

[0039] In another aspect, this disclosure provides a method for enriching a sample for polynucleotides comprising a breakpoint of a fusion gene, comprising: a) contacting a probe set of claim 20 with a mixture of polynucleotides under hybridization conditions to produce probe-captured polynucleotides; and b) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with polynucleotides comprising breakpoint fragments of the fusion gene. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides. In another embodiment the polynucleotides comprise cell-free DNA or fragmented genomic DNA. In another embodiment the method further comprises isolating captured polynucleotides from the probes. In another embodiment the method further comprises sequencing the isolated polynucleotides.

[0040] In another aspect, this disclosure provides method of diagnosing cancer in a subject comprising: a) providing a sample comprising polynucleotides from a subject; b) contacting the cell-free DNA (cfDNA) from the sample with a probe set of claim 20 under hybridization conditions to produce probe-captured polynucleotides; c) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with polynucleotides comprising breakpoint fragments of the fusion gene; d) sequencing the isolated polynucleotides to produce sequences; e) detecting polynucleotides comprising breakpoints of fusion genes based on the sequences; and f) diagnosing cancer based on the detection of breakpoint fragments. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

[0041] Another aspect of 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.

[0042] Another aspect of the present disclosure provides a system comprising one or more computer processors and a non-transitory computer-readable medium coupled thereto. The non-transitory computer readable medium comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0042a] In another aspect, this disclosure provides a method for providing a diagnostic or therapeutic intervention to a subject having or suspected of having cancer, comprising:

(a) contacting cell-free deoxyribonucleic acid (cfDNA) molecules obtained from a biological sample of a subject or derivatives thereof with a probe set comprising one or more locked nucleic acid (LNA) nucleotides under hybridization conditions sufficient to produce probe-captured polynucleotides, which probe set comprises a plurality of polynucleotide probes, wherein each of the plurality of polynucleotide probes has (i) sequence complementarity with a fusion gene and (ii) affinity for the fusion gene that is greater than a polynucleotide having the sequence complementarity with the fusion gene and containing only unmodified nucleotides;

(b) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with the isolated polynucleotides comprising breakpoint fragments of the fusion gene;

(c) sequencing the isolated polynucleotides to produce sequences;

(d) detecting the polynucleotides comprising breakpoints of fusion genes based on the sequences; and

(e) providing the diagnostic or therapeutic intervention based on the detection of breakpoint fragments.

[0042b] In another aspect, this disclosure provides a method for capturing a breakpoint fragment of a fusion gene, comprising:

(a) contacting cell-free deoxyribonucleic acid (cfDNA) molecules or obtained from a biological sample of a subject or derivatives thereof with a polynucleotide probe, which comprises one or more locked nucleic acid (LNA) nucleotides, under conditions sufficient to:

i. permit hybridization between the polynucleotide probe and the breakpoint fragment to provide a probe-captured polynucleotide in a mixture, which polynucleotide probe has sequence complementarity with the breakpoint fragment and has affinity for the fusion gene that is greater than a polynucleotide having the sequence complementary and containing only unmodified nucleotides; and

ii. enrichment or isolation of the probe-captured polynucleotide from the mixture, wherein the polynucleotide probe has sequence complementarity with the breakpoint fragment,

(b) eluting the probe-captured polynucleotides to isolate the captured polynucleotides from the probes; and

(c) directly sequencing the eluted polynucleotides or using the eluted polynucleotides to produce sequencing libraries,

wherein the biological sample contains or is suspected of containing a cell-free nucleic acid molecule comprising the breakpoint fragment of the fusion gene.

[0043] Additional 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 embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different 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

WHAT IS CLAIMED IS:

1. A method for providing a diagnostic or therapeutic intervention to a subject having or suspected of having cancer, comprising:
 - (a) contacting cell-free deoxyribonucleic acid (cfDNA) molecules obtained from a biological sample of a subject or derivatives thereof with a probe set comprising one or more locked nucleic acid (LNA) nucleotides under hybridization conditions sufficient to produce probe-captured polynucleotides, which probe set comprises a plurality of polynucleotide probes, wherein each of the plurality of polynucleotide probes has (i) sequence complementarity with a fusion gene and (ii) affinity for the fusion gene that is greater than a polynucleotide having the sequence complementarity with the fusion gene and containing only unmodified nucleotides;
 - (b) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with the isolated polynucleotides comprising breakpoint fragments of the fusion gene;
 - (c) sequencing the isolated polynucleotides to produce sequences;
 - (d) detecting the polynucleotides comprising breakpoints of fusion genes based on the sequences; and
 - (e) providing the diagnostic or therapeutic intervention based on the detection of breakpoint fragments.
2. The method of claim 1, wherein each of the plurality of polynucleotide probes comprises a plurality LNA nucleotides, wherein at least two of the LNA nucleotides are spaced no more than 30 nucleotides apart.
3. The method of claim 2, wherein the at least two of the LNA nucleotides are spaced no more than 15 apart.
4. The method of claim 1, wherein at least 50% of the nucleotides of each of at least a subset of the plurality of polynucleotide probes are LNA nucleotides.
5. The method of claim 4, wherein at least 75% of the nucleotides of each of at least a subset of the plurality of polynucleotide probes are LNA nucleotides.
6. The method of claim 1, wherein each of the plurality of polynucleotide probes has a melting temperature that is at least about 1 °C higher than the polynucleotide having sequence complementarity with the fusion gene and containing only unmodified nucleotides.

