DIAGNOSTIC METHODS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/155,755, filed May 1, 2015, which application is incorporated herein by reference.

BACKGROUND

[0002] Cancer is a major cause of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it. In many countries, cancer ranks the second most common cause of death following cardiovascular diseases.

[0003] To detect cancer, several screening tests are available. A physical exam and history surveys general signs of health, including checking for signs of disease, such as lumps or other unusual physical symptoms. A history of the patient's health habits and past illnesses and treatments will also be taken. Laboratory tests are another type of screening test and may require medical procedures to procure samples of tissue, blood, urine, or other substances in the body before conducting laboratory testing. Imaging procedures screen for cancer by generating visual representations of areas inside the body. Genetic tests detect certain gene deleterious mutations linked to some types of cancer. Genetic testing is particularly useful for a number of diagnostic methods.

[0004] One approach for cancer screening may include the monitoring of a sample derived from cell free nucleic acids, a population of polynucleotides that can be found in different types of bodily fluids. In some cases, disease may be characterized or detected based on detection of genetic variations, such as a change in copy number variation and/or sequence variation of one or more nucleic acid sequences, or the development of other certain rare genetic alterations. Cell free DNA ("cfDNA") may contain genetic variations associated with a particular disease. With improvements in sequencing and techniques to manipulate nucleic acids, there is a need in the art for improved methods and systems for using cell free DNA to detect and monitor disease.

SUMMARY

[0005] In an example~~aspect,~~ the present disclosure provides 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.

[0005a] In an aspect, the present disclosure provides a method for analyzing a disease state of a subject, comprising:

(a) using a genetic analyzer to generate genetic data from cell-free nucleic acid molecules in a plurality of biological samples of the subject obtained at (i) two or more time points or (ii) substantially the same time point, wherein the genetic data comprises sequence information corresponding to said cell-free nucleic acid molecules;

(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 indicative of one or more genetic variations associated with the disease state of the subject by processing the sequence information of ~~a sample~~ of the plurality of biological samples,

wherein the adjusted test result comprises a reduction of noise compared to a test result produced from genetic data generated from only one biological sample of the plurality of biological samples,

wherein the genetic data comprises sequence reads of the sample of the plurality of biological samples and sequence reads from one or more additional samples of the plurality of biological samples, and a first co-variate variation is detected in ~~at least~~ the sequence reads of the one or more additional samples,

wherein the processing comprises comparing the sequence reads of the sample of the plurality of biological samples with the sequence reads of one or more additional samples of the plurality of biological samples, and updating a diagnostic confidence indication accordingly by:

(i) increasing the diagnostic confidence indication if the information from a first time point corroborates information from a second time point, or

(ii) decreasing the diagnostic confidence indication if the information from the first time point conflicts with information from the second time point, or

(iii) leaving as is the diagnostic confidence indication in a subsequent characterization for de novo information,

which diagnostic confidence indication is indicative of a probability of accurately identifying the one or more genetic variations in the sample of the subject, and

wherein ~~the~~ updating the diagnostic confidence indication further comprises increasing the diagnostic confidence indication in a subsequent characterization ~~subsequent to the~~

~~detection of the first co-variate variation~~ if a second co-variate variation is detected in the sequence reads of the sample; and

(d) outputting the adjusted test result into computer memory.

[0006] In some examples~~embodiments~~, 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.

[0007] In some examples~~embodiments~~, the method further comprises generating a confidence interval for the current sequence reads. In some examples~~embodiments~~, the method further comprises comparing the confidence interval with one or more prior confidence intervals and determining a disease progression based on overlapping confidence intervals.

[0008] In some examples~~embodiments~~, 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. In some examples~~embodiments~~, 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.

[0009] In some examples~~embodiments~~, 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.

[0010] In some examples~~embodiments~~, 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.

[0011] In some examples~~embodiments~~, the method further comprises obtaining a subsequent characterization and leaving as is a diagnostic confidence indication in the subsequent characterization for de novo information. In some examples~~embodiments~~, the method further comprises 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.

In some examples~~embodiments~~, the method further comprises 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. In some examples~~embodiments~~, the method further comprises using the adjusted test result to provide (i) a therapeutic intervention or (ii) a diagnosis of a health or disease to the subject.

[0012] In some examples~~embodiments~~, the genetic data comprises sequence data from potions of a genome comprising disease-associated or cancer associated genetic variants.

