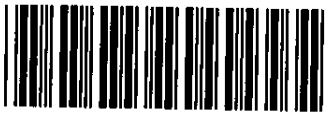

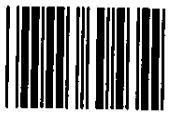

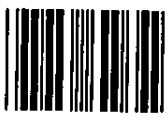

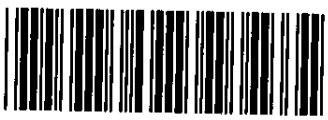

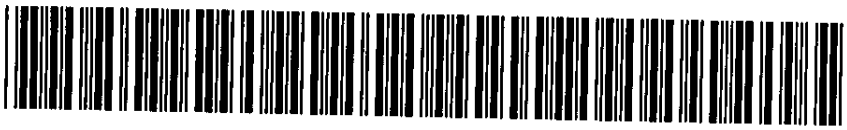

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ORCHID BIOSCIENCES, INC.

CLERK U.S. DISTRICT COURT
SOUTHERN DISTRICT OF CALIFORNIA
BY: [illegible] DEPUTY

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MAR 12 2001

ORIGINAL

UNITED STATES DISTRICT COURT

FOR THE SOUTHERN DISTRICT OF CALIFORNIA

ORCHID BIOSCIENCES, INC., a Delaware corporation,

Plaintiff,

vs.

ST. LOUIS UNIVERSITY, a non-profit organization,

Defendant.

Civil Action No. 00cv1558 L (JFS)

FIRST AMENDED COMPLAINT

DEMAND FOR JURY TRIAL

CR

Plaintiff Orchid BioSciences, Inc. ("Plaintiff Orchid") by its First Amended Complaint against Defendant St. Louis University ("Defendant SLU") alleges as follows:

PARTIES

1. Plaintiff Orchid is a corporation organized under the laws of the State of Delaware, having a place of business at 303 College Road East, Princeton, New Jersey 08540.

2. On information and belief, Defendant SLU is a non-profit organization having a place of business at 221 North Grand Boulevard, St. Louis, Missouri 63103.

3. On information and belief, Defendant SLU is the assignee and owner of United States Patent No. 5,846,710 ("'710 patent"), to S. Paul Bajaj, entitled "Method for The Detection of Genetic Diseases and Gene Sequence Variations by Single Nucleotide Primer Extension," a true and correct copy of which is attached hereto as Exhibit A.

4. Defendant SLU is transacting and/or soliciting business in the State of California and specifically in this judicial district.

5. Defendant SLU has licensed the '710 patent to Nanogen, Inc. having a place of business at 10398 Pacific Center Court, San Diego, California 92121.

6. Defendant SLU has licensed the '710 patent to Illumina, Inc. having a place of business at 9390 Towne Centre Drive, #200, San Diego, California 92121.

7. Defendant SLU has entered into over 100 agreements with California entities over the past two years.

/ / /

JURISDICTION AND VENUE

8. This is an action for: a declaratory judgment of non-infringement, invalidity and non-enforceability of the '710 patent; violation of 35 U.S.C. § 1125; and violation of California Business & Profession Code § 17200 *et seq.*

9. Jurisdiction of this Court arises under 28 U.S.C. §§ 2201, 2202, 1338(a)and (b) and 1367. Venue is based upon 28 U.S.C. §§ 1391(b) and/or (c).

DEFENDANT SLU THREATENS TO SUE PLAINTIFF ORCHID

10. Defendant SLU has demanded, in writing and orally in several conversations with Plaintiff Orchid's patent counsel, that Plaintiff Orchid agree to license the '710 patent from Defendant SLU and to pay license fees to Defendant SLU.

11. On May 10, 2000, patent counsel for Plaintiff Orchid sent a letter to Defendant SLU summarizing the reasons why Plaintiff Orchid believed that it was not obligated to license the '710 patent. Exhibit B. The letter stated that Plaintiff Orchid intended to seek an opinion from independent counsel, and asked for a response to the substantive concerns discussed in his letter. The letter concluded as follows:

> ...in light of the above issues it would be premature to begin negotiating the terms of a license until and unless these concerns regarding the proper interpretation of Bajaj [the '710 patent] and the validity of claim 16 are resolved satisfactorily.

Exh. B at 3.

12. Defendant SLU responded by letter from its outside counsel dated July 26, 2000, indicating that Defendant SLU intends to sue Plaintiff Orchid for patent infringement within

ten (10) days if Plaintiff Orchid fails to agree to a license on the terms demanded by Defendant SLU. Exhibit C. At no time has Defendant SLU or its outside counsel fully responded to Plaintiff Orchid's concerns regarding the validity of Claim 16. Defendant SLU has persistently refused to discuss possible alternative terms to its proposed form of license.

13. On, August 3, 2000, Plaintiff Orchid filed the original Complaint in this action. At that time, the only count was for declaratory judgement.

14. At the time that Plaintiff Orchid originally brought this action, it was under the belief and reasonable apprehension that it would be sued for infringement of the '710 patent by Defendant SLU in the very near future. In the time since Plaintiff Orchid originally brought this action, Defendant SLU has neither answered the Complaint nor provided any indication that it would not sue Plaintiff Orchid for patent infringement.

15. As a result of Defendant SLU's positions and actions, Plaintiff Orchid is under the reasonable apprehension that Defendant SLU intends to bring suit against it.

16. Defendant SLU's positions have created uncertainties that have caused harmful effects with Plaintiff Orchid's continuing and planned expansion of its business activities.

DEFENDANT SLU'S FALSE AND MISLEADING MARKETING OF ITS TECHNOLOGY

17. After Plaintiff Orchid filed its original Complaint in this action, Defendant SLU served a motion to dismiss for lack of jurisdiction, or in the alternative to transfer the case to the United States District Court for the Eastern District of

27. Defendant SLU is estopped by the express language of the specification, the claims and the file wrapper history of the '710 patent, as well as by statements made during the prior interference proceeding, from arguing that the claims of the '710 patent are sufficiently broad to cover Plaintiff Orchid's activities.

28. An actual controversy within the meaning of 28 U.S.C. §§ 2201 and 2202 exists between Plaintiff Orchid and Defendant SLU with respect to the validity, enforceability and infringement of the '710 patent.

COUNT II: VIOLATION OF 35 U.S.C. § 1125

29. Plaintiff Orchid realleges and incorporates by reference the allegations set forth in paragraphs 1 to 28 of this First Amended Complaint.

30. Defendant SLU made false and misleading statements of fact in commercial advertisements and/or promotions, including written documents sent to California commercial entities as to the scope of the claims of the '710 patent. Included in these statements was the false and misleading statement that the '710 patent dominates Orchid's '819 patent and that in order to practice the technology of the '819 patent, one must have a license under the '710 patent. These statements were made to commercial California entities with the intent of inducing those entities to license the '710 patent.

31. Defendant SLU's false and misleading statements also include statements that the claims of the '710 patent cover certain methods for determining nucleotide sequences that use different nucleotides during the primer extension reaction to

detect gene sequence variations.

32. Defendant SLU made these false and misleading statements with knowledge that the '710 patent is unenforceable, and the knowledge that the '710 patent does not dominate Orchid's '819 patent. The making of these statements with this knowledge is in bad faith.

33. Defendant SLU's false and misleading statements were made with knowledge that the claims of the '710 patent as Defendant SLU now construes them are not supported by the specification. The making of these statements with this knowledge is in bad faith.

34. Defendant SLU's false and misleading statements were made in connection with interstate commerce.

35. Defendant SLU's false and misleading statements are likely to deceive a substantial segment of the intended recipients of these statements.

36. Defendant SLU's statements are likely to influence the decisions of potential licensees as to whether to license the technology of the '710 patent, as well as rights to other patents in the field held by competitors of Defendant SLU.

37. Defendant SLU's false and misleading statements resulted in actual or probable injury to Plaintiff Orchid, who also holds and licenses patents in this field, by diminishing the perceived value of Plaintiff Orchid's patent portfolio in the marketplace.

38. Defendant SLU's false and misleading commercial advertising and promotion, which was made in bad faith, violates 35 U.S.C. § 1125.

COUNT III: VIOLATION OF CALIFORNIA BUSINESS & PROFESSION CODE § 17200 *ET SEQ.*

39. Plaintiff Orchid realleges and incorporates by reference the allegations set forth in paragraphs 1 to 38 of this First Amended Complaint.

