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(71) Applicant: **GUARDANT HEALTH, INC.** [US/US]; 505 Penobscot Drive, Redwood City, CA 94063 (US).

(72) Inventor: **MORTIMER, Stefanie Ann, Ward**; 2000 Willow Springs Road, Morgan Hill, CA 95037 (US).

(74) Agents: **STRONCEK, Jacqueline** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (US).

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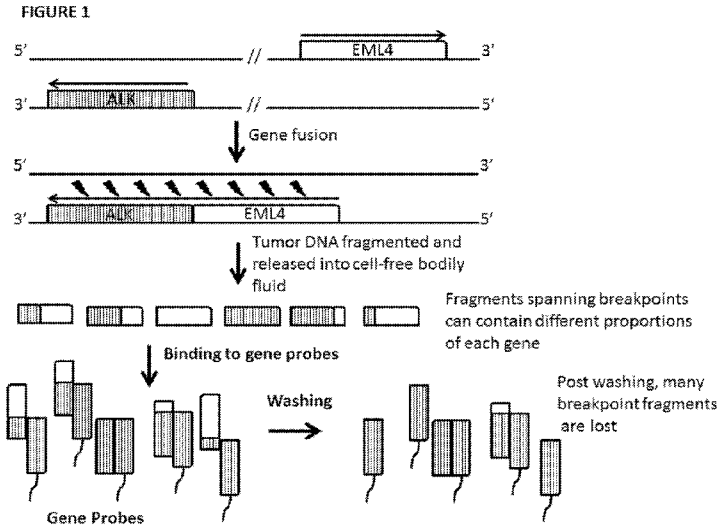
(54) Title: LOCKED NUCLEIC ACIDS FOR CAPTURING FUSION GENES

FIGURE 1



(57) Abstract: Provided herein is a method for enriching a sample for polynucleotides comprising a breakpoint of a fusion gene, comprising: a) contacting a probe set comprising a plurality of polynucleotide probes, each probe configured to specifically hybridize to a fusion gene, wherein the set comprises one or more high affinity polynucleotide probes (e.g., a polynucleotide comprising one or more locked nucleic acid nucleotides), 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.

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LOCKED NUCLEIC ACIDS FOR CAPTURING FUSION GENES

CROSS-REFERENCE

[001] This application claims priority to U.S. Provisional Patent Application No. 62/195,280, filed July 21, 2015, which is entirely incorporated herein by reference.

BACKGROUND

[002] Gene fusion events are chromosomal rearrangements that bring together formerly separate portions of at least two genes in a genome. Gene fusion events can result in cancer fusion genes, where the aberrant juxtaposition of two or more genes can encode a fusion protein, or the regulatory elements of one gene can drive the aberrant expression of an oncogene. Detecting such cancer fusion genes can be difficult. Breakpoint fragments are less likely to hybridize to probes to the same extent as fragments that do not contain breakpoints. Therefore, hybridization methods for enrichment of breakpoint fragments can lack efficacy.

[003] Fusion genes are a form of somatic mutation found in cancer cells. The ability to detect such fusion genes is useful in the diagnosis and monitoring of cancer.

[004] Fusion genes known to be found in cancer include, for example, the following: APIP/SLC1A2 in colon cancer, ATG7/RAF1 in pancreatic cancer, BCL6/RAF1 in astrocytoma, BCR-ABL in chronic myeloid leukemia, BRD4-NUT in midline carcinomas, CEP85L/ROS1 in angiosarcoma, CLTC/VMP1 in breast cancer, ELM4-ALK in lung cancer, EWSR1/CREM in in melanoma, FAM133B/CDK6 in T-cell acute lymphoblastic leukemia, KIAA1549–BRAF (at 7q34) in low-grade astrocytoma, MECT1-MAML2 in mucoepidermoid carcinoma, PAX8-PPARG in follicular thyroid carcinoma, RET-NTRK1 in papillary thyroid carcinoma, SEC16A-NOTCH1 in breast cancers, SRGAP3–RAF1 (at 3p25) in low-grade astrocytoma, TFE3-TFEB in kidney cancer.

[005] Breakpoints can occur at many different locations in a gene involved in gene fusion. Such breakpoint may be clustered at certain parts of the gene.

[006] One method of detecting gene fusions is by FISH (fluorescent in situ hybridization). Another is by deoxyribonucleic acid (DNA) sequencing.

SUMMARY

[007] Recognized herein is the need for methods to enrich breakpoint fragments in order to detect and characterize cancer fusion genes.