7. The method of claim 6, wherein the melting temperature is at least about 10 °C higher.
8. The method of claim 1, wherein each of the plurality of polynucleotide probes has a melting temperature that is at least about 2 % higher than the polynucleotide having sequence complementarity with the fusion gene and containing only unmodified nucleotides.
9. The method of claim 8, wherein the melting temperature is at least about 10% higher.
10. The method of claim 1, wherein the fusion gene is a cancer fusion gene.
11. The method of claim 1, wherein each of the plurality of polynucleotide probes has sequence complementarity with a gene of a fusion gene pair of FIGs. 2A-2B or a fusion gene between two or more genes selected from FIG. 3.
12. The method of claim 1, wherein each of the plurality of polynucleotide probes has sequence complementarity with a breakpoint region no more than 500 nucleotides away from a breakpoint of the fusion gene.
13. The method of claim 1, wherein each of the plurality of polynucleotide probes has sequence complementarity with a sequence across a breakpoint in the fusion gene.
14. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length less than about 500 nucleotides.
15. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length between about 20 and about 200 nucleotides.
16. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length between about 80 and about 160 nucleotides.
17. The method of claim 1, wherein each of the breakpoint fragments has a length between about 140 nucleotides and 180 nucleotides.
18. The method of claim 1, wherein the plurality of polynucleotide probes is coupled to a solid support.
19. The method of claim 1, wherein the probe set comprises one or more natural polynucleotide probes.
20. The method of claim 1, wherein the plurality of polynucleotide probes comprises at least one polynucleotide probe that hybridizes to a breakpoint region of a nucleic acid sequence included in the fusion gene, and at least one natural polynucleotide probe that hybridizes to a non-breakpoint region of the nucleic acid sequence included in the fusion gene.

21. The method of claim 1, wherein each of the plurality of polynucleotide probes provides at least 50% coverage of a breakpoint region no more than 500 nucleotides away from a breakpoint of a nucleic acid sequence included in the fusion gene.
22. The method of claim 1, wherein (d) comprises attaching, to the isolated polynucleotides, tags comprising barcodes having distinct barcode sequences to generate tagged parent polynucleotides.
23. The method of claim 22, further comprising amplifying the tagged parent polynucleotides to produce tagged progeny polynucleotides.
24. The method of claim 23, further comprising (i) sequencing the tagged progeny polynucleotides to produce sequence reads, wherein each sequence read comprises a barcode sequence and a sequence derived from a given one of the isolated polynucleotides, and (ii) grouping the sequence reads into families based at least on the barcode sequence.
25. The method of claim 24, further comprising 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.
26. A method for capturing a breakpoint fragment of a fusion gene, comprising:
- (a) contacting cell-free deoxyribonucleic acid (cfDNA) molecules or obtained from a biological sample of a subject or derivatives thereof with a polynucleotide probe, which comprises one or more locked nucleic acid (LNA) nucleotides, under conditions sufficient to:
 - i. permit hybridization between the polynucleotide probe and the breakpoint fragment to provide a probe-captured polynucleotide in a mixture, which polynucleotide probe has sequence complementarity with the breakpoint fragment and has affinity for the fusion gene that is greater than a polynucleotide having the sequence complementary and containing only unmodified nucleotides; and
 - ii. enrichment or isolation of the probe-captured polynucleotide from the mixture, wherein the polynucleotide probe has sequence complementarity with the breakpoint fragment,
 - (b) eluting the probe-captured polynucleotides to isolate the captured polynucleotides from the probes; and
 - (c) directly sequencing the eluted polynucleotides or using the eluted polynucleotides to produce sequencing libraries,

wherein the biological sample contains or is suspected of containing a cell-free nucleic acid molecule comprising the breakpoint fragment of the fusion gene.

27. The method of claim 26, wherein the polynucleotide probe comprises a plurality LNA nucleotides, wherein at least two of the LNA nucleotides are spaced no more than 30 nucleotides apart.
28. The method of claim 27, wherein the at least two of the LNA nucleotides are spaced no more than 15 nucleotides apart.