGTTCATA	CTTTGAGCAC	AAAGATAAAG	CCTTTTGCTG	3300
TAAAAGGAGG	CAAAGGTAA	CCCCGCTTTT	ATGTTCTTAA	CAGTCTCATG	AATATGAAAT	3360
TGTTTCAGTT	GACTCTGCAG	TCAAATTTT	AATTCATTG	ATTTTATTGA	TCCATAATTT	3420
CTTCTGGTGA	GTTTCCGTAG	AATCGTTCAC	GGTCCTAGAT	TAGTGGTTTTT	GGTCACTAGA	3480
TTTCTGGCAC	TAATAACTAT	AATACATATA	CATATATATG	TGTGAGTAA	GGCTAATGGT	3540
TAGGCAAGAT	TTTGATTGAC	CTGTGATATA	AACTTAGATT	GGATGCCACT	AAAGTTTGCT	3600
TATCACAGAG	GGCAAGTAGC	ACATTATGGC	CTTGAAGTAC	TTATTGTTCT	CTTCCAGCAA	3660
CTTATGATTT	GCTCCAGTGA	TTTTCTTGCA	CAC T GACTGG	AATATAAGAA	ATGCCTTCTA	3720
TTTTTGCTAT	TAATTCCTCT	CTTTTTTGTT	TTGTTTTGTA	ACGAAGTTGT	TTAACTTGAA	3780
GGTGAATGAA	GAATAGGTTG	GTTGCCCTTT	AGTTCCTTGA	GGAGAAATGT	TAATACTTGA	3840
ACAAGTGTGT	GTCAGACAAA	TTGCTGTTAT	GTTTATTTAA	TTAAGTTTGA	TTTCTAAGAA	3900

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AATCTCAAAT GGTCTGCACT GATGGAAGAA CAGTTTCTGT AACAAAAAAG CTGAAATTT 3960
TTATATGACT TATAAATACTG CTGTGAGTTT TAAAAGTAAA GCAAAAGTAA ACTGAGTTGC 4020
TTGTCCAGTG GGATGGACAG GAAAGATGTG AAATAAAAAC CAATGAAAAA TGAAGTCTG 4080
TGGAGAAGTG TTACATTTAT GGAAAAAGAA ATAGGAACCT TGTTCATCAA ATTGATAGAA 4140
AAGCTTTTAA AACTAAACAA ATCAAACAAC TTGAGTATAA TGGAATTC 4188

```

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 229 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```

GAATTCAGGT AAGCTATCTT GAAAGGGGAA ATATCAAAG CTAGAGATCA GAGTAAGGCT 60
GAGACTCAGA GTCAAGTGGG GAAGACTAAG TTGCAGTATG TACTGGCAGT GAAGATAAGT 120
ATTTATTTCAT TCATTGAACA TACCTTGAAA TCAACCACTT TTAATGTGCC AGGGACACAA 180
AGATAGAAAA GACATTTGCC CTGTCTGGAA GGTACTAATA ATCCAATAA 229

```

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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CTTGCCAACC GTTCAGCCAC 20

```

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```

ATTTCTGGA GCACAGACTG 20

```

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
AGAGCTTCAC TATGCAATGG AATC 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
GTACATTAGA GTCACCTGTG GTGC 24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
TAGCTAACCA CCAACAGCAA GGC 23

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
AACTGGCAGC CTGATAGGCA GATTC 25

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
GCTCAGCTCC GTTCGGTTT CACTTCCGGT 30

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
AGCCCCGCAC TTCCACCACC AGCTCCTCCA 30

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
CGTGGGGTCC TTTTACCAG CAAG 24

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
AATTATGGAC AGGACTGAAC GTC 23

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 657 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Asp Gly Gly Ala Arg Ala Arg Gly Arg Ala Ala Ala Arg Arg Arg Arg
1 5 10 15

Arg
20 25 30

Arg Leu Gly Leu Glu Arg Pro
35 40 45

Gln Pro Thr Ser Arg Gly Arg Ala Pro Gly Ala Ser Arg Ala Glu Glu
50 55 60

Lys Met Glu Glu Leu Val Val Glu Val Arg Gly Ser Asn Gly Ala Phe
65 70 75 80

Tyr Lys Ala Phe Val Lys Asp Val His Glu Asp Ser Ile Thr Val Ala
85 90 95

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Phe Glu Asn Asn Trp Gln Pro Asp Arg Gln Ile Pro Phe His Asp Val
 100 105 110

Arg Phe Pro Pro Val Gly Tyr Asn Lys Asp Ile Asn Glu Ser Asp
 115 120 125

Glu Val Glu Val Tyr Ser Arg Ala Asn Glu Lys Glu Pro Cys Cys Trp
 130 135 140

Trp Leu Ala Lys Val Arg Met Ile Lys Gly Glu Phe Tyr Val Ile Glu
 145 150 155 160

Tyr Ala Ala Cys Asp Ala Thr Tyr Asn Glu Ile Val Thr Ile Glu Arg
 165 170 175

Leu Arg Ser Val Asn Pro Asn Lys Pro Ala Thr Lys Asp Thr Phe His
 180 185 190

Lys Ile Lys Leu Asp Val Pro Glu Asp Leu Arg Gln Met Cys Ala Lys
 195 200 205

Glu Ala Ala His Lys Asp Phe Lys Lys Ala Val Gly Ala Phe Ser Val
 210 215 220

Thr Tyr Asp Pro Glu Asn Tyr Gln Leu Val Ile Leu Ser Ile Asn Glu
 225 230 235 240

Val Thr Ser Lys Arg Ala His Met Leu Ile Asp Met His Phe Arg Ser
 245 250 255

Leu Arg Thr Lys Leu Ser Leu Ile Met Arg Asn Glu Glu Ala Ser Lys
 260 265 270

Gln Leu Glu Ser Ser Arg Gln Leu Ala Ser Arg Phe His Glu Gln Phe
 275 280 285

Ile Val Arg Glu Asp Leu Met Gly Leu Ala Ile Gly Thr His Gly Ala
 290 295 300

Asn Ile Gln Gln Ala Arg Lys Val Pro Gly Val Thr Ala Ile Asp Leu
 305 310 315 320

Asp Glu Asp Thr Cys Thr Phe His Ile Tyr Gly Glu Asp Gln Asp Ala
 325 330 335

Val Lys Lys Ala Arg Ser Phe Leu Glu Phe Ala Glu Asp Val Ile Gln
 340 345 350

Val Pro Arg Asn Leu Val Val Ile Gly Lys Asn Gly Lys Leu Ile Gln
 355 360 365

Glu Ile Val Asp Lys Ser Gly Val Val Arg Val Arg Ile Glu Ala Glu
 370 375 380

Asn Glu Lys Asn Val Pro Gln Glu Glu Glu Ile Met Pro Pro Asn Ser
 385 390 395 400

Leu Pro Ser Asn Asn Ser Arg Val Gly Pro Asn Ala Pro Glu Glu Lys
 405 410 415

Lys His Leu Asp Ile Lys Glu Asn Ser Thr His Phe Ser Gln Pro Asn
 420 425 430

Ser Thr Lys Val Gln Arg Gly Met Val Pro Phe Val Phe Val Gly Thr
 435 440 445

Lys Asp Ser Ile Ala Asn Ala Thr Val Leu Leu Asp Tyr His Leu Asn
 450 455 460

Tyr Leu Lys Glu Val Asp Gln Leu Arg Leu Glu Arg Leu Gln Ile Asp
 465 470 475 480

Glu Gln Leu Arg Gln Ile Gly Ala Ser Ser Arg Pro Pro Pro Asn Arg
 485 490 495

Thr Asp Lys Glu Lys Ser Tyr Val Thr Asp Asp Gly Gln Gly Met Gly
 500 505 510

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Arg Gly Ser Arg Pro Tyr Arg Asn Arg Gly His Gly Arg Arg Gly Pro
 515 520 525

Gly Tyr Thr Ser Gly Thr Asn Ser Glu Ala Ser Asn Ala Ser Glu Thr
 530 535 540

Glu Ser Asp His Arg Asp Glu Leu Ser Asp Trp Ser Leu Ala Pro Thr
 545 550 555 560

Glu Glu Glu Arg Glu Ser Phe Leu Arg Arg Gly Asp Gly Arg Arg Arg
 565 570 575

Gly Gly Gly Gly Arg Gly Gln Gly Gly Arg Gly Arg Gly Gly Gly Phe
 580 585 590

Lys Gly Asn Asp Asp His Ser Arg Thr Asp Asn Arg Pro Arg Asn Pro
 595 600 605

Arg Glu Ala Lys Gly Arg Thr Thr Asp Gly Ser Leu Gln Asn Thr Ser
 610 615 620

Ser Glu Gly Ser Arg Leu Arg Thr Gly Lys Asp Arg Asn Gln Lys Lys
 625 630 635 640

Glu Lys Pro Asp Ser Val Asp Gly Gln Gln Pro Leu Val Asn Gly Val
 645 650 655

Pro

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGAACAGCGT TGATCACGTG ACGTGGTTTC 30

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ACCGAAGTG AAACCGAAAC GGAGCTGAGC 30

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCACGCCCC TGGCAGCGGC GCCTCCGTC A 30

(2) INFORMATION FOR SEQ ID NO: 18:

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
 TGGGCCTCGA GCGCCCGCAG CCCACCTCTC 30
- (2) INFORMATION FOR SEQ ID NO: 19:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
 AGTGCGACCT GTCACCGCCC TTCAGCCTTC 30
- (2) INFORMATION FOR SEQ ID NO: 20:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
 GAAACCACGT CACGTGATCA ACGCTGTTC 30
- (2) INFORMATION FOR SEQ ID NO: 21:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
 ACCAGGTAGC CTGTGGGGCC TCTACGATGG GC 32
- (2) INFORMATION FOR SEQ ID NO: 22:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
 CCAGAGCGTG CGCGAAGTGA TCCAGAACCC GG 32
- (2) INFORMATION FOR SEQ ID NO: 23:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5222 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GAATTCAGGT	AAGCTATCTT	GAAAGGGGAA	ATATCAAAAAG	CTAGAGATCA	GAGTAAGGCT	60
GAGACTCAGA	GTCAAGTGGG	GAAGACTAAG	TTGCAGTATG	TACTGGCAGT	GAAGATAAGT	120
ATTTATTTCAT	TCATTGAAAC	TACCTTGAAA	TCAACCACCTT	TTAATGTGCC	AGGGACACAA	180
AGATAGAAA	GACATTTGCC	CTGCTGCGAA	GGTACTAATA	ATCCAATAAG	GAAAACAGAA	240
ATATAAATAA	ATTATTCTAG	TACACTAACC	ATCATAGTAG	AGGTATTCAA	CATTTGTTGA	300
GTCTCTGCTA	TATGCCAAGC	AGTGTAAATGA	GGAAGCAGAG	GGTATGCACA	AAGTTCTACA	360
AGAGCACAAA	ATAAGTTCTG	GCAAAGGTTT	GTAAAGACAT	TCACAAGGGT	TTTCACCACA	420
GTATGACTTC	AGGGAGTTGG	CAGTAACCTA	GATGCCCGAT	CAGTAGGGAT	ATGTATGAAT	480
AAAAATTTCTG	GCATACTCGG	TAGCAAACCTA	GGTGTACACA	CAGCAATGTG	GGTATAGCTC	540
AAAAACAGAC	TGTTGAGTAA	AACAGTGGGA	AATAGAGATT	TACAGTCCAA	TACCATCTCT	600
GTAAATGCAA	GAGGCATAAA	CAAAACATTA	TCTGTGTAA	ATTATCAAGG	ATCTCTATCG	660
AACATATTGC	AGCTTGTTGC	TAGAAGAATG	AGAGTGGGGA	TCGAGAAAAG	TGAGGAAAAA	720
ATAATATAAA	CACTATAAAA	TAATGTAAC	AAGGACCCTG	TAGGGACTGA	TATGACAATG	780
TGCTGAAAAAT	TGAGGAGCAA	AGTTAACTCT	CTGTACCTGA	GATAAAATAA	CTAGCTAATA	840
GGAATCCAGC	TGAAAACCTT	AAGGTGCAGG	GCCTCTATGG	GGCCCAGGAA	GGATGTGTAG	900
AGACATGAAC	GGATGAAAGT	GCATCACAGG	TTCAGGGAAC	AACACAGGTT	GAGTGTGGCT	960
TGTAGTAAAA	ATGGTTGTGA	AGAGTTGACA	TATTTTAAAG	CCCTGGGTAA	ATTGAACAAC	1020
AGCTTACACT	TGGAGGGGTA	TAATCATTCT	AATCAATGTG	TCCCCTTTTA	CTATAATACA	1080
TTGGAGTTGC	AGCTAATGCT	CTGCTCCCAT	TCAGCCTATG	ATGAGATTCT	CTTTCAGCCC	1140
TATTGGGTTCT	TTGGCCTCAT	GTGACTACTC	CAAAGACCCT	AGTCCAAAAG	GTCTTTCCTG	1200
TTTGCTATGG	CCTTGAGGAA	TGTGGCCCTA	GATCCACCGC	TTTAAAGCTG	GAGTTCACC	1260
AGCAGCAACA	TCCTCTCATT	CTGGGGCACC	TGCCTGGGGC	AGGTCAATCCT	GCCTCTGCCA	1320
ACTCAGTGCT	ATTAGTTAAC	TCTCACCTGC	CATATTCCAG	CTGGAATCAT	CTCCCCTTCT	1380
CCACCCAGA	CTAGGTCATG	TTCCGCCATC	ATGGAAGCGC	CTATTCTTCA	TACCCCTTAT	1440
CACAGCTGCA	ACTACTCATT	TACTTGTCTG	ACAATTTGAT	TTATGTCCAC	CTACTTTGCT	1500
AGGTACTAAG	TTCAATGCTG	GCACTCGTTT	CTTCTTTTTT	TTTCTTTTCT	GTTTGTCTCA	1560
CCGATTTCTC	GTTAGCACTT	AGCACAGTGT	CTGGCACACG	ATAGATGCTC	CGTCAACTTC	1620
TCAGTTGGAT	ACCAGCATCC	CGAAGGGGAC	ATGGATTAAG	GCAGCTATAA	GCACGGTGTA	1680
AAAACAGGAA	TAAGAAAAAG	TTGAGGTTTG	TTTCACAGTG	GAATGTAAAG	GTTTGCAAGG	1740
AGGTGCATCG	GCCCCGTGG	ACAGGACGCA	TGACTGCTAC	ACACGTGTTT	ACCCCACCCT	1800
CTGGCACAGG	GTGCACATAC	AGTAGGGGCA	GAAATGAACC	TCAAGTGCTT	AACACAATTT	1860
TTAAAAAATA	TATAGTCAAG	TGAAAGTATG	AAAATGAGTT	GAGGAAAGGC	GAGTACGTGG	1920
GTCAAAGCTG	GGTCTGAGGA	AAGGCTCACA	TTTTGAGATC	CCGACTCAAT	CCATGTCCCT	1980
TAAAGGGCAC	AGGGTGTCTC	CACAGGGCCG	CCCAAAATCT	GGTGAGAGAG	GGCGTAGACG	2040
CCTCACCTTC	TGCCCTACG	GGTCACAAAA	GCCTGGGTCA	CCCTGGTTGC	CACTGTTCCCT	2100
AGTTCAAAGT	CTTCTTCTGT	CTAATCCTTC	ACCCCTATTC	TCGCCTTCCA	CTCCACCTCC	2160
CGCTCAGTCA	GACTGCGCTA	CTTTGAACCG	GACCAAACCA	AACCAAACCA	AACCAAACCA	2220