[008] The present disclosure provides methods to detect fusion genes, which may be used to detect a disease, such as cancer. Provided herein are methods for enrichment of breakpoint

fragments, such as to detect and characterize fusion genes, which may be associated with a disease, such as cancer.

[009] In an aspect, the present disclosure provides a method for providing a diagnostic or therapeutic intervention to a subject having or suspected of having cancer, comprising (a) providing a biological sample comprising cell-free nucleic acid molecules from a subject; (b) contacting the cell-free nucleic acid molecules from the biological sample with a probe set 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 sequence complementary with the fusion gene and containing only unmodified nucleotides; (c) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with isolated 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) providing the diagnostic or therapeutic intervention based on the detection of breakpoint fragments.

[0010] In some embodiments, each of the plurality of polynucleotide probes comprises one or more locked nucleic acid (LNA) nucleotides. In some embodiments, 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. In some embodiments, the at least two of the LNA nucleotides are spaced no more than 15 apart.

[0011] In some embodiments, at least 50% of the nucleotides of each of at least a subset of the plurality of polynucleotide probes are locked nucleic acid (LNA) nucleotides. In some embodiments, at least 75% of the nucleotides of each of at least a subset of the plurality of polynucleotide probes are locked nucleic acid (LNA) nucleotides.

[0012] In some embodiments, each of the plurality of polynucleotide probes has a melting temperature that is at least about 1 °C higher than the polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides. In some embodiments, the melting temperature is at least about 10 °C higher.

[0013] In some embodiments, each of the plurality of polynucleotide probes has a melting temperature that is at least about 2 % higher than the polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides. In some embodiments, the melting temperature is at least about 10% higher.

[0014] In some embodiments, the fusion gene is a cancer fusion gene. In some embodiments, 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. In some embodiments, 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. In some embodiments, each of the plurality of polynucleotide probes has sequence complementarity with a sequence across a breakpoint in the fusion gene.

[0015] In some embodiments, each of the plurality of polynucleotide probes has a length less than about 500 nucleotides. In some embodiments, each of the plurality of polynucleotide probes has a length between about 20 and about 200 nucleotides. In some embodiments, each of the plurality of polynucleotide probes has a length between about 80 and about 160 nucleotides.

[0016] In some embodiments, each of the breakpoint fragments has a length between about 140 nucleotides and 180 nucleotides.

[0017] In some embodiments, the plurality of polynucleotide probes is coupled to a solid support. In some embodiments, the probe set comprises one or more natural polynucleotide probes. In some embodiments, 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.

[0018] In some embodiments, each of the plurality of polynucleotide probes provides at least 50% coverage of a breakpoint region of a nucleic acid sequence included in the fusion gene.

[0019] In some embodiments, (d) comprises attaching, to the isolated polynucleotides, tags comprising barcodes having distinct barcode sequences to generate tagged parent polynucleotides. In some embodiments, the method further comprises amplifying the tagged parent polynucleotides to produce tagged progeny polynucleotides.

[0020] In some embodiments, the method further comprises (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.

[0021] In some embodiments, 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.

[0022] In another aspect, the present disclosure provides a method for capturing a breakpoint fragment of a fusion gene, comprising (a) providing a biological sample containing or suspected of containing a cell-free nucleic acid molecule comprising the breakpoint fragment of the fusion gene; and (b) contacting the biological sample with a polynucleotide probe 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 sequence complementary with the fusion gene 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.

[0023] In some embodiments, the polynucleotide probe comprises one or more locked nucleic acid (LNA) nucleotides. In some embodiments, the polynucleotide probe comprises a plurality LNA nucleotides, wherein at least two of the LNA nucleotides are spaced no more than 30 nucleotides apart. In some embodiments, the at least two of the LNA nucleotides are spaced no more than 15 nucleotides apart.

[0024] Another aspect of the present disclosure provides a probe set comprising a plurality of polynucleotide probes, wherein each of the polynucleotide probes has (i) sequence complementarity with a fusion gene as part of a cell-free nucleic acid molecule and (ii) affinity for the fusion gene that is greater than a polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides.

[0025] In some embodiments, each of the plurality of polynucleotide probes comprises one or more locked nucleic acid nucleotides. In some embodiments, the probe set further comprises one or more natural polynucleotide probes. In some embodiments, each of 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.

[0026] In some embodiments, each of the plurality of polynucleotide probes provides at least 50% coverage of a breakpoint region of a nucleic acid sequence included in the fusion gene.