[0013] In some examples~~embodiments~~, the method further comprises 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.

[0014] In some examples~~embodiments~~, 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. In some examples~~embodiments~~, the detection threshold is a noise threshold. In some examples~~embodiments~~, the method further comprises, 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. In some examples~~embodiments~~, the method further comprises, 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.

[0015] In some examples~~embodiments~~, the disease state is cancer and the genetic analyzer is a nucleic acid sequencer.

[0016] In some examples~~embodiments~~, the biological samples include at least two different types of biological samples. In some examples~~embodiments~~, the biological samples include the same type of biological sample. In some examples~~embodiments~~, the biological samples are blood samples. In some examples~~embodiments~~, the nucleic acid molecules are cell-free deoxyribonucleic acid (DNA).

[0017] In another example~~aspect~~, the present disclosure provides 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.

[0018] In some examples~~embodiments~~, the cancer polynucleotides are deoxyribonucleic acid (DNA) molecules. In some examples~~embodiments~~, the DNA is cell-free DNA.

[0019] In some examples~~embodiments~~, the frequency at each of the plurality of time points is determined by sequencing nucleic acid molecules in biological samples of the subject. In some examples~~embodiments~~, the biological samples are blood samples. In some examples~~embodiments~~, the nucleic acid molecules are cell-free deoxyribonucleic acid (DNA).

[0020] In another example~~aspect~~, the present disclosure provides 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.

[0021] In some examples~~embodiments~~, the method further comprises obtaining the cell-free nucleic acid molecules from the subject.

[0022] In some examples~~embodiments~~, the method further comprises sequencing additional cell-free nucleic acid molecules of the subject to generate a third set of sequence reads at a third time point subsequent to the first time point, and detecting a presence or absence of the one or more genetic variations and/or amount of genetic variation in the additional cell-free nucleic acid molecules of the subject based on the diagnostic confidence indication.

[0023] In some examples~~embodiments~~, the method further comprises 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.

[0024] In some examples~~embodiments~~, the method further comprises 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. In some examples~~embodiments~~, the method further comprises leaving as is the diagnostic confidence indication in a subsequent characterization for de novo information.

[0025] In another example~~aspect~~, the present disclosure provides 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.

[0026] In some examples~~embodiments~~, the method further comprises prior to (a), providing a plurality of sets of tagged parent polynucleotides derived from the cell-free nucleic acid sample, wherein each set is mappable to a different reference sequence.

[0027] In some examples~~embodiments~~, the method further comprises: 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.

[0028] In another example~~aspect~~, the present disclosure provides 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.

[0029] In some examples~~embodiments~~, the method further comprises increasing the diagnostic confidence indication if the set of sequencing reads is identified in the at least one prior time period. In some examples~~embodiments~~, the method further comprises decreasing the diagnostic

confidence indication if the set of sequencing reads is not identified in the at least one prior time period. In some examples~~embodiments~~, the method further comprises 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.

[0030] In some examples~~embodiments~~, the set of sequencing reads comprises at least one sequencing read.

[0031] In some examples~~embodiments~~, the biological sample is a blood sample. In some examples~~embodiments~~, the biological sample comprises cell-free nucleic acid molecules, and at least one set of tagged parent polynucleotides are generated from the cell-free nucleic acid molecules.

[0032] In some examples~~embodiments~~, the method further comprises generating a genetic profile of polynucleotides of the subject, which genetic profile includes an analysis of one or more genetic variants of the subject. In some examples~~embodiments~~, the polynucleotides include extracellular polynucleotides.

[0033] In another example~~aspect~~, the present disclosure provides 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.

[0034] In some examples~~embodiments~~, the bodily sample is a blood sample. In some examples~~embodiments~~, the extracellular polynucleotides include cell-free deoxyribonucleic acid (DNA) molecules.

[0035] In another example~~aspect~~, the present disclosure provides 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.

[0036] In some examples~~embodiments~~, the bodily sample is a blood sample. In some examples~~embodiments~~, the initial starting genetic material includes cell-free deoxyribonucleic acid (DNA).

[0037] In some examples~~embodiments~~, the set of consensus sequences for each set of tagged parent molecules is analyzed separately.