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AACCAGACCA GACACCCCT CCCGCGGAAT CCCAGAGAGG CCGAACTGGG ATAACCGGAT 2280
GCATTTGATT TCCCACGCCA CTGAGTGCAC CTCTGCAGAA ATGGGCGTTC TGGCCCTCGC 2340
GAGGCAGTGC GACCTGTAC CGCCCTTCAG CCTTCCCGCC CTCCACCAAG CCCGCGCAGC 2400
CCCGGCCCGC GCGTCTGTCT TTCGACCCGG CACCCCGGCC GGTTCCCAGC AGCGCGCATG 2460
CGCGCGCTCC CAGGCCACTT GAAGAGAGAG GCGGGGGCCG AGGGGCTGAG CCCGCGGGGG 2520
GAGGGAACAG CGTTGATCAC GTGACGTGGT TTCAGTGTTC ACACCCGCGC GGGGCCGGGG 2580
GTTCCGGCCT AGTCAGGCGC TCAGCTCCGT TTCGGTTTCA CTTCCGGTGG AGGGCCGCCT 2640
CTGAGCGGGC GCGGGCCGA CGGCGAGCGC GGGCGCGGCC GGTGACGGAG GCGCCGCTGC 2700
CAGGGGGCGT GCGGCAGCGC GCGGCGGGC GCGGCGGGC GCGGCGGGC GCGGCGGGC 2760
GCGGCGGGTG GGCCTCAGC GCCCGCAGCC CACCTCTCGG GGGCGGGCTC CCGGCGCTAG 2820
CAGGGCTGAA GAGAAGATGG AGGAGCTGGT GGTGGAAGTG CGGGGCTCCA ATGGCGCTTT 2880
CTACAAGGTA CTTGGCTCTA GGGCAGGCC CATCTTCGCC CTTCCCTCCC TCCCTTTTCT 2940
TCTTGGTGTC GCGGGGAGG AGGCCCGGG CCCTCTTCCC GAGCACCGCG CCTGGGTGCC 3000
AGGGCACGCT GCGCGGATG TTGTTGGGAG GGAAGGACTG GACTTGGGGC CTGTTGGAAG 3060
CCCCCTCCG ACTCCGAGAG GCCCTAGCGC CTATCGAAAT GAGAGACCAG CGAGGAGAGG 3120
GTTCTCTTTC GCGCCGAGC CCCGCCGGG TGAGCTGGG ATGGGCGAGG GCCGGCGGCA 3180
GGTACTAGAG CCGGGCGGA AGGGCCGAAA TCGGCGCTAA GTGACGGCGA TGGCTTATTC 3240
CCCCTTTCT AAACATCATC TCCCAGCGG ATCCGGGCTT GTCGTGTGG TAGTTGTGGA 3300
GGAGCGGGG GCGCTTCAGC CGGGCCGCCT CCTGCAGCG CAAGAGGGCT TCAGGTCTCC 3360
TTTGGCTTCT CTTTTCCGGT CTAGCATTGG GACTTCGGAG AGCTCCACTG TTCTGGGCGA 3420
GGGCTGTGAA GAAAGAGTAG TAAGAAGCGG TAGTCGGCAC CAAATCACAA TGGCAACTGA 3480
TTTTTAGTGG CTCTCTTTG TGGATTTCCG AGGAGATTTT AGATCCAAAA GTTTCAGGAA 3540
GACCCTAACA TGGCCAGCA GTGCATTGAA GAAGTTGATC ATCGTGAATA TTCGCGTCCC 3600
CCTTTTTGTT AAACGGGTA AATTCAGGAA TGCACATGCT TCAGCGTCTA AAACCATTAG 3660
CAGCGCTGCT ACTTAAAAAT TGTGTGTGTG TGTTTAAGTT TCCAAAGACC TAAATATATG 3720
CCATGAAACT TCAGGTAATT AACTGAGAGT ATATTATTAC TAGGGCATT TTTTTTAAAC 3780
TGAGCGAAAA TATTTTTGTG CCCCTAAGAA CTTGACCACA TTTCTTTGA ATTTGTGGTG 3840
TTGCAGTGGG CTGAATTGTT GAGGCTTTAT ATAGGCATTC ATGGGTTTAC TGTGCTTTTT 3900
AAAGTTACAC CATTCAGAT CAACTAACAC CTTTCAGTTT TAAAAGGAAG ATTTACAAAT 3960
TTGATGTAGC AGTAGTGCCT TTGTTGGTAT GTAGGTGCTG TATAAATTC TCTATAAATT 4020
CTCATTTTCT TTTGAATGTC TATAACCTCT TTCAATAATA TCCCACCTTA CTACAGTATT 4080
TTGGCAATAG AAGGTGCGTG TGAAGGAAG GCTGGAAAAT AGCTATTAGC AGTGTCCAAC 4140
ACAATTTCTA AATGTATTGT AGAATGGCTT GAATGTTTCA GACAGGACAC GTTTGGCTAT 4200
AGGAAAATAA ACAATTGACT TTATTCTGTG TTTACCAATT TTATGAAGAC ATTTGGAGAT 4260
CAGTATATTT CATAAATGAG TAAAGTATGT AAAGTGTTC ATACTTTGAG CACAAAGATA 4320
AAGCCTTTTG CTGTAAAAGG AGGCAAAAAG TAACCCCGCG TTTATGTTCT TAACAGTCTC 4380
ATGAATATGA AATTGTTTCA GTTACTCTG CAGTCAAAAT TTTAATTTC TTGATTTTAT 4440
TGATCCATAA TTTCTTCTG TGAGTTTGGC TAGAATCGTT CACGGTCCCTA GATTAGTGGT 4500
TTTGGTCACT AGATTTCTG CACTAATAAC TATAATACAT ATACATATAT ATGTGTGAGT 4560

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AACGGCTAAT	GGTTAGGCAA	GATTTTGATT	GACCTGTGAT	ATAAACTTAG	ATTGGATGCC	4620
ACTAAAGTTT	GCTTATCACA	GAGGGCAAGT	AGCACATTAT	GGCCTTGAAG	TACTTATTGT	4680
TCTCTCCAG	CAACTTATGA	TTTGCTCCAG	TGATTTTGCT	TGCACACTGA	CTGGAATATA	4740
AGAAATGCCT	TCTATTTTGG	CTATTAATTC	CCTCCTTTTT	TGTTTTGTTT	TGTAACGAAG	4800
TTGTTTAACT	TGAAGGTGAA	TGAAGAATAG	GTTGGTTGCC	CCTTAGTTCC	CTGAGGAGAA	4860
ATGTTAATAC	TTGAACAAGT	GTGTGTCAGA	CAAATGCTG	TTATGTTTAT	TTAATTAAGT	4920
TTGATTTCTA	AGAAAATCTC	AAATGGTCTG	CACTGATGGA	AGAACAGTTT	CTGTAACAAA	4980
AAAGCTTGAA	ATTTTTATAT	GACTTATAAT	ACTGCTGTGA	GTTTTAAAAG	TAAAGCAAAA	5040
GTAACCTGAG	TTGCTTGTC	AGTGGGATGG	ACAGGAAAAG	TGTGAAATAA	AAACCAATGA	5100
AAAATGAACT	GCTGTGGAGA	AGTGTTCACAT	TTATGAAAAA	AGAAATAGGA	ACCTTGTTC	5160
TCAAATTGAT	AGAAAAGCTT	TTAAAACATA	ACAAATCAAA	CAACTTGAGT	ATAATGGAAT	5220
TC						5222

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1026 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTGCAGAAAT	GGCGTTCTG	GCCCTCGCGA	GGCAGTTGCG	ACCTGTCACC	GCCCTTCAGC	60
CTTCCCGCCC	TCCACCAAGC	CGCGCACGCG	CCGCCCCGCG	CGTCTGTCTT	TCGACCCGGC	120
ACCCCGGCCG	GTTCCACGCA	GCGCGCATGC	GCGCGCTCCC	AGGCCACTTG	AAGAGAGAGG	180
GCGGGGCCGA	GGGCTGAGC	CCGCGGGGGG	AGGGAACAGC	GTTGATCAGC	TGACGTGGTT	240
TCAGTGTTTA	CACCCGACG	GGGCCGGGGG	TTCGGCCCTA	GTCAGGCGCT	CAGCTCCGTT	300
TCGGTTTAC	TTCCGGTGG	GGCCGCCTC	TGAGCGGGCG	GCGGGCCGAC	GGCAGCGCG	360
GGCGCGGCG	GTGACGGAG	CGCCGCTGCC	AGGGGCGGTG	CGGCAGCGCG	GCGGCGGCGG	420
CGCGCGGCG	GGCGCGGCG	GCGCGGCGG	CGGCGGCTGG	GCCTCGAGCG	CCCAGCGCC	480
ACCTCTCGGG	GGCGGGCTCC	CGCGCTAGC	AGGCTGAAG	AGAAGATGGA	GGAGCTGGTG	540
GTGGAAGTGC	GGGCTCCAA	TGGCGTTTC	TACAAGGTAC	TTGGCTCTAG	GGCAGCCCC	600
ATCTTCGCC	TTCTTCCCT	CCCTTTTCTT	CTTGGTGTG	GCGGGAGGCA	GGCCCGGGC	660
CCTCTTCCCG	AGCACCGCG	CTGGGTGCCA	GGGCACGCTC	GCGGGATGT	TGTTGGGAGG	720
GAAGACTGG	ACTTGGGGCC	TGTTGGAAGC	CCCTCTCCGA	CTCCGAGAGG	CCCTAGCGCC	780
TATCGAAATG	AGAGACCAGC	GAGGAGAGGG	TTCCTTTTCG	GCGCCGAGCC	CCGCCGGGT	840
GAGCTGGGGA	TGGCGGAGG	CCGGCGGCAG	GTAAGTAGAGC	CGGGCGGAA	GGCCGAAAT	900
CGGCGCTAAG	TGACGGCGAT	GGCTTATTCC	CCCTTTCCTA	AACATCATCT	CCCAGCGGGA	960
TCCGGGCGTG	TCGTGTGGGT	AGTTGTGGAG	GAGCGGGGGG	CGCTTCAGCC	GGGCCGCCTC	1020
CTGCAG						1026

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What is claimed is:

1. A method of detecting a mutation for fragile X syndrome comprising the step of measuring and comparing the expression of the FMR-1 gene in normal and affected individuals, wherein variation in the expression in affected individuals compared with that in normal individuals indicates a mutation for the fragile-X syndrome.

