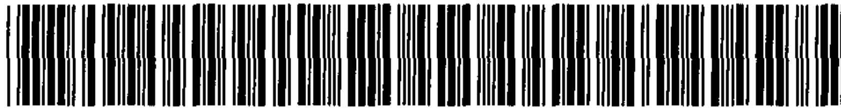

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3:01-CV-02317 SEQUENOM INC V. MYRIAD GENETICS INC

*1*

*CMP.*

BROBECK, PHLEGER & HARRISON LLP
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Attorney for Plaintiff SEQUENOM, INC.

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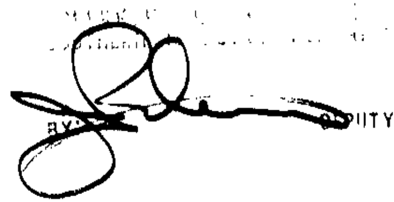

ORIGINAL

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

| | |
|---|---|
| SEQUENOM, INC, a Delaware Corporation,<br><br>Plaintiff,<br><br>v.<br><br>MYRIAD GENETICS, INC., a Delaware Corporation, MYRIAD GENETICS LABORATORIES, a Delaware Corporation<br><br>Defendants. | Case No. '01 CV 2317 BTM (JAH)<br><br>COMPLAINT FOR DECLARATORY JUDGMENT OF PATENT NON-INFRINGEMENT AND INVALIDITY<br><br>DEMAND FOR JURY TRIAL |

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Plaintiff, by and through its designated attorneys, complains and alleges as follows:

## PARTIES

1. Plaintiff SEQUENOM, INC. ("SEQUENOM") is a corporation organized under the laws of the State of Delaware and has its principal place of business at 3595 Johns Hopkins Court, San Diego, California, 92121.

2. Upon information and belief, Defendant MYRIAD GENETICS, INC. is a corporation organized and existing under the laws of Delaware and has a place of business at 320 Wakara Way, Salt Lake City, Utah 84108. Upon information and belief, Defendant MYRIAD GENETICS, INC. has a wholly owned subsidiary Myriad Genetics Laboratories. Defendant MYRIAD GENETICS INC., and its wholly owned subsidiary Myriad Genetics Laboratories will hereinafter be referred to collectively as "MYRIAD".

## JURISDICTION AND VENUE

3. The Court has jurisdiction over all claims for declaratory judgment of patent non-infringement and invalidity under 28 U.S.C. §§ 1331 and 1338(a), the Declaratory Judgment Act, 28 U.S.C. §§ 2201, 2202, and under the laws of the United States concerning patents, 35 U.S.C. §§ 101 et seq.

4. Venue is proper in this district under 28 U.S.C. §§ 1391(b) -- the venue statute applicable to declaratory judgment actions -- for several alternative reasons. Venue is proper in this district under § 1391(b)(2) because a substantial part of the events or omissions giving rise to the claim – the alleged infringing activity – occurred or will occur in this judicial district. Alternatively, venue is proper in this district under § 1391(b)(1) because all defendants "reside" in the State of California and at least one defendant "resides" in this judicial district under the definition of "resides" found in § 1391(c) because its contacts with this judicial district are sufficient to render it amenable to personal jurisdiction in this district.

5. Defendant MYRIAD has engaged in substantial contact with SEQUENOM, which is located in San Diego, CA (which is in the Southern District of California) including at least sending letters to SEQUENOM on August 20, 1999, September 16, 1999, May 25, 2000 and December 12, 2001 relating to the use of mass spectrometry to detect DNA polymorphisms, and

1 more specifically, the alleged infringement of and/or licensing of rights to United States Letters
2 Patent No. 5,869,242 (hereinafter "the '242 Patent"), which is the patent in suit, and also meeting
3 in San Diego with representatives of SEQUENOM regarding the same in San Diego on at least the
4 following dates: on or about April 6, 2001 and January 14, 2000.

5 6. More specifically, on or about January 14, of 2000, there were meetings in San Diego
6 California between SEQUENOM and MYRIAD personnel, including at least Arnold Oliphant,
7 Vice President of Functional Genomics for Myriad Genetics Inc. and Gregory C. Critchfield,
8 President of Myriad Genetic Laboratories, Inc., to discuss details of SEQUENOM's business and
9 their respective patents and products, including the '242 patent.

10 7. Myriad Genetics asked and had SEQUENOM perform a feasibility study involving
11 DNA samples provided by Myriad Genetics that were tested for Myriad Genetics using
12 SEQUENOM's technology. This testing determined the presence of genetic polymorphous and
13 generated data that was given to Myriad. Myriad and SEQUENOM had several meetings to discuss
14 the data generated by this feasibility study including phone calls and at least one face-to-face
15 meeting at SEQUENOM's facility in San Diego. Specifically, a meeting was held on or about
16 April 6, 2001 at SEQUENOM's facilities in San Diego in which Myriad's Program Director of
17 Technology Development, Satish Bhatnagar, and other Myriad employees came to San Diego to
18 discuss the feasibility study which was later performed by SEQUENOM. SEQUENOM invoiced
19 MYRIAD for the feasibility study.

20 8. On or about July 18 of 2001, Satish Bhatnagar and Chris Wight, General Counsel for
21 Myriad Genetics discussed the intellectual property of SEQUENOM and SEQUENOM's
22 productswith SEQUENOM employees and specifically discussed various patents and patent
23 applications that involved the analysis of DNA samples with mass spectrometry.

24 9. Additional contacts render MYRIAD amenable to personal jurisdiction in this district.
25 Upon information and belief, Defendant MYRIAD has engaged in a collaboration with Torrey
26 Mesa Research Institute ("TMRI"), a subsidiary of Syngenta to sequence the entire rice genome.
27 TMRI is located in the district at 3115 Merryfield Row, San Diego, CA 92121. As a direct result of
28 its substantial business collaboration with San Diego based-TMRI, Defendant MYRIAD has

received or will receive at least $3 million dollars.

10. Still further contacts render MYRIAD amenable to personal jurisdiction in this district. Upon information and belief, individuals of Defendant MYRIAD attended the American Society for Human Genetics Meeting in the district at held at the San Diego Convention Center on October 12 through 16 of 2001.

## FACTUAL BACKGROUND

### United States Letters Patent No. 5,869,242

11. On February 9, 1999, United States Letters Patent No. 5,869,242 (hereinafter "the '242 Patent") entitled "MASS SPECTROMETRY TO ASSESS DNA SEQUENCE POLYMORPHISMS" issued to assignee defendant MYRIAD. The named inventor on the '242 Patent was Alexander Kamb. A copy of the '242 Patent is attached as Exhibit 1.

12. On information and belief, defendant MYRIAD GENETICS, INC. is still the assignee of record of the '242 Patent. Thus, on information and belief, defendant MYRIAD GENETICS, INC. has had and still maintains substantial rights in the '242 Patent.

### Facts Leading Up to the Filing of Suit

13. Plaintiff SEQUENOM is a discovery genetics company that is a leader in efforts to determine the medical impact of genes and genetic variations. Plaintiff SEQUENOM is identifying potential disease-related genes that affect the most significant portions of the overall population. This approach is possible due to the pinpoint accuracy and unique specificity of the Company's MassARRAY™ system, which is largely accepted as the most powerful high-throughput screening technology of its kind in the industry.

14. Plaintiff SEQUENOM is a leader in the development and application of products, technologies and services to analyze genetic variations and to determine their association with disease.

15. Plaintiff SEQUENOM's products include the MassARRAY™ system, disposable MassARRAY™ kits consisting of SpectroCHIPs™ chips and reagents.

16. Plaintiff SEQUENOM's services include assay design for MassARRAY™ customers, collaborative and in-house validation projects using its MassARRAY™ system, a SNP

assay portfolio and disease association studies using its proprietary DNA banks.