40. Defendant SLU's aforementioned conduct is an attempt to assert broader coverage of the '710 patent than it is entitled to assert, falsely denigrates the value of Plaintiff Orchid's '819 patent, and violates the public's right to protection from fraud and deceit. Defendant SLU's conduct constitutes an anticompetitive business practice and unfair competition in violation of California Business & Profession Code § 17200 *et seq.*

WHEREFORE, Plaintiff Orchid prays:

a. for entry of judgment declaring that Defendant SLU is without right or authority to threaten or to maintain suit against Plaintiff Orchid for alleged infringement of the '710 patent; that claims 16, 17, 18, 19 and 20 of the '710 patent are invalid and void in law; that the '710 patent is unenforceable; and that the '710 patent is not infringed by Plaintiff Orchid;

b. for a preliminary and permanent injunction enjoining Defendant SLU, its officers, directors, agents, servants, employees and attorneys, and those persons in active concert or participation with them who receive actual notice thereof, from initiating litigation to enforce the '710 patent, and from threatening to initiate such proceedings, against Plaintiff Orchid or any of its licensees, customers, dealers, officers, directors, agents, servants, or employees, or any

prospective or present sellers, dealers or users of Plaintiff Orchid's research, development, manufacture and sale of certain products, services and technologies for nucleotide sequence analysis;

c. for an injunction prohibiting Defendant SLU from continuing to violate 35 U.S.C. § 1125;

d. for an injunction prohibiting Defendant SLU form continuing to violate California Business & Profession Code § 17200 *et seq.*;

e. for damages in an amount sufficient to compensate Plaintiff Orchid for the diminished value of its patent portfolio due to Defendant SLU's false and misleading statements;

f. for assessment of costs against Defendant SLU;

g. for reasonable attorneys' fees; and

h. for such other relief as the Court may deem just and proper.

Respectfully Submitted,

Dated: March 12, 2001 CAMPBELL & FLORES LLP

By: _____
David M. Beckwith, Esq.
Attorneys for Plaintiff
ORCHID BIOSCIENCES, INC.

OF COUNSEL:
KALOW & SPRINGUT LLP
David A. Kalow
William D. Schmidt
488 Madison Avenue
New York, New York 10022
Phone: (212) 813-1600

DEMAND FOR JURY TRIAL

Pursuant to Fed. R. Civ. P. 38(b), Plaintiff Orchid BioSciences, Inc. hereby demands a jury trial on all issues raised by the Complaint herein and triable of right to jury.

Dated this 12th day of March, 2001.

David M. Beckwith

FIRST AMENDED COMPLAINT FOR DECLARATORY JUDGMENT
DEMAND FOR JURY TRIAL

INDEX OF EXHIBITS

Docket No. 00cv1558 L (JFS)
Orchid BioSciences, Inc. v. Saint Louis University

PROOF OF SERVICE

I, Rosalind E. Brady, hereby certify and declare under penalty of perjury that the following statements are true and correct:

1. I am over the age of 18 years and am not a party to the within cause.

2. My business and mailing address is Campbell & Flores LLP, 4370 La Jolla Village Drive, Suite 700, San Diego, California 92122.

3. On March 12, 2001, I served a true copy of the attached document titled exactly:

FIRST AMENDED COMPLAINT; DEMAND FOR JURY TRIAL

On the party in this action, address as follows:

Counsel for Defendant Saint Louis University:

Winthrop B. Reed, III, Esq.
LEWIS, RICE & FINGERSH, L.C.
500 N. Broadway, Suite 2000
St. Louis, MO 63102-2147
Tel: (314) 444-7600
Fax: (314) 612-7617

☒ (By MAIL) I placed a true copy thereof in a sealed envelope(s) and personally placed such envelope(s) with postage fully prepaid for collection and mailing on the above referenced date following the ordinary business practices of this office. I am readily familiar with the practice of Campbell & Flores LLP for collection and processing of correspondence, said practice being that in the ordinary course of business, correspondence is deposited in the United States Postal Service the same day as it is placed for collection.

☐ (By HAND DELIVERY) I delivered by hand each sealed envelope(s) to an attorney service for hand delivery to the counsel listed above at the addresses shown above.

☐ (By FEDERAL EXPRESS) I am readily familiar with the practice of Campbell & Flores LLP for collection and processing correspondence for overnight delivery and know that the document(s) described herein will be deposited in a box or other facility regularly maintained by Federal Express for overnight delivery.

[X] (By FACSIMILE) I caused to be transmitted the document(s) described herein via the fac numbers listed above.

Executed at San Diego, California on March 12, 2001.

Rosalind E. Brady

Docket No. 00cv1558 L (JFS)
Orchid BioSciences, Inc. v. Saint Louis University

PROOF OF SERVICE

I, Rosalind E. Brady, hereby certify and declare under penalty of perjury that the following statements are true and correct:

1. I am over the age of 18 years and am not a party to the within cause.

2. My business and mailing address is Campbell & Flores LLP, 4370 La Jolla Village Drive, Suite 700, San Diego, California 92122.

3. On March 12, 2001, I served a true copy of the attached document titled exactly:

FIRST AMENDED COMPLAINT; DEMAND FOR JURY TRIAL

On the party in this action, address as follows:

Counsel for Defendant Saint Louis University:

Joseph J. McCann, Jr., Esq.
Michael J. Hickman, Esq.
MUSICK, PEELER & GARRETT LLP
225 Broadway, Suite 1900
San Diego, CA 92101-5028
Tel: (619) 525-2500
Fax: (619) 231-1234

☐ (By MAIL) I placed a true copy thereof in a sealed envelope(s) and personally placed such envelope(s) with postage fully prepaid for collection and mailing on the above referenced date following the ordinary business practices of this office. I am readily familiar with the practice of Campbell & Flores LLP for collection and processing of correspondence, said practice being that in the ordinary course of business, correspondence is deposited in the United States Postal Service the same day as it is placed for collection.

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☐ (By FACSIMILE) I caused to be transmitted the document(s) described herein via the fac numbers listed above.

Executed at San Diego, California on March 12, 2001.

Rosalind E. Brady


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[54] METHOD FOR THE DETECTION OF GENETIC DISEASES AND GENE SEQUENCE VARIATIONS BY SINGLE NUCLEOTIDE PRIMER EXTENSION

[75] Inventor: S. Paul Bajaj, St. Louis, Mo.

[73] Assignee: St. Louis University, St. Louis, Mo.

[21] Appl. No.: 103,408

[22] Filed: Aug. 6, 1993

Related U.S. Application Data

[63] Continuation of Ser. No. 608,225, Nov. 2, 1990, abandoned.

[51] Int. Cl.[6] C12Q 1/68; C12P 19/34; C07H 21/04; C12N 15/00

[52] U.S. Cl. 435/6; 435/91.1; 435/91.2; 536/23.1; 536/23.5; 536/24.33; 935/76; 935/77; 935/78

[58] Field of Search 435/6, 91, 91.1, 435/91.2, 183; 536/23.1, 24.3, 24.33, 25.4; 935/6, 17, 19, 78, 80, 76, 77

[56] References Cited

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Primary Examiner—Bradley L. Sisson
Attorney, Agent, or Firm—Senniger, Powers, Leavitt & Roedel

[57] ABSTRACT

Method for screening a sample oligonucleotide for a variation in sequence at a predetermined position thereof relative to a nucleic acid the sequence of which is known, wherein the sample oligonucleotide is provided as a single stranded molecule, the single stranded molecule is mixed with an inducing agent, a labeled nucleotide, and a primer having a sequence identical to a region flanking the predetermined position to form a mixture, the mixture having an essential absence of nucleotides constituted of bases other than the base of which the labeled nucleotide is constituted, the mixture is subjected to conditions conducive for the annealing of the primer to the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide, and the mixture is analyzed for the presence of primer extension product containing labeled nucleotide.