2. The method of claim 1 wherein the expression is measured by determining the amount of FMR-1 mRNA expressed.

3. The method of claim 2, wherein the amount of mRNA is determined by the steps of:

extracting RNA from affected individuals to be tested and normal individuals;

preparing FMR-1 cDNA and control gene cDNA from said extracted RNA;

quantifying the FMR-1 cDNA by comparing the amount of FMR-1 cDNA with the amount of control gene cDNA; and

comparing the variation in the amount of FMR-1 cDNA from tested individuals with the amount of FMR-1 cDNA in normal individuals, wherein variation in the

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amount of FMR-1 cDNA from affected individuals compared with that in normal individuals indicates a mutation for the fragile X syndrome.

4. The method of claim 3, wherein the quantification step includes PCR of the FMR-1 cDNA, PCR of the control gene cDNA, electrophoresis of the PCR products, ethidium bromide staining of the products and quantification of FMR-1 products versus control gene products.

5. The method of claim 4, wherein the oligonucleotide primers SEQ ID NO: 8 and SEQ ID NO: 9 are used to amplify the cDNA from the fragile X site.

6. The method of claim 5, wherein the control gene is HPRT and the oligonucleotide primers are SEQ ID NO: 12 and SEQ ID NO: 13.

7. The method of claim 1, wherein the expression is measured by determining the amount of predicted FMR-1 protein.

8. The method of claim 7, wherein the predicted FMR-1 protein is SEQ ID NO: 14.

9. A 657 amino acid peptide sequence of protein FMR-1 having the sequence of SEQ ID NO: 14.

* * * * *

EXHIBIT 14



US006143504A

United States Patent [19]

[11] **Patent Number:** **6,143,504**

Das et al.

[45] **Date of Patent:** **Nov. 7, 2000**

[54] **METHODS AND COMPOSITIONS FOR THE DIAGNOSIS OF FRAGILE X SYNDROME**

[75] Inventors: **Soma Das; David H. Ledbetter**, both of Chicago, Ill.

[73] Assignee: **Arch Development Corporation**, Chicago, Ill.

[21] Appl. No.: **09/429,499**

[22] Filed: **Oct. 27, 1999**

[51] **Int. Cl.**⁷ **C07H 21/04**; C12Q 1/68; C12P 19/34

[52] **U.S. Cl.** **435/6**; 536/22.1; 536/24.3; 435/91.2

[58] **Field of Search** 435/6, 91.2; 536/22.1, 536/24.3

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(List continued on next page.)

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Assistant Examiner—Arun Chakrabarti
Attorney, Agent, or Firm—Fulbright & Jaworski L.L.P.

[57] **ABSTRACT**

The present invention relates generally to the field of diagnostics. More particularly, it concerns the use of methylation-specific PCR in order to identify those males having Fragile X syndrome. The present invention provides a method in which amplification specific for the methylated FMR1 sequence is observed in all individuals with a full mutation, while all normal and premutation individuals show only amplification specific for the unmethylated sequence, thus allowing affected and unaffected males to be distinguished. A full mutation in the presence of mosaicism also may be detectable by this method. Thus, methylation-specific PCR is demonstrated as a rapid and reliable tool for the diagnosis of fragile X.

24 Claims, 2 Drawing Sheets

UNTREATED SEQUENCE (ANTISENSE STRAND; SEQ ID NO:1)

3'-CGGCCAAGGGTCGTCGCGCGTACGCGCGAGGGTCC...CAGTCCGCGAGTCGAGGCAAAGCC
 ...GCGCCCGCCGCCCACTGCCTC-5'

TREATED METHYLATED SEQUENCE (FRAGILE X AFFECTED; SEQ ID NO:2)

FX-BS-for
 →

3'-TGGCTAAGGGTTGTTGCGCGTATGCGCGCGAGGGTTT...TAGTTTGCGAGTTGAGGCAAAGCT

FX-BS-rev
 ←

...GCGCTTGCTGCTGCTIATGCTTT-5'

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UNTREATED SEQUENCE (ANTIENSE STRAND; SEQ ID NO:1)

3´ - CCGCCAAGGTCGTCGGCGGTACGGCGCGGAGGGTCC...CAGTCCGCGAGTCGAGGCAAAGCC

... GCGCCCGCCCGCCACTGCCCTC - 5´

TREATED METHYLATED SEQUENCE (FRAGILE X AFFECTED; SEQ ID NO:2)

FX - BS - for



3´ - TGGCTAAGGTTGTTGGCGGTATGCGCGCGGAGGGTTT...TAGTTTGGAGTTGAGGCAAAGCT

FX - BS - rev



... GCGCTTGCTGCTATTGCCTT - 5´

FIG. 1A

TREATED METHYLATED SEQUENCE (FRAGILE X UNAFFECTED; SEQ ID NO:3)

NM-BS - rev

NM-BS - for



$3' - \underline{\text{TGGTTAAGGGTTGTTGTTGTA}} \underline{\text{TGTTGTTGTTGAGGGTTT}} \dots \underline{\text{TAGTTTGTGAGTTGAGGTTAAAGTT}}$
 $\dots \underline{\text{GTGTTTGTGTTGTTATTTGTTT}} - 5'$

FIG. 1B

METHODS AND COMPOSITIONS FOR THE DIAGNOSIS OF FRAGILE X SYNDROME

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of priority from U.S. Provisional Application Ser. 60/105,892, filed Oct. 27, 1998.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of diagnostics. More particularly, it concerns the use of methylation-specific PCR in order to identify those individuals having Fragile X syndrome.

2. Description of Related Art

The fragile X syndrome is the most common inherited form of mental retardation and developmental disability. This condition afflicts approximately 1 in 2500 males and 1 in 5000 females. Males with fragile X syndrome usually have mental retardation and often exhibit characteristic physical features and behavior (Hagerman and Silverman, 1992; Warren and Nelson, 1994). Perhaps the most debilitating clinical feature noted in individuals with Fragile X is that of behavior, these characteristics include behavior similar to autism and attention deficit disorder, obsessive-compulsive tendencies, hyperactivity, slow development of motor skills and anxiety fear disorder. When these disabilities are severe and occur simultaneously, the condition is sometimes described as autism, and may be associated with any degree of intelligence. Minorities of individuals with fragile X have autism. Many more have some of the above features. Particularly common in fragile X (and much less so in other conditions) is the combination of a likable, happy, friendly personality with a limited number of autistic-like features such as hand-flapping, finding direct eye contact unpleasant, and some speech and language problems.

Generally speaking, the problems experienced by girls and women with fragile X are similar to those of boys and men. Girls and women with more average intellectual functioning may still have large discrepancies between different ability areas and may show similar concentration problems with impulsiveness, distractibility and difficulty sticking to tasks even if they are not overactive. Shyness and anxiety in social situations can occur.

Fragile X is an X chromosome-linked condition that is characterized by a visible constriction near the end of the X chromosome, at locus q27.3, and there is a tendency for the tip of the X-chromosome to break off under certain conditions in tissue culture. The pattern of inheritance of this condition is atypical of that associated with X-linked conditions. In typical X-linked genetic defects, there is a 50% probability that the male offspring of a female carrier will be afflicted by the defect. Additionally, all males who carry the abnormal gene are afflicted by the X-linked disorder in the typical pattern. Furthermore, since females have two X chromosomes, they normally do not suffer the effects of a single damaged X chromosome. In fragile X syndrome, however, carrier males are phenotypically normal. Certain individuals are carriers of fragile X in that they have a premutation in the FMR1 gene but do not show symptoms of fragile X. Carrier men (transmitting males) pass the premutation to all their daughters but none of their sons. Each child of a carrier woman has a 50% chance of inheriting the gene. The fragile X premutation can be passed silently down through generations in a family before a child

is affected by the syndrome. Moreover, about one-third of the females inheriting the fragile X chromosome are afflicted with the disease. Daughters of carrier males are generally non-expressing carriers, but may have afflicted sons. Afflicted daughters occur more frequently among the offspring of carrier mothers than among the offspring of carrier fathers (Brown, 1990).

The genomic region associated with this condition has been identified (Oberle et al., 1991; Kremer et al., 1991; Bell et al., 1991). Researchers have sequenced a cDNA clone derived from this region, called FMR1 (Verkerk et al., 1991). FMR1 has been recognized since 1991 as the gene that causes fragile X (Verkerk et al., 1991; Richards et al., 1991; Eichler et al., 1993; Hirst et al., 1995). The fragile X syndrome is predominantly caused by a large expansion of a CGG trinucleotide repeat in the promoter region of the FMR1 gene, which is associated with methylation and down regulation of transcription. However, it appears that the mutation that ultimately results in the fragile X phenotype occurs in stages. In the early stages, the gene is not fully defective; rather there is a "pre-mutation" of the gene. Carriers of the pre-mutation have a normal phenotype. A further expansion of the premutation occurs in carrier females-that produces the phenotype in their offspring. In individuals who have fragile X syndrome, a defect in FMR1 (a full mutation) of a CGG trinucleotide repeat correlates with methylation of the gene.

Individuals who are not carriers have approximately 30 CGG repeats in their FMR1. Carriers, however, have between about 50 and about 200 CGG repeats. This amplification of the FMR1 CGG sequence is the pre-mutation. Patients with fragile X syndrome have an expansion to the full mutation, which is greater than 200 repeats (Verkerk et al., 1991; Kremer et al., 1991), with as many as several thousand CGG repeats having been reported in afflicted individuals (Oberle et al., 1991). A CpG island, located upstream of the CGG repeat region is methylated when the number of CGG repeats is above a threshold of about 200 copies (Oberle et al., 1991; Kremer et al., 1991, Bell et al., 1991). This methylation results in an inactivation of the gene and silencing of gene transcription which is believed to result in the fragile X phenotype (Verkerk et al., 1991; Oberle et al., 1991; Sutcliffe et al., 1992). Most affected individuals do not express the FMR-1 mRNA (Pieretti et al., 1991). Full mutations also can exist with premutation and normal alleles, and such individuals are known as "mosaic" (Verkerk et al., 1991; Kremer et al., 1991).

A molecular diagnosis of this disorder is based on repeat size and methylation analysis of the FMR1 gene. As methylation has a direct effect on the fragile X phenotype and does not always correlate with repeat expansion, its analysis is an important part of fragile X diagnostics. Molecular testing of the fragile X syndrome is predominantly performed by Southern blot (Rousseau et al., 1991) and/or PCR analysis (Fu et al., 1991; Pergolizzi et al., 1992; U.S. Pat. No. 5,658,764). The advantage of Southern analysis is that methylation status is obtained in addition to repeat expansion. The main disadvantages of this technique are the time taken to perform the procedure, the large amounts of DNA necessary for analysis, and the use of radioisotopes. In PCR-based methods, carriers of the fragile X genotype are identified based on molecular structure of the gene defect. These methods determine whether the number of CGG repeats in the test individual's X-chromosome are characteristic of a normal, carrier or afflicted person. The PCR test, which provides information on repeat size, usually employs radioactivity based assays and has shown limited success in

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diagnosing full mutations. Other PCR based methods that serve as rapid screening tools for fragile X have been described (Wang et al., 1995; Haddad et al., 1996; Larsen et al., 1997). These methods, however, depend on the non-amplification of a full mutation as an indicator of fragile X and require confirmation by Southern analysis, additionally, these assays are unable to detect a full mutation in the presence of mosaicism.

Additional methods for the diagnosis of fragile X syndrome use microscopy, in which an afflicted individual's chromosomes are examined after cell growth and treatment in tissue culture. The X chromosome is examined to ascertain whether it was characteristically constricted, or had a broken tip. This method is both costly and unreliable. A more recent approach to the identification of fragile X is by assay of FMRP, where a lack of protein is indicative of fragile X (Willemsen et al., 1995). This is a promising technique particularly for large screening studies; however it cannot be used to identify premutations and has a high false negative rate in females.