[0027] In some embodiments, the plurality of polynucleotide probes hybridize to portions of one or both of the different genes in the fusion gene.

[0028] In some embodiments, the probe set further comprises a solid support, wherein the plurality of polynucleotide probes is coupled to the solid support.

[0029] In some embodiments, each of the plurality of polynucleotide probes has a melting temperature that is at least about 1 °C higher than the polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides. In some embodiments, the melting temperature is at least about 10 °C higher.

[0030] In some embodiments, each of the plurality of polynucleotide probes has a melting temperature that is at least about 2 % higher than the polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides. In some embodiments, the melting temperature is at least about 10% higher.

[0031] In some embodiments, the fusion gene is a cancer fusion gene.

[0032] In some embodiments, 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.

[0033] In another aspect, disclosed herein is a high affinity polynucleotide, comprising a sequence that is configured to specifically hybridize to a nucleic acid sequence associated with a fusion gene in a cell-free nucleic acid molecule.

[0034] In another aspect, disclosed herein is a high affinity polynucleotide configured to specifically hybridize to a fusion gene. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides. In another embodiment the high affinity polynucleotide has a melting temperature that is at least any of 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 10 °C, 15 °C or 20 °C higher than a polynucleotide with the same sequence comprising only natural nucleotides. In another embodiment the high affinity polynucleotide has a melting temperature that is at least any of 2%, 4%, 6%, 8%, or 10% higher than a polynucleotide with the same sequence comprising only natural nucleotides. In another embodiment the high affinity polynucleotide is configured to specifically hybridize to a cancer fusion gene. In another embodiment the high affinity polynucleotide is configured to specifically hybridize to a gene of a fusion gene pair of FIGs. 2A-2B or a fusion gene between at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more genes selected from FIG. 3. In another embodiment the high affinity polynucleotide is configured to hybridize within a breakpoint region no more than 500 nucleotides away from a breakpoint of the fusion gene. In another embodiment the high affinity polynucleotide is configured to hybridize across a breakpoint in the fusion gene. In another embodiment the high affinity polynucleotide has a length less than about 500 nucleotides, between about 20 and about 200 nucleotides, or between about 80 and about 160 nucleotides. In another embodiment the high affinity polynucleotide comprises a plurality of locked nucleic acid (LNA) nucleotides, wherein at least two of the LNA nucleotides are spaced no more than 30, 20, 15, 10 or 5 nucleotides apart. In another embodiment 100%, or at least any of 90%, 75%, 50%, 20%, 10%, or 5% or 1% of the nucleotides in the polynucleotide are locked nucleic acid nucleotides. In another embodiment the high affinity polynucleotide is has a nucleotide sequence perfectly or substantially complementary to a nucleotide sequence of the fusion gene.

[0035] In another aspect this disclosure provides a high affinity polynucleotide probe comprising a high affinity polynucleotide configured to specifically hybridize to a fusion gene. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides. In another embodiment the probe comprises a functionality selected from a detectable label, a binding moiety or a solid support. In another embodiment the probe is configured to hybridize to a breakpoint fragment of a fusion gene. In another embodiment the breakpoint fragment has a length between about 140 nucleotides and about 180 nucleotides. In another embodiment the fragment is cell-free deoxyribonucleic acid (DNA) or genomic DNA. In another embodiment the high affinity polynucleotide is bound to a solid support.

[0036] In another aspect this disclosure provides a probe set comprising a plurality of polynucleotide probes, each probe configured to specifically hybridize to a fusion gene, wherein the set comprises one or more high affinity polynucleotide probes. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides. In another embodiment the set comprises one or more natural polynucleotide probes. In another embodiment the probe set comprises at least one high affinity polynucleotide probe that specifically hybridizes to a breakpoint region of a gene involved in the fusion gene, and at least one natural polynucleotide probe that hybridizes to a non-breakpoint region of the gene involved in the fusion gene. In another embodiment the one or more high affinity polynucleotide probes in the probe set provide at least 50% (e.g., at least 0.5X to 5X) coverage of a breakpoint region of a gene involved in the fusion gene. In another embodiment the probes hybridize to portions of one or both of the different genes in the fusion gene. In another embodiment the probe set is configured as an oligonucleotide chip. In another embodiment a target sequence is targeted by both high affinity polynucleotide probes and standard affinity polynucleotide probes.

