[0004] In some examples, an MSI status may vary (e.g., increase or decrease) over a duration of time (e.g., over two or more different time points). In some examples, this duration of time may correspond to, e.g., a course of treatment for the cancer of the subject or a monitoring period after surgical resection or other treatment of a tumor for (e.g., to detect recurrence of the tumor in the subject). In some examples, generation of an MSI status may comprise generating a quantitative measure of cfDNA sequencing reads for each of a plurality of genetic loci corresponding to microsatellites. The plurality of genetic loci may comprise microsatellites, such as the entire set of microsatellite repeats in the human reference genome (or a subset thereof), a set of microsatellite repeats optimized to minimize noise in microsatellite stable (MSS) data (or a subset thereof), a set of microsatellite repeats all of the same class (such as all repeats whose repeated unit is of length one, or a subset thereof), a set of microsatellite repeat units that are within a certain range of sizes (e.g., lengths), a set of microsatellite repeats where the sequencing data indicate the lack of a confounding germline insertions or deletions (indels) (or a subset thereof), a set of microsatellite repeats optimized to maximize the performance of the algorithm given a set of training data (or a subset thereof), or a union or intersection of a combination thereof. In some cases, the quantitative measure of cfDNA (e.g., sequencing reads) may comprise a count of sequencing reads that align with each of the plurality of genetic loci. Alternatively, obtaining the quantitative measure of cfDNA may comprise performing binding measurements of the plurality of cfDNA molecules at each of the plurality of microsatellite repeat elements. In some examples, generation of an MSI status may comprise generating a comparison (e.g., a difference or a ratio) of quantitative measures for cfDNA (e.g., sequencing reads). By assessing a comparison of counts of sequencing reads across different sets of genetic loci corresponding to microsatellites, methods provided herein may allow generation of MSI statuses, which can be useful for diagnosis, prognosis, or treatment selection for a subject through a non-invasive lab test (e.g., a blood-based test).

[0005] In an aspect, the present disclosure provides a computer-implemented method of assessing microsatellite instability of a subject, comprising: obtaining, by one or more processors, a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject , wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules; processing, by the one or more processors, the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and detecting, by the one or more processors, a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

about 0.98. In some examples, the presence or absence of the microsatellite stability of the subject is detected with an area under the curve (AUC) of at least about 0.99.

[0021] In another aspect, the present disclosure provides a system, comprising a controller comprising or capable of accessing, a non-transitory computer-readable medium comprising machine-executable instructions which, upon execution by one or more computer processors, perform a method for assessing microsatellite instability of a subject, the method comprising: obtaining a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject, wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules; processing the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and detecting a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

[0022] In some examples, the quantitative measure of the plurality of microsatellite repeat elements is selected from the group consisting of a mean length at each of the plurality of microsatellite repeat elements (or a subset thereof), a number, frequency, or fraction of the plurality of microsatellite repeat elements having a length that falls within a predetermined size range (or a subset thereof), and a mean insertion or deletion (indel) length of each of the plurality of microsatellite repeat elements (or a subset thereof). In some examples, the subject is diagnosed with cancer. In some examples, the subject is asymptomatic for cancer. In some examples, the subject has one or more risk factors for cancer (e.g., age, sex, race, ethnicity, family history, history of tobacco or alcohol use, presence of genetic variants, or other clinical health characteristics). In some examples, the plurality of quantitative measures is measured from a plurality of cell-free DNA (cfDNA) molecules. In some examples, the plurality of quantitative measures is measured from a set of sequencing reads at each of the plurality of microsatellite repeat elements in the plurality of cfDNA molecules. In some examples, the method of the system further comprises sequencing the plurality of cfDNA molecules to generate the set of sequencing reads. In some examples, the sequencing comprises whole genome sequencing (WGS). In some examples, the sequencing is performed at a depth of no more than about 50X, no more than about 48X, no more than about 46X, no more than about 44X, no more than about 42X, no more than about 40X, no more than about 38X, no more than about 36X, no more than about 34X, no more than about 32X, no more than about 30X, no more than about 28X, no more than about 24X, no more than about 22X, no more than about 20X, no more than about 18X, no more than about 16X, no more than about 14X, or no more than about 12X.

[0032] In another 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 a method for assessing microsatellite instability of a subject, the method comprising: obtaining a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject, wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules; processing the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and detecting a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

[0033] In some examples, the quantitative measure of the plurality of microsatellite repeat elements is selected from the group consisting of a mean length at each of the plurality of microsatellite repeat elements (or a subset thereof), a number, frequency, or fraction of the plurality of microsatellite repeat elements having a length that falls within a predetermined size range (or a subset thereof), and a mean insertion or deletion (indel) length of each of the plurality of microsatellite repeat elements (or a subset thereof). In some examples, the subject is diagnosed with cancer. In some examples, the subject is asymptomatic for cancer. In some examples, the subject has one or more risk factors for cancer (e.g., age, sex, race, ethnicity, family history, history of tobacco or alcohol use, presence of genetic variants, or other clinical health characteristics). In some examples, the plurality of quantitative measures is measured from a plurality of cell-free DNA (cfDNA) molecules. In some examples, the plurality of quantitative measures is measured from a set of sequencing reads at each of the plurality of microsatellite repeat elements in the plurality of cfDNA molecules. In some examples, the method of the non-transitory computer-readable medium further comprises sequencing the plurality of cfDNA molecules to generate the set of sequencing reads. In some examples, the sequencing comprises whole genome sequencing (WGS). In some examples, the sequencing is performed at a depth of no more than about 50X, no more than about 48X, no more than about 46X, no more than about 44X, no more than about 42X, no more than about 40X, no more than about 38X, no more than about 36X, no more than about 34X, no more than about 32X, no more than about 30X, no more than about 28X, no more than about 24X, no more than about 22X, no more than about 20X, no more than about 18X, no more than about 16X, no more than about 14X, or no more than about 12X. In some examples, the sequencing is performed at a depth of no more than about 10X. In some examples, the sequencing is performed at a depth of no more than about 8X. In some examples, the sequencing is performed at a depth of no more

CLAIMS

WHAT IS CLAIMED IS:

1. A computer-implemented method for assessing microsatellite instability of a subject, comprising:

obtaining, by one or more processors, a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject, wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules;

processing, by the one or more processors, the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and

detecting, by the one or more processors, a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

2. The method of claim 1, wherein the quantitative measure of the plurality of microsatellite repeat elements is a mean length at each of the plurality of microsatellite repeat elements, a number or fraction of the plurality of microsatellite repeat elements having a length in a predetermined size range, or a mean insertion or deletion (indel) length of each of the plurality of microsatellite repeat elements.

3. The method of claim 1, wherein the subject is diagnosed with cancer.

4. The method of claim 1, wherein the plurality of quantitative measures is measured from a set of sequencing reads at each of the plurality of microsatellite repeat elements in the plurality of cfDNA molecules.

5. The method of claim 4, further comprising sequencing the plurality of cfDNA molecules to generate the set of sequencing reads.

6. The method of claim 4, wherein the sequencing comprises whole genome sequencing (WGS).

7. The method of claim 1, wherein measuring the plurality of quantitative measures comprises performing binding measurements of the plurality of cfDNA molecules at each of the plurality of microsatellite repeat elements.

8. The method of claim 1, further comprising, based on the detected presence or absence of the microsatellite instability of the subject, identifying a treatment for the subject.

9. The method of claim 8, wherein the treatment is a chemotherapy, a radiation therapy, or an immunotherapy.

10. The method of claim 1, further comprising enriching the plurality of cfDNA molecules for at least a subset of the plurality of microsatellite repeat elements.

11. The method of claim 10, wherein the enrichment comprises (a) amplifying the plurality of cfDNA molecules, or (b) selectively isolating at least a portion of the plurality of cfDNA molecules.

12. The method of claim 1, wherein the statistical measure of deviation is a mean z-score.

13. The method of claim 1, wherein the statistical measure of deviation is a mean z-score relative to a reference blood sample.

14. The method of claim 13, wherein the reference blood sample is obtained from a subject having microsatellite instability.

15. The method of claim 13, wherein the reference blood sample is obtained from a subject not having microsatellite instability.

16. The method of claim 12, wherein the predetermined criterion is the absolute value of the mean z-score being greater than a predetermined number.

17. The method of claim 1, wherein the plurality of microsatellite repeat elements comprises mononucleotides or dinucleotides.

18. The method of claim 17, wherein the plurality of microsatellite repeat elements comprises mononucleotides and dinucleotides.

19. The method of claim 1, wherein the plurality of microsatellite repeat elements comprises at least 1 million distinct microsatellite repeat elements.

20. The method of claim 1, wherein the presence or absence of the microsatellite instability of the subject is detected with a sensitivity of at least 90%.

21. The method of claim 1, wherein the presence of the microsatellite instability of the subject is detected with a positive predictive value (PPV) of at least 90%.

22. The method of claim 1, wherein the presence or absence of the microsatellite instability of the subject is detected with an area under the curve (AUC) of at least 0.90.

23. The method of claim 1, further comprising detecting a presence of a microsatellite stability (MSS) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

24. A system, comprising a controller comprising or capable of accessing, a non-transitory computer-readable medium comprising machine-executable instructions which, upon execution

by one or more computer processors, perform a method for assessing microsatellite instability of a subject, the method comprising:

obtaining a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject, wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules;

processing the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and

detecting a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.

25. A non-transitory computer-readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements a method for assessing microsatellite instability of a subject, the method comprising:

obtaining a quantitative measure of a plurality of microsatellite repeat elements from a blood sample of a subject, wherein the plurality of quantitative measures is measured from a plurality of tumor-derived cell-free DNA (cfDNA) molecules;

processing the plurality of quantitative measures to obtain a statistical measure of deviation of the plurality of quantitative measures; and

detecting a presence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures satisfies a predetermined criterion, or detecting an absence of the microsatellite instability (MSI) of the subject when the statistical measure of deviation of the plurality of quantitative measures does not satisfy the predetermined criterion.