20 Claims, 4 Drawing Sheets

EXHIBIT A

Case 3:00-cv-01558-L-JFS Document 30 Filed 03/15/01 Page 18 of 37

FIG.1A STANDARD DNA

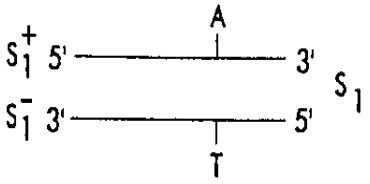


FIG.1B SAMPLE DNA

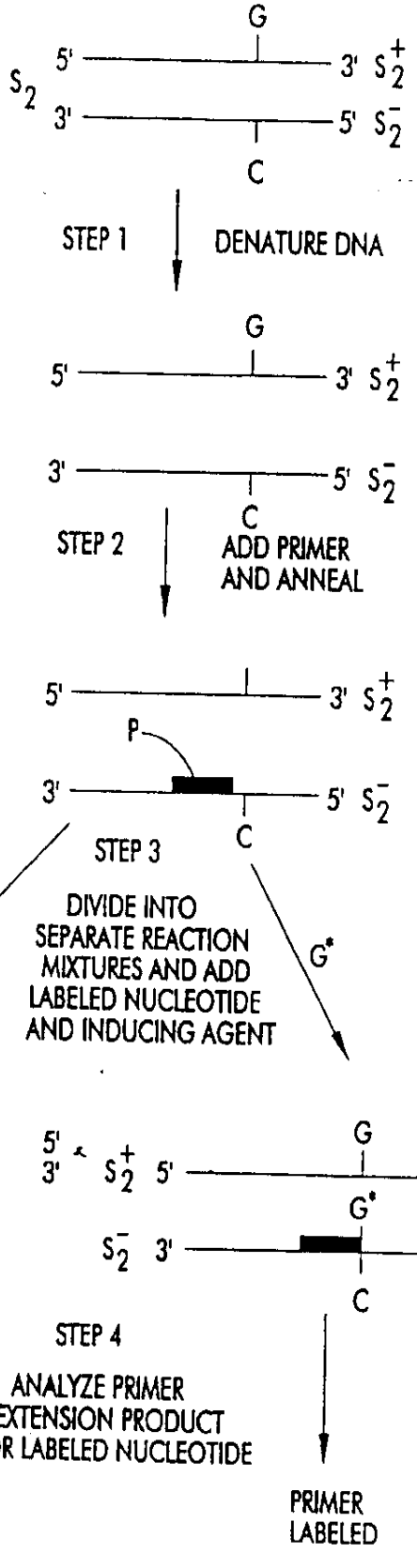


EXHIBIT A
2

Case 3:00-cv-01558-L-JFS Document 30 Filed 03/15/01 Page 19 of 37

FIG. 2A

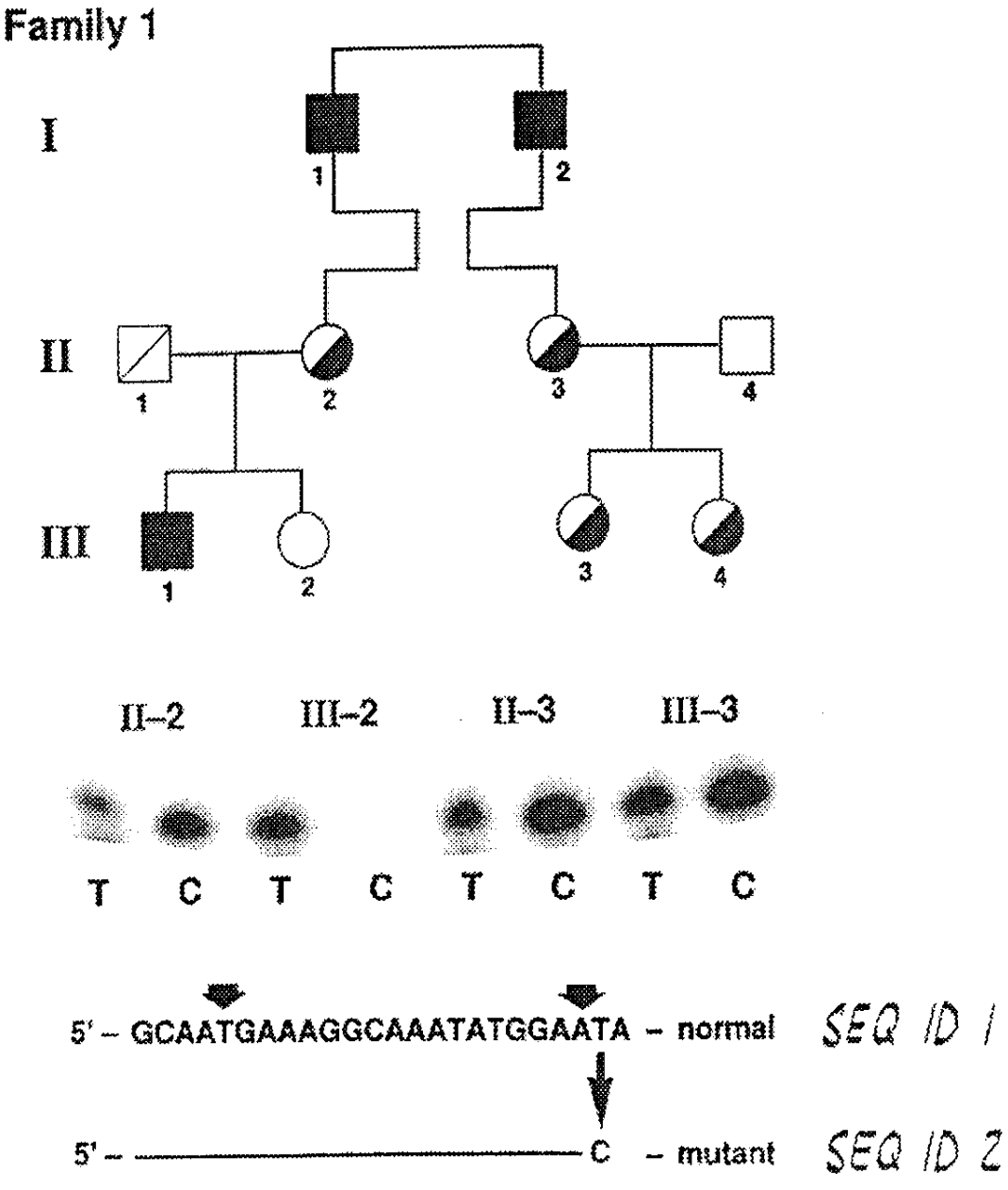
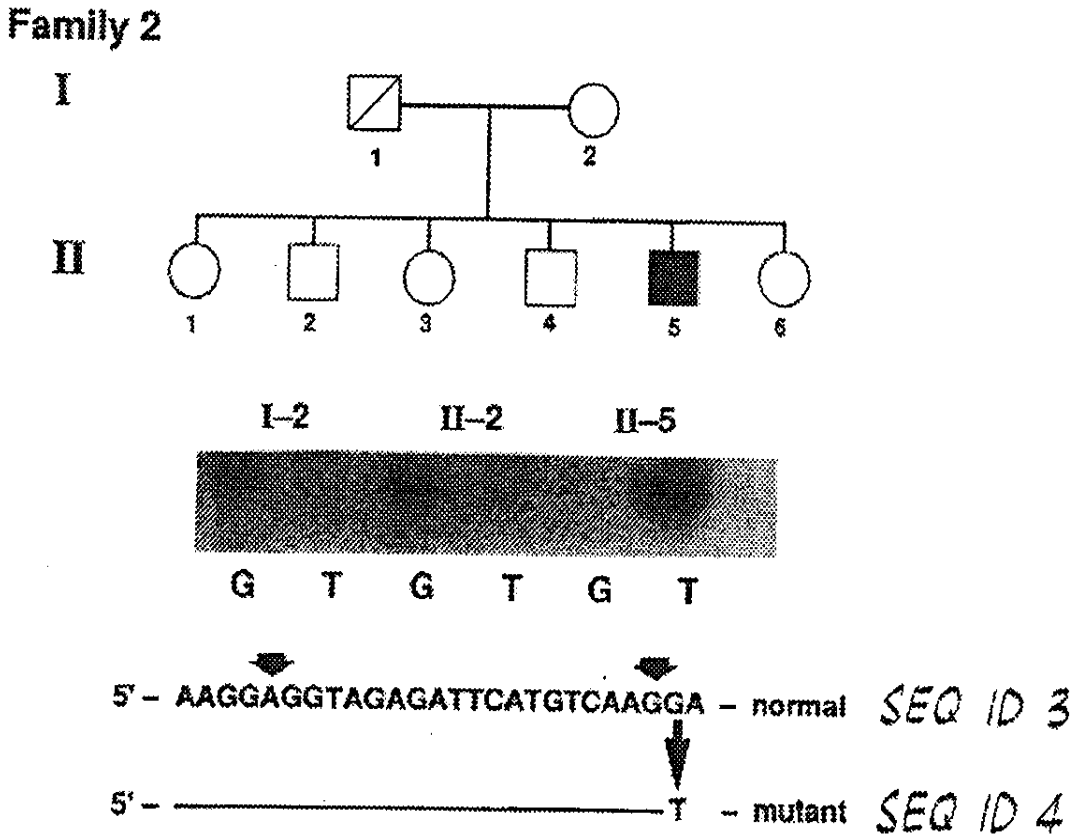