There currently is no cure for fragile X syndrome, although appropriate education and medications can help maximize the potential of each child. However, most boys and many girls remain significantly affected throughout their lives. The cost to society for treatment, special education, and lost income is staggering. Diagnoses of this syndrome will be helpful in designing appropriate therapies and counseling for affected individuals and carriers of the syndrome. There still exists a need for rapid and reliable assays for Fragile X syndrome to aid those suffering from or carrying the disorder.

SUMMARY OF THE INVENTION

The present invention provides a reliable tool for the diagnosis of fragile X syndrome in males. More particularly, the present invention provides a method for determining the methylation state of an FMR1 gene promoter of a male subject comprising the steps of (a) denaturing a DNA sample from the subject; (b) subjecting the denatured DNA to bisulfite modification; (c) amplifying the DNA using primers pairs having the sequences ACCGATTCCTCAA-CAACGCGCATA and TTTCGTTATCGTCGTCGTTTCGC, and ACACACATACACACTCCCAA and TTGAAATGGAGTTGAGTGTGTTGAT; and (d) detecting amplification products from step (c).

In specific embodiments, the method further may comprise the step, before step (a), of obtaining a DNA sample from the subject. In particular embodiments, the sample is blood, amniotic fluid or a buccal smear. In specific embodiments, the denaturing comprises treatment with NaOH and heat. In particular embodiments, the bisulfite modification comprises treatment with hydroquinone and sodium bisulfite, followed by treatment with NaOH. In certain embodiments, the denaturing comprises treatment with NaOH and heat. In certain embodiments, it is contemplated that the method further comprises the step of purifying the bisulfite modified DNA. In particular embodiments, the amplification comprises PCR. In more defined embodiments, the PCR comprises 35 cycles at 94° C. for 30 seconds, 64° C. for 30 seconds and 72° C. for 30 seconds, followed by a final extension at 72° C. for 10 minutes. In certain embodiments, the amplification products are unlabeled. In alternative embodiments, the amplification products are labeled with a detectable label.

More particularly, the label may be is radioactive, fluorescent, chemiluminescent or colorimetric. In certain

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embodiments, the detection comprises gel electrophoresis and visualization of size-separated PCR products. In other embodiments, the primer may comprise an additional sequence that is not complementary to a region of FMR1 gene promoter. In more defined embodiments, the non-complementary region comprises a restriction enzyme site.

The present invention further provides an isolated primer comprising the sequence ACCGATTCCTCAAACGCGCATA. In other embodiments, the present invention provides an isolated primer comprising the sequence TTTCGTTATCGTCGTCGTTTCGC. In yet another embodiment, the present invention provides an isolated primer comprising the sequence ACACACATACACACTCCCAA. In still a further embodiment, the invention provides an isolated primer comprising the sequence TTGAAATGGAGTTGAGTGTGTTGAT.

Also contemplated by the present invention is a set of two primer pairs comprising the following sequences: ACCGATTCCTCAAACGCGCATA and TTTCGTTATCGTCGTCGTTTCGC, and ACACACATACACACTCCCAA and TTGAAATGGAGTTGAGTGTGTTGAT.

Also provided by the present invention is a kit comprising, in suitable container means, primer pairs comprising the following sequences: ACCGATTCCTCAAACGCGCATA and TTTCGTTATCGTCGTCGTTTCGC, and ACACACATACACACTCCCAA and TTGAAATGGAGTTGAGTGTGTTGAT. In certain embodiments, the kit further may comprise a thermostable DNA polymerase. In other preferred embodiments, the kit further may comprise sodium bisulfite and hydroquinone. In additional embodiments, the kit also comprises a DNA denaturing agent. In other embodiments, the kit may also include dNTP's.

Following longstanding patent law convention, the word "a" and "an", when used in conjunction with the word comprising, mean "one or more" in this specification, including the claims.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Bisulfite modification of the 5' untranslated region of the FMR1 gene. Depicted is the antisense strand from nucleotides 13551 to 13803 (Genbank accession no. L29074). Changes in nucleotide sequence between the bisulfite treated and untreated versions are underlined. Changes in nucleotide sequence between the methylated and unmethylated versions after bisulfite treatment are indicated by the arrowhead. Note that those residues denoted by the arrowhead reflect cytosines that are methylated in fragile X affected as opposed to unaffected individuals. Primers designed to the bisulfite modified sequence contain nucle-

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otides that differ between the methylated and unmethylated versions of the FMR1 gene to allow for their discrimination. Primers also contain nucleotide sequence that allows for the discrimination between the treated and untreated versions of the FMR1 gene. The sequences in this figure are given in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 SEQ ID NO:4 SEQ ID NO:5 SEQ ID NO:6 SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

1. The Present Invention

The present invention describes a PCR-based method for the analysis of methylation of the FMR1 gene, which involves bisulfite treatment of DNA prior to amplification. Methylation-specific PCR of the present invention is a rapid and reliable tool for the diagnosis of fragile X males.

Methylation-PCR is a rapid assay that can be completed in two days and requires very little DNA for analysis, two important factors for prenatal diagnosis. Other advantages of the test are that it is non-radioactive, cost and labor efficient, making it amenable for routine diagnostics and screening studies. The methylation-PCR assay produces amplification specific for either presence or absence of methylation (or both), and thus provides an advantage over the other screening methods described above, where a positive result is dependent on an absence of product. A possible disadvantage of the methylation-PCR test is the inability to analyze females, as the inactive X chromosome will always result in amplification specific for the methylated FMR1 gene. However, the majority of tests for fragile X are initiated from males with mental retardation. It may be desirable to perform methylation-PCR along with amplification by conventional PCR across the triplet repeat for all male samples sent for fragile X testing, as this will allow normal, pre-mutation and full mutation males to be identified. Female samples are best analyzed by conventional Southern blot or PCR analysis.

Methylation-PCR provides an alternative method for the molecular testing of fragile X and can be used to identify affected and unaffected males even in the presence of mosaicism. This is an important factor, as about 20% of affected fragile X individuals are mosaic (Rousseau et al., 1991). Two types of mosaicism have been described in fragile X, those where full mutations are present with pre-mutation or normal alleles (Verkerk et al., 1991; Oberle et al., 1991), as well as the rarer instance of full mutations mosaic for methylation (Rousseau et al., 1991). In a retrospective study, two cases out of 100 samples where methylation was not identified fall into the second category: both were borderline pre-mutation/full mutation individuals with less than 10% methylation. Both patients were ascertained by analysis of families with a positive history of fragile X syndrome. They had none of the physical features characteristic of fragile X and demonstrated only cognitive features of very mild fragile X. All other methylation mosaic individuals in the study had detectable methylation by the assay. The methylation-PCR assay cannot distinguish between an individual with a full mutation and an individual mosaic for a full mutation/pre-mutation. Male individuals with a full mutation, may be further tested by Southern analysis to determine if mosaicism is present.

Fifty-two normal and 48 affected (pre-mutation or mosaic) males were analyzed in a blinded study by this method. A prospective study of 30 males suspected of fragile X also was performed. Amplification specific for the methylated FMR1 sequence was readily observed in all individuals with a full mutation, while all normal and pre-mutation individu-

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als showed only amplification specific for the unmethylated sequence, thus allowing affected and unaffected males to be distinguished. A full mutation in the presence of mosaicism also was detectable by this method.

The present invention also describes a PCR assay that tests for methylation to diagnose the fragile X syndrome. Using this assay, FX-specific amplification (amplification specific for the methylated sequence) is seen in full mutation individuals and not in normal or pre-mutation individuals.

The latter two groups of patients show only NM-specific amplification (amplification specific for the unmethylated sequence) in over 70 normal males tested. Some amount of NM-specific amplification is seen in most full mutation carriers as well. This does not appear to be due to non-specific priming, as it is not seen in all cases and as other NM-specific primers tested have given similar results. This may either reflect a low level of mosaicism and/or a low level of unmethylated FMR1 sequence in fragile X individuals not detectable by Southern analysis. The finding of different levels of NM-specific amplification in full mutation carriers is consistent with the observation of expression of some Fragile X mental retardation 1 protein (FMRP) in a proportion of affected males (Willemsen et al., 1995).

The use of methylation-PCR to distinguish between affected and unaffected males with fragile X demonstrates the value of this method as a diagnostic and screening tool for genetic diseases that involve methylation. Methods and compositions relating the use of this assay for the diagnostic purposes are described in further detail herein below.

2. The FMR1 Gene and Fragile X Syndrome

The fragile X mental retardation gene, FMR1, is well known to those of skill in the art, and FMR1 nucleic acid sequences may be found at computerized databases known to those of ordinary skill in the art. One such database, for example, is the National Center for Biotechnology Information's Genbank database (<http://www.ncbi.nlm.nih.gov/>). Non-limiting examples of FMR1 sequences include Genbank accession No. Q06787; Genbank accession No L29074; Genbank accession No M67468; Genbank accession No A39530, each incorporated herein by reference.

The present invention provides methods for the diagnosis and prognosis of fragile X syndrome in individuals suspected of having fragile X. One embodiment of the instant invention comprises a method for detecting variation in CpG islands of FMR1. The methods may use a biological sample in analysis. The biological sample can be any tissue or fluid. In a particular embodiment, the fluid used is blood. In genetic testing amniotic fluid and blood may be assayed. Skin tissue, buccal smears, hair root follicles or any other material from which DNA may be extracted also may be used.

Nucleic acids are isolated from cells contained in the biological sample, according to standard methodologies (Sambrook et al., 1989). The nucleic acid may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to convert the RNA to a complementary DNA. In one embodiment, the RNA is whole cell RNA; in another, it is poly-A RNA. Normally, the nucleic acid is amplified.

Depending on the format, the specific nucleic acid of interest is identified in the sample directly using amplification or by comparison with a second, known nucleic acid following amplification. Next, the identified product is detected. In certain applications, the detection may be performed by visual means (e.g., ethidium bromide staining of a gel). Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radio-

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active scintigraphy of radiolabel or fluorescent label or even via a system using electrical or thermal impulse signals (Affymax Technology; Bellus, 1994).

Following detection, one may compare the results seen in a given patient with a statistically significant reference group of normal individuals (normal reference group) and individuals that have fragile X syndrome (positive reference group). In this way, it is possible to correlate the amount or kind of FMR1 mutation.

3. Methylation Specific PCR

The present invention describes a PCR-based method for the detection of methylation in fragile X. This method is based on the bisulfite modification of DNA where unmethylated cytosine residues are converted to uracil, while methylated residues remain unconverted (Frommer et al., 1992; Clark et al., 1994). The subsequent change in the sequence of the FMR1 promoter between fragile X affected and unaffected individuals after bisulfite treatment is monitored by PCR. The use of methylation-specific PCR for the detection of methylation in tumor suppressor genes (Herman et al., 1996) and genetic disorders of imprinting such as Prader-Willi syndrome and Angelmans syndrome (Kubota et al., 1997) have previously been demonstrated. The present invention demonstrates the successful identification of affected and unaffected males with fragile X by methylation-specific PCR.

a. Conventional PCRTM

The polymerase chain reaction (referred to as PCRTM) has been extensively described in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159, and in Innis et al., 1990, each incorporated herein by reference.

Briefly, in PCR, two primer sequences are prepared that are complementary to regions on opposite complementary strands of the marker sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase, e.g., Taq polymerase. If the marker sequence is present in a sample, the primers will bind to the marker and the polymerase will cause the primers to be extended along the marker sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the marker to form reaction products, excess primers will bind to the marker and to the reaction products and the process is repeated.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh et al., 1989; Gingeras et al, PCT Application WO 88/10315). In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a clinical sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has target specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double-stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double-stranded by addition of second target specific primer, followed by polymerization. The double-stranded DNA molecules are then multiply transcribed by an RNA polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNA's are reverse transcribed into single stranded DNA, which is then converted to double-stranded DNA, and then transcribed once again with an RNA polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target specific sequences.

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b. Methylation-specific PCRTM

Methylation specific PCTTM combines the use of methylation sensitive enzymes and PCRTM (Singer-Sam et al., 1990). After digestion of DNA with the enzymes, PCR amplification of the primers flanking a restriction site occurs only if the DNA cleavage has been prevented by methylation (Razin and Cedar, 1991; Stoger et al., 1993). The chemical modification of cytosine to uracil by bisulfite treatment provides a useful modification of traditional PCR techniques. Using this modification, the need for restriction enzymes is circumvented (Frommer et al., 1992).

