

## CLAIMS

### WHAT IS CLAIMED IS:

1. A method of identifying a sequence variant in a nucleic acid sample comprising a plurality of polynucleotides, each polynucleotide of the plurality having a 5' end and a 3' end, the method comprising:

(a) circularizing individual polynucleotides of said plurality to form a plurality of circular polynucleotides, each of which having a junction between the 5' end and 3' end;

(b) amplifying the circular polynucleotides of (a);

(c) sequencing the amplified polynucleotides to produce a plurality of sequencing reads;

(d) identifying sequence differences between sequencing reads and a reference sequence; and

(e) calling a sequence difference that occurs in at least two circular polynucleotides having different junctions as the sequence variant.

2. A method of identifying a sequence variant, the method comprising: identifying sequence differences between sequencing reads and a reference sequence, and calling a sequence difference that occurs in at least two circular polynucleotides having different junctions as the sequence variant, wherein:

(a) the sequencing reads correspond to amplification products of the at least two circular polynucleotides; and

(b) each of the at least two circular polynucleotides comprises a different junction formed by ligating a 5' end and 3' end of the respective polynucleotides.

3. The method of claim 1 or 2, wherein the plurality of polynucleotides are single-stranded.

4. The method of claim 1 or 2, wherein circularizing is effected by subjecting the plurality of polynucleotides to a ligation reaction.

5. The method of claim 1 or 2, wherein an individual circular polynucleotide has a junction that is unique among the circularized polynucleotides.

6. The method of claim 1 or 2, wherein the sequence variant is a single nucleotide polymorphism.

7. The method of claim 1 or 2, wherein the reference sequence is a consensus sequence formed by aligning the sequencing reads with one another.

8. The method of claim 1 or 2, wherein the reference sequence is a known reference sequence.

9. The method of claim 1, wherein circularizing comprises the step of joining an adapter polynucleotide to the 5' end, the 3' end, or both the 5' end and the 3' end of a polynucleotide in the plurality of polynucleotides.

10. The method of claim 1, wherein amplifying is effected by using a polymerase having strand-displacement activity.

11. The method of claim 1, wherein amplifying comprises subjecting the circular polynucleotides to an amplification reaction mixture comprising random primers.

12. The method of claim 1, wherein amplifying comprises subjecting the circular polynucleotides to an amplification reaction mixture comprising one or more primers, each of which specifically hybridizes to a different target sequence via sequence complementarity.

13. The method of claim 11 or 12, wherein the amplified polynucleotides are subjected to the sequencing step without enrichment.

14. The method of claim 11 or 12, further comprising enriching one or more target polynucleotides among the amplified polynucleotides by performing an enrichment step prior to sequencing.

15. The method of claim 14, wherein the enrichment step comprises hybridizing amplified polynucleotides to a plurality of probes attached to a substrate.

16. The method of claim 14, wherein the enrichment step comprises amplifying a target sequence comprising sequence A and sequence B oriented in a 5' to 3' direction in an amplification reaction mixture comprising:

(a) the amplified polynucleotides;

(b) a first primer comprising sequence A', wherein the first primer specifically hybridizes to sequence A of the target sequence via sequence complementarity between sequence A and sequence A';

(c) a second primer comprising sequence B, wherein the second primer specifically hybridizes to sequence B' present in a complementary polynucleotide comprising a complement of the target sequence via sequence complementarity between B and B'; and

(d) a polymerase that extends the first primer and the second primer to produce amplified polynucleotides;

wherein the distance between the 5' end of sequence A and the 3' end of sequence B of the target sequence is 75nt or less.

17. The method of claim 1 or 2, wherein a microbial contaminant is identified based on the calling step.

18. A system for detecting a sequence variant, the system comprising:

(a) a computer configured to receive a user request to perform a detection reaction on a sample;

(b) an amplification system that performs a nucleic acid amplification reaction on the sample or a portion thereof in response to the user request, wherein the amplification reaction comprises the steps of (i) circularizing individual polynucleotides to form a plurality of circular polynucleotides, each of which having a junction between the 5' end and 3' end; and (ii) amplifying the circular polynucleotides;

(c) a sequencing system that generates sequencing reads for polynucleotides amplified by the amplification system, identifies sequence differences between sequencing reads and a reference sequence, and calls a sequence difference that occurs in at least two circular polynucleotides having different junctions as the sequence variant; and

(d) a report generator that sends a report to a recipient, wherein the report contains results for detection of the sequence variant.

19. The system of claim 18, wherein the recipient is the user.

20. A computer-readable medium comprising codes that, upon execution by one or more processors, implement a method of detecting a sequence variant, the method comprising:

(a) receiving a customer request to perform a detection reaction on a sample;

(b) performing a nucleic acid amplification reaction on the sample or a portion thereof in response to the customer request, wherein the amplification reaction comprises the steps of (i) circularizing individual polynucleotides to form a plurality of circular polynucleotides, each of which having a junction between the 5' end and 3' end; and (ii) amplifying the circular polynucleotides;

(c) performing a sequencing analysis comprising the steps of (i) generating sequencing reads for polynucleotides amplified in the amplification reaction; (ii) identifying sequence differences between sequencing reads and a reference sequence; and (iii) calling a sequence difference that occurs in at least two circular polynucleotides having different junctions as the sequence variant; and

(d) generating a report that contains results for detection of the sequence variant.

21. The method of claim 1 or 2, wherein the sample is a sample from a subject.

22. The method of claim 21, wherein the sample is urine, stool, blood, saliva, tissue, or bodily fluid.

23. The method of claim 21, wherein the sample comprises tumor cells.

24. The method of claim 21, wherein the sample is a formalin-fixed paraffin embedded (FFPE) sample.

25. The method of claim 21, further comprising diagnosing said subject based on the calling step.
26. The method of claim 1 or 2, wherein the sequence variant is a causal genetic variant.
27. The method of claim 1 or 2, wherein the sequence variant is associated with a type or stage of cancer.
28. The method of claim 1 or 2, wherein the plurality of polynucleotides comprise cell-free polynucleotides.
29. The method of claim 28, wherein the cell-free polynucleotides comprise circulating tumor DNA.
30. The method of claim 1, wherein the polynucleotides of the sample comprise cell-free polynucleotides.