17. Defendant MYRIAD is the owner of United States Letters Patent No. 5,869,242 (hereinafter "the '242 Patent") which defendant MYRIAD alleges covers methods of detecting polymorphisms, including mutations, in DNA using mass spectrometry.

18. In August of 1999, MYRIAD expressed to SEQUENOM its belief that SEQUENOM was engaging in activities that MYRIAD believed might constitute infringement of the '242 Patent. Plaintiff SEQUENOM and Defendant MYRIAD engaged in various discussions to resolve this matter, which concluded on December 12, 2001, when attorneys for Defendant MYRIAD wrote a letter to informing plaintiff SEQUENOM that the above-referenced discussions failed to achieve any resolution. The letter also informed SEQUENOM that MYRIAD had decided that MYRIAD was not interested in an acquisition of SEQUENOM products or a license under its patents, and further informed SEQUENOM that MYRIAD had retained legal counsel to ensure that SEQUENOM ceased all activities that MYRIAD believed were infringing, activities characterized by MYRIAD as a "serious invasion of its rights." Finally, the letter requested that SEQUENOM confirm in writing by December 19, 2001 that SEQUENOM had ceased its allegedly infringing activities and further informed SEQUENOM that the attorneys for MYRIAD were "prepared and authorized to initiate litigation" if such confirmation were not received.

19. Based on the totality of the circumstances outlined above, plaintiff SEQUENOM is reasonably apprehensive that defendants will file suit against SEQUENOM alleging infringement of the '242 Patent.

## CLAIM

**(Declaration of Non-Infringement and Invalidity of U.S. Patent No. 5,869,242)**

20. Plaintiff hereby incorporates by this reference paragraphs 1 through 19 inclusive.

21. As a result of defendants' actions and statements and the totality of circumstances detailed above, an actual controversy now exists between plaintiff SEQUENOM and defendants concerning whether SEQUENOM's actions including sale of its products and providing services infringe any valid claim of the '242 Patent.

22. Therefore, Plaintiff SEQUENOM hereby seeks entry of a declaratory judgment that

1 SEQUENOM's actions including sale of its products and providing services and its internal
2 activities does not infringe any claim of the '242 Patent, and/or such claims are invalid for failing
3 to satisfy one or more of the conditions of patentability set forth in 35 U.S.C. § 101, 102, 103 and
4 112.

5 **PRAYER FOR RELIEF**

6 WHEREFORE, Plaintiff SEQUENOM prays for Judgment for the following:

7 a) The entry of a Declaratory Judgment that Plaintiff SEQUENOM does not infringe
8 any of the claims of the '242 Patent and/or such claims are invalid;

9 b) A finding that this case is an exceptional case and awarding plaintiff its reasonable
10 attorneys fees pursuant to 35 U.S.C. § 285;

11 c) An award to plaintiff of its costs and expenses in bringing and prosecuting this
12 action; and

13 d) An award to plaintiff for such other relief as this Court may deem just and proper.

14 **DEMAND FOR TRIAL BY JURY**

15 Pursuant to the Federal Rules of Civil Procedure, plaintiff SEQUENOM respectfully
16 demands a trial by jury.

17

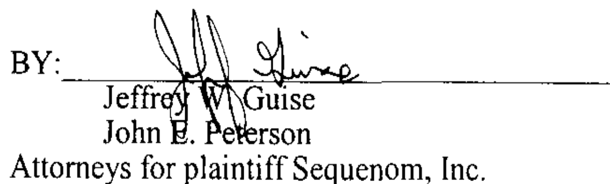
18 Dated: December 17, 2001

Respectfully submitted,

19 BROBECK, PHLEGER & HARRISON, LLP

20

21 BY:



22 Jeffrey W. Guise
John E. Peterson
23 Attorneys for plaintiff Sequenom, Inc.

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**United States Patent** [19] | [11] **Patent Number:** 5,869,242
Kamb | [45] **Date of Patent:** Feb. 9, 1999

[54] **MASS SPECTROMETRY TO ASSESS DNA SEQUENCE POLYMORPHISMS**

[75] Inventor: **Alexander Kamb**, Salt Lake City, Utah

[73] Assignee: **Myriad Genetics, Inc.**, Salt Lake City, Utah

[21] Appl. No.: **529,879**

[22] Filed: **Sep. 18, 1995**

[51] **Int. Cl.**[6] ........................ C12Q 1/68; G01N 24/00; B01D 59/44

[52] **U.S. Cl.** ........................ 435/6; 435/91.53; 436/173

[58] **Field of Search** ........................ 435/6, 91.2, 91.21, 435/91.53, 172.3, 320.1; 436/94, 173, 174; 935/76, 77; 250/282

[56] **References Cited**

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*Primary Examiner*—George C. Elliott
*Assistant Examiner*—Thomas G. Larson
*Attorney, Agent, or Firm*—Rothwell, Figg, Ernst & Kurz, P.C.

[57] **ABSTRACT**

A method for determining the presence of polymorphisms, including mutations, in nucleic acids by using mass spectrometry is presented. The method requires amplification of the nucleic acid region to be analyzed followed by analysis by mass spectrometry and comparison of the obtained spectrum with spectra obtained from wild-type sequences and/or sequences known to contain the polymorphism. Differences between the spectra, either the appearance or disappearance of one or more peaks indicating a change in mass or a change in the height of one or more peaks indicating a change in the amount of nucleic acid of a specific mass, indicate the presence of a polymorphism. Variations of the method involve digestion of the amplified nucleic acid, e.g., by using restriction enzymes, nucleases or chemical methods, prior to analysis by mass spectrometry. The method can be applied to any type of nucleic acid including genomic DNA, CDNA and RNA. The method is especially well suited for performing routine genetic screening on a large scale for mutations known to be associated with a disease. The method is also appropriate for determining the presence of polymorphisms for other purposes, e.g., for genotyping or screening for mutations in a positional cloning project. A preferred approach is to amplify then digest the nucleic acid and then to analyze it via matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) using a neodymium-garnet laser and a 3-hydroxypicolinic acid matrix.

**12 Claims, No Drawings**

# MASS SPECTROMETRY TO ASSESS DNA SEQUENCE POLYMORPHISMS

## BACKGROUND OF THE INVENTION

The determination of the presence of polymorphisms, especially mutations, in DNA has become a very important and useful tool for a variety of purposes. Detecting mutations which are known to cause or to predispose persons to disease is one of the more important uses of determining the possible presence of a mutation. One example is the analysis of the gene named BRCA1 which may result in breast cancer if it is mutated (Miki et al., 1994; Futreal et al., 1994). Several known mutations in the BRCA1 gene have been causally linked with breast cancer. With this knowledge in hand it is now possible to screen women for these known mutations to determine whether they are predisposed to develop breast cancer. Some other uses for determining polymorphisms or mutations are for genotyping and for mutational analysis for positional cloning experiments.

A few different methods are commonly used to analyze DNA for polymorphisms or mutations. The most definitive method is to sequence the DNA to determine the actual base sequence (Maxam and Gilbert, 1977; Sanger et al., 1977). Although such a method is the most definitive it is also the most expensive and time consuming method. Restriction mapping analysis has some limited use in analyzing DNA for polymorphisms. If one is looking for a known polymorphism at a site which will change the recognition site for a restriction enzyme it is possible simply to digest DNA with this restriction enzyme and analyze the fragments on a gel or with a Southern blot to determine the presence or absence of the polymorphism. This type of analysis is also useful for determining the presence or absence of gross insertions or deletions. Hybridization with allele specific oligonucleotides is yet another method for determining the presence of known polymorphisms. These latter methods require the use of hybridization techniques which are time consuming and costly.

In recent years some breakthroughs have been made which allow the use of mass spectrometry to analyze macromolecules (Hillenkamp et al., 1991; Schneider and Chait, 1995; Wang and Biemann, 1994; Nordhoffet al., 1993; Siuzdak, 1994; Wu et al., 1994; Nelson et al., 1989; Nordhoff et al., 1994; Kirpekar et al., 1994; and Pieles et al., 1993). Many papers have now been published which establish that mass spectrometry can be used to analyze DNA fragments. Some papers have focused simply on the ability to measure the mass of a single fragment of DNA or RNA whereas others have gone so far as to show the utility of mass spectrometry for sequencing short fragments of nucleic acids (Pieles et al., 1993; Kirpekar et al., 1994). In all of the papers published to date the analysis has been limited to polynucleotides on the order of about 50 nucleotides or fewer. The appeal of mass spectrometry is the tremendous speed in obtaining data. Once samples have been prepared the throughput can be as fast as 1–2 seconds per sample. Analysis of the data is then done off-line. This time of 1–2 seconds is a tremendous advantage when compared with the many hours needed for running gels and/or hybridizing samples for analysis if the more classical methods of nucleic acid polymorphism analysis are used.

The present invention applies mass spectrometry to the determination of the presence of polymorphisms within known genes. The method uses mass spectrometry to compare the mass spectrum of a fragment of DNA from a sample to be analyzed with known reference mass spectra of DNA, e.g., spectra for wild-type DNA and DNA with a known polymorphism. Determination of the presence of a polymorphism in the sample being tested is rapid and accurate. The use of mass spectrometry with its very rapid analysis is especially useful for routine screening of large numbers of samples.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the text and respectively grouped in the appended List of References.

## SUMMARY OF THE INVENTION

The invention is directed to determining the presence of polymorphisms in nucleic acids by analyzing the nucleic acid using mass spectrometry. Many different reasons exist for wanting to determine the presence of polymorphisms. One popular reason for doing this analysis is to determine if a person's genome contains a mutation known to be associated with a disease, e.g., cancer or heart disease. Such mutations are known and by analyzing for their presence, persons found to have such mutations can take preemptive action to treat or cure the disease associated with the mutation. A typical analysis involves sequencing the genes of interest. As more gene mutations associated with various diseases are discovered it is certain that more genetic analyses will be performed. The time and cost of present sequencing methods will limit the amount of genetic testing which can be done because DNA sequencing as done presently requires trained technicians and many hours of preparation and analysis. Newer methods which are faster and less costly to perform are necessary to allow for routine screening of mutations known to be associated with disease. As more disease causing mutations are found this need will become even more critical.

The present invention uses mass spectrometry rather than requiring the use of gels and/or nucleic acid hybridization techniques to analyze for polymorphisms. Once a sample has been prepared, data acquisition via mass spectrometry requires only a few seconds. This removes the hours of time required for techniques which require use of gel electrophoresis or nucleic acid hybridization. Time for sample preparation is still required just as for analysis by the established methods, but the analysis of the samples once prepared is tremendously faster using mass spectrometry.

One method of using the present invention is to prepare amplified DNA from a patient's sample in the region of a known mutation. The amplified DNA is then analyzed in a mass spectrometer to determine the mass of the amplified fragment.

The mass spectrum obtained is compared to the mass spectrum of fragments obtained from known samples of either wild-type genes or genes containing the known mutation. These known spectra are referred to as "signature" spectra. A simple comparison of the sample spectrum vs. signature spectra will reveal whether the patient's DNA contains a mutation. Although sequencing of fragments of nucleic acids is possible using mass spectrometry, actual sequencing of the nucleic acid is not required for this mutational analysis. Less preparation and analysis is needed to prepare and analyze a complete, intact fragment as compared to treating a sample for actual sequencing.

A variation of the above technique may also be used to analyze for polymorphisms. In this variation the fragments of nucleic acid are digested via any one of several techniques

to smaller fragments which may range from one base up to approximately 50 bases. The resulting mix of fragments is then analyzed via mass spectrometry. The resulting spectrum contains several peaks and is compared with signature spectra of samples known to be wild-type or to contain a known polymorphism. A comparison of the locations (mass) and heights (relative amounts) of peaks in the sample with the known signature spectra indicate what type of polymorphism, if any, is present.

## DESCRIPTION OF THE INVENTION

The invention is directed to a rapid method for analyzing for the presence of polymorphisms in a nucleic acid sample. The method utilizes mass spectrometry to analyze the nucleic acid. This method is much more rapid than other nucleic acid analysis techniques, e.g., DNA sequencing using polyacrylamide gel electrophoresis or hybridization techniques such as using allele specific oligonucleotides. A rapid technique is very desirable for routine screening of many samples. As more and more genes are identified, purified and sequenced, and determined to be associated with disease states when mutated, the number of requests for mutational analysis will grow. The present method, by greatly increasing the rate of analysis, will help meet the need for the vast amount of genetic testing expected in the near future.

One aspect of the present invention is the realization that it is unnecessary to analyze a complete gene for genetic or hereditary testing. Often it is found that only one or a few specific mutations are the cause of a disease in most, if not all, cases of the disease. For routine genetic screening for the disease it is necessary to analyze only the regions of nucleic acid in the immediate region of the known mutation. Knowledge of the nucleic acid composition in these relatively short regions will enable one to determine if the patient contains a harmful mutation. There is no need to analyze fully the complete gene sequence of a gene associated with a disease. It is another aspect of this invention that it is also now realized that one need not do an actual sequence analysis of even these short regions of nucleic acid to determine the presence of a polymorphism. Rather one can use a method which is exquisitely precise in determining the total composition of the fragments of DNA. Mass spectrometry is one such method which yields very precise results and is applicable to short nucleotide fragments. This invention teaches that a simple comparison of a mass spectrum of a total nucleic acid sample fragment with spectra of known fragments quickly yields data capable of determining the presence of a polymorphism in the sample. Alternatively, one can digest the fragment to yield a spectrum of several peaks, rather than a single peak representing the whole fragment, and use this spectrum to determine whether a polymorphism is present in the sample. This is again simpler and faster than sequencing the sample.

The disclosed methods are useful for determining the presence of a polymorphism in a nucleic acid sample for any purpose, it is not limited to testing for mutations in genes associated with a disease.

## EXAMPLE I

### Amplification of Nucleic Acid to be Analyzed

Relatively pure nucleic acid fragments must be obtained in sufficient amounts to be detectable by mass spectrometry. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is currently sensitive at the femtomole level (Siuzdak, 1994). Typically picomole amounts of nucleic acids are used in MALDI-MS analyses. A wide variety of techniques for preparing large amounts of purified fragments of nucleic acids are known to those of skill in the art. Several amplification techniques are commonly used. Polymerase chain reaction (PCR) is one very powerful technique for amplifying specific fragments of nucleic acids and is especially appropriate for the present invention. PCR requires knowledge of two small portions of about 15 bases or more each of the DNA sequence. Two primers are made, one corresponding to each known region, and these primers are designed such they will each prime synthesis of a different strand of DNA such that synthesis will be in the direction of one primer towards the other primer. The primers, DNA to be amplified, a thermostable DNA polymerase, a mix of the four deoxynucleotides, and a buffer are combined. DNA synthesis will occur. The solution is then denatured by heating, then it is cooled to allow annealing of new primer and another round of DNA synthesis occurs. This process is typically repeated for about 30 cycles resulting in an amplification of several million fold of the region of DNA internal to the two primers (including the region of the two primers). Many variations of PCR are known. One can begin with RNA, reverse transcribe the RNA to synthesize cDNA, and use the cDNA for the amplification template rather than using genomic DNA. Another alternative is to synthesize RNA rather than DNA to be analyzed via mass spectrometry. One method for preparing large quantities of RNA is to clone a fragment of DNA into a vector which has promoters specific for an RNA polymerase, e.g., a T7 or SP6 RNA polymerase promoter. These vectors can be linearized and RNA transcripts can be synthesized yielding a large quantity of homogeneous RNA. This amplified RNA can be used for the mass spectrometry analysis.

In synthesizing these amplified nucleic acids, one can incorporate a variety of nucleotide analogs into the nucleic acid if desired. One useful substitution is to incorporate deoxyuridine into amplified DNA. This is useful for producing small fragments by later digesting the amplified DNA with uracil-N-glycosidase. One other example of a useful substitution is to incorporate 7-deaza-guanosine and 7-deaza-adenosine into the amplified DNA since these compounds are reported to stabilize the nucleic acid during mass spectrometry (Schneider and Chait, 1995).

## EXAMPLE II

### Mass Spectrometry of a Nucleic Acid Sample

Advances in the art of mass spectrometry over about the last eight years have enabled mass spectrometry to be applied to biopolymers including nucleic acids (for an early review see Hillenkamp et al., 1991). One breakthrough was the use of a matrix to embed the sample to be tested. This technique is referred to as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Several different matrices have been developed which yield good results with biopolymers. One of the more useful matrices is 3-hydroxypicolinic acid. Some other matrices which have been used with polynucleotides are a mixture of anthranilic acid and nicotinic acid, succinic acid, 2,4,6-trihydroxy acetophenone, 2,5-dihydroxybenzoic acid, etc (Hillenkamp et al., 1991; Bing and Biemann, 1994). An ion source is needed to desorb the sample. Pulsed lasers are used for this purpose. The type of laser used produces specific wavelengths and these must be appropriate for the matrix which is being used. A preferred combination for polynucleotides

is to use a neodymium-garnet solid state laser in combination with a 3-hydroxypicolinic acid matrix.

## EXAMPLE III

### Amplification and Analysis of a Gene Fragment To Determine the Possible Presence of an Insertion Known to be Associated with a Disease

A patient is to be tested for the possible presence of a mutation in a gene which can cause breast cancer if it is mutated. Much data is presently available for such a gene, BRCA1, and several mutations in this gene associated with breast cancer have been discovered. The cDNA for BRCA1 has been completely sequenced and the locations of several mutations which cause breast cancer have been determined (Miki et al., 1994; Futreal et al., 1994). This cDNA encodes a protein of 1863 amino acids and consists of 24 exons. Codon 1756 which is encoded by exon 20 in the wild-type gene has been found to contain an insertion of a C in some patients with breast cancer. The DNA sequence centered around this codon is as follows:

CAAAGCGAGCAAGAGAATCCCAGGACA-GAAAGATCTTCA (SEQ ID NO: 1).

Insertion of a C yields the following sequence:

CAAAGCGAGCAAGAGAATCCCCAGGACA-GAAAGATCTTCA (SEQ ID NO: 2). Because this region is known, primers may be made complementary to the 5' and 3' ends of this sequence and a polymerase chain reaction performed to amplify the region, yielding an amplified product of 39 or 40 base pairs. This amplified product is purified away from the primers by any suitable method well known to those in the art. The amplified fragment is denatured and the strands are separated. Techniques for purifying a single strand are known to those of skill in the art. One of the more commonly used techniques is to label one of the primers used for amplification with biotin. The biotin labeled strand is then captured by binding to streptavidin. The amplified single-stranded fragment is analyzed via mass spectrometry, e.g., via MALDI-MS using a 3-hydroxypicolinic acid matrix and a neodymium-garnet laser. The molecular weight for each phosphorylated deoxynucleotide is approximately as shown in Table I.

TABLE I

| | |
|---|---|
| pA | 329 |
| pC | 305 |
| pG | 345 |
| pT | 320 |

The insertion of a C in codon 1756 adds a G:C base pair to the amplified double-stranded fragment. This results in the amplified mutated fragment having a mass which is 305 Daltons more in the sense strand and 345 Daltons more in the antisense strand than the wild-type fragment. This difference of either 305 or 345 Daltons (depending on which strand is selected for analysis) is very readily detected from the mass spectrum obtained.

If a mutational insertion or deletion of an A:T base pair were to be observed, the difference in mass for a single strand would be approximately 329 or 320 Daltons. This would be just as easily seen as the insertion or deletion of a G:C base pair.

Insertions or deletions of more than a single base pair would also be easily identified.

## EXAMPLE IV

### Determination of a Transitional or Transversional Mutation

The method used in Example III is not useful for determining the presence of a polymorphism which is simply a result of a base change, i.e., a transition or a transversion. This is because regardless of the change, the wild-type DNA fragment and the mutated fragment will have nearly identical masses. A change of an A:T to a T:A will increase the mass of one strand by approximately 9 Daltons and decrease the mass of the complementary strand by approximately 9 Daltons. If a 40 base fragment were to be analyzed the total mass would be approximately 13,000 Daltons. A change of only 9 Daltons is likely to be undetectable. Assuming one wants to amplify the DNA using PCR, primers of at least 13 base pairs in length will be necessary. Therefore the shortest amplified fragment will consist of greater than 26 base pairs. Even at only a length of 30 bases (mass of about 9750 Daltons) it is unlikely that a change of only 9 Daltons will be observable. The present invention overcomes this problem by using any one of several techniques as detailed below.

A) Polymorphism Causes a Change in a Restriction Enzyme Site: Purifying a Single-stranded Fragment for Analysis

A polymorphism may cause a change in a recognition sequence for a restriction endonuclease. An example can be given using a mutation in BRCA1 known to be associated with causing breast cancer. A G to T transversion in codon 1541 changes a GAG codon which encodes glutamic acid to a TAG stop codon. The DNA sequence in the immediate region of codon 1541 in the wild-type is:

TGGAGGAGCAACAGCTGGAA-GAGTCTGGGCCACACGATTT (SEQ ID NO: 3). Codon 1541 is shown in underlined bold-faced type. The mutation changes the first G of the codon to a T. The restriction enzyme Mbo II recognizes the sequence

5' . . . GAAGA($N_8$)↓. . . 3'

3' . . . CTTCT($N_7$)↑. . . 5'

This restriction site is present just at the site of the mutation. The wild-type sequence contains the restriction site but the mutated sequence will not be recognized by the enzyme. By amplifying this DNA fragment (using a biotin labeled primer for the sense strand), digesting with Mbo II, and purifying the sense strand, wild-type DNA will yield a strand of 30 bases in length (Mbo II cutting 8 bases 3' of the last A of the recognition site). The mutated version will not be recognized by Mbo II and a complete length strand of 40 bases will be seen. The other fragments, e.g. the 10 base 3' fragment of wild-type, will not be present because it will not be labeled with biotin and will not be captured by the streptavidin capture step. The difference between fragments of 30 and 40 bases is easily detected via MALDI-MS.

B) Polymorphism Causes a Change in a Restriction Enzyme Site: Denaturation and Analysis of Both Strands

One need not purify the single strands of DNA as in Example IV-A. An alternative is simply to amplify the 40 base pair fragment, digest with Mbo II, purify the DNA, then denature the purified DNA and analyze it via mass spectrometry. In mutated DNA with no recognition sequence, the two single strands will each be 40 bases in length. The sense strand will consist of 7 Ts, 14 Gs, 1 As and 8 Cs and have a mass of approximately 13,129 Daltons. The antisense strand will consist of 11 Ts, 8 Gs, 7 As and 14 Cs and have a mass of approximately 12,853 Daltons. The mass spectrum will show two major peaks in the region of the corresponding masses. Other minor peaks may occur due to multiply charged species or degradation products. These will help create a "signature" spectrum for the mutated fragment. The wild-type fragment will yield a completely different spectrum. The 40 base pair double stranded fragment will be

digested by Mbo II and upon denaturation there will be 4 single-stranded fragments present. These will consist of 30 base and 10 base fragments resulting from the sense strand and 29 base and 11 base fragments resulting from the antisense strand. The masses of these four fragments are approximately 9922, 3207, 9196 and 3632 Daltons, respectively. Again there will likely be minor bands appearing due to multiply charged fragments and degradation products. There may also be larger bands resulting from incomplete digestion with Mbo II. The signature spectrum of the wild-type digested DNA will obviously be quite different from that of the mutated DNA. The major peaks which will be seen in the spectrum from amplifying the 40 base pair fragment, digesting with Mbo II and then analyzing via MALDI-MS are as shown in Table II.

TABLE II

| Masses of the Major Peaks Observed | |
|---|---|
| Wild-type | Mutated |
| 3207 | 12828 |
| 3632 | 13104 |
| 9196 | |
| 9922 | |

This last technique is very simple and will likely have wide although probably not universal applicability. Because one is screening DNA in regions of known mutations, the sequences are already known and it is simple to determine proper primers for PCR. There is a very large number of known restriction enzymes with different recognition sequences to choose from and there is a reasonable chance of finding one which will recognize the wild-type but not the mutated DNA or vice versa. Again, because one is dealing with known sequences it is simply a matter of looking through a catalog of available restriction enzymes to find an appropriate one.

If desired, this last method can be modified. Larger fragments of DNA may be generated via PCR or other amplification method. This may be necessary if no suitable primers can be designed to give a smaller fragment. It may also be done to examine more than one mutation at a time if two or more mutations are relatively close to each other. These long fragments are then digested with a series of restriction enzymes to produce a mix of several sizes of DNA fragments. This complete mix is then analyzed via mass spectrometry. The resulting signature spectrum will consist of several major peaks. As an example, assume an initial fragment of 1,500 base pairs was synthesized via PCR and digested with a mix of restriction enzymes, e.g., Bam HI, Eco RI and Hind III. The digestion products in this hypothetical are 20 fragments of sizes shown in Table III arranged by size, not by location along the fragment.

TABLE III

| Fragment No. | Size in Base Pairs |
|---|---|
| 1 | 20 |
| 2 | 24 |
| 3 | 25 |
| 4 | 30 |
| 5 | 32 |
| 6 | 35 |
| 7 | 35 |
| 8 | 40 |
| 9 | 50 |
| 10 | 55 |

TABLE III-continued

| Fragment No. | Size in Base Pairs |
|---|---|
| 11 | 65 |
| 12 | 66 |
| 13 | 68 |
| 14 | 70 |
| 15 | 85 |
| 16 | 100 |
| 17 | 120 |
| 18 | 140 |
| 19 | 180 |
| 20 | 260 |

Further, for this hypothetical example assume that there is one mutation known to occur in fragment I and one mutation known to occur in fragment 6. One mutation affects a Hind III site and the other affects a Bam HI site present in the wild-type. Analyzing this complete mix will give one spectrum (as shown in Table III) for wild-type DNA. A different spectrum will be seen if one or both of the mutations are present. If the mutation affecting fragment 1 is present the 20 base fragment will no longer appear. If the mutation affecting fragment 6 is present, the peak at a size representing 35 nucleotides will be one half of its initial height. (Note that this assumes that the two 35 base single stranded fragments (fragments 6 and 7) are of equal mass. If they are of unequal mass then one of the peaks will disappear.) One other peak will also disappear with the occurrence of each mutation. If the mutation affecting fragment 1 also affects fragment 10 (the Hind III site is at the junction of fragments 1 and 10) then the 55 base peak will also disappear and a new peak corresponding to the sum of fragments 1 and 10 (20+55=75 base pairs) will appear. It will be irrelevant whether the large fragments of DNA can be properly analyzed because the enzymes are chosen to produce short fragments which will be affected by the mutations.

EXAMPLE V

Completely Digesting an Amplified DNA Fragment with DNAse

Another variation for determining the presence of a polymorphism using a mass spectrometry analysis is to amplify the DNA of interest and then totally digest it with an exonuclease such as deoxyribonuclease II (DNAse II). This enzyme completely cleaves polynucleotides to mononucleotides. Using this method to analyze the mutation occurring at codon 1541 of BRCA1 (see Example IV-A above) gives the following results:

A) Analyzing the 40 base pair double-stranded fragment

If both strands are present, digestion with DNAse II will result in the presence of 18 Ts, 22 Gs, 18 As and 22 Cs in the wild-type DNA. When the mutation is present the breakdown is 19 Ts, 21 Gs, 19 As and 21 Cs. Four major peaks will be seen, one for each nucleotide. These will be at approximately masses of 320 (Tp), 345 (Gp), 329 (Ap), and 305 (Cp). The relative peak heights or intensities will change depending on the composition of the DNA. When mutated DNA is analyzed for this example, the peak heights for T and A will be larger while the peak heights for G and C will be smaller as compared with wild-type DNA. In this example the change in peak height is approximately 5% for each of the peaks. Use of fragments shorter than 40 base pairs will usually yield a greater relative change in peak intensity.

This method is also applicable to analyzing for insertions or deletions. Again a difference in peak height will be seen

when wild-type and mutant spectra are compared. For the BRCA1 mutation discussed in Example III, there would be an addition of 1 extra C and 1 extra G thereby increasing those two peaks and having no effect on the A and T peaks.

B) Analyzing Only a Single-Stranded DNA

The method used in part A above can be modified to yield a more sensitive technique. As always, amplified DNA is prepared. For this example use one primer labeled with biotin and then purify the single strand - here the sense strand of the BRCA1 fragment containing codon 1541. This single strand of 40 bases (with the 5' T being biotinylated) is digested with deoxyribonuclease I (DNAse I) which is an enzyme which will cleave single-stranded DNA preferentially next to pyrimidines to yield 5'-phosphate terminated polynucleotides with 3'-OH termin. In the case of the 40 base fragment from BRCA1, the fragments which will result from wild-type DNA are shown in Table IV.

TABLE IV

| | Wild Type | | Mutated | |
|---|---|---|---|---|
| Fragment | Sequence | Mass | Sequence | Mass |
| 1 | T(biotinylated) | 563 | T(biotinylated) | 563 |
| 2 | GGAGGAGC | 2688 | GGAGGAGC | 2688 |
| 3 | AAC | 963 | AAC | 963 |
| 4 | AGC | 979 | AGC | 979 |
| 5 | T | 320 | T | 320 |
| 6 | GGAAGAGT | 2687 | GGAAT | 1668 |
| 7 | C | 305 | AGT | 994 |
| 8 | T | 320 | C | 305 |
| 9 | GGGC | 1340 | T | 320 |
| 10 | C | 305 | GGGC | 1340 |
| 11 | AC | 634 | C | 305 |
| 12 | AC | 634 | AC | 634 |
| 13 | GAT | 994 | AC | 634 |
| 14 | T | 320 | GAT | 994 |
| 15 | T | 320 | T | 320 |
| 16 | — | — | T | 320 |

Rearranging this data by mass and number of fragments of the particular mass yields Table V.

TABLE V

| | Number of Fragments | |
|---|---|---|
| Mass | Wild-type | Mutated |
| 305 | 2 | 2 |
| 320 | 4 | 4 |
| 563 | 1 | 1 |
| 634 | 2 | 2 |
| 963 | 1 | 1 |
| 979 | 1 | 1 |
| 994 | 1 | 2 |
| 1340 | 1 | 1 |
| 1668 | — | 1 |
| 2687 | 1 | — |
| 2688 | 1 | 1 |

Analysis of this data reveals differences between the two spectra at masses of 994, 1668 and 2687. The peak at 994 is twice as large in the mutated sample as in the wild-type, the peak at 1668 is present only for the mutated sample, and the peak at 2687 is present only in the wild-type. The "signature" of each spectrum is distinctive and clearly different from the other spectrum.

EXAMPLE VI

Analysis of Ribonucleic Acid

Analysis for the presence of polymorphisms using mass spectrometry need not be limited to using DNA. RNA is just as suitable and has actually been reported to be more stable and less prone to degradation during mass spectrometry. Those of skill in the art will know a number of ways in which to prepare RNA fragments for the region of interest to be analyzed. Amplified fragments of DNA may be cloned into vectors which contain RNA polymerase promoters. These vectors are linearized at the end of the insert away from the promoter and RNA transcripts can be produced in large quantities. This yields a single-stranded RNA which can be assayed essentially in the same manner as single-stranded DNA as discussed above. It is also possible to "transcribe" an amplified DNA fragment to produce RNA. Since genes of known sequence are to be analyzed, the sequence is already known (except for the possibility of a mutation or polymorphism in the region to be tested) and it is a simple matter to design an appropriate primer. For assays involving digesting the RNA with nucleases, rather than restriction enzymes or DNAses one will of course use RNAses. These may be used singly or in combinations. Some ribonucleases which are available commercially are ribonuclease A (hydrolyzes the 3' side of pyrimidines), ribonuclease $T_1$ (hydrolyzes the 3' side of G), ribonuclease $T_2$ (hydrolyzes the 3' side of purines and at pyrimidines), and ribonuclease $U_2$ (cleaves the 3' side of purines).

EXAMPLE VII

Analysis of cDNA

Complementary DNA (cDNA) may be analyzed exactly as genomic DNA was analyzed above. In the examples above, mutations occurred in the middle of exons and genomic DNA was amplified by PCR using primers complementary to the exon around the region of the mutation. One may also prepare cDNA to accomplish this same purpose. Preparation of cDNA is well known to those of skill in the art. Briefly, one purifies messenger RNA (mRNA), reverse transcribes this mRNA to produce an RNA:DNA hybrid, hydrolyzes the mRNA to leave a single-stranded DNA, and finally synthesizes the complementary strand to form a double stranded DNA. This cDNA is a mixture of all of the expressed genes. The cDNA may then be specifically amplified via PCR or some other amplification technique. The use of cDNA limits one to analyzing mutations which occur in exons. To analyze for a polymorphism which occurs in an intron it is necessary to analyze genomic DNA.

The above examples are clearly not all encompassing. Many variations will be readily apparent to those skilled in the art. Amplification need not be performed by PCR but can be done by any other suitable amplification technique. Digestion of samples may be done in a variety of ways—either using enzymes other than those specified in the examples or simply by chemical cleavage of the polynucleotides. Purification of single strands can also be accomplished by a number of techniques known to those skilled in the art. Use of biotin and streptavidin is only one such technique. Also, as noted earlier, a large variety of nucleotide analogs may be used. These will have different purposes. Increased stability of nucleic acids has been reported for those containing 7-deaza-guanosine and 7-deaza-adenosine. Incorporation of deoxyuridine into the amplified DNA allows one to remove these bases using uracil-N-glycosidase digestion. The matrix used for the mass spectrometry need not be limited to 3-hydroxy-picolinic acid. Other matrices are also suitable, although many researchers have found 3-hydroxy-picolinic acid to be most suitable for polynucleotide analysis. The choice of exactly what type of mass spectrometer or laser source is also a variable. Again, MALDI-MS using a neodymium-garnet laser is a favored choice.

The invention as described above enhances the rapidity of analysis for polymorphisms in known genes. The method does not require DNA sequencing or hybridization techniques and therefore does away with the need for running gels or hybridizing samples. The nucleic acid is prepared, amplified and purified, possibly digested, and then analyzed via mass spectrometry. Differences in the signature of the spectrum obtained as compared to known signature spectra make the presence or absence of a polymorphism readily apparent. The throughput of samples in the mass spectrometer is estimated to be 1–2 seconds with analysis occurring off-line. The BRCA1 gene encodes 1863 amino acids corresponding to 5589 base pairs. Many different mutations associated with breast cancer have now been found in this gene. It will require analysis of many different fragments if one is concerned with analyzing the complete gene. (If a relative has a known mutation one may simply wish to analyze for that single mutation.) Mass spectrometers are presently available which have target slides with 64 sample spots that can be deposited by robot. Using a 100 Hz laser for 1 second of collection time (about 50 samplings) and 1 second for slide movement requires 128 seconds for the 64 samples. Thus one can analyze for at least 64 distinct mutations in about 2 minutes of mass spectrometry time. The sample preparation time is not included here, but sample preparation is just as time consuming in other techniques such as DNA sequencing. The off-line analysis is quite simple and quick, a comparison of the spectra with known signature spectra for either the wild-type sequence or for a sequence with a known polymorphism.

The utility of the method need not be limited to analyzing for mutations or polymorphisms in known genes associated with diseases. It may be used to screen candidate gene sequences for mutations in a positional cloning project. It may also be used for other types of comparative DNA analyses such as genotyping.

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SEQUENCE LISTING

( 1 ) GENERAL INFORMATION:

( i i i ) NUMBER OF SEQUENCES: 3

( 2 ) INFORMATION FOR SEQ ID NO:1:

( i ) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 39 base pairs
( B ) TYPE: nucleic acid
( C ) STRANDEDNESS: double

-continued

( 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:1:

CAAAGCGAGC AAGAGAATCC CAGGACAGAA AGATCTTCA 39

( 2 ) INFORMATION FOR SEQ ID NO:2:

( i ) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 40 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:2:

CAAAGCGAGC AAGAGAATCC CCAGGACAGA AAGATCTTCA 40

( 2 ) INFORMATION FOR SEQ ID NO:3:

( i ) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 40 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:

TGGAGGAGCA ACAGCTGGAA GAGTCTGGGC CACACGATTT 40

What is claimed is:

1. A method to analyze for a polymorphism or a mutation in a gene or a portion of said gene encoded by a nucleic acid by

a) denaturing said nucleic acid or a portion of said nucleic acid to produce a denatured nucleic acid,

b) performing mass spectrometry on said denatured nucleic acid to obtain a mass spectrum,

c) comparing the obtained mass spectrum with reference mass spectra obtained of the nucleic acid in its wild-type, polymorphic, or mutated state, and

d) determining whether the obtained mass spectrum matches a reference spectrum for either the wild-type nucleic acid or the nucleic acid having said polymorphism or mutation, wherein a match with said wild-type nucleic acid indicates that said gene is wild-type and a match with said nucleic acid having said polymorphism or mutation indicates that said gene has said polymorphism or mutation, a match being indicated by identity of peak locations (representing mass) and relative peak heights (representing quantity), with the proviso that said method does not comprise sequencing said nucleic acid.

2. The method according to claim 1 wherein a portion of said nucleic acid is analyzed.

3. A method to analyze for a polymorphism or a mutation in a portion of a gene encoded by a nucleic acid by

a) denaturing said nucleic acid encoding said portion of said gene to produce a denatured nucleic acid,

b) amplifying said denatured nucleic acid to produce an amplified nucleic acid,

c) performing mass spectrometry on said amplified nucleic acid to obtain a mass spectrum,

d) comparing the obtained mass aspect with reference mass spectra obtained of the nucleic acid in its wild-type, polymorphic, or mutated state, and

e) determining whether the obtained mass spectrum matches a reference spectrum for either the wild-type nucleic acid or the nucleic acid having said polymorphism or mutation, wherein a match with said wild-type nucleic acid indicates that said gene is wild-type and a match with said nucleic acid having said polymorphism or mutation indicates that said gene has said polymorphism or mutation, a match being indicated by identity of peak locations (representing mass) and relative peak heights (representing quantity), with the proviso that said method does not comprise sequencing said nucleic acid.

4. The method according to claim 3 wherein said amplified nucleic acid is digested prior to denaturation and analysis.

5. The method according to claim 4 wherein digestion is performed using one or more restriction endonucleases.

6. The method according to claim 4 wherein digestion is performed using a deoxyribonuclease.

7. The method according to claim 4 wherein digestion is performed using chemical cleavage.

8. The method according to claim 4 wherein following digestion the digested nucleic acid is denatured and a single-stranded nucleic acid fragment is purified and analyzed.

9. The method according to claim 5 wherein following digestion the digested nucleic acid is denatured and a single-stranded nucleic acid fragment is purified and analyzed.

10. The method according to claim 3 wherein the amplified nucleic acid is denatured and a single-stranded nucleic acid fragment is purified and analyzed.

11. The method according to claim 3 wherein said amplification produces an RNA fragment.

12. A method to analyze for a polymorphism or mutation in a portion of a gene encoded by a nucleic acid by

a) cloning said nucleic acid into a vector containing one or more RNA promoters,

b) synthesizing RNA using said vector with said nucleic acid as a template,

c) performing mass spectrometry on said RNA to obtain a mass spectrum,

d) comparing the obtained mass spectrum with reference mass spectra obtained of the RNA in its wild-type, polymorphic, or mutated state, and

e) determining whether the obtained mass spectrum matches a reference spectrum for either the wild-type RNA or the RNA having said polymorphism or mutation, wherein a match with the wild-type RNA indicates that said gene is wild-type and a match with said RNA having said polymorphism or mutation indicates that said gene has said polymorphism or mutation, a match being indicated by identity of peak locations (representing mass) and relative peak heights (representing quantity), with the proviso that said method does not comprise sequencing said RNA.

* * * * *

**AFTER COMPLETING THE FRONT SIDE OF FORM JS-44C, COMPLETE THE INFORMATION REQUESTED BELOW.**

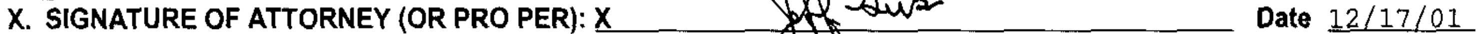
**VIII(b). RELATED CASES:** Have any cases been previously filed that are related to the present case? __x__ No _____ Yes

If yes, list case number(s): __________

CIVIL CASES ARE DEEMED RELATED IF A PREVIOUSLY FILED CASE AND THE PRESENT CASE:

(CHECK ALL BOXES THAT APPLY)

- [ ] A. Appear to arise from the same or substantially identical transactions, happenings, or events;
- [ ] B. Involve the same or substantially the same parties or property;
- [ ] C. Involve the same patent, trademark or copyright;
- [ ] D. Call for determination of the same or substantially identical questions of law, or
- [ ] E. Likely for other reasons may entail unnecessary duplication of labor if heard by different judges.



**IX. VENUE:** List the California County, or State if other than California, in which **EACH** named plaintiff resides. (Use an additional sheet if necessary)

- [ ] CHECK HERE IF THE US GOVERNMENT, ITS AGENCIES OR EMPLOYEES IS A NAMED PLAINTIFF.

SEQUENOM, INC.: San Diego County

List the California County, or State if other than California, in which **EACH** named defendant resides. (Use an additional sheet if necessary).

- [ ] CHECK HERE IF THE US GOVERNMENT, ITS AGENCIES OR EMPLOYEES IS A NAMED DEFENDANT.

MYRIAD GENETICS, INC.: San Diego County
MYRIAD GENETICS LABORATORIES: San Diego County

List the California County, or State if other than California, in which **EACH** claim arose. (Use an additional sheet if necessary).

**NOTE:** In land condemnation cases, use the location of the tract of land involved.

San Diego County

**X. SIGNATURE OF ATTORNEY (OR PRO PER): X** __________ **Date** 12/17/01

**NOTICE TO COUNSEL/PARTIES:** The CV-71 (JS-44) Civil Cover Sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law. This form, approved by the Judicial Conference of the United States in September 1974, is required pursuant to Local Rule 3.3 is not filed but is used by the Clerk of the Court for the purpose of statistics, venue and initiating the civil docket sheet. (For more detailed instructions, see separate instructions sheet.)

Key to Statistical codes relating to Social Security Cases:

| NATURE OF SUIT CODE | ABBREVIATION | SUBSTANTIVE STATEMENT OF CAUSE OF ACTION |
|---|---|---|
| 861 | HIA | All claims for health insurance benefits (Medicare) under Title 18, Part A, of the Social Security Act, as amended. Also, include claims by hospitals, skilled nursing facilities, etc., for certification as providers of services under the program. (42 U.S.C. 1935FF(b)) |
| 862 | BL | All claims for "Black Lung" benefits under Title 4, Part B, of the Federal Coal Mine Health and Safety Act of 1969. (30 U.S.C. 923) |
| 863 | DIWC | All claims filed by insured workers for disability insurance benefits under Title 2 of the Social Security Act, as amended; plus all claims filed for child's insurance benefits based on disability. (42 U.S.C. 405 (g)) |
| 863 | DIWW | All claims filed for widows or widowers insurance benefits based on disability under Title 2 of the Social Security Act, as amended. (42 U.S.C. 405 (g)) |
| 864 | SSID | All claims for supplemental security income payments based upon disability filed under Title 16 of the Social Security Act, as amended. |
| 865 | RSI | All claims for retirement (old age) and survivors benefits under Title 2 of the Social Security Act, as amended. (42 U.S.C. (g)) |

| TO: Commissioner of Patents and Trademarks Washington, D.C. 20231 | REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT |
|---|---|

In compliance with the Act of July 19, 1952 (66 Stat. 814; 35 U.S.C. 290) you are hereby advised that a court action has been filed on the following patent(s) in the U.S. District Court:

| DOCKET NO. | DATE FILED | U.S. DISTRICT COURT |
|---|---|---|
| 01-CV*2317 BTM(JAH) | 12-17-01 | United States District Court, Southern District of California |
| PLAINTIFF<br>Sequenom Inc. | | DEFENDANT<br>Myriad Genetics, Inc |
| **PATENT NO.** | **DATE OF PATENT** | **PATENTEE** |
| 1 01-CV-2317 BTM(JAH) | 01-14-00 | Sequenom Inc. |
| 2. | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above-entitled case, the following patent(s) have been included:

| DATE INCLUDED | INCLUDED BY<br>☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading | |
|---|---|---|
| **PATENT NO.** | **DATE OF PATENT** | **PATENTEE** |
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above-entitled case, the following decision has been rendered or judgment issued:

| DECISION/JUDGMENT | | |
|---|---|---|
| CLERK | (BY) DEPUTY CLERK | DATE |

Copy 1 - Upon initiation of action, mail this copy to Commissioner Copy 3 - Upon termination of action, mail this copy to Commissioner
Copy 2 - Upon filing document adding patent(s), mail this copy to Commissioner Copy 4 - Case file copy

| TO: Commissioner of Patents and Trademarks Washington, D.C. 20231 | **REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT** |
|---|---|

In compliance with the Act of July 19, 1952 (66 Stat. 814; 35 U.S.C. 290) you are hereby advised that a court action has been filed on the following patent(s) in the U.S. District Court:

| DOCKET NO. | DATE FILED | U.S. DISTRICT COURT |
|---|---|---|
| 01-CV*2317 BTM(JAH) | 12-17-01 | United States District Court, Southern District of California |

| PLAINTIFF | DEFENDANT |
|---|---|
| Sequenom Inc. | Myriad Genetics, Inc |

| PATENT NO. | DATE OF PATENT | PATENTEE |
|---|---|---|
| 1 01-CV-2317 BTM(JAH) | 01-14-00 | Sequenom Inc. |
| 2. | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above-entitled case, the following patent(s) have been included:

| DATE INCLUDED | INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading |
|---|---|
| | |

| PATENT NO. | DATE OF PATENT | PATENTEE |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

In the above-entitled case, the following decision has been rendered or judgment issued:

DECISION/JUDGMENT

| CLERK | (BY) DEPUTY CLERK | DATE |
|---|---|---|
| | | |

Copy 1 - Upon initiation of action, mail this copy to Commissioner Copy 3 - Upon termination of action, mail this copy to Commissioner
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| **PATENT NO.** | **DATE OF PATENT** | **PATENTEE** |
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| DECISION/JUDGMENT | | |
|---|---|---|
| CLERK | (BY) DEPUTY CLERK | DATE |

Copy 1 - Upon initiation of action, mail this copy to Commissioner Copy 3 - Upon termination of action, mail this copy to Commissioner
Copy 2 - Upon filing document adding patent(s), mail this copy to Commissioner Copy 4 - Case file copy

**I (a) PLAINTIFFS**
SEQUENOM, INC., a Delaware Corporation,

**DEFENDANTS**
MYRIAD GENETICS, INC., a Delaware Corporation, MYRIAD GENETICS LABORATORIES, a Delaware Corporation

**(b)** COUNTY OF RESIDENCE OF FIRST LISTED PLAINTIFF San Diego County
(EXCEPT IN U.S. PLAINTIFF CASES)

COUNTY OF RESIDENCE OF FIRST LISTED DEFENDANT San Diego County
(IN U.S. PLAINTIFF CASES ONLY)

**(c)** ATTORNEYS (FIRM NAME, ADDRESS, AND TELEPHONE NUMBER)
JEFFREY W. GUISE (State Bar No. 164203)
JOHN E. PETERSON (State Bar No. 197978)
BROBECK, PHLEGER & HARRISON LLP
12390 El Camino Real
San Diego, CA 92130-2081
(858) 720-2500

ATTORNEYS (IF KNOWN)

'01 CV 2317 BTM (JAH)

## II. BASIS OF JURISDICTION (PLACE AN x IN ONE BOX ONLY)

- [ ] 1 U.S. Government Plaintiff
- [X] 3 Federal Question (U.S. Government Not a Party)
- [ ] 2 U.S. Government Defendant
- [ ] 4 Diversity (Indicate Citizenship of Parties in Item III)

## III. CITIZENSHIP OF PRINCIPAL PARTIES (PLACE AN x IN ONE BOX FOR PLAINTIFF AND ONE FOR DEFENDANT)
(For Diversity Cases Only)

| | PTF | DEF | | PTF | DEF |
|---|---|---|---|---|---|
| Citizen of This State | 1 | 1 | Incorporated or Principal Place of Business In This State | 4 | 4 |
| Citizen of Another State | 2 | 2 | Incorporated and Principal Place of Business In Another State | 5 | 5 |
| Citizen or Subject of a Foreign Country | 3 | 3 | Foreign Nation | 6 | 6 |

## IV. ORIGIN *(PLACE AN x IN ONE BOX ONLY)*

- [X] 1 Original Proceeding
- [ ] 2 Removed from State Court
- [ ] 3 Remanded from Appellate Court
- [ ] 4 Reinstated or Reopened
- [ ] 5 Transferred from another district (specify)
- [ ] 6 Multidistrict Litigation
- [ ] 7 Appeal to District Judge from Magistrate Judgment

## V. REQUESTED IN COMPLAINT:

[ ] CHECK IF THIS IS A **CLASS ACTION** UNDER F.R.C.P. 23

**DEMAND $** Declaratory judgment

Check YES only if demanded in complaint:
**JURY DEMAND:** [X] YES [ ] NO

## VI. CAUSE OF ACTION (CITE THE U.S. CIVIL STATUTE UNDER WHICH YOU ARE FILING AND WRITE A BRIEF STATEMENT OF CAUSE. DO NOT CITE JURISDICTIONAL STATUTES UNLESS DIVERSITY.)

Declaratory judgment of patent non-infringement and patent invalidity, 35 USC 101 ET SEQ., 28 USC 2201, 2202. 35:0145 (ser)

## VII. NATURE OF SUIT (PLACE AN x IN ONE BOX ONLY)

**OTHER STATUTES**
- 400 State Reapportionment
- 410 Antitrust
- 430 Banks and Banking
- 450 Commerce/ICC Rates/etc.
- 460 Deportation
- 470 Racketeer Influenced and Corrupt Organizations
- 810 Selective Service
- 850 Securities/Commodities/ Exchange
- 875 Customer Challenge 12 USC 3410
- 891 Agricultural Act
- 892 Economic Stabilization Act
- 893 Environmental Matters
- 894 Energy Allocation Act
- 895 Freedom of Information Act
- 900 Appeal of Fee Determination Under Equal Access to Justice
- 950 Constitutionality of State Statutes
- 890 Other Statutory Actions

**CONTRACT**
- 110 Insurance
- 120 Marine
- 130 Miller Act
- 140 Negotiable Instrument
- 150 Recovery of Overpayment & Enforcement of Judgment
- 151 Medicare Act
- 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- 153 Recovery of Overpayment Of Veteran's Benefits
- 160 Stockholders' Suits
- 190 Other Contract
- 195 Contract Product Liability

**REAL PROPERTY**
- 210 Land Condemnation
- 220 Foreclosure
- 230 Rent Lease & Ejectment
- 240 Torts to Land
- 245 Tort Product Liability
- 290 All Other Real Property

**TORTS**

**PERSONAL INJURY**
- 310 Airplane
- 315 Airplane Product Liability
- 320 Assault, Libel & Slander
- 330 Federal Employers' Liability
- 340 Marine
- 345 Marine Product Liability
- 350 Motor Vehicle
- 355 Motor Vehicle Product Liability
- 360 Other Personal Injury

**PERSONAL INJURY**
- 362 Personal Injury-Med Malpractice
- 365 Personal Injury-Product Liability
- 368 Asbestos Personal Injury Product Liability

**PERSONAL PROPERTY**
- 370 Other Fraud
- 371 Truth in Lending
- 380 Other Personal Property Damage
- 385 Property Damage Product Liability

**CIVIL RIGHTS**
- 441 Voting
- 442 Employment
- 443 Housing/ Accommodations
- 444 Welfare
- 440 Other Civil Rights

**PRISONER PETITIONS**
- 510 Motions to Vacate Sentence Habeas Corpus
- 530 General
- 535 Death Penalty
- 540 Mandamus&Other
- 550 Civil Rights
- 555 Prison Condition

**FORFEITURE/PENALTY**
- 610 Agriculture
- 620 Other Food & Drug
- 625 Drug Related Seizure of Property 21 USC 881
- 630 Liquor Laws
- 640 R.R. & Truck
- 650 Airline Regs
- 660 Occupational Safety/Health
- 690 Other

**LABOR**
- 710 Fair Labor Standards Act
- 720 Labor/Mgmt. Relations
- 730 Labor/Mgmt. Reporting & Disclosure Act
- 740 Railway Labor Act
- 790 Other Labor Litigation
- 791 Empl. Ret. Inc. Security Act

**BANKRUPTCY**
- 422 Appeal 28 USC 158
- 423 Withdrawal 28 USC 157

**PROPERTY RIGHTS**
- 820 Copyrights
- [X] 830 Patent
- 840 Trademark

**SOCIAL SECURITY**
- 861 HIA (1395ff)
- 862 Black Lung (923)
- 863 DIWC/DIWW (405(g))
- 864 SSID Title XVI
- 865 RSI (405(g))

**FEDERAL TAX SUITS**
- 870 Taxes (U.S. Plaintiff or Defendant)
- 871 IRS - Third Party 26 USC 7609

**VIII(a). IDENTICAL CASES:** Has this action been previously filed and dismissed, remanded or closed? x No ____ Yes

If yes, list case number(s): ____________



*FOR OFFICE USE ONLY:* [ ] *Pro Hac Vice fee:* [ ] *paid* [ ] *not paid*

*Applying IFP* ________ *Judge* ________ *Mag. Judge* ________

**ORIGINAL**

CCD-JS44

(#7770) Pd $150

UNITED STATES
DISTRICT COURT
Southern District of California
San Diego Division

# 077770 - 03
December 17, 2001

| Code | Case # | Qty | Amount |
|---|---|---|---|
| [illegible]086900 | | | [illegible]0.00 CH |
| CV510000 | | | [illegible]0.00 CH |

Total [illegible] 350.00

FROM: CIVIL FILING
[illegible]ZUENAN ET AL VS [illegible]
GENETIC[illegible] ET AL [illegible] CV2317
BC#1[illegible]

ORIGINAL