In this reaction, all the cytosines in a given nucleic acid sequence are converted to uracil but those cytosines that are methylated (5-methylcytosine) are resistant to this modification and remains as cytosine (Wang et al., 1980). The sequence under investigation is then amplified by PCR with two sets of strand-specific primers to yield a pair of fragments, one from each strand, in which all uracil and thymine residues have been amplified as thymine and only 5-methylcytosine residues have been amplified as cytosine. The PCR products can be sequenced directly to provide a strand-specific average sequence for the population of molecules or can be cloned and sequenced to provide methylation maps of single DNA molecules (Frommer et al., 1992).

A methylation-specific PCR has been described by Herman et al. (1996), which can rapidly assess the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-sensitive restriction enzymes. This assay entails initial modification of DNA by sodium bisulfite, converting all unmethylated, but not methylated, cytosines to uracil, and subsequent amplification with primers specific for methylated versus unmethylated DNA. This assay requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Methylation-specific PCR eliminates the false positive results inherent to previous PCR-based approaches which relied on differential restriction enzyme cleavage to distinguish methylated from unmethylated DNA.

The following is a brief description of the methylation specific PCR method. In bisulfite modification, an appropriate amount of DNA (e.g., 1 μ g in a 50 μ l volume) is denatured by adding 0.2M NaOH and incubating at 37° C. for 10 minutes. For samples with nanogram quantities of DNA, a carrier DNA may be added to spike the sample before the modification reaction. The DNA may be extracted from any tissue of the individual being examined, for example, the DNA may be extracted from blood, amniotic fluid and the like.

30 μ l of 10 mM hydroquinone and 520 μ l of 3M sodium bisulfite at pH 5 are added and the samples are incubated under mineral oil at 50° C. for 16 hours. Modified DNA is then purified as described in Herman et al., 1996. The modification is completed by NaOH treatment for 5 minutes at room temperature followed by ethanol precipitation. DNA is resuspended in water and used immediately or stored at -20° C.

PCR typically employs two primers that bind to a selected nucleic acid template. The primers are combined with the other PCR reagents under conditions that induce primer extension, i.e., with four different nucleoside triphosphates (or analogues thereof), an appropriate polymerase and an appropriate buffer ("buffer" includes pH, ionic strength, cofactors, etc.) at a suitable temperature. In a PCR method where the polymerase is Taq polymerase, the buffer preferably contains 1.5-2 mM of a magnesium salt, preferably

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MgCl₂, 150–200 μM of each nucleoside, triphosphate (or analog thereof), 1 μM of each primer, preferably with 50 mM KCl, 10 mM Tris buffer at pH 8.4, and 100 μg/ml gelatin.

In specific embodiments, PCR primers are prepared from the FMR1 gene and PCR was carried out in a 50 μl volume containing 1 X PCR buffer II (Perkin-Elmer), 4.5 mM MgCl₂, 200 μM dNTPs, 0.5 μM NM-BS primers, 1.0 μM FX-BS primers (sequences in table 1), 1 unit of Amplitaq Gold enzyme (Perkin-Elmer) and approximately 30 ng of bisulfite-modified DNA. The polymerase was activated at 95° C. for 10 mins, and DNA amplified in a Perkin-Elmer model 9600 thermocycler for 35 cycles at 94° C. for 30s, 64° C. for 30s and 72° C. for 30s, followed by a final extension at 72° C. for 10 mins.

4. Primers and Probes

The present invention will use PCR primers designed specific for FMR-1 to be used to determine whether the gene is mutated or wild-type. The primer is preferably single stranded for maximum efficiency in amplification, but it may be double stranded. Double stranded primers are first “denatured”, i.e. treated to separate its strands before being used to prepare extension products. A preferred means of denaturing double stranded nucleic acids is by heating.

a. Primer Design

The term primer, as defined herein, is meant to encompass any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Typically, primers are oligonucleotides from about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78, about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87, about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96, about 97, about 98, about 99, to about 100 bases in length, including all intermediate ranges. It will be readily understood that “intermediate ranges”, as used herein, means any length or range including or between the quoted values (i.e. all integers including and between such values). A non-limiting example of such a range would be from about 10 to about 50 bases in length. Additionally, sequences greater than 100 bases or base pairs in length can be employed.

Primers may be provided in double-stranded or single-stranded form, although the single-stranded form is preferred. Probes are defined differently, although they may act as primers. Probes, while perhaps capable of priming, are designed to binding to the target DNA or RNA and need not be used in an amplification process.

In the present invention, a primer needs to be sufficiently long to “prime” the synthesis of extension products in the presence of an appropriate polymerase and other reagents. The primer length depends on many factors, including the temperature and source of the primer and the use of the method. The particular length of the primer is not believed to be critical, with the primer sequence ranging from about 10 to about 25 bases. Short primer molecules generally

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require lower reaction temperatures to form and maintain the primer-template complexes which support the chain extension reaction. In some embodiments, the primers are labeled with radioactive species (e.g. ³²P, ¹⁴C, ³⁵S, ³H, or other isotope), with a fluorophore (including, but not limited to, rhodamine, fluorescein or GFP) or a chemiluminescent label (including, but not limited to, luciferase).

The primers used in the present method are “substantially” complementary to a nucleic acid containing the selected sequence to be amplified, i.e. the primers must bind to, or hybridize with, a nucleic acid containing the selected sequence (or its complement). Nonetheless, the primer sequence need not be an exact complement of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the nucleic acid containing the selected sequence. Alternatively, one or more non-complementary bases can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the nucleic acid containing the selected sequence to (i) hybridize therewith and (ii) support a chain extension reaction. Notwithstanding the above, primers which are fully complementary to the nucleic acid containing the selected sequence are preferred to obtain the best results.

b. Primer Synthesis

Oligonucleotide synthesis is well known to those of skill in the art, and may be performed according to standard methods. See, for example, Itakura and Riggs (1980). Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Pat. No. 4,659,774, U.S. Pat. No. 4,816,571, U.S. Pat. No. 5,141,813, U.S. Pat. No. 5,264,566, U.S. Pat. No. 4,959,463, U.S. Pat. No. 5,428,148, U.S. Pat. No. 5,554,744, U.S. Pat. No. 5,574,146, U.S. Pat. No. 5,602,244, each of which is incorporated herein by reference. Additionally, U.S. Pat. No. 4,704,362; U.S. Pat. No. 5,221,619 U.S. Pat. No. 5,583,013 each describe various methods of preparing synthetic structural genes, and are incorporated herein by reference. Chemical synthesis can be achieved by the diester method, the triester method polynucleotides phosphorylase method and by solid-phase chemistry. These methods are discussed in further detail below.

Diester Method

The basic step of the diester method is the joining of two suitably protected deoxynucleotides to form a dideoxynucleotide containing a phosphodiester bond. The diester method is well established and has been used to synthesize DNA molecules (Khorana, 1979).

Triester Method

The main difference between the diester and triester methods is the presence in the latter of an extra protecting group on the phosphate atoms of the reactants and products (Itakura et al., 1975). The phosphate protecting group is usually a chlorophenyl group, which renders the nucleotides and polynucleotide intermediates soluble in organic solvents. Therefore purification's are done in chloroform solutions. Other improvements in the method include the block coupling of trimers and larger oligomers, the extensive use of high-performance liquid chromatography for the purification of both intermediate and final products, and solid-phase synthesis.

Polynucleotide Phosphorylase Method

This is an enzymatic method of DNA synthesis that can be used to synthesize many useful oligodeoxynucleotides

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(Gillam et al., 1978; Gillam et al., 1979). Under controlled conditions, polynucleotide phosphorylase adds predominantly a single nucleotide to a short oligodeoxynucleotide. Chromatographic purification allows the desired single adduct to be obtained. At least a trimer is required to start the procedure, and this primer must be obtained by some other method.

Solid-phase Methods

Drawing on the technology developed for the solid-phase synthesis of polypeptides, it has been possible to attach the initial nucleotide to solid support material and proceed with the stepwise addition of nucleotides. All mixing and washing steps are simplified, and the procedure becomes amenable to automation. These syntheses are now routinely carried out using automatic DNA synthesizers.

Phosphoramidite chemistry (Beaucage and Lyer, 1992) has become by far the most widely used coupling chemistry for the synthesis of oligonucleotides. As is well known to those skilled in the art, phosphoramidite synthesis of oligonucleotides involves activation of nucleoside phosphoramidite monomer precursors by reaction with an activating agent to form activated intermediates, followed by sequential addition of the activated intermediates to the growing oligonucleotide chain (generally anchored at one end to a suitable solid support) to form the oligonucleotide product.

5. Enzymes and Reagents

The present section provides examples of enzymes and reagents used in the present invention to carry out methylation specific PCR, these include RT enzymes, DNA polymerases, RNase inhibitors and other compositions required or helpful for optimizing reaction conditions. Of course, the enzymes and reagents discussed below are exemplary and non-limiting, as it is understood that any additional enzymes or reagents that possess similar activities may substitute for those specifically described.

M-MLV Reverse Transcriptase

M-MLV (Moloney Murine Leukemia Virus Reverse Transcriptase) is an RNA-dependent DNA polymerase requiring a DNA primer and an RNA template to synthesize a complementary DNA strand. The enzyme is a product of the pol gene of M-MLV and consists of a single subunit with a molecular weight of 71 kDa. M-MLV RT has a weaker intrinsic RNase H activity than Avian Myeloblastosis Virus (AMV) reverse transcriptase which is important for achieving long full-length complementary DNA (>7 kb).

M-MLV can be used for first strand cDNA synthesis and primer extensions. Storage is recommended at -20° C. in 20 mM Tris-HCl (pH 7.5), 0.2M NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% Nonidet® P-40 and 50% glycerol. The standard reaction conditions are 50 mM Tris-HCl (pH 8.3), 7 mM MgCl₂, 40 mM KCl, 10 mM DTT, 0.1 mg/ml BSA, 0.5 mM ³H-dTTP, 0.025 mM oligo(dT)₅₀ and 0.25 mM poly(A)₄₀₀ at 37° C.

M-MLV Reverse Transcriptase, RNase H Minus

This is a form of Moloney murine leukemia virus reverse transcriptase which has been genetically altered to remove the associated ribonuclease H activity (Tanese and Goff, 1988). It can be used for first strand cDNA synthesis and primer extension. Storage is typically at 20° C. in 20 mM Tris-HCl (pH 7.5), 0.2M NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% Nonidet® P-40 and 50% glycerol.

AMV Reverse Transcriptase

Avian Myeloblastosis Virus reverse transcriptase is a RNA dependent DNA polymerase that uses single-stranded

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RNA or DNA as a template to synthesize the complementary DNA strand (Houts et al., 1979). It has activity at high temperature (42° C.-50° C.). This polymerase has been used to synthesize long cDNA molecules.

Standard reaction conditions are 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 10 mM MgCl₂, 500 μM of each dNTP, 5 mM dithiothreitol, 200 μg/ml oligo-dT₍₁₂₋₁₈₎, 250 μg/ml polyadenylated RNA, 6.0 pMol ³²P-dCTP and 30 U enzyme in a 7 μl volume. The reaction mixture is then incubated 45 min at 42° C. Typical storage buffer is 200 mM KPO₄ (pH 7.4), 2 mM dithiothreitol, 0.2% Triton X-100 and 50% glycerol. AMV may be used for first strand cDNA synthesis, RNA or DNA dideoxy chain termination sequencing, and fill-ins or other DNA polymerization reactions for which Klenow polymerase is not satisfactory (Maniatis et al., 1976).

Superscript™ II RNase H

Reverse Transcriptase (U.S. Pat. No. 5,244,797, incorporated herein by reference) is purified to near homogeneity from *E. coli* containing the pol gene of Moloney Murine Leukemia Virus. The enzyme is used to synthesize first strand cDNA and will generally give higher yields of cDNA and more full length product than other reverse transcriptases.

An exemplary RT PCR that employs SUPERSCRIPT™ can be found in the Gibco catalog. Briefly, a 20-μl reaction volume can be used for 1-5 μg of total RNA or 50-500ng of mRNA. The following components are added to a nuclease-free microcentrifuge tube: 1 μl Oligo (dT)₁₂₋₁₈ (500 μg/ml) 1-5 μg total RNA and sterile, distilled water is added to a final volume of 12 μl. The reaction mixture is heated to 70° C. for 10 min and quickly chilled on ice. The contents of the tube are collected by brief centrifugation. To this precipitate is added: 4 μl 5X First Strand Buffer, 2 μl 0.1 M DTT and 1 μl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP and dTTP at neutral pH). The contents are mixed gently and incubate at 42° C. for 2 min. Then 1 μl (200 units) of Superscript II™ is added and the reaction mixture is mixed by pipetting gently up and down. This mixture is then incubated for 50 min at 42° C. and then inactivated by heating at 70° C. for 15 min. The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (those >1 kb) may require the removal of RNA complementary to the cDNA. RNA complementary to the cDNA may be removed by adding 1 μl (2 units) of *E. coli* RNase H and incubating at 37° C. for 20 min.

