

CLAIMS

WHAT IS CLAIMED IS:

1. A method for analyzing a disease state of a subject, comprising:
 - (a) using a genetic analyzer to generate genetic data from nucleic acid molecules in biological samples of the subject obtained at (i) two or more time points or (ii) substantially the same time point, wherein the genetic data relates to genetic information of the subject, and wherein the biological samples include a cell-free biological sample;
 - (b) receiving the genetic data from the genetic analyzer;
 - (c) with one or more programmed computer processors, using the genetic data to produce an adjusted test result in a characterization of the genetic information of the subject; and
 - (d) outputting the adjusted test result into computer memory.
2. The method of claim 1, wherein the genetic data comprises current sequence reads and prior sequence reads, and wherein (c) comprises comparing the current sequence reads with the prior sequence reads and updating a diagnostic confidence indication accordingly with respect to the characterization of the genetic information of the subject, which diagnostic confidence indication is indicative of a probability of identifying one or more genetic variations in a biological sample of the subject.
3. The method of claim 2, further comprising generating a confidence interval for the current sequence reads.
4. The method of claim 3, further comprising comparing the confidence interval with one or more prior confidence intervals and determining a disease progression based on overlapping confidence intervals.
5. The method of claim 1, wherein the biological samples are obtained at two or more time points including a first time point and a second time point, and wherein (c) comprises increasing a diagnostic confidence indication in a subsequent or a previous characterization if the information from the first time point corroborates information from the second time point.
6. The method of claim 1, wherein the biological samples are obtained at two or more time points including a first time point and a second time point, and wherein (c) comprises increasing a diagnostic confidence indication in the subsequent characterization if the information from the first time point corroborates information from the second time point.

7. The method of claim 1, wherein a first co-variate variation is detected in the genetic data, and wherein (c) comprises increasing a diagnostic confidence indication in the subsequent characterization if a second co-variate variation is detected.

8. The method of claim 1, wherein the biological samples are obtained at two or more time points including a first time point and a second time point, and wherein (c) comprises decreasing a diagnostic confidence indication in the subsequent characterization if the information from a first time point conflicts with information from the second time point.

9. The method of claim 1, further comprising obtaining a subsequent characterization and leaving as is a diagnostic confidence indication in the subsequent characterization for de novo information.

10. The method of claim 1, further comprising determining a frequency of one or more genetic variants detected in a collection of sequence reads included in the genetic data and producing the adjusted test result at least in part by comparing the frequency of the one or more genetic variants at the two or more time points.

11. The method of claim 1 further comprising determining an amount of copy number variation at one or more genetic loci detected in a collection of sequence reads included in the genetic data and producing the adjusted test result at least in part by comparing the amount at the two or more time points.

12. The method of claim 1, further comprising using the adjusted test result to provide (i) a therapeutic intervention or (ii) a diagnosis of a health or disease to the subject.

13. The method of claim 1, wherein the genetic data comprises sequence data from portions of a genome comprising disease-associated or cancer associated genetic variants.

14. The method of claim 1, further comprising using the adjusted test result to increase a sensitivity of detecting genetic variants by increasing read depth of polynucleotides in a sample from the subject.

15. The method of claim 1, wherein the genetic data comprises a first set of genetic data and a second set of genetic data, wherein the first set of genetic data is at or below a detection threshold and the second set of genetic data is above the detection threshold.

16. The method or claim 15, wherein the detection threshold is a noise threshold.

17. The method of claim 15, further comprising, in (c), adjusting a diagnosis of the subject from negative or uncertain to positive when the same genetic variants are detected in the first set of genetic data and the second set of genetic data in a plurality of sampling instances or time points.

18. The method of claim 15, further comprising, in (c), adjusting a diagnosis of the subject from negative or uncertain to positive in a characterization from an earlier time point when the same genetic variants are detected in the first set of genetic data at an earlier time point and in the second set of genetic data at a later time point.