[0038] In some examples~~embodiments~~, analyzing comprises detecting mutations, indels, copy number variations, transversions, translocations, inversion, deletions, aneuploidy, partial aneuploidy, polyploidy, chromosomal instability, chromosomal structure alterations, gene fusions, chromosome fusions, gene truncations, gene amplification, gene duplications, chromosomal lesions, DNA lesions, abnormal changes in nucleic acid chemical modifications, abnormal changes in epigenetic patterns, abnormal changes in nucleic acid methylation infection or cancer.

[0039] In some examples~~embodiments~~, (vi) comprises increasing diagnostic confidence indication in the current sequence reads if information from the prior sequence reads corroborates information from the current sequence reads. In some examples~~embodiments~~, (vi) comprises 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. In some examples~~embodiments~~, (vi) comprises 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.

[0040] In some examples~~embodiments~~, (v) comprises comparing one or more current sequence read variations with one or more prior sequence read variations.

[0041] In another example~~aspect~~, the present disclosure provides 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.

[0042] In some examples~~embodiments~~, said first set of genetic data and said second set of genetic data are within said detection threshold. In some examples~~embodiments~~, said first set of genetic data is within said detection threshold and said second set of genetic data is above said detection threshold. In some examples~~embodiments~~, said detection threshold is a noise threshold.

[0043] In some examples~~embodiments~~, the method further comprises identifying said one or more genetic variants in said first set of genetic data, and increasing said diagnostic confidence indication.

[0044] In some examples~~embodiments~~, subsets of said nucleic acid molecules are assayed at different time points. In some examples~~embodiments~~, said nucleic acid molecules are obtained from a plurality of cell-free biological samples at the same time point or different time points.

[0045] In some examples~~embodiments~~, said nucleic acid molecules are deoxyribose nucleic acid (DNA). In some examples~~embodiments~~, said DNA is cell-free DNA (cfDNA).

[0046] In some examples~~embodiments~~, the method further comprises generating a genetic profile for said subject, wherein said genetic profile comprises said diagnostic confidence indication for identifying said one or more genetic variants.

[0047] In some examples~~embodiments~~, 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. In some examples~~embodiments~~, the method further comprises increasing said diagnostic confidence indication in (c) if said first set of genetic data is observed in said second set of genetic data. In some examples~~embodiments~~, the method further comprises decreasing said diagnostic confidence indication in (c) if said first set of genetic data differs from said second set of genetic data.

[0048] In some examples~~embodiments~~, said detection threshold comprises errors introduced by sequencing or amplification.

[0049] In some examples~~embodiments~~, said detection threshold comprises a per-base error rate of 0.5 % to 5%. In some examples~~embodiments~~, said detection threshold comprises a per-base error rate of 0.5 % to 1%.

[0050] In some examples~~embodiments~~, said nucleic acid molecules are obtained from a second cell-free biological sample of said subject. In some examples~~embodiments~~, said second cell-free biological sample

is obtained after obtaining said cell-free biological sample of (a). In some examplesembodiments, said second cell-free biological sample is obtained prior to obtaining said cell-free biological sample of (a). In some examplesembodiments, said second cell-free biological sample is obtained concurrent with obtaining said cell-free biological sample of (a). In some examplesembodiments, 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.

[0051] In some examplesembodiments, the method further comprises: attaching tags to said nucleic acid molecules to generate tagged parent polynucleotides; amplifying said tagged parent polynucleotides to produce tagged progeny polynucleotides; and sequencing said tagged progeny polynucleotides to produce sequencing reads.

[0052] In some examplesembodiments, the attaching comprises uniquely tagging the nucleic acid molecules. In some examplesembodiments, the attaching comprises non-uniquely tagging said nucleic acid molecules such that no more than 5% of said nucleic acid molecules are uniquely tagged.

[0053] In some examplesembodiments, the method further comprises selectively enriching sequences of interest prior to the sequencing.

[0054] In some examplesembodiments, the method further comprises grouping said sequence reads into families based at least on a sequence tag. In some examplesembodiments, grouping the sequence reads is further based on one or more of: sequence information at a beginning of a sequence read derived from the nucleic acid molecule, sequence information at an end of said sequence derived from the nucleic acid molecule, and a length of said sequence read.

[0055] In some examplesembodiments, the method further comprises comparing the sequence reads grouped within each family to determine consensus sequences for each family, wherein each of the consensus sequences corresponds to a unique polynucleotide among the tagged parent polynucleotides.

[0056] In some examplesembodiments, the method further comprises obtaining less than 100 ng of the nucleic acid molecules.

[0057] In another exampleaspect, the present disclosure provides 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.

[0058] In some examples~~embodiments~~, the method further comprises (f) detecting a trend, wherein, at the first time point, the genetic variant is detected below the diagnostic limit and called as positive, and, at one or more subsequent time points, the genetic variant is detected above the diagnostic limit whereby the genetic variant is increasing.

[0059] In some examples~~embodiments~~, the diagnostic limit is less than or equal to about 1.0%.

[0060] In another example~~aspect~~, the present disclosure provides 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.

[0061] In another example~~aspect~~, the present disclosure provides a non-transitory computer readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0062] In another example~~aspect~~, the present disclosure provides a computer system comprising one or more computer processors and memory coupled thereto. The memory comprises a non-transitory computer readable medium comprising machine-executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0063] Additional examples ~~aspects~~ and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples ~~embodiments~~ of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples~~embodiments~~, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

CLAIMS

1. A method for analyzing a disease state of a subject, comprising:

(a) using a genetic analyzer to generate genetic data from cell-free nucleic acid molecules in a plurality of biological samples of the subject obtained at (i) two or more time points or (ii) substantially the same time point, wherein the genetic data comprises sequence information corresponding to said cell-free nucleic acid molecules;

(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 indicative of one or more genetic variations associated with the disease state of the subject by processing the sequence information ~~of a sample~~ of the plurality of biological samples,

wherein the adjusted test result comprises a reduction of noise compared to a test result produced from genetic data generated from only one biological sample of the plurality of biological samples,

wherein the genetic data comprises sequence reads of the sample of the plurality of biological samples and sequence reads from one or more additional samples of the plurality of biological samples, and a first co-variate variation is detected in ~~at least~~ the sequence reads of the one or more additional samples,

wherein the processing comprises comparing the sequence reads of the sample of the plurality of biological samples with the sequence reads of one or more additional samples of the plurality of biological samples, and updating a diagnostic confidence indication accordingly by:

(i) increasing the diagnostic confidence indication if the information from a first time point corroborates information from a second time point, or

(ii) decreasing the diagnostic confidence indication if the information from the first time point conflicts with information from the second time point, or

(iii) leaving as is the diagnostic confidence indication in a subsequent characterization for *de novo* information,

which diagnostic confidence indication is indicative of a probability of accurately identifying the one or more genetic variations in the sample of the subject, and

wherein ~~the~~ updating the diagnostic confidence indication further comprises increasing the diagnostic confidence indication in a subsequent characterization ~~subsequent to the detection of the first co-variate variation~~ if a second co-variate variation is detected in the sequence reads of the sample; and

(d) outputting the adjusted test result into computer memory.

2. The method of claim 1, further comprising generating a confidence interval for the detection of the one or more genetic variants in the sequence reads of the sample.

3. The method of claim 2, further comprising comparing the confidence interval with one or more prior confidence intervals and determining a disease progression based on overlapping confidence intervals.

4. The method of claim 1, wherein the plurality of biological samples is 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.

~~5. The method of claim 4, wherein the plurality of biological samples is 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 characterization subsequent to the second time~~

~~point if the information from the first time point corroborates information from the second time point.~~

~~6. The method of claim 4, wherein the plurality of biological samples is 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 the first time point conflicts with information from the second time point.~~

~~7. 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.~~

~~8.~~5. 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.

~~9.~~6. 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 of copy number variation at the two or more time points.

~~10.~~7. 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.

~~11.~~8. 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.

~~12.~~9. The method of claim ~~8~~11, wherein the detection threshold is a noise threshold.

~~13.~~10. The method of claim ~~8~~11, 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.

~~14.~~11. The method of claim ~~8~~11, 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.

~~15.~~12. The method of claim 1, wherein the disease state is cancer and the genetic analyzer is a nucleic acid sequencer.

~~16.~~13. The method of claim 1, wherein the plurality of biological samples includes at least two different types of biological samples.

~~17.~~14. The method of claim 1, wherein the plurality of biological samples includes the same type of biological sample.

~~18.~~15. The method of claim 14~~17~~, wherein the biological samples are blood samples.

~~19.~~16. The method of claim 15~~18~~, wherein the nucleic acid molecules are cell-free deoxyribonucleic acid (DNA).