[0037] In another aspect this disclosure provides a kit comprising a plurality of probe sets, wherein each probe set specifically hybridizes to a different gene and at least one of the probe sets is a probe set of this disclosure. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

[0038] In another aspect, this disclosure provides a method for capturing a breakpoint fragment of a fusion gene comprising contacting the breakpoint fragment with a high affinity polynucleotide probe under stringent hybridization conditions and allowing hybridization, wherein the polynucleotide probe is bound to a solid support and wherein the polynucleotide probe has a nucleotide sequence that is substantially or perfectly complementary to a nucleotide sequence of the breakpoint fragment. In one embodiment the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

[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.

[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.

INCORPORATION BY REFERENCE

[0044] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0046] **FIG. 1** depicts breakpoint fragments derived from a fusion gene and the loss of such fragments during standard probe capture protocols;

[0047] **FIG. 2A** provides a list of cancer fusion gene pairs; **FIG. 2B** provides another list of cancer fusion gene pairs;

[0048] **FIG. 3** provides a list of genes detected in cancer fusion genes;

[0049] **FIGS. 4A-4U** provide exemplary breakpoints for cancer fusion gene pairs;

[0050] **FIGS. 5A-B** shows different coverage depths and tiling for probes and/or polynucleotides;

[0051] **FIGS. 6A-6D** shows different exemplary mixtures of high affinity probe sequence subsets and standard affinity probe sequence subsets;

[0052] **FIG. 7** shows a 64 gene panel, including four genes, ALK, NKRT1, RET and ROS1, involved in gene rearrangements;

[0053] **FIG. 8** shows eight genomic regions of the ALK gene that may be targeted for deeper coverage; and

[0054] **FIG. 9** shows a computer control system that is programmed or otherwise configured to implement methods provided herein.

DETAILED DESCRIPTION

[0055] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

I. Definitions

[0056] "High affinity polynucleotide", as used herein, refers to a polynucleotide comprising at least one chemical modification that provides the polynucleotide with a higher melting temperature in a hybridization reaction compared with a same sequence polynucleotide not so modified. In embodiments, the higher melting temperature can be at least any of 1°, 2°, 3°, 4°, 5°, 10°, 15° or 20° C higher. The polynucleotide can comprise one or more nucleotide analogs, an LNA nucleotide.

[0057] "Locked nucleic acid" ("LNA") (sometimes referred to as "inaccessible RNA"), as used herein, refers to a high affinity polynucleotide comprising at least one locked nucleic acid (LNA) nucleotide.

[0058] "Locked nucleic acid nucleotide" ("LNA nucleotide") as used herein, refers to a modified RNA nucleotide that provides the polynucleotide with greater thermodynamic stability during hybridization as compared with a polynucleotide that differs from the LNA only by having a natural ribonucleotide in place of the modified RNA nucleotide. In certain embodiments, the ribose moiety of a modified RNA nucleotide is modified with an extra bridge connecting the 2' oxygen and 4' carbon.

[0059] LNA nucleotides can comprise any type of extra bridge between the 2'O and 4'C of the RNA that increases the thermodynamic stability of the duplex between the LNA and its complement. In some cases, BNA, the 2' oxygen and 4' carbon are bridged by a methylene group. In some cases, 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), the 2' oxygen and 4' carbon are bridged by an ethylene group. Other examples of BNA can include, but are not limited to, 2',4'-BNA^{NC}[NH], 2',4'-BNA^{NC}[NMe], and 2',4'-BNA^{NC}[NBn].

[0060] "Bridged nucleic acid" ("BNA") refers to 2'-O,4'-C-methylene-modified nucleic acids.

[0061] Other 2'O-modified nucleotides, such as 2'O-Me, demonstrate greater stability, as well.

[0062] "Fusion gene", as used herein, refers to a gene that results from a chromosomal rearrangement (inversion, deletion, translocation) that brings together formerly separate portions of at least two different genes in a genome.

[0063] "Cancer fusion gene", as used herein, refers to a fusion gene resulting from somatic mutation in a cancer cell.

[0064] "Breakpoint", as used herein, refers to a nucleotide position in a fusion gene at which portions of two different genes are fused.

[0065] "Breakpoint region", as used herein, refers to a region of a gene that can be involved in gene fusions at which a breakpoint can occur.

[0066] "Breakpoint fragment" of a fusion gene, as used herein, refers to a fragment of a fusion gene that includes sequences from two different genes making up the fusion gene.

[0067] "Probe", as used herein, refers to a polynucleotide comprising a functionality. The functionality can be a detectable label (fluorescent), a binding moiety (biotin), or a solid support (a magnetically attractable particle or a chip).