EXHIBIT A

Case 3:00-cv-01558-L-JFS Document 30 Filed 03/15/01 Page 20 of 37

FIG. 2B



Case 3:00-cv-01558-L-JFS Document 30 Filed 03/15/01 Page 23 of 37

FIG. 3

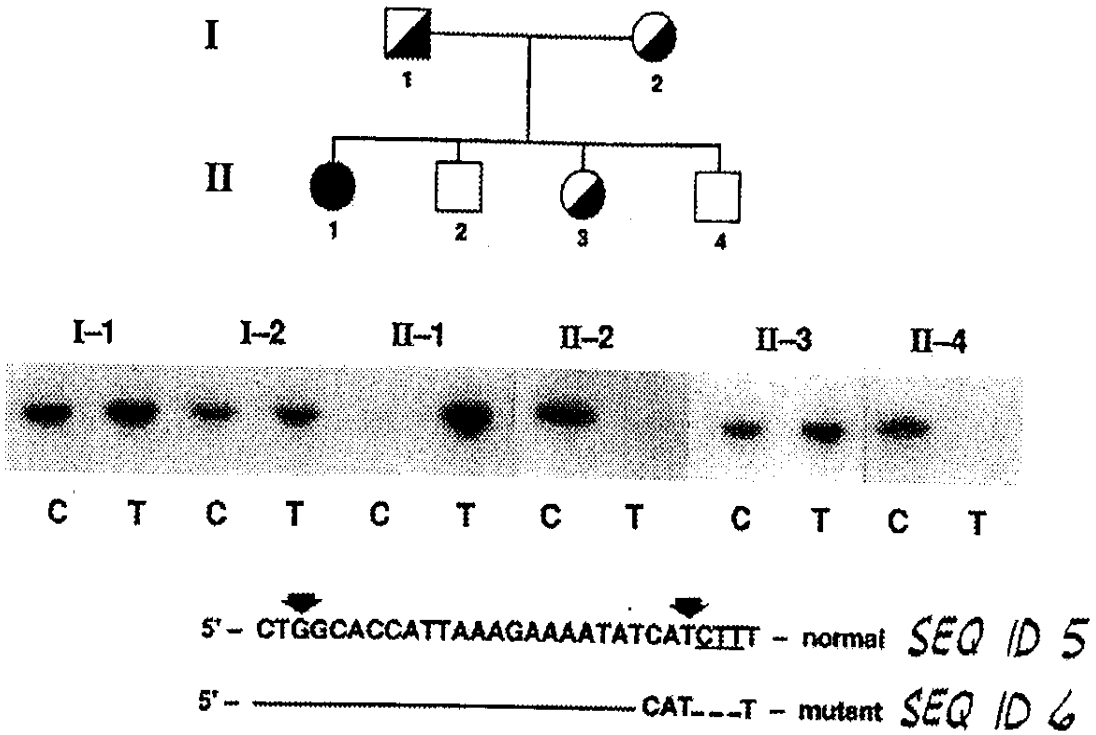


EXHIBIT A
5

METHOD FOR THE DETECTION OF GENETIC DISEASES AND GENE SEQUENCE VARIATIONS BY SINGLE NUCLEOTIDE PRIMER EXTENSION

This is a continuation of application Ser. No. 07/608,255 filed on Nov. 2, 1990, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates, in general, to a method for determining the sequence of a sample DNA fragment at a predetermined position, and in particular, to a method for the detection of known genetic abnormalities or gene sequence variations resulting from nucleotide substitutions, translocations, insertions or deletions of as little as one nucleotide.

One goal of clinical molecular biology is to identify the mutations that cause genetic diseases and to develop strategies and related technologies to diagnose them. Towards this end, in the last decade or so many methodological advances have been made to detect human genetic abnormalities at the DNA level. These include indirect methods such as linkage analysis using the Southern blotting technique where the inheritance of a disorder is associated with the presence of a restriction fragment-length polymorphism (RFLP), e.g., Duchenne muscular dystrophy. Other indirect methods include ribonuclease A cleavage at mismatches in probe RNA:sample DNA duplexes or denaturing gradient gel electrophoresis for mismatches in probe DNA:sample DNA duplexes, e.g., β-thalassemia. The direct methods include detection with the restriction enzymes or with the allele specific oligonucleotide (ASO) probes, e.g., the sickle cell mutation. See, for example Landegren et al., *Science* 242:229–237, 1988; Rossiter et al, *J. Biol. Chem.* 265:12753–12756, 1990.

A majority of the above approaches have now been combined with the polymerase chain reaction (PCR) for diagnosis of the sequence variations. Initially, the target DNA is amplified by PCR followed by the analysis of the sequence variation by ASO hybridization, e.g., the sickle cell mutation, restriction enzyme analysis, e.g., the sickle cell mutation and some hemophilia B mutations, ribonuclease A cleavage, e.g., α1-antitrypsin gene Z mutation, denaturing gradient gel electrophoresis, e.g., hemophilia A mutations, chemical cleavage, e.g., hemophilia B mutations, and the ligation of oligonucleotide pairs or the ligation amplification, e.g., the sickle cell mutation. Recently, an allele specific PCR (ASPCR) amplification technique to diagnose point mutations has also been introduced, Wu et al., *Proc. Natl. Acad. Sci.* 86:2757–2760, 1989.

Some of the above techniques do not detect all mutations that involve single nucleotides and are technically quite demanding. Others require optimization of conditions that allows specific hybridization of the ASO probe or specific amplification of the selected allele by ASPCR.

SUMMARY OF THE INVENTION

Among the objects of the invention, therefore, may be noted the provision of a method for the detection of abnormal alleles in those genetic diseases where the frequency of occurrence of the same mutation is high, and in other genetic diseases where multiple mutations cause the disease and the sequence variation in an affected member of a given family is known; the provision of a method for determining the sequence of a genomic DNA sample at a predetermined position thereof; the provision of such a method in which nucleotide sequence variations of as little as one nucleotide can be detected; and the provision of such a method which is relatively rapid and not technically demanding.

Briefly, therefore, the present invention is directed to a method for detecting a known genetic abnormality or gene sequence variation resulting from a nucleotide substitution, translocation, insertion or deletion at a predetermined position in a sample DNA fragment by single nucleotide primer extension. The method comprises providing the sample DNA fragment as a denatured molecule and mixing it with an inducing agent, a primer having a sequence complementary to a region flanking the predetermined position, and a labeled nucleotide to form a mixture. The mixture has an essential absence of nucleotides constituted of bases other than the base of which the labeled nucleotide is constituted. The mixture is subjected to conditions conducive for the annealing of primer to the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide. The mixture is thereafter analyzed for the presence of primer extension product which has incorporated the labeled nucleotide.

Other objects will be in part apparent, and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B are schematic diagrams of a preferred embodiment of the method of the present invention.

FIG. 2 is a diagram depicting the results of Example 1.

FIG. 3 is a diagram depicting the results of Example 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "DNA" or "DNA fragment" as used herein is defined as a molecule comprised of two complementary strands of approximately 30 or more deoxyribonucleotides.

The term "primer" as used herein refers to an oligonucleotide which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH. The primer must be sufficiently long to prime the synthesis of an extension product in the presence of the inducing agent. The primer is preferably an oligodeoxyribonucleotide and typically contains about 18 nucleotides.

The term "inducing agent" as used herein is defined as any compound or system which will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for example, E. Coli. DNA polymerase I, Klenow fragment of E. Coli. DNA polymerase, other available DNA polymerases, and other enzymes, including heat-stable enzymes which will facilitate addition of the labeled nucleotide in the proper manner to form the primer extension product.

In accordance with the present invention, it has been found that the nucleotide sequence of a genomic DNA sample can be analyzed for sequence variations and that variations of as little as one nucleotide can be detected relatively rapidly according to a method which is not technically demanding. This method may be conveniently used to detect the presence of abnormal alleles in those genetic diseases where frequency of occurrence of the same mutation is high (e.g., cystic fibrosis and sickle cell disease), and in others where multiple mutations cause the disease and the

sequence variation in an affected member of a given family is known (e.g., hemophilia B).