19. The method of claim 1, wherein the disease state is cancer and the genetic analyzer is a nucleic acid sequencer.

20. The method of claim 1, wherein the biological samples include at least two different types of biological samples.

21. The method of claim 1, wherein the biological samples include the same type of biological sample.

22. The method of claim 21, wherein the biological samples are blood samples.

23. The method of claim 22, wherein the nucleic acid molecules are cell-free deoxyribonucleic acid (DNA).

24. A method of detecting a trend in the amount of cancer polynucleotides in a biological sample from a subject over time, comprising:

determining, using or more programmed computer processors, a frequency of the cancer polynucleotides at each of a plurality of time points;

determining an error range for the frequency at each of the plurality of time points to provide at least a first error range at a first time point and a second error range at a second time point subsequent to the first time point; and

determining whether (1) the first error range overlaps with the second error range, which overlap is indicative of stability of frequency of the cancer polynucleotides at a plurality of time points, (2) the second error range is greater than the first error range, thereby indicating an increase in frequency of the cancer polynucleotides at a plurality of time points, or (3) the second error range is less than the first error range, thereby indicating a decrease in frequency of the cancer polynucleotides at a plurality of time points.

25. The method of claim 24, wherein the DNA is cell-free DNA.

26. A method to detect one or more genetic variations and/or amount of genetic variation in a subject, comprising:
- sequencing nucleic acid molecules in a cell-free nucleic acid sample of the subject with a genetic analyzer to generate a first set of sequence reads at a first time point;
 - comparing the first set of sequence reads with at least a second set of sequence reads obtained at least at a second time point before the first time point to yield a comparison of first set of sequence reads and the at least the second set of sequence reads;
 - using the comparison to update a diagnostic confidence indication accordingly, which diagnostic confidence indication is indicative of a probability of identifying one or more genetic variations in a cell-free nucleic acid sample of the subject; and
 - detecting -a presence or absence of the one or more genetic variations and/or amount of genetic variation in nucleic acid molecules in a cell-free nucleic acid sample of the subject based on the diagnostic confidence indication.
27. The method of claim 26, further comprising obtaining the cell-free nucleic acid molecules from the subject.
28. The method of claim 26, further (i) comprising increasing the diagnostic confidence indication if information obtained from the first set of sequence reads at the first time point corroborates information obtained from the at least the second set of sequence reads at the second time point, (ii) decreasing the diagnostic confidence indication if information obtained from the first set of sequence reads at the first time point does not corroborate or conflicts with information obtained from the at least the second set of sequence reads at the second time point, or (iii) leaving as is the diagnostic confidence indication in a subsequent characterization for de novo information.
29. A method for detecting a mutation in a cell-free nucleic acid sample of a subject, comprising:
- (a) determining consensus sequences by comparing current sequence reads obtained from a genetic analyzer with prior sequence reads from a prior time period to yield a comparison, and updating a diagnostic confidence indication based on the comparison, wherein each consensus sequence corresponds to a unique polynucleotide among a set of tagged parent polynucleotides derived from the cell-free nucleic acid sample, and
 - (b) based on the diagnostic confidence, generating a genetic profile of extracellular polynucleotides in the subject, wherein the genetic profile comprises data resulting from copy number variation or mutation analyses.

30. The method of claim 29, further comprising:

using the consensus sequences to normalize ratios or frequency of variance for each mappable base position and determining actual or potential rare variant(s) or mutation(s); and

comparing a resulting number for each region with potential rare variant(s) or mutation(s) to similarly derived numbers from a reference sample.

31. A method to detect abnormal cellular activity, comprising:

providing at least one set of tagged parent polynucleotides derived from a biological sample of a subject;

amplifying the tagged parent polynucleotides in the set to produce a corresponding set of amplified progeny polynucleotides;

using a genetic analyzer to sequence a subset of the set of amplified progeny polynucleotides to produce a set of sequencing reads; and

collapsing the set of sequencing reads to generate a set of consensus sequences by comparing current sequence reads with prior sequence reads from at least one prior time period and updating a diagnostic confidence indication accordingly, which diagnostic confidence indication is indicative of a probability of identifying one or more genetic variations in a biological sample of the subject, wherein each consensus sequence corresponds to a unique polynucleotide among the set of tagged parent polynucleotides.

32. The method of claim 31, further comprising (i) increasing the diagnostic confidence indication if the set of sequencing reads is identified in the at least one prior time period, (ii) decreasing the diagnostic confidence indication if the set of sequencing reads is not identified in the at least one prior time period, or (iii) keeping the diagnostic confidence indication unchanged if the set of sequencing reads is identified in the at least one prior time period but is nonconclusive.

33. A method for detecting a mutation in a cell-free or substantially cell free sample of a subject comprising:

(a) sequencing extracellular polynucleotides from a bodily sample of the subject with a genetic analyzer;

(b) for each of the extracellular polynucleotides, generating a plurality of sequencing reads;

(c) filtering out reads that fail to meet a set threshold;

(d) mapping sequence reads derived from the sequencing onto a reference sequence;

- (e) identifying a subset of mapped sequence reads that align with a variant of the reference sequence at each mappable base position;
- (f) for each mappable base position, calculating a ratio of (i) a number of mapped sequence reads that include a variant as compared to the reference sequence, to (ii) a number of total sequence reads for each mappable base position; and
- (g) using one or more programmed computer processors to compare the sequence reads with other sequence reads from at least one previous time point and updating a diagnostic confidence indication accordingly, which diagnostic confidence indication is indicative of a probability of identifying the variant.

34. The method of claim 33, wherein the extracellular polynucleotides include cell-free deoxyribonucleic acid (DNA) molecules.

35. A method for operating a genetic test equipment, comprising:

providing initial starting genetic material obtained from a bodily sample obtained from a subject;

converting double stranded polynucleotide molecules from the initial starting genetic material into at least one set of non-uniquely tagged parent polynucleotides, wherein each polynucleotide in a set is mappable to a reference sequence; and

for each set of tagged parent polynucleotides:

(i) amplifying the tagged parent polynucleotides in the set to produce a corresponding set of amplified progeny polynucleotides;

(ii) sequencing the set of amplified progeny polynucleotides to produce a set of sequencing reads;

(iii) collapsing the set of sequencing reads to generate a set of consensus sequences, wherein collapsing uses sequence information from a tag and at least one of: (1) sequence information at a beginning region of a sequence read, (2) an end region of the sequence read and (3) length of the sequence read, wherein each consensus sequence of the set of consensus sequences corresponds to a polynucleotide molecule among the set of tagged parent polynucleotides; and

(iv) analyzing the set of consensus sequences for each set of tagged parent molecules;

(v) comparing current sequence reads with prior sequence reads from at least one other time point; and

(vi) updating a diagnostic confidence indication accordingly, which diagnostic confidence indication is indicative of a probability of identifying one or more genetic variations in a bodily sample of the subject.

36. The method of claim 35, wherein the initial starting genetic material includes cell-free deoxyribonucleic acid (DNA).

37. The method of claim 35, wherein the set of consensus sequences for each set of tagged parent molecules is analyzed separately.

38. The method of claim 35, wherein (vi) comprises (1) increasing diagnostic confidence indication in the current sequence reads if information from the prior sequence reads corroborates information from the current sequence reads, (2) decreasing a diagnostic confidence indication in the current sequence reads if information from the prior sequence reads conflicts with information from the current sequence reads, or (3) keeping a diagnostic confidence indication the same in the current sequence reads if information from the prior sequence reads is inconclusive with respect to information from the current sequence reads.

39. The method of claim 35, wherein (v) comprises comparing one or more current sequence read variations with one or more prior sequence read variations.

40. A method for detecting one or more genetic variants in a subject, comprising:
- (a) obtaining nucleic acid molecules from one or more cell-free biological samples of said subject;
 - (b) assaying said nucleic acid molecules to produce a first set of genetic data and a second set of genetic data, wherein said first set of genetic data and/or said second set of genetic data is within a detection threshold;
 - (c) comparing said first set of genetic data to said second set of genetic data to identify said one or more genetic variants in said first set of genetic data or said second set of genetic data; and
 - (d) based on said one or more genetic variants identified in (c), using one or more programmed computer processors to update a diagnostic confidence indication for identifying said one or more genetic variants in a cell-free biological sample of said subject.

41. The method of claim 40, wherein said first set of genetic data and said second set of genetic data are within said detection threshold.

42. The method of claim 40, wherein said first set of genetic data is within said detection threshold and said second set of genetic data is above said detection threshold.

43. The method of claim 40, wherein said detection threshold is a noise threshold.
44. The method of claim 43, further comprising identifying said one or more genetic variants in said first set of genetic data, and increasing said diagnostic confidence indication.
45. The method of claim 40, wherein subsets of said nucleic acid molecules are assayed at different time points.
46. The method of claim 40, wherein said nucleic acid molecules are obtained from a plurality of cell-free biological samples at the same time point or different time points.
47. The method of claim 40, wherein said nucleic acid molecules are cell-free deoxyribose nucleic acid.
48. The method of claim 40, wherein a co-variate variant is identified in said first set of genetic data in (c), and further comprising updating said diagnostic confidence indication for identifying a second co-variate variant in a cell-free biological sample of said subject.
49. The method of claim 40, further comprising (1) increasing said diagnostic confidence indication in (c) if said first set of genetic data is observed in said second set of genetic data, or (2) decreasing said diagnostic confidence indication in (c) if said first set of genetic data differs from said second set of genetic data.
50. The method of claim 40, wherein said detection threshold comprises a per-base error rate of 0.5 % to 5%.
51. The method of claim 40, wherein said first set of genetic data corresponds to said cell-free biological sample of (a) and said second set of genetic data corresponds to said second cell-free biological sample.
52. A method for calling a genetic variant in cell-free deoxyribose nucleic acids (cfDNA) from a subject comprising:
- (a) using a DNA sequencing system to sequence cfDNA from a sample taken at a first time point from a subject;
 - (b) detecting a genetic variant in the sequenced cfDNA from the first time point, wherein the genetic variant is detected at a level below a diagnostic limit;
 - (c) using the DNA sequencing system to sequence cfDNA from a sample taken from the subject at one or more subsequent time points;
 - (d) detecting the genetic variant in the sequenced cfDNA from the one or more subsequent time points, wherein the genetic variant is detected at level below the diagnostic limit;

(e) calling the samples as positive for the genetic variant based on detecting the genetic variant below the diagnostic limit in samples taken at a plurality of the time points.

53. A method for calling a genetic variant in cell-free deoxyribose nucleic acids (cfDNA) from a subject comprising:

(a) using a deoxyribonucleic acid (DNA) sequencing system to sequence cfDNA from a sample from a subject;

(b) detecting a genetic variant in the sequenced cfDNA, wherein the genetic variant is detected at a level below a diagnostic limit;

(c) using the DNA sequencing system to sequence cfDNA from the sample taken from the subject, wherein the sample is re-sequenced one or more times;

(d) detecting the genetic variant in the sequenced cfDNA from the one or more re-sequenced samples, wherein the genetic variant is detected at level below the diagnostic limit; and

(e) calling the samples as positive for the genetic variant based on detecting the genetic variant below the diagnostic limit in re-sequenced samples.

54. A method for detecting one or more genetic variants in a subject, comprising:

(a) obtaining nucleic acid molecules from one or more cell-free biological samples of said subject;

(b) assaying said nucleic acid molecules to produce a set of genetic data, wherein a genetic variant is in said set of genetic data within a detection threshold;

(c) identifying one or more co-variate genetic variations associated with the genetic variation by querying said set of genetic data within a detection threshold for one or more co-variate genetic variations associated with the genetic variation; and

(d) based on said one or more genetic variants identified in (c), using one or more programmed computer processors to update a diagnostic confidence indication for identifying said one or more genetic variants in a cell-free biological sample of said subject.

55. A method for processing genetic data, comprising:

(a) receiving genetic data from genetic analyzer, which genetic data is generated from a cell-free nucleic acid sample of a subject;

(b) using a programmed computer processor to analyze the genetic data to identify one or more genetic variants in the genetic data; and

(c) providing an output corresponding to the one or more genetic variants identified in (b) on an electronic portal.

56. The method of claim 55, wherein the one or more results are outputted on a web portal or graphical user interface.

57. A method comprising:

- (a) using a gene sequencing system to sequence cfDNA from a subject and generate sequencing data;
- (b) receiving into computer memory the sequence data;
- (c) using a processor to execute logic to:
 - (i) determine the frequency genetic variants at one or more genetic loci;
 - (ii) determining whether the frequency of a genetic variant is above or below a predetermined diagnostic limit;
 - (iii) if the frequency is above the diagnostic limit, calling the genetic variant as present in the sample;
 - (iv) or, if the frequency is below the diagnostic limit, accessing sequence days from the subject taken at another time and if the variant is detected at the other time point, calling to genetic variant as present;
 - (v) generating a report identifying genetic variants called as present;
- (d) transmitting the report to website where in the report is viewable on a graphical user interface.