[0068] "Natural polynucleotide" or "natural oligonucleotide", as used herein, refers to a polynucleotide or an oligonucleotide in which all of the nucleotides in the probe are natural nucleotides.

[0069] "Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (Watson-Crick base pairing) with a second nucleic acid sequence (5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary, respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

[0070] "Substantially complementary" as used herein refers to a degree of complementarity that is at least any of 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions. Sequence identity, such as for the purpose of assessing percent complementarity, may be measured by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (see e.g. the EMBOSS Needle aligner available at the world wide web site: ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html, optionally with default settings), the BLAST algorithm (see e.g. the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), or the Smith-Waterman algorithm (see e.g. the EMBOSS Water aligner available at the world wide web site: ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

[0071] "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogstein binding, or in any other sequence specific manner according to base complementarity. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded

complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the enzymatic cleavage of a polynucleotide by an endonuclease. A second sequence that is complementary to a first sequence is referred to as the "complement" of the first sequence. The term "hybridizable" as applied to a polynucleotide refers to the ability of the polynucleotide to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues in a hybridization reaction.

[0072] "Specifically hybridize to" or "hybridizing specifically to" or "specific hybridization" refers to the formation of a stable duplex between two polynucleotides under conditions of 50% formamide, 5 x SSC and 1% SDS incubated at 42° C or 5 x SSC and 1% SDS incubated at 65° C, with a wash in 0.2 x SSC and 0.1% SDS at 65° C.

[0073] The term "stringent hybridization conditions" refers to conditions under which a polynucleotide will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" in the context of nucleic acid hybridization experiments are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York.

[0074] Generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe.

[0075] Stringent hybridization conditions include a buffer comprising water, a buffer (a phosphate, tris, SSPE or SSC buffer at pH 6-9 or pH 7-8), a salt (sodium or potassium), and a denaturant (SDS, formamide or tween) and a temperature of 37° C -70° C, 60° C -65° C.

[0076] An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42° C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCI at 72° C for about 15 minutes. An example of stringent wash conditions is a 0.2X SSC wash at 65° C for 15 minutes (see, Sambrook et al. for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, more than 100 nucleotides, is 1x SSC at 45° C for 15 minutes.

An example low stringency wash for a duplex of, e. g., more than 100 nucleotides, is 4-6x SSC at 40° C for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

II. Overview

[0077] Provided herein are compositions and methods for detecting polynucleotides comprising one or more fusion genes. The polynucleotides can be deoxyribonucleic acid (DNA). The compositions and methods provided herein can detect fusion genes with high sensitivity in heterogeneous polynucleotide samples, such as cell-free DNA ("cfDNA").

[0078] DNA from cells, including cancer cells, can be shed into the blood in the form of cell-free DNA. Cell-free DNA has an average length of about 160 nucleotides. Because fragmentation does not occur at pre-specified points, for any genomic locus, fragments may be found in a sample that tile across that locus.

[0079] In cancer, certain genes are commonly involved in gene fusions with other genes. For example, the EML4 and ALK genes commonly undergo gene fusion with each other in cancer. The breakpoint of each gene involved in a fusion can occur at breakpoint regions ("hot spots") in each of the genes. When cells containing these fusion genes die, their DNA is shed into the blood in the form of cfDNA. As shown in FIG. 1, the position in the fragment mapping to the breakpoint may occur anywhere in the fragment, near the 5' end, in the middle, or near the 3' end. Accordingly, the cfDNA polynucleotide can have a very short or a very long nucleotide sequence from either gene involved in the fusion.

[0080] Certain DNA sequencing methods use sequence capture to enrich for sequences of interest. Sequence capture typically involves the use of oligonucleotide probes that hybridize to the sequence of interest. The probe set strategy can involve tiling the probes across a region of interest. Such probes can be, about 120 bases long. The set can have a depth of about 2x. The effectiveness of sequence capture depends, in part, on the length of the sequence in the target molecule that is complementary (or nearly complementary) to the sequence of the probe.

[0081] However, in the case of fusion genes, polynucleotides mapping to the breakpoint may contain a sequence from the target gene that is shorter than optimal for hybridization and capture. For example, a cfDNA fragment mapping to a fusion involving an ALK-EML4 fusion may have, for example, a 150 nucleotide sequence of the ALK gene, a 100 nucleotide sequence, a 50 nucleotide sequence, a 25 nucleotide sequence or a 10 nucleotide sequence. In this case, there is a lower probability of capturing the polynucleotide if it has a shorter ALK sequence than of capturing a polynucleotide with a sequence fully complementary to the ALK probe. The

problem is more acute when sequence capture is multiplex, targeting sequences from many different genes.