A preferred embodiment of the method of the present invention is depicted schematically in FIG. 1 in the context of a hypothetical wild-type, "standard" DNA fragment S_1 and a hypothetical sample DNA fragment S_2. For purposes of this illustration, the standard and sample DNA fragments S_1 and S_2 are assumed to be identical except that the sample fragment S_2 has a nucleotide substitution at a predetermined position B relative to the standard fragment; the standard fragment has base pairs A and T (strands S_1^+ and S_1^-, respectively) whereas the sample fragment has base pairs G and C (strands S_2^+ and S_2^-, respectively) at the predetermined position.

To confirm that (or determine whether) sample DNA fragment S_2 has a sequence variation at the predetermined position B relative to the standard, the sample DNA fragment S_2 is denatured into single stranded molecules S_2^+ and S_2^- (step 1). Primer P which is complementary to single stranded molecule S_2^- at a position immediately flanking the 3' end of the predetermined position B is then annealed to the single stranded molecule S_2^- (step 2). The resulting product is then divided into separate aliquots, and an inducing agent and a labeled nucleotide are added to each aliquot to form separate reaction mixtures (step 3). The labeled nuclueotide added to one of the reaction mixtures is labeled adenine whereas the labeled nucleotide added to other reaction mixture is labeled guanine. Each reaction mixture is allowed to undergo single nucleotide primer extension with the labeled nucleotide, i.e., extension with nucleotide constituted of only one of the four types of bases (adenine in one of the reaction mixtures in this illustration and guanine in the other) and thereafter analyzed for the presence of primer extension product incorporating labeled nucleotide (step 4). In this illustration, the incorporation of labeled guanine and the lack of incorporated labeled adenine positively identifies the sequence of the sample DNA fragment S_2 at the predetermined position B as being a variant of the standard DNA.

It should be noted that the primer P in FIG. 1 may have alternatively comprised a nucleotide sequence which is complementary to single stranded molecule S_2^+ at a position immediately flanking the 3' end of the predetermined position B. If such a primer were selected, labeled cytosine would be substituted for labeled guanine in step 3.

Unlike standard PCR, the object of single nucleotide primer extension is not amplification of oligodeoxyribonucleotides. Rather, the object is to identify the nucleotide at a predetermined point of potential variation or mutation in a sample gene, the sequence of which is otherwise known. Thus, it is preferred that only one primer be included in the reaction mixture. Similarly, it is also preferred that the reaction mixture comprise nucleotide constituted of only one type of base. Inclusion of a plurality of primers and/or nucleotides would tend to eliminate the specificity of the reactions.

The rapidity and ease with which a sample DNA fragment is analyzed at a predetermined position relative to a known, standard DNA sequence is thus achieved by the composition of the reaction mixture and by the fact that only one cycle of chain extension is necessary. Because the reaction mixture contains nucleotide constituted of only one type of base and a primer having a sequence complementary to only one of the strands of the sample DNA fragment, the incorporation of labeled nucleotide into a primer extension product directly and positively confirms the sequence of the sample DNA at the predetermined position. In addition, by using separate reaction mixtures each containing a different labeled nucleotide, the extent of variation in the sample DNA at the predetermined position is positively identified.

Preferably, the reaction mixture comprises about 50 to 100 ng (depending upon the length of the fragment) of the DNA sample fragment containing the putative variation site. Conveniently, the DNA sample fragment may be provided as a PCR product of copy DNA obtained from RNA or of genomic DNA. Irrespective of source, the sample DNA is provided as single stranded molecules (preferably by denaturation where genomic DNA is the source). The reaction mixture is then subjected to conditions suitable for the annealing of the primer to the single stranded molecules and the formation of primer extension product incorporating the labeled nucleotide. Upon completion of that cycle, the primer extension product is separated from free labeled nucleotide preferably by denaturing polyacrylamide gel electrophoresis. The oligomers are then analyzed for the presence of label.

If it is desired to analyze a sample DNA fragment for purposes of determining whether the sample DNA fragment donor carries a mutant gene, two reaction mixtures can be prepared. Each contains the sample DNA fragment, a primer whose sequence is complementary to the sequence of the gene immediately flanking the 3' end of the putative mutation site and an inducing agent. One of the reaction mixtures contains a labeled nucleotide corresponding to the normal coding sequence at the putative mutation site and the other contains a labeled nucleotide corresponding to a mutant sequence. Each reaction mixture has an essential absence of nucleotides other than labeled nucleotide. Primer extension is carried out in each reaction mixture and the products are analyzed for the presence of a primer extension product containing labeled nucleotide. According to the Watson-Crick base pair rule, in the wild type only the normal base, in an affected member only the mutant base, and in carriers both the normal and the mutant base will be incorporated into the primer.

For use in the reaction mixture, nucleotides may be labeled by any appropriate means. Preferably, the nucleotides are radioactively labeled by means of incorporation of ^3H, ^{32}S or ^{32}P and are detected by means of autoradiography. However, other methods for labeling nucleotides presently known or hereafter developed may be used in accordance with the present invention.

Similarly, upon completion of one cycle of chain extension the primer may be separated from labeled nucleotide for detection of signal by any means standard in the art. Preferably and conveniently, the reaction mixture components may be separated by denaturing polyacrylamide gel electrophoresis. When the nucleotides are radioactively labeled, this technique can be combined with audioradiography to provide rapid and convenient detection of primer extension product incorporating labeled nucleotide.

The method of the present invention is rapid and has utility in carrier detection and prenatal diagnosis of genetic diseases with a known sequence variation. For instance, Cystic Fibrosis (CF) is the most common severe autosomal recessive disorder in the Caucasian population; its clinical, physiologic and genetic aspects have been discussed recently, and a three-base pair deletion which removes Phe^{508} from the putative CF protein of 1480 amino acids has been identified as the mutation which causes CF in a majority (60%) of the chromosomes Lemna et al., *N. Engl. J. Med* 322:291–296, 1990. The single nucleotide primer extension method of the present invention provides a rapid

and convenient technique for determining an individual's status as a carrier of this mutation. If the index patient is fully informative, i.e., has haplotype 2/2 (see Example 2), single nucleotide primer extension alone can be used for carrier detection and prenatal diagnosis. If the index patient is partly informative, i.e., has haplotype 1/2, single nucleotide primer extension can still be of value in diagnosing some of the carriers (normal phenotype but one chromosome having haplotype 2) in the same family and all of the carriers in either the maternal or the paternal side of the family. Although the remainder of the CF mutations cannot presently be detected using single nucleotide primer extension because the causative mutations are unknown, upon identification of these mutations single nucleotide primer extension will be useful on a more general basis for the CF gene screening.

Similarly, hemophilia B is an X-linked bleeding disorder caused by the absence of factor IX coagulant activity. Among others, hemophilia B point mutations in the protease domain (exon VIII) of factor IX have recently been described which lead to impaired macromolecular catalysis by the mutated enzymes (Bajaj et al., *J. Biol. Chem.* 265:2956–2961, 1990; Spitzer et al., *Biochem. J.* 265:219–245, 1990). Knowing the causative base change in each of these families, the single nucleotide primer extension method of the present invention serves as a powerful screening tool to determine whether an individual bears this particular mutation. If so, prenatal diagnosis and determination of the carrier status of females members in such pedigrees could be accurately carried out.

In addition to detection of point mutations in hemophilia B and a deletion in the CF gene, the single nucleotide primer extension technique should also be applicable to the detection of other genetic diseases of known sequence variations, particularly the sickle cell mutation (A→T) and the α1-antitrypsin gene Z mutation (G→A). Recently, PCR in combination with ASO hybridization has been employed for determination of the HLA-DR, DQ, and DP alleles Angelini et al., *Human Immunol.* 23:77, 1988; Scharf et al., *Human Immunol.* 23:143, 1988. Similarly, the human platelet alloantigens Pl^{A1} and Pl^{A2} have been shown to differ only by one nucleotide (C→T) Newman et al., *J. Clin. Invest.* 83:1778–1781, 1989. Therefore, it should be possible to use single nucleotide primer extension for the direct analysis of the HLA types as well as the human platelet alloantigen (C→T) polymorphism. Moreover, single nucleotide primer extension may also find application in the early detection of Codon 12 and Codon 61 mutations in ras oncogenes which are estimated to cause as much as 30% of human tumors Kumar et al., *Science* 248:1101–1104, 1990.

Single nucleotide primer extension can also be useful in many research settings. For example, in an autosomal genetic disorder, if an investigator finds a mutation (or sequence variation) in a PCR amplified segment of the DNA (obtained from the patient) cloned into PUC 18 or M13 vector, he (or she) can readily check, using single nucleotide primer extension, whether both chromosomes carry the same mutation and, thus, distinguish between the homozygous and the compound heterozygous mutations. The mutations can also be verified in the PCR amplified fragments relatively easily by the use of the method of the present invention, thus eliminating the need for sequencing the fragments in their entirety.

The following examples illustrate the invention.

EXAMPLE 1

The method of the present invention was used to identify members from two separate families as being afflicted with or carriers of hemophilia B.

Experimental Procedures

Materials. Taq polymerase was obtained from Cetus Corporation. [α-^{32}P]-labeled nucleotides (10 μCi/μL, 3000 Ci/mmol) were obtained from DuPont-New England Nuclear. Genomic DNA was isolated from the blood leukocytes by standard techniques. Use of volunteer blood donor was approved by the human subjects committee of St. Louis University and of the University of Southern California.

PCR Amplification and Isolation of the Amplified DNA. The set of primers employed for PCR amplification of exon VIII corresponded to the nucleotides 30760–30780 and 31360–31379 of factor IX gene. Target sequences in the genomic DNA were amplified by standard PCR technique. Following amplification, the DNA was electrophoresed on 1% agarose gel in Tris-acetate-EDTA buffer. The segment of the gel containing the amplified region was cut out and mixed with an equal volume of phenol, pH 8.0 and frozen at −70° C. for a minimum period of 10 minutes. The sample was thawed at 37° C. for 10 minutes and briefly centrifuged in an eppendorf tube. The DNA in the upper aqueous layer was ethanol-precipitated and stored till used.

Single Nucleotide Primer Extension ("SNuPE"). Each SNuPE reaction was carried out in a 50-μL volume containing ~100 ng of the amplified DNA fragment, 1 μM of the SNuPE primer, 2 units of Taq polymerase and 1 μL of the [α-^{32}P]-labeled appropriate nucleotide (10 μCi/μL, 3000 Ci/mmol). The buffer used was 10 mM Tris-HCl, pH 8.3 containing 50 mM KCl, 5 mM $MgCl_2$ and 0.001% (w/v) gelatin. The samples were subjected to one cycle consisting of 2-minutes denaturation period at 94° C., 2-minutes annealing period at 60° C., and 2-minutes primer extension period at 72° C. The sequence of the SNuPE primer for each family is given in FIG. 2. Details of gel electrophoresis and autoradiography for detection of the extended primer are also given in legends to FIG. 2.

Figure Legends

Family 1 has Ile397Thr (nucleotide 31,311 T→C) mutation Spitzer et al., *Biochem. J.* 265:219–225, 1990 and family 2 has Gly363Val (nucleotide 31,209 G→T) mutation Bajaj et al., *J. Biol. Chem.* 265:2956–2961. Affected members in both families have hemophilia B. Initially, exon VIII from each subject was amplified from the genomic DNA by the standard PCR using the two primers corresponding to nucleotides 30760–30780 and 31360–31379 of factor IX gene. The isolated amplified fragments were used for SNuPE reactions (for details see "Experimental Procedures"). The extension of the SNuPE primer for each reaction was then analyzed by gel electrophoresis and autoradiography. A 5 μl-aliquot of each sample was mixed with an equal volume of gel loading buffer (80% formamide, 50 mM Tris-borate pH 8.3, 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue), heat denatured at 90° C. for 1 min, and loaded onto a 6% polyacrylamide gel containing 8M urea. Gels (17 cm) were run at 300 V for 2 hours to obtain adequate resolution of the extended primer from the free nucleotide. Autoradiographs of gels were made by overlaying Kodak X-AR5 film and exposing for 20 to 30 minutes at room temperature. The sequence of SNuPE primer for each family is indicated by the top two arrows. The causative base change in each family is also depicted. For each individual two SNuPE reactions were carried out; the single radiolabeled nucleotide included in the SNuPE reaction was either T (normal) or C (mutant) for family 1 and was either G (normal) or T (mutant) for family 2. When only the wild-type base was incorporated into the SNuPE primer, the subject was considered normal; when only the mutant base was incorporated into the primer, the subject was considered

a hemophiliac and when both bases (one in each reaction) were incorporated into the primer, the subject was considered a carrier of the disease. The autoradiographs depicted in this figure show results of limited subjects only. Symbols: square, male; circle, female; filled symbol, hemophiliac; half-filled symbol, carrier of hemophilia; slashed symbol, deceased.

RESULTS

In both families, prior to defining the mutation sites within exon VIII of the factor IX gene, attempts to establish the carrier status of the female members in these pedigrees employing IX:C/IX:Ag ratios and linked RFLPs analyses were not successful. After identification of the causative base change (T→C at position 31,311) in family 1, SNuPE was used to determine the carrier status of the females in this family; III_2 was identified a noncarrier and III_3 and III_4 were identified as carriers of the disease (FIG. 2). The obligatory carrier status of II_2 and II_3 was also confirmed. Since it has been estimated that approximately 1 out of 5 hemophilia B patients (with factor IX sequence changes) carries the T→C mutation at position 31,311, SNuPE could serve as a powerful screening tool to determine whether or not the index patient bears this particular mutation. If so, prenatal diagnosis and determination of the carrier status of females members in such pedigrees could be accurately carrier out using SNuPE.

In family 2 (FIG. 2), there is no prior history of bleeding. Subject I_2 in this family had unilateral ovariectomy before any of her children were conceived; thus, all of her offspring are the product of ova from one ovary. Again, attempts to establish the carrier status in this pedigree using IX:C/IX:Ag ratios and linked RFLP's were not successful. Once the mutation was identified, SNuPE was applied to determine the carrier status of females in this family. Results are given in FIG. 2. None of the females, including the mother of the patient, had the mutated allele. Thus, the mutation causing hemophilia B in Subject II_5 is a de novo mutation and in all probability occured in a single ovum that resulted in the II5 zygote.

EXAMPLE 2

Using the procedures outlined in Example 1, individuals were screened for the Phe^{508} deletion mutation present in 60% of cystic fibrosis chromosomes. The exon region containing the 1611–1708 bp segment of the CF gene containing the most common Phe^{508} deletion mutation was amplified using the two PCR primers (C16B and C16D) used earlier by other groups Lemna et al., *N. Engl. J. Med.* 322:291–196. Following amplification, the DNA was electrophoresed on 6% polyacrylamide gel in Tris-borate-EDTA buffer. The segment of the gel containing the amplified fragment (~100 bp) was cut out and the DNA was extracted by electroelution (40 V/12 h) using the dialysis membrane tubing (Spectra/por 2) in Tris-borate-EDTA buffer. Execution of the SNuPE reaction was carried out as detailed in Example 1. The ^{32}P-labeled single nucleotide included in the SNuPE reaction was either C (normal) or T (mutant). In a fully informative family, when only the C base is incorporated into the primer, the subject is considered normal; when only the T base is incorporated into the primer, the subject is considered a CF patient; and when both C and T were incorporated into the primer, the subject is considered a carrier of the CF gene.

Using the single nucleotide primer extension method, 34 chromosomes of 17 unrelated individuals were analyzed and it was found that none of them had the three-base pair deletion corresponding to the amino acid Phe^{508} (Table 1). 74 CF chromosomes (37 CF unrelated patients) were also analyzed and it was found that 46 of them had the putative three base-pair deletion. This finding further establishes that indeed ~60% of the CF chromosomes carry the three-base pair deletion.

Using single nucleotide primer extension several new families homozygous for this mutation were also identified. Results of one family are shown in FIG. 3. (Symbols: square, male; circle, female; filled symbol, CF patient; half-filled symbol, carrier of CF gene). Each parent has one CF and one normal chromosome and the affected child (II_1) has two CF chromosomes, one derived from each parent. Two children (II_2 and II_4) have inherited the normal chromosome from each parent. Another child (II_3) inherited one normal and one CF chromosome and is a carrier of the CF disease.

TABLE 1

Prevalence of Phe^{508} Deletion Mutation in Normal and CF Chromosomes as Detected by SNuPE

	Total	Phe^{508} Deletion	% Phe^{508} Deletion
Normal Chromosomes	34	0	0
CF Chromosomes (unrelated CF patients)	74	46	62
1/1 genotype CF patients[a]	10	0	—
1/2 genotype CF patients[a]	36	18	—
2/2 genotype CF patients[a]	28	28	—

[a]Haplotype 1 is defined as that in which CF mutation is at a region other than the Phe^{508} deletion and haplotype 2 is defined as that in which the CF mutation is the Phe^{508} deletion.

In view of the above, it will be seen that the several objects of the invention are achieved.

As various changes could be made in the above methods without departing from the scope of the invention, it is intended that the above description shall be interpreted as illustrative and not in a limiting sense.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

EXHIBIT A

-continued

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCAATGAAAG GCAAATATGG AATA 24

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCAATGAAAG GCAAATATGG AACA 24

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGGAGGTAG AGATTCATGT CAAGGA 26

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGGAGGTAG AGATTCATGT CAAGTA 26

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGGCACCAT TAAAGAAAAT ATCATCTTT 29

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTGGCACCAT TAAAGAAAAT ATCATT

26

What is claimed:

1. A method for screening a DNA fragment of a gene for variation of nucleotide sequence at a predetermined position relative to the nucleotide sequence of the corresponding wild-type gene, the sequence of the wild-type gene being known at the predetermined position, the method comprising the steps of:

(a) providing the DNA fragment as a single stranded molecule,

(b) mixing the single stranded molecule with an inducing agent, an unlabeled primer having a nucleotide sequence complementary to a region flanking the predetermined position, and a labeled nucleotide to form a mixture, the mixture having an essential absence of nucleotides constituted of bases other than the base of which the labeled nucleotide is constituted,

(c) subjecting the mixture to conditions conducive for the annealing of the primer to the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide,

(d) after step, analyzing the mixture for the presence or absence of primer extension product incorporating the labeled nucleotide, the analysis being carried out under conditions such that any primer which did not form a primer extension product incorporating the labeled nucleotide in step (c) is present throughout the analysis, and

(e) determining whether the sequence of the DNA fragment at the predetermined position is the same as or a variant of that of the wild-type gene based upon the presence or absence of the labeled nucleotide in the primer.

2. The method of claim 1 wherein the DNA fragment is being screened for a mutation relative to the wild-type gene at the predetermined position and wherein prior to step the sample DNA fragment is separated into at least two aliquots and then in step (b), labeled nucleotide corresponding to the sequence of the wild-type gene at the predetermined position is mixed with one of the aliquots to form a first mixture and labeled nucleotide corresponding to a mutation of the wild-type gene at the predetermined position is mixed with another one of the aliquots to form a second mixture.

3. The method of claim 1 wherein the sample DNA fragment is being screened for a mutation responsible for cystic fibrosis.

4. The method of claim 1 wherein the sample DNA is being screened for a mutation responsible for hemophilia B.

5. The method of claim 1 wherein prior to step (b) the sample DNA fragment is separated into at least two aliquots and then in step (b), labeled nucleotide corresponding to the sequence of a first variant of the wild-type gene at the predetermined position is mixed with one of the aliquots to form a first mixture and labeled nucleotide corresponding to a second variant of the wild-type gene at the predetermined position is mixed with another one of the aliquots to form a second mixture.

6. A method for screening an organism for genetic diseases or gene sequence variation resulting from a nucleotide substitution, translocation, insertion or deletion at a predetermined position, the sequence in the corresponding wild-type gene and normal variations thereof at that position being known, the method comprising

(a) providing a sample DNA fragment from the genome of that organism which contains the predetermined position,

(b) providing the sample DNA fragment as a single stranded molecule,

(c) mixing the single stranded molecule with an inducing agent, a primer having a nucleotide sequence complementary to a region flanking the predetermined position, and a labeled nucleotide to form a mixture, the mixture having an essential absence of nucleotides constituted of bases other than the base of which the labeled nucleotide is constituted,

(d) subjecting the mixture to conditions conducive for the formation of double stranded hybrids comprising the primer and the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide,

(e) denaturing the double stranded hybrids formed in the mixture in step (d),

(f) analyzing the denatured double stranded hybrids for the presence of primer extension product incorporating labeled nucleotide, and

(g) determining whether the sequence of the DNA fragment at the predetermined position is the same as or a variant of the wild-type gene.

7. The method of claim 6 wherein the sample DNA fragment is being screened for a mutation relative to the wild-type gene at the predetermined position and wherein prior to step (c) the sample DNA fragment is separated into at least two aliquots and then in step (c), labeled nucleotide corresponding to the sequence of the wild-type gene at the predetermined position is mixed with one of said at least two aliquots to form a first mixture and labeled nucleotide corresponding to a mutation of the wild-type gene at the predetermined position is mixed with another one of said at least two aliquots to form a second mixture.

8. The method of claim 6 wherein the organism is being screened for cystic fibrosis.

9. The method of claim 6 wherein the organism is being screened for hemophilia B.

10. The method of claim 6 wherein prior to step (c) the sample DNA fragment is separated into at least two aliquots and then in step (c), labeled nucleotide corresponding to the sequence of a first variant of the wild-type gene at the predetermined position is mixed with one of said at least two aliquots to form a first mixture and labeled nucleotide corresponding to a second variant of the wild-type gene at the predetermined position is mixed with another one of said at least two aliquots to form a second mixture.

11. A method for comparing the nucleotide sequence of a DNA fragment of a gene at a predetermined position relative to the nucleotide sequence of the corresponding wild-type gene, the sequence of the wild-type gene being known, the method consisting essentially of the steps of:

(a) providing the DNA fragment as a single stranded molecule in solution,

(b) mixing the single stranded molecule with an inducing agent, a primer having a nucleotide sequence complementary to a region flanking the predetermined position, and a labeled nucleotide to form a mixture, the mixture having all essential absence of nucleotides constituted of bases other than the base of which the labeled nucleotide is constituted,

(c) subjecting the mixture to conditions conducive for the annealing of the primer to the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide,

(d) after step (c), subjecting the mixture to gel electrophoresis under denaturing conditions, and

(e) determining whether the sequence of the DNA fragment at the predetermined position is the same as or a variant of the wild-type gene.

12. The method of claim 11 wherein the DNA fragment is being screened for a mutation relative to the wild-type gene at the predetermined position and wherein prior to step (b) the sample DNA fragment is separated into at least two aliquots and then in step (b), labeled nucleotide corresponding to the sequence of the wild-type gene at the predetermined position is mixed with one of the aliquots to form a first mixture and labeled nucleotide corresponding to a mutation of the wild-type gene at the predetermined position is mixed with another one of the aliquots to form a second mixture.

13. The method of claim 11 wherein the sample DNA fragment is being screened for a mutation responsible for cystic fibrosis.

14. The method of claim 11 wherein the sample DNA is being screened for a mutation responsible for hemophilia B.

15. The method of claim 11 wherein prior to step (b) the sample DNA fragment is separated into at least two aliquots to and then in step (b), labeled nucleotide corresponding to the sequence of a first variant of the wild-type gene at the predetermined position is mixed with one of the aliquots to form a first mixture and labeled nucleotide corresponding to a second variant of the wild-type gene at the predetermined position is mixed with another one of the aliquots to form a second mixture.

16. A method for screening a DNA fragment of a gene for variation of nucleotide sequence at a predetermined position relative to the nucleotide sequence of the corresponding wild-type gene, the sequence of the wild-type gene being known at the predetermined position, the method comprising:

(a) providing the DNA fragment as a single stranded molecule;

(b) mixing the single stranded molecule with an inducing agent, an unlabeled primer having a nucleotide sequence complementary to a region flanking the predetermined position, and a labeled nucleotide to form a mixture, the mixture having an essential absence of nucleotides that would eliminate the specificity of a primer extension reaction of step (c);

(c) subjecting the mixture to conditions conducive for annealing of the primer to the single stranded molecule and the formation of a primer extension product incorporating the labeled nucleotide at a position complementary to the predetermined position;

(d) after step (c), analyzing the mixture for the presence or absence of primer extension product incorporating the labeled nucleotide, the analysis being carried out under conditions such that any primer which did not form a primer extension product incorporating the labeled nucleotide in step (c) is present throughout the analysis; and

(e) determining whether the sequence of the DNA fragment at the predetermined position is the same as or a variant of that of the wild-type gene based upon the presence or absence of the labeled nucleotide in the primer.