Retrotherm™ RT

(Epicentre technologies) is a thermostable reverse transcriptase and DNA polymerase derived from a thermophilic bacterium. This thermostable enzyme has both RNA- and DNA-dependent DNA polymerase activities under the same reaction conditions. These characteristics enable researchers to synthesize both strands of a specific cDNA in a single tube with no buffer changes. The only components needed are Retrotherm™ RT, the Retrotherm Reaction Buffer™ supplied with the enzyme, deoxynucleoside-triphosphates (dNTPs), an RNA template and specific primers for synthesis of each strand of cDNA. After first-strand synthesis, the RNA:DNA hybrid is thermally denatured to allow the second-strand primer to hybridize to the cDNA for second-strand synthesis in the same buffer. The high reaction temperatures possible with Retrotherm RT minimize secondary structure in templates. Thus, when primers are available for both strands, single-tube cDNA synthesis with

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Retrotherm RT is easy, fast and powerful, even when working with mixed populations of RNA. Retrotherm™ RT has no RNase H activity.

If specific primers are available for priming synthesis of both cDNA strands from a target RNA, then single-tube cDNA synthesis with Retrotherm™ RT is fast and convenient, even when working with mixed populations of RNA. In these cases, the enzyme's thermostability and its combination of RNA- and DNA-dependent DNA polymerase activities that function well in the same buffer give Retrotherm™ RT an advantage over other reverse transcriptases.

The amount of RNA needed depends on the application and whether the sample consists of a single RNA species or a mixture of different RNAs. Similarly, the optimal enzyme concentration will vary with the amount and nature of the template. A typical 50 µl reaction contains 0.5 to 5.0 units of Retrotherm™ RT. Insufficient enzyme may fail to produce full-length product. Excess enzyme may result in failure to produce discrete bands. Two templates of the same size but differing in sequence, or different amounts of the same template, may have different optimal enzyme concentrations.

RetroAmp™

RetroAmp™ RT DNA Polymerase (Epicentre Technologies), is a highly efficient, thermally stable enzyme. The use of a thermal stable polymerase allows reverse transcription to take place at an elevated temperature, minimizing the effects of RNA secondary structure. RetroAmp™ is available in a commercial preparation with a 10X PCR Enhancer (with betaine) referred to as MasterAmp™. The presence of betaine (trimethyl glycine) in the MasterAmp™ 10X PCR Enhancer substantially improves the yield and specificity of amplification of many target sequences, especially those containing a high G+C content or secondary structure. Betaine lowers the melting temperature of G+C rich regions to a temperature more similar to A+T(U) rich regions. This results in destabilization of double-stranded regions which limits polymerase pausing, thereby increasing the yield of full-length product. In addition, betaine also may enhance PCR by protecting DNA polymerases from thermal denaturation.

Typically in the RT-PCR reaction, 50 µl reactions are assembled on ice as two separate 25 µl premixes and combined just before the reverse transcription step to minimize RNA sample degradation. One premix includes the dNTPs, primers, and the RNA template. The other premix included all other reaction components. The reactions contain 1X RT-PCR Buffer that comprises 3.0 mM MgCl₂, 1X MasterAmp™ PCR Enhancer, 0.5 mM MnSO₄, 400 µM each dNTP, 12.5 pmoles of each primer, 100 ng of total RNA template and 2.5 units of RetroAmp™ RT DNA Polymerase. Standard reactions are incubated at 60° C. for 20 minutes for first strand cDNA synthesis, followed by 30–35 cycles of PCR. Annealing temperatures vary depending on the primer pair used; typically samples are denatured at 92° C. for 30 seconds, annealed at 60° C. for 30–60 seconds, and extended at 72° C. for 60 seconds. Ten percent of each reaction (5 µl) may be separated by agarose gel electrophoresis and visualized with ethidium bromide staining.

RetroAmp™ RT DNA

Polymerase can efficiently reverse transcribe RNA into cDNA at the high temperatures for such reactions. The RetroAmp™ RT-PCR produces abundant specific products

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with reverse transcription temperatures up to 70° C., depending on the primer sequences and template abundance in the reaction. In the manufacture's specification the ability of RetroAmp™ RT DNA Polymerase to perform high-temperature RT-PCR, is demonstrated by performing RT-PCR using four different first-strand synthesis incubation temperatures (55° C., 60° C., 65° C., and 70° C.) with two different templates. Primers that amplify a 479 bp region of *E. coli* 16S rRNA were used in a standard reaction with the following cycling conditions: RNA was reverse transcribed at the specified temperature for 20 minutes, then 20 cycles of 92° C. for 30 seconds and 68° C. for 60 seconds were performed. Primers that amplify a 250 bp region of the [beta]-actin message from human placental RNA were also used in a standard reaction with the following cycling profile: RNA was reverse transcribed at the specified temperature for 20 minutes, then 35 cycles of 92° C. for 40 seconds and 70° C. for 60 seconds were performed. These high annealing temperatures were possible because of the primer sequences chosen and the optimized buffer conditions used, including the presence of MasterAmp™ PCR Enhancer. The 16S rRNA product was optimally amplified with a reverse transcription temperature of 65° C. and the [beta]-actin message amplifies well under all temperatures tested.

Thermoscript™

Thermoscript™ (Gibco-BRL) is an avian reverse transcriptase that has been shown to be useful for high temperature cDNA synthesis to improve RT-PCR (Schwabe et al., 1998). It is a cloned RT in which the active site of the RNase H domain has been mutated thereby reducing the RNase H by 99.5% as compared to native AMV. Thermoscript™ is operative in the temperature range between about 50° C. and about 70° C., a description of the efficacy of the Thermoscript™ at this temperature range is given in a FIG. 2 of the product description on the manufacturer's web site at http://www2.lifetech.com/catalog/techline/molecular_biology/product_description/thrmsc_rp.html. The optimized conditions for first strand synthesis by Thermoscript™ have been described by Schwabe et al., 1998. Briefly, the 20 µl reaction mixture for the synthesis contains 50 mM Tris-acetate (pH 8.4); 75 mM K-acetate; 8 mM Mg-acetate; 5 mM dithioerythritol; 1 mM each of dATP, dTTP, dCTP and dGTP; 0.5 µg oligo(dT); 2.5mg RNA; 40 units RNase inhibitor and 15 units Thermoscript RT. The RT-PCR procedure, total cell RNA and oligo(dT) are incubated at 65° C. for 5 minutes and cooled on wet ice and cDNA synthesis reaction mixture is added. The reaction tubes are transferred to a prewarmed heating block and incubated for 50 minutes. Following RT inactivation, RNA is degraded by an RNase H. For PCR 20 µl cDNA reaction mixture is added to a 50 µl PCR mixture and incubated for 2 minutes at 94° C. PCR conditions involved 35 cycles of 94° C. for 30s, 55–60° C. for 30s and 68–72° C. for 1 to 15 minutes. Exemplary polymerases used for this method were Platinum Taq™ and eLONGase®.

rTth Reverse Transcriptase

The GeneAmp Thermostable rTth Reverse Transcriptase (Perkin-Elmer) catalyses the reverse transcription of RNA to cDNA at elevated temperature (60–70° C.) and subsequently amplifies cDNA using the same recombinant thermostable enzyme—rTth DNA Polymerase. The procedure begins with first strand cDNA synthesis from RNA, with rTth DNA Polymerase acting as a reverse transcriptase in the presence of MnCl₂ (Myers and Gelfand, 1991; Young et al., 1993).

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Subsequently, in the presence of MgCl₂, Chelating Buffer and the second primer, synthesis of second strand cDNA and amplification of cDNA is initiated.

The ability of thermostable rTth DNA Polymerase to efficiently reverse transcribe RNA templates at 70° C. is useful in the present invention because the secondary structures are unstable at the higher reaction temperatures. An additional advantage of performing reverse transcription at higher temperatures is increased specificity of primer hybridization and subsequent extension by the rTth DNA Polymerase and therefore sensitivity of the reaction.

Reverse transcription using rTth DNA Polymerase is accomplished using a single specific oligonucleotide primer complementary to the 3'-terminus of the RNA. Subsequent PCR amplifications are achieved using specific oligonucleotide primer pairs at intervals progressively 3' to the resultant first-strand cDNA. The reverse transcription is performed at 60° C. for 2 hours, followed by a 1 minute predenaturation step at 95° C. then 40 cycles of 95° C. for 15s and 65° C. for 30s, for each primer pair. Starting template can be a poly(A) RNA or RNA from a given tissue with a target copy number of approximately 10⁸ copies. The tissue RNA can be isolated from any desired tissues by techniques well known to those of skill in the art and also by techniques described elsewhere is the specification.

Having produced the first strand of the cDNA species using reverse transcription, the present invention also contemplates the use of various DNA polymerases, either described herein or known to those of ordinary skill in the art, to produce the second strand of the double-stranded cDNA moiety. Exemplary, but not limiting, polymerases are described below.

Bst DNA Polymerase Large Fragment

Bst DNA Polymerase Large Fragment is the portion of the *Bacillus stearothermophilus* DNA Polymerase protein that contains the 5'→3' polymerase activity, but lacks the 5'→3' exonuclease domain. BST Polymerase Large Fragment is prepared from an *E. coli* strain containing a genetic fusion of the *Bacillus stearothermophilus* DNA Polymerase gene, lacking the 5'→3' exonuclease domain, and the gene coding for *E. coli* maltose binding protein (MBP). The fusion protein is purified to near homogeneity and the MBP portion is cleaved off in vitro. The remaining polymerase is purified free of MBP (Iiyy et al., 1991).

Bst DNA polymerase can be used in DNA sequencing through high GC regions (Hugh and Griffin, 1994; McClary et al., 1991) and rapid sequencing from nanogram amounts of DNA template (Mead et al., 1991). The reaction buffer is 1X ThermoPol Buffer [20 mM Tris-HCl (pH 8.8 at 25° C.), 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1% Triton X-100], typically supplied with enzyme as a 10X concentrated stock.

Bst DNA Polymerase does not exhibit 3'→5' exonuclease activity. 100 μl/ml BSA or 0.1% Triton X-100 is required for long term storage. Reaction temperatures above 70° C. are not recommended. Heat inactivated by incubation at 80° C. for 10 min. Bst DNA Polymerase cannot be used for thermal cycle sequencing. Unit assay conditions are 50 mM KCl, 20 mM Tris-HCl (pH 8.8), 10 mM MgCl₂, 30 nM M13mp18 ssDNA, 70 nM M13 sequencing primer (-47) 24 mer, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 100 μM ³H-dTTP, 100 μg/ml BSA and enzyme. Incubate at 65° C. Storage buffer is 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 0.1 mM EDTA, 0.1% Triton-X-100 and 50% glycerol. Storage is at -20° C.

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VENT_R® DNA Polymerase and VENT_R® (exo-) DNA Polymerase

VENT_R® DNA Polymerase is a high-fidelity thermophilic DNA polymerase. The fidelity of VENT_R® DNA Polymerase is 5–15-fold higher than that observed for Taq DNA Polymerase (Mattila et al., 1991; Eckert and Kunkel, 1991). This high fidelity derives in part from an integral 3'→5' proofreading exonuclease activity in VENT_R® DNA Polymerase (Mattila et al., 1991; Kong et al., 1993). Greater than 90% of the polymerase activity remains following a 1 h incubation at 95° C.

VENT_R® (exo-) DNA Polymerase has been genetically engineered to eliminate the 3'→5' proofreading exonuclease activity associated with VENT_R® DNA Polymerase (Kong et al., 1993). This is the preferred form for high-temperature dideoxy sequencing reactions and for high yield primer extension reactions. The fidelity of polymerization by this form is reduced to a level about 2-fold higher than that of Taq DNA Polymerase (Mattila et al., 1991; Eckert and Kunkel, 1991). VENT_R® (exo-) DNA Polymerase may be used for DNA sequencing.

Both VENT_R® and VENT_R® (exo-) are purified from strains of *E. coli* that carry the Vent DNA Polymerase gene from the archaea *Thermococcus litoralis* (Perler et al., 1992). The native organism is capable of growth at up to 98° C. and was isolated from a submarine thermal vent (Belkin and Jannasch, 1985). They are useful in primer extension, thermal cycle sequencing and high temperature dideoxy-sequencing.