[0082] Provided herein are materials and methods for capturing polynucleotide fragments mapping to a breakpoint in a fusion gene. Such polynucleotides are captured using high affinity polynucleotide probes, such locked nucleic acids. Such probes have higher melting temperature than probes of the same sequence made from natural nucleotides. Consequently, they produce higher yield of captured products from the same sample.

[0083] Such probes can be included in a probe set targeting both fusion genes and non-fused genes. In this way, captured polynucleotides are enriched for those including fusion genes, compared with a population captured using only probes made from natural nucleotides.

[0084] An exemplary probe set can contain, for example, a subset of LNA probes. The LNA probes can be configured to tile across breakpoint regions of genes involved in fusion genes.

[0085] Every nucleotide in an LNA probe can be an LNA nucleotide. Alternatively, a fraction of the nucleotides can be LNA nucleotides. In certain embodiments, the LNA nucleotides can be spaced a predetermined number of nucleotides apart.

[0086] The present invention provides high-affinity polynucleotides that can be used to enrich a sample containing nucleic acid fragments for those nucleic acid fragments that contain gene fusion events. These high-affinity polynucleotides can contain LNA nucleotides. Substituting LNA nucleotides for standard nucleotides can increase the melting temperature of the high-affinity polynucleotide, thereby increasing the stability of the duplex between the high-affinity polynucleotide and a nucleic acid fragment that contains a fusion gene.

[0087] Gene fusions can be associated with, and in some cases contribute to, the development of a healthy cell into a neoplasm (a tumor or an adenoma). Detecting these gene fusion events may provide a useful approach for detecting and/or monitoring the presence of a neoplasm in a patient. Breakpoint fragments, however, will have less sequence derived from either gene flanking the breakpoint than a nucleic acid fragment of a similar length comprising sequence from just one of the genes. For this reason, a breakpoint fragment is often only capable of binding to a reduced section of a gene probe or gene-specific oligonucleotide. If the hybridization and wash conditions have been optimized for full-length or near full-length binding, the nucleic acid fragment containing the breakpoint can hybridize with insufficient affinity and be lost (see FIG. 1). Furthermore, in a heterogeneous sample containing nucleic acid fragments from cells that have and have not undergone gene fusion events, nucleic acid fragments from those that have not undergone gene fusion events can bind more stably to the gene probe or gene-specific oligonucleotide and competitively inhibit the hybridization of nucleic acid fragments containing breakpoints.

[0088] Tumor-derived nucleic acid can be found in cell-free bodily fluids. Tumor-derived nucleic acids from such cell-free bodily fluids can be assayed for nucleic acid fragments containing fusion genes in order to detect neoplasms. Cell-free bodily fluids can contain small amounts of tumor-derived nucleic acid, and the tumor-derived nucleic acid can be admixed with nucleic acid that is derived from healthy tissue. The present disclosure also provides approaches for enriching for nucleic acid fragments that contain fusion genes from nucleic acid derived from a cell-free bodily fluid.

III. Test Samples

A. Subject Types

[0089] Samples are collected from subjects, e.g. patients at risk for developing cancer. The subjects may be patients with no known risk factors for cancer. The subjects can be patients whose only risk factors for cancer are age and/or gender. In some cases, the subjects can have known risk factors for cancer, e.g. smoking or familial history of cancer. In some cases, the subjects can be patients with symptoms of cancer.

[0090] Other subjects can be patients with neoplasms that have previously been detected, by colonoscopy or imaging. The samples derived from patients with previously detected neoplasms can be assayed for nucleic acid fragments containing breakpoints in order to recommend a course of treatment or therapy. The samples derived from patients with neoplasms can be assayed for nucleic acids fragments containing breakpoints in order to determine the effectiveness of the treatment or therapy they are receiving.

[0091] Other subjects can be patients with neoplasms that have been previously detected, but in whom the neoplasm is no longer detectable (patients in remission or who have no evidence of disease). The samples derived from patients in whom the neoplasm is no longer detectable can be assayed for nucleic acid fragments containing breakpoints in order to detect a relapse or reemergence of the neoplasm.

[0092] Other subjects can be women with a familial history of cancer, wherein the genetic defect responsible for the familial cancer is known or suspected to be a fusion gene. In some cases, a woman with a family history of cancer may be pregnant and want to determine whether the fetus she is carrying has the fusion gene. In some cases, a sample containing fetal nucleic acids from such a subject can be assayed for the gene fusion event.