17. The method of claim 16 wherein the mixture formed in step (b) has an essential absence of deoxynucleotides constituted of bases other than the base of which the labeled nucleotide is constituted.

18. The method of claim 16 wherein the labeled nucleotide is a deoxynucleotide.

19. The method of claim 16 wherein the labeled nucleotide is a deoxynucleotide and the mixture formed in step (b) has an essential absence of deoxynucleotides constituted of bases other than the base of which the labeled deoxynucleotide is constituted.

20. The method of claim 16 wherein the primer has a nucleotide sequence complementary to a region immediately flanking the predetermined position.

* * * * *

EXHIBIT B

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ORCHID

May 10, 2000

VIA FACSIMILE

E.J. Brandt, PhD
Technology Development Consultant
For St. Louis University
635 Westborough Place
St. Louis, MO 63119

Dear EJ:

As promised, I write in response to your demand that Orchid BioSciences, Inc. license the Bajaj patent assigned to St. Louis University. For the reasons explained below, at this time I believe that Orchid does not infringe any valid claims of the Bajaj patent. Before making a final determination in this regard, however, I intend to have an outside law firm complete an independent analysis.

I summarize below the principal reasons why I believe that Orchid does not need to license the Bajaj patent. There are significant other issues, but these are not worth discussing until and unless we resolve the matters set forth below. I solicit your comments. It would be helpful if you would provide them to me in the very near future, so that our outside law firm can be made aware of them early in their analysis.

As you know, Orchid practices a method for the detection of single nucleotide polymorphisms that involves more than one type of nucleotide in the reaction mixture. In contrast, the Bajaj patent teaches that if more than one type of nucleotide is used the method will not work. See, for example, column 3 of the Bajaj patent at lines 29 through 34 and at lines 50 through 55.

Given that Bajaj did not disclose -- indeed denied -- possibility that multiple types of nucleotides could be used in his assay at the time he filed his application for a patent, it necessarily follows that he cannot obtain a patent claim covering a method for using more than one type of nucleotide. Therefore, to the extent that the University seeks to interpret Claim 16 of the Bajaj patent to cover the use of multiple terminating nucleotides, that claim is necessarily invalid. All of the other claims of the Bajaj patent expressly require the use of only a single labeled

EXHIBIT B

nucleotide, and therefore there is no possibility that Orchid's activities infringe any of them.

The Administrative Patent Judge in the *Bajaj - Goelet - Soderlund* Interference before the United States Patent & Trademark Office (Interference No. 103,562) has ruled that Bajaj is not entitled to claims covering the use of multiple nucleotides. Bajaj's Preliminary Motion No. 3 sought to redefine the interference count by adding a new independent claim. In that proposed claim, Bajaj sought to distinguish between the use of natural deoxynucleotides and dideoxy terminating nucleotides. The newly-proposed claim would have restricted the "single nucleotide" requirement of the former claims to a single type of "deoxynucleotides." Bajaj asserted that the proposed claim encompassed the use of more than one dideoxynucleotide nucleotide as invented by Goelet *et* al.

In opposing the addition of the proposed Claim 16, both Goelet and Soderlund pointed out that Bajaj had expressly stated in his patent application that it was essential to use one, and only one, labeled nucleotide in his method. Bajaj agreed that the term "nucleotide" as used in the Bajaj patent encompassed both deoxynucleotides and terminating nucleotides. Given Bajaj's insistence in his patent application that the use of multiple nucleotides would not work, Goelet and Soderlund argued that Bajaj was not in possession of any invention involving the use of multiple terminating nucleotides. The Administrative Patent Judge agreed, and denied Bajaj's motion for lack of written description for the reasons set forth in the Goelet and Soderlund Oppositions to the motion of Bajaj to add the proposed Claim 16.

I have reviewed the papers submitted in support and in opposition to Bajaj's Preliminary Motion No 3 and believe that the arguments submitted in opposition, and subsequently adopted by the Patent Law Judge and grounds for denial, are compelling. I have also examined Bajaj's motion for reconsideration, and find nothing in that submission that undermines the obvious conclusion that Bajaj did not believe multiple nucleotides could be used and therefore cannot obtain patent claims covering such use. The fact that the Administrative Patent Judge vacated his decision in order to allow the Patent Examiner to consider the proprietary of adding Bajaj's proposed claim 16 in no way undermines the substantive correctness of his decision.

Further, in his order vacating the denial of Bajaj's Preliminary Motion No. 3, the Administrative Patent Judge expressly required Bajaj to "bring to the examiner's attention the relevant papers in the interference." We have reviewed the file history, and found no indication that Bajaj complied with this requirement.

After the close of this Interference, the Bajaj application was transferred back to *ex parte* prosecution before the Patent Examiner. At this point, Bajaj again submitted a proposed new Claim 16 using language that is somewhat different from the proposed claim rejected by the Administrative Patent Judge. This change appears to have been designed to extend patent coverage to the use of more than one type of nucleotide. However, Bajaj did not inform the Patent Examiner that it was this very same additional subject matter that the Administrative Patent Judge had determined Bajaj was not entitled to under the law. This omission was a significant oversight.

In support for the revised proposed Claim 16, Bajaj pointed to a portion of his patent which mentions the use of multiple nucleotides but taught away from their use (without distinction between dideoxynucleotide and deoxynucleotide). Significantly, however, Bajaj failed to cite other portions of his patent which expressly state that one, and only one, type of labeled nucleotide may be used for the method to work. See, for example, the Bajaj patent at column 3, lines 28 through 34 and lines 61 through 67. This omission, and the failure to refer the Examiner to the decision of the Administrative Patent Judge, was materially misleading.

In sum, Bajaj cannot claim what he did not invent. Further, unless you can point to evidence that Bajaj complied with the order of the Administrative Patent Judge requiring disclosure of the interference papers relevant to a claim encompassing the use of multiple nucleotides, it would appear that there was a failure to disclose material facts. Given Bajaj's knowledge of his obligation to disclose, there would appear to be good reason to infer that, in addition to all of the other flaws noted above, the entire Bajaj patent is very probably unenforceable for fraud on the patent office.

While I have tried to present my reasoning as directly and succinctly as possible, please understand that it is not my intent to give offense by the tone of this letter. I am seriously interested in your viewpoints on this matter and will keep an open mind. Nevertheless, I am sure you understand that in light of the above issues it would be premature to begin negotiating the terms of a license until and unless these concerns regarding the proper interpretation of Bajaj and the validity of claim 16 are resolved satisfactorily.

I look forward to hearing from you to arrange a meeting in St. Louis.

Very truly yours,

ORCHID BIOSCIENCES, INC.

Kevin Nash
Sr. Director, Licensing
Intellectual Property Counsel

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July 26, 2000

VIA FACSIMILE AND
CERTIFIED MAIL
Mr. Kevin Nash
Sr. Director, Licensing
Intellectual Property Counsel
Orchid Biocomputer, Inc.
303 College Road East
Princeton, New Jersey 08540

Re: St. Louis University v. Orchid BioSciences
Our File SLU 4518

Dear Mr. Nash:

St. Louis University has asked me to write to you regarding licensing of St. Louis University's U.S. Patent No. 5,846,710 issued to Dr. Paul Bajaj (the "Bajaj patent") by Orchid BioSciences.

As you know, St. Louis University has endeavored to negotiate a license with Orchid as to its products and services which practice the Bajaj patent for some time now. St. Louis University has previously proposed a detailed license to Orchid which, in my opinion, is highly favorable to Orchid. However, Orchid has refused to discuss a license under these or similar terms.

After due consideration and review of the positions taken by Orchid, St. Louis University remains firmly convinced that, as explained in Dr. Brandt's letter to you of last August 12, 1999, the Bajaj patent is properly interpreted to cover methods employing single nucleotide extension reactions in which the reaction is carried out using a labeled nucleotide and which reaction has an essential absence of nucleotides that would eliminate the specificity of the primer extension reaction. This interpretation is consistent with the specification, the file history and the ordinary

EXHIBIT C

meaning of the claim terms. Further Orchid has sold, and continues to offer to sell, products and services which are covered by the Bajaj patent, without authorization or the benefit of a license.

St. Louis University continues to believe that a business resolution of this matter is in both parties' interest. However, it has other remedies available to it, and will not hesitate to exercise them.

Accordingly, this letter serves as notice to Orchid that unless this matter is resolved within the next ten days, I have been authorized to pursue such alternative remedies.

Yours truly,

G. Harley Blosser

GHB/bk