DEEP VENT_R™ DNA Polymerase and DEEP VENT_R™(exo-) DNA Polymerase

DEEP VENT_R™ DNA Polymerase is the second high-fidelity thermophilic DNA polymerase available from New England Biolabs. The fidelity of DEEP VENT_R™ DNA Polymerase is derived in part from an integral 3'→5' proofreading exonuclease activity. DEEP VENT_R™ is even more stable than VENT_R™ at temperatures of 95° C. to 100° C.

DEEP VENT_R™ (exo-) DNA Polymerase has been genetically engineered to eliminate the 3'→5' proofreading exonuclease activity associated with DEEP VENT_R™ DNA Polymerase. This exo- version can be used for DNA sequencing but requires different dNTP/ddNTP ratios than those used with VENT_R™(exo-) DNA Polymerase. Both DEEP VENT_R™ and DEEP VENT_R™ (exo-) are purified from a strain of *E. coli* that carries the DEEP VENT_R™ DNA Polymerase gene from *Pyrococcus* species GB-D (Perler et al., 1996). The native organism was isolated from a submarine thermal vent at 2010 meters (Jannasch et al., 1992) and is able to grow at temperatures as high as 104° C. Both enzymes can be used in primer extension, thermal cycle sequencing and high temperature dideoxy-sequencing.

T7 DNA Polymerase (unmodified)

T7 DNA polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3'→5' exonuclease (Hori et al., 1979; Engler et al., 1983; Nordstrom et al., 1981). The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kilodaltons) and *E. coli* thioredoxin (12 kilodaltons) (Hori et al., 1979; Studier et al., 1990; Grippo and Richardson, 1971; Modrich and Richardson, 1975;

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Adler and Modrich, 1979). Each protein is cloned and overexpressed in a T7 expression system in *E. coli* (Studier et al., 1990). It can be used in second strand synthesis in site-directed mutagenesis protocols (Bebenek and Kunkel, 1989).

The reaction buffer is 1X T7 DNA Polymerase Buffer [20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂ and 1 mM dithiothreitol]. Supplement with 0.05 mg/ml BSA and dNTPs. Incubate at 37° C. The high polymerization rate of the enzyme makes long incubations unnecessary. T7 DNA Polymerase is not suitable for DNA sequencing.

Unit assay conditions are 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, 0.05 mg/ml BSA, 0.15 mM each dNTP, 0.5 mM heat denatured calf thymus DNA and enzyme. Storage conditions are 50 mM KPO₄ (pH 7.0), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol. Store at -20° C.

DNA Polymerase I (*E. coli*)

DNA Polymerase I is a DNA-dependent DNA polymerase with inherent 3'→5' and 5'→3' exonuclease activities. The 5'→3' exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick-translation. It is isolated from *E. coli* CM 5199, a lysogen carrying λpolA transducing phage (Murray and Kelley, 1979). The phage in this strain was derived from the original polA phage encoding wild-type Polymerase I.

Applications include nick translation of DNA to obtain probes with a high specific activity (Meinkoth and Wahl, 1987) and second strand synthesis of cDNA (Gubler and Hoffmann, 1983; D'Alessio and Gerard, 1988). The reaction buffer is *E. coli* Polymerase I/Klenow Buffer [10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂ and 7.5 mM dithiothreitol]. Supplement with dNTPs.

DNase I is typically not included with this enzyme and must be added for nick translation reactions. Heat inactivation is for 20 min at 75° C. Unit assay conditions are 40 mM KPO₄ (pH 7.5), 6.6 mM MgCl₂, 1 mM 2-mercaptoethanol, 20 μM dATP copolymer, 33 μM dATP and 33 μM ³H-dTTP. Storage conditions are 0.1 M KPO₄ (pH 6.5), 1 mM dithiothreitol and 50% glycerol. Store at -20° C.

DNA Polymerase I, Large (Klenow) Fragment

Klenow fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3'→5' exonuclease activity, but has lost 5'→3' exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5' termini.

A genetic fusion of the *E. coli* polA gene, that has its 5'→3' exonuclease domain genetically replaced by maltose binding protein (MBP). Klenow Fragment is cleaved from the fusion and purified away from MBP. The resulting Klenow fragment has the identical amino and carboxy termini as the conventionally prepared Klenow fragment.

Applications include DNA sequencing by the Sanger dideoxy method (Sanger et al., 1977), fill-in of 3' recessed ends (Sambrook et al., 1989), second-strand cDNA synthesis, random priming labeling and second strand synthesis in mutagenesis protocols (Gubler, 1987)

Reactions conditions are 1X *E. coli* Polymerase I/Klenow Buffer (10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 7.5 mM dithiothreitol). Supplement with dNTPs. Klenow fragment is also 50% active in all four standard NEBuffers when supplemented with dNTPs. Klenow may be heat inactivated by incubating at 75° C. for 20 min. Fill-in conditions: DNA

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should be dissolved, at a concentration of 50 μg/ml, in one of the four standard NEBuffers (1X) supplemented with 33 μM each dNTP. Add 1 unit Klenow per μg DNA and incubate 15 min at 25° C. Stop reaction by adding EDTA to 10 mM final concentration and heating at 75° C. for 10 min. Unit assay conditions 40 mM KPO₄ (pH 7.5), 6.6 mM MgCl₂, 1 mM 2-mercaptoethanol, 20 μM dAT copolymer, 33 μM dATP and 33 μM ³H-dTTP. Storage conditions are 0.1 M KPO₄ (pH 6.5), 1 mM dithiothreitol, and 50% glycerol. Store at -20° C.

Klenow Fragment (3'→5' exo-)

Klenow Fragment (3'→5' exo-) is a proteolytic product of DNA Polymerase I which retains polymerase activity, but has a mutation which abolishes the 3'→5' exonuclease activity and has lost the 5'→3' exonuclease (Derbyshire et al., 1988). It is a genetic fusion of the *E. coli* polA gene that has its 3'→5' exonuclease domain genetically altered and 5'→3' exonuclease domain replaced by maltose binding protein (MBP). Klenow Fragment exo- is cleaved from the fusion and purified away from MBP. Applications include random priming labeling, DNA sequence by Sanger dideoxy method (Sanger et al., 1977), second strand cDNA synthesis and second strand synthesis in mutagenesis protocols (Gubler, 1987).

Reaction buffer is 1X *E. coli* Polymerase I/Klenow Buffer [10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂ and 7.5 mM dithiothreitol]. The reaction mixture is supplemented with dNTPs. Klenow Fragment exo- is also 50% active in all four standard NEBuffers when supplemented with dNTPs. Klenow Fragment exo- may be heat inactivated by incubating at 75° C. for 20 min. When using Klenow Fragment (3'→5' exo-) for sequencing DNA using the dideoxy method of Sanger et al. (1977), an enzyme concentration of 1 unit/5 μl is recommended.

Unit assay conditions are 40 mM KPO₄ (pH 7.5), 6.6 mM MgCl₂, 1 mM 2-mercaptoethanol, 20 μM dAT copolymer, 33 μM dATP and 33 μM ³H-dTTP. Storage conditions are 0.1 M KPO₄ (pH 7.5), 1 mM dithiothreitol and 50% glycerol. Store at -20° C.

T4 DNA Polymerase

T4 DNA Polymerase catalyzes the synthesis of DNA in the 5'→3' direction and requires the presence of template and primer. This enzyme has a 3'→5' exonuclease activity which is much more active than that found in DNA Polymerase I. Unlike *E. coli* DNA Polymerase I, T4 DNA Polymerase does not have a 5'→3' exonuclease function.

T4 DNA Polymerase is purified from a strain of *E. coli* that carries a T4 DNA Polymerase overproducing plasmid. Applications include removing 3' overhangs to form blunt ends (Tabor and Struhl, 1989; Sambrook et al., 1989), 5' overhang fill-in to form blunt ends (Tabor and Struhl, 1989; Sambrook et al., 1989), single strand deletion subcloning (Dale et al., 1985), second strand synthesis in site-directed mutagenesis (Kunkel et al., 1987), and probe labeling using replacement synthesis (Tabor and Struhl, 1989; Sambrook et al., 1989).

The reaction buffer is 1X T4 DNA Polymerase Buffer [50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂ and 1 mM dithiothreitol (pH 7.9 at 25° C.)]. Supplement with 40 μg/ml BSA and dNTPs, which are typically not included in supplied 10X buffer. The reaction temperature varies for the protocol being used, but such conditions are known to those of skill in the art (Tabor and Struhl, 1989; Sambrook et al., 1989).

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It is recommended to use 100 μ M of each dNTP, 1–3 units polymerase/ μ g DNA and incubation at 12° C. for 20 min in the above reaction buffer (Tabor and Struhl, 1989; Sambrook et al., 1989). T4 DNA Polymerase may be heat inactivated by incubating at 75° C. for 10 min. T4 DNA Polymerase is active in all four standard NEBuffers when supplemented with dNTPs.

Unit assay conditions are 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9 at 25° C.), 33 μ M dATP, dCTP and dGTP, 33 μ M ³H dTTP, 70 μ g/ml denatured calf thymus DNA, and 170 μ g/ml BSA. These reaction conditions differ from those used in the Reaction Buffer. Storage conditions are 100 mM KPO₄ (pH 6.5), 10 mM 2-mercaptoethanol and 50% glycerol. Store at –20° C.

Taq Polymerases

Native Taq™ (Perkin-Elmer) DNA Polymerase is a thermostable, 94-kDa DNA polymerase isolated from *Thermus aquaticus* YT1. It is primarily used for exact replication of studies performed prior to the availability of recombinant AmpliTaq DNA Polymerase. AmpliTaq DNA Polymerase is a 94-kDa, gelatin-free, thermostable, recombinant DNA polymerase obtained by expression of a modified form of the Taq DNA Polymerase gene cloned in *E. coli* (Lawyer et al., 1989; Lawyer et al., 1993).

The thermal activity profile of AmpliTaq DNA Polymerase is ideal for PCR applications because its optimal activity is in the same range at which stringent annealing of primers occurs (55° C.–75° C.). The enzyme's PCR cycling half-life is 50 cycles at 95° C., providing sufficient thermostability such that there is no substantial loss of enzymatic activity, even after repeated exposure to the highest temperatures recommended in most PCR protocols. The enzyme has a 5'→3' exonuclease activity which has been exploited in development of a homogeneous simultaneous signal generation assay (Holland et al., 1991) and it lacks 3'→5' exonuclease activity.

Tfl DNA Polymerase

Tfl is yet another polymerase enzyme with an apparent molecular weight of approximately 94 kDa. It was isolated from *Thermus flavus* (Kaledin et al., 1981). The isolated enzyme is thermostable and has a temperature optimum on the DNA templates at 70° degrees and that on RNA templates at 50 degrees. The enzyme does not appear to contain contaminant endo- and exonuclease activities. For maximal activity, the enzyme requires the presence of template, four deoxyribonucleoside triphosphates and monovalent and bivalent cations in the incubation mixture. The enzyme is highly active when "activated" DNA, poly(dA)-poly(dT), poly(dA)-oligo(dT) 10 and poly(rA)-oligo(dT)10 are used as templates, moderately active on single-stranded and double-stranded DNAs and inactive on poly(rC)-oligo(dG) 12–18 and native RNA molecules. Tfl is commercially available from a variety of sources including Promega.

Tth DNA Polymerase was isolated from *Thermus thermophilus* HB-8 (Ruttimann et al., 1985). This enzyme catalyzes the DNA polymerization, of nucleotides into duplex DNA in the 5'→3' direction in the presence of MgCl₂. Also the enzyme catalyzes RNA polymerization in the presence of MgCl₂. The ability of Tth DNA polymerase to act as an RT at elevated temperatures is particularly useful in the context of the present invention.

Tli DNA Polymerase

Tli DNA polymerase is an extremely thermostable polymerase that replicates DNA at 75° C. and remains functional

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even after incubation at 100° C. Tli DNA polymerase has an integral 3'→5' exonuclease activity (proofreading) function. The enzyme has a molecular weight of approximately 90 kDa (Mattila et al., 1991) and is commercially available from a variety of sources.

UITma™ DNA Polymerase

Is a thermostable DNA polymerase specifically designed, thoroughly optimized and tested for its ability to repair 3'-mismatches in PCR amplification, to provide high yield of specific PCR product, and to produce blunt-ended PCR products suitable for cloning and gene expression. Ultma™ DNA Polymerase, a 70-kDa recombinant enzyme, is encoded by a modified form of a *Thermotoga maritima* DNA polymerase gene which has been cloned and expressed in *E. coli* (U.S. Pat. No. 5,310,652, incorporated herein by reference). The enzyme has been specifically engineered to achieve an optimal balance between polymerase and proofreading activity. It has also been optimized for higher yield by using a hot start reaction.

6. Separation Methods

It normally is desirable, at one stage or another, to separate the amplification products from reagents, such as the template or excess primers, or from other amplification products. In one embodiment, amplification products are separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using standard methods. See Sambrook et al., 1989. When working with nucleic acids, denaturing PAGE is preferred.

7. Blotting Methods

In certain embodiments, blotting techniques will be used to examine the size of cDNAs made or to verify the completion of a PCR reaction. Blotting techniques are well known to those of skill in the art. Southern blotting involves the use of DNA as a target, whereas Northern blotting involves the use of RNA as a target. Each provide different types of information, although cDNA blotting is analogous, in many aspects, to blotting of RNA species.

Briefly, a probe is used to target a DNA or RNA species that has been immobilized on a suitable matrix, often a filter of nitrocellulose. The different species should be spatially separated to facilitate analysis. This often is accomplished by gel electrophoresis of nucleic acid species followed by "blotting" on to the filter.

Subsequently, the blotted target is incubated with a probe (usually labeled) under conditions that promote denaturation and rehybridization. Because the probe is designed to base pair with the target, the probe will bind a portion of the target sequence under renaturing conditions. Unbound probe is then removed, and detection is accomplished as described above.

8. Kits

All the essential materials and reagents required for performing the methylation specific PCR method of the present invention may be assembled together in a kit. Such kits generally will comprise preselected primers and may include other oligo- and polynucleotides, such as probes and expression vectors. Also included may be enzymes suitable for amplifying nucleic acids including various polymerases (reverse transcriptases, polymerase, etc.), dNTPs and buffers to provide the necessary reaction mixture for amplification. Such kits also generally will comprise, in suitable means, distinct containers for each individual primer, probe, vector, dNTPs, buffer and enzyme(s).

9. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appre-

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ciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Methods

DNA was extracted from 3–5 ml of blood using the Puregene kit (Gentra systems). 1 µg of DNA was subjected to bisulfite treatment as described (Clark et al., 1994; Herman et al., 1996) using the modifications described in Kubota et al., 1997. PCR was carried out in a 50 µl volume containing 1 X PCR buffer II (Perkin-Elmer), 4.5 mM MgCl₂, 200 µM dNTPs, 0.5 µM NM-BS primers, 1.0–1.5 µM FX-BS primers (sequences in table 1), 1 unit of Ampli-taq Gold enzyme (Perkin-Elmer) and approximately 30 ng of bisulfite-modified DNA. The polymerase was activated at 95° C. for 10 mins, and DNA amplified in a Perkin-Elmer model 9600 thermocycler for 35 cycles at 94° C. for 30s, 64° C. for 30s and 72° C. for 30s, followed by a final extension at 72° C. for 10 mins. Negative controls using untreated DNA and no DNA were also performed.

For non-radioactive amplification across the triplet repeat, modifications of the protocol by Papp et al., 1996 were carried out as follows: 400 ng of DNA was used for digestion with HindIII restriction enzyme. Reactions were set up in a 6 µl volume and incubated for 3 hours at 37° C. The digested DNA was subjected to PCR using primers that flank the triplet repeat, FXF-1 (nt. pos. 13708, 5'-AGGCGCTCAGCTCCGTTTCG-3' SEQ ID NO:10) and FXR-1 (nt. pos. 13922, 5'-AGAGGTGGGCTGCGGGCGCT-3' SEQ ID NO:11). Nucleotide positions are based on the numbering of Verkerk et al., 1991 (Genbank accession no. L29074). PCR was carried out in a 50 µl volume containing 1 X PCR buffer II (Perkin-Elmer), 2.5 mM MgCl₂, 200 µM dNTPs, 0.4 µM of each primer, 1 unit of Ampli-taq Gold enzyme and 2.5M betaine. A negative control using no DNA was performed. PCR products were visualized by electrophoresis of 10 µl of reaction on a 2.7% agarose gel, stained with ethidium bromide, under UV illumination.

EXAMPLE 2

Sodium Bisulfite Treatment of FMR1 DNA

Methylation studies of the 5' untranslated region of the FMR1 gene have shown that CpG sites within and around the triplet repeat are methylated in fragile X individuals and are unmethylated in normal and premutation individuals (Hansen et al., 1992; Homstra et al., 1993). Sodium bisulfite treatment of DNA, which converts unmethylated cytosine to uracil, therefore results in a changed 5' untranslated FMR-1 sequence in affected individuals compared to normal and premutation individuals (FIG. 1). PCR primers were designed that were specific for the antisense strand of the methylated and unmethylated versions of the FMR-1 gene and that were also specific for the treated version of the gene (FIG. 1 and Table 1).

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TABLE 1

Primers Used for Methylation-Specific PCR			
Primers	Sequence (5'→3')	Size (bp)	cDNA ID position NO
FX-BS- <i>for</i>	ACCGATTCCCAACAACGCGCATA	255	13551 12
FX-BS- <i>rev</i>	TTTCGTTATCGTCGTCGTTCCG		13803 13
NM-BS- <i>for</i>	ACACACATACACACTCCCAAA	163	13565 14
NM-BS- <i>rev</i>	TTGAAATGGAGTTGAGTGTGGAT		13728 15

The FX-BS primers are specific for the methylated version of FMR-1 gene and the NM-BS primers for the unmethylated versions. Sequence differences between primers designed to the treated DNA strand and the untreated DNA strand are in bold and italics. Differences between the methylated and unmethylated sequences are underlined. cDNA position is the location of the 5' nucleotide of the primer according to the numbering of Verkerk et al., 1991 (Genbank Accession No. L29074).

DNA samples of full mutation, premutation and normal male individuals (mutation status previously determined by Southern blot analysis) were treated with sodium bisulfite and amplified with primers specific for the methylated sequence (FX primers) and unmethylated sequence (NM primers) in a duplex PCR reaction. A 163 bp amplification product specific for the unmethylated sequence (NM-specific) was observed in normal and premutation individuals. A 255 bp amplification product specific for the methylated sequence (FX-specific) was observed in individuals who carried a full mutation, and in individuals mosaic for a full mutation. The 163 bp NM-specific amplification product was additionally observed in some cases. No amplification was observed with untreated DNA. Normal and premutation males could be distinguished by amplification across the triplet repeat using conventional PCR (as described in Example 1). Individuals with repeat lengths in the normal range (5–50 repeats) showed amplification products of between 169–304 bps, whereas individuals with repeat lengths in the premutation range (50–200 repeats) did not show any amplification.

EXAMPLE 3

Retrospective Diagnostic Study

A retrospective blinded study was performed on 100 DNA samples previously analyzed by Southern blot. These samples were obtained from 52 normal, 31 full mutation, 10 premutation, 6 premutation/full mutation mosaic and 1 normal/full mutation mosaic male individuals. The results of the methylation PCR assay corresponded with the Southern blot results.

All normal and premutation individuals showed NM-specific amplification only. 29 individuals with full mutations showed FX-specific amplification with some also showing NM-specific amplification, while 2 individuals showed NM-specific amplification only. Investigation of these 2 patients showed that both had borderline premutation/full mutation and demonstrated less than 10%

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methylation by Southern blot analysis. All 7 individuals mosaic for full mutations showed both FX- and NM-specific amplification. Amplification across the triplet repeat by conventional PCR was performed for all the individuals and used to distinguish between normal and premutation carriers. All 52 normal individuals presented with an amplification product, while the premutation, full mutation and mosaic individuals did not produce any amplification. By this criteria, the two patients with borderline premutation/full mutation expansions who did not show FX-specific amplification, were classified as premutation carriers.

Additional patients were analyzed to further test the sensitivity of methylation-specific PCR in the presence of mosaicism. 5 fragile X patients mosaic for the full mutation and 4 patients with full mutations mosaic for methylation were analyzed. FX-specific amplification product was observed in all cases. In addition, mosaicism for methylation was also present in a number of individuals included in the blinded study. For example, 2 of the full mutations and 3 of the premutation/full mutations were mosaic for methylation. FX-specific amplification was observed for all these cases.

EXAMPLE 4

Prospective Diagnostic Study

A prospective study also was undertaken on 30 male blood samples referred for diagnosis of fragile X. Methylation-specific PCR showed the presence of methylation in two cases and no amplification across the triplet repeat by conventional PCR, consistent with a full mutation and therefore positive for fragile X. An absence of methylation was observed in the remaining 28 cases with a corresponding normal size triplet repeat amplification product, indicative of normal for fragile X. These results corresponded to those obtained by Southern blot analysis performed in parallel and demonstrate that the methylation-specific PCR described in the present invention may be used as a fast reliable diagnostic tool for identifying males with fragile X syndrome.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the [compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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 U.S. Pat. No. 5,264,566
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 U.S. Pat. No. 5,428,148
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What is claimed is:

1. A method for determining the methylation state of an FMR1 gene promoter of a male subject comprising the steps of:

- (a) denaturing a DNA sample from said subject;
- (b) subjecting the denatured DNA to bisulfite modification;
- (c) amplifying said DNA using primers pairs having the sequences
 ACCGATTCCCAACAACGCGCATA (SEQ ID NO:12) and
 TTTCGTTATCGTCGTCGTTTCGC (SEQ ID NO:13),
 and
 ACACACATACACACTCCCAA (SEQ ID NO:14) and
 TTGAAATGGAGTTGAGTGTGTTGAT (SEQ ID NO:15); and

(d) detecting amplification products from step (c).

2. The method of claim 1, further comprising the step, before step (a), of obtaining a DNA sample from said subject.

3. The method of claim 2, wherein said sample is blood, amniotic fluid, buccal smears.

4. The method of claim 1, wherein denaturing comprises treatment with NaOH and heat.

5. The method of claim 1, wherein bisulfite modification comprises treatment with hydroquinone and sodium bisulfite, followed by treatment with NaOH.

6. The method of claim 1, further comprising the step of purifying said bisulfite modified DNA.

7. The method of claim 1, wherein amplification comprises PCR.

8. The method of claim 7, wherein said PCR comprises 35 cycles at 94° C. for 30 seconds, 64° C. for 30 seconds and

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72° C. for 30 seconds, followed by a final extension at 72° C. for 10 minutes.

9. The method of claim 1, wherein said amplification products are unlabeled.

10. The method claim 1, wherein said amplification products are labeled with a detectable label. 5

11. The method of claim 10, wherein said label is radioactive, fluorescent, chemiluminescent or colorimetric.

12. The method of claim 1, wherein said detection comprises gel electrophoresis and visualization of size-separated PCR products. 10

13. The method of claim 1, wherein said primer comprises an additional sequence that is not complementary to a region of FMR1 gene promoter.

14. The method of claim 13, wherein the non-complementary region comprises a restriction enzyme site. 15

15. An isolated primer comprising the sequence ACCGATTCCCAACAACGCGCATA (SEQ ID NO:12).

16. An isolated primer comprising the sequence TTTCGTTATCGTCGTCGTTTCGC (SEQ ID NO:13). 20

17. An isolated primer comprising the sequence ACACACATACACACACTCCCAAA (SEQ ID NO:14).

18. An isolated primer comprising the sequence TTGAAATGGAGTTGAGTGTTTGAT (SEQ ID NO:15). 25

19. A set of two primer pairs comprising the following sequences:

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ACCGATTCCCAACAACGCGCATA (SEQ ID NO:12) and

TTTCGTTATCGTCGTCGTTTCGC (SEQ ID NO:13), and

ACACACATACACACACTCCCAAA (SEQ ID NO:14) and

TTGAAATGGAGTTGAGTGTTTGAT (SEQ ID NO:15).

20. A kit comprising, in suitable container means, primer pairs comprising the following sequences:

ACCGATTCCCAACAACGCGCATA (SEQ ID NO:12) and

TTTCGTTATCGTCGTCGTTTCGC (SEQ ID NO:13), and

ACACACATACACACACTCCCAAA (SEQ ID NO:14) and

TTGAAATGGAGTTGAGTGTTTGAT (SEQ ID NO:15).

21. The kit of claim 20, further comprising a thermostable DNA polymerase.

22. The kit of claim 20, further comprising sodium bisulfite and hydroquinone.

23. The kit of claim 20, further comprising a DNA denaturing agent.

24. The kit of claim 20, further comprising dNTP's.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,143,504
DATED : Nov. 7, 2000
INVENTOR(S) : Das et al.

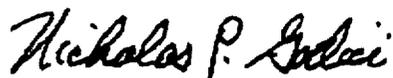
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item [60], Related U.S. Application Data, please insert -- Provisional Application Ser. No. 60/105,892, Oct. 27, 1998.-- therefor.

In claim 3, column 30, line 56, please delete "fluid" and insert -- fluid or -- therefor.

Signed and Sealed this
Twenty-ninth Day of May, 2001

Attest:



NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office