B. Sample Types

[0093] Samples can be nucleic acids extracted from various sources. Nucleic acids can be, but are not limited to, genomic DNA, RNA, mitochondrial DNA, fetal DNA, and miRNA.

[0094] Samples may be extracted from a variety of bodily fluids containing cell-free nucleic acids, including but not limited to blood, serum, plasma, vitreous, sputum, urine, tears,

perspiration, saliva, semen, mucosal excretions, mucus, spinal fluid, amniotic fluid, lymph fluid and the like. The collection of bodily fluids can be achieved using a variety of techniques. In some cases, collection may comprise aspiration of a bodily fluid from a subject using a syringe. In other cases collection may comprise pipetting or direct collection of fluid into a collecting vessel.

[0095] After collection of bodily fluid, cell-free nucleic acids may be isolated and extracted using a variety of techniques. In some cases, cell-free nucleic acids may be isolated, extracted and prepared using commercially available kits such as the Qiagen Qiamp® Circulating Nucleic Acid Kit protocol. In other examples, Qiagen Qubit™ dsDNA HS Assay kit protocol, Agilent™ DNA 1000 kit, or TruSeq™ Sequencing Library Preparation; Low-Throughput (LT) protocol may be used to quantify nucleic acids. Cell-free nucleic acids may be fetal in origin (via fluid taken from a pregnant subject), or may be derived from tissue of the subject itself. Cell-free nucleic acids can be derived from a neoplasm (e.g. a tumor or an adenoma).

[0096] Generally, cell-free nucleic acids are extracted and isolated from bodily fluids through a partitioning step in which cell-free nucleic acids, as found in solution, are separated from cells and other non-soluble components of the bodily fluid. Partitioning may include, but is not limited to, techniques such as centrifugation or filtration. In other cases, cells are not partitioned from cell-free nucleic acids first, but rather lysed. In one example, the genomic DNA of intact cells is partitioned through selective precipitation. Cell-free nucleic acids, including DNA, may remain soluble and may be separated from insoluble genomic DNA and extracted. Generally, after addition of buffers and other wash steps specific to different kits, nucleic acids may be precipitated using isopropanol precipitation. Further clean up steps may be used such as silica based columns to remove contaminants or salts. General steps may be optimized for specific applications. Non-specific bulk carrier nucleic acids, for example, may be added throughout the reaction to optimize certain aspects of the procedure such as yield.

[0097] Cell-free nucleic acids can be at most 500 nucleotides in length, at most 400 nucleotides in length, at most 300 nucleotides in length, at most 250 nucleotides in length, at most 225 nucleotides in length, at most 200 nucleotides in length, at most 190 nucleotides in length, at most 180 nucleotides in length, at most 170 nucleotides in length, at most 160 nucleotides in length, at most 150 nucleotides in length, at most 140 nucleotides in length, at most 130 nucleotides in length, at most 120 nucleotides in length, at most 110 nucleotides in length, or at most 100 nucleotides in length.

[0098] Cell-free nucleic acids can be at least 500 nucleotides in length, at least 400 nucleotides in length, at least 300 nucleotides in length, at least 250 nucleotides in length, at least 225 nucleotides in length, at least 200 nucleotides in length, at least 190 nucleotides in

length, at least 180 nucleotides in length, at least 170 nucleotides in length, at least 160 nucleotides in length, at least 150 nucleotides in length, at least 140 nucleotides in length, at least 130 nucleotides in length, at least 120 nucleotides in length, at least 110 nucleotides in length, or at least 100 nucleotides in length. In particular, cell-free nucleic acids can be between 140 and 180 nucleotides in length.

[0099] A sample may be extracted from tissue from the subject. A sample can be a tumor biopsy. The tumor biopsy can contain a mixture of tumor and healthy tissue. The tumor biopsy can be formaldehyde-fixed and paraffin-embedded. The tumor can be at least 0.1% of the biopsy, at least 0.2% of the biopsy, at least 0.5% of the biopsy, at least 0.7% of the biopsy, at least 1% of the biopsy, at least 2% of the biopsy, at least 3% of the biopsy, at least 4% of the biopsy, at least 5% of the biopsy, at least 10% of the biopsy, at least 15% of the biopsy, at least 20% of the biopsy, at least 25% of the biopsy, or at least 30% of the biopsy. A sample can be a biopsy from healthy tissue.

[00100] Nucleic acids extracted from tissue can be at most 10 kb in length, at most 7 kb in length, at most 5 kb in length, at most 4 kb in length, at most 3 kb in length, at most 2 kb in length, at most 1 kb in length, at most 500 nucleotides in length, at most 400 nucleotides in length, at most 300 nucleotides in length, at most 250 nucleotides in length, at most 225 nucleotides in length, at most 200 nucleotides in length, at most 190 nucleotides in length, at most 180 nucleotides in length, at most 170 nucleotides in length, at most 160 nucleotides in length, at most 150 nucleotides in length, at most 140 nucleotides in length, at most 130 nucleotides in length, at most 120 nucleotides in length, at most 110 nucleotides in length, or at most 100 nucleotides in length.

[00101] Nucleic acids extracted from tissue can be at least 5 kb in length, at least 4 kb in length, at least 3 kb in length, at least 2 kb in length, at least 1 kb in length, at least 500 nucleotides in length, at least 400 nucleotides in length, at least 300 nucleotides in length, at least 250 nucleotides in length, at least 225 nucleotides in length, at least 200 nucleotides in length, at least 190 nucleotides in length, at least 180 nucleotides in length, at least 170 nucleotides in length, at least 160 nucleotides in length, at least 150 nucleotides in length, at least 140 nucleotides in length, at least 130 nucleotides in length, at least 120 nucleotides in length, at least 110 nucleotides in length, or at least 100 nucleotides in length.

[00102] In some cases, nucleic acids can be sheared during the extraction process and comprise fragments between 100 and 400 nucleotides in length. In some cases, nucleic acids can be sheared after extraction can comprise nucleotides between 100 and 400 nucleotides in length.

[00103] Isolation and purification of cell-free and tissue-derived nucleic acids may be accomplished using various approaches, including, but not limited to, the use of commercial kits

and protocols provided by companies such as Sigma Aldrich, Life Technologies, Promega, Affymetrix, IBI or the like. Kits and protocols may also be non-commercially available.

IV. Genetic Analysis

[00104] Genetic analysis includes detection of nucleotide sequence variants, copy number variations, and fusion genes. Genetic variants can be determined by sequencing. The sequencing method can be massively parallel sequencing, that is, simultaneously (or in rapid succession) sequencing any of at least 100,000, 1 million, 10 million, 100 million, or 1 billion polynucleotide molecules. Sequencing methods may include, but are not limited to: high-throughput sequencing, pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, RNA-Seq (Illumina), Digital Gene Expression (Helicos), Next-generation sequencing, Single Molecule Sequencing by Synthesis (SMSS)(Helicos), massively-parallel sequencing, Clonal Single Molecule Array (Solexa), shotgun sequencing, Maxam-Gilbert or Sanger sequencing, primer walking, sequencing using PacBio, SOLiD, Ion Torrent, nanopore-based platforms or other sequencing methods.

[00105] Sequencing can be made more efficient by performing sequence capture, that is, the enrichment of a sample for target sequences of interest, sequences of cancer fusion genes and cancer fusion gene breakpoints as described herein. Sequence capture can be performed using immobilized probes that hybridize to the targets of interest. Sequence capture can be performed using probes attached to functional groups, biotin, that allow probes hybridized to specific sequences to be enriched for from a sample by pulldown. In some cases, prior to hybridization to functionalized probes, specific sequences such as adapter sequences from library fragments can be masked by annealing complementary, non-functionalized polynucleotide sequences to the fragments in order to reduce non-specific or off-target binding.

[00106] In some cases the cell-free nucleic acid fragments or tissue-derived nucleic acid fragments are inputs to produce sequencing libraries. In some cases, the fragments are enriched for specific sequence prior to preparing a sequencing library. The enriched fragmented nucleic acids can be attached to any sequencing adaptor suitable for use on any sequencing platform disclosed herein. For example, a sequence adaptor can comprise a flow cell sequence, a sample barcode, or both. In another example, a sequence adaptor can be a hairpin shaped adaptor and/or comprise a sample barcode. Further, the resulting fragments can be amplified and sequenced. In some cases, the adaptor does not comprise a sequencing primer region. In some cases, the sequencing libraries are enriched for specific sequences prior to sequencing.

[00107] Cell-free nucleic acids can include small amounts of tumor nucleic acids mixed with germline nucleic acids. In some cases, tumor biopsies can include small amounts of tumor tissue

mixed in with healthy tissue, and nucleic acids extracted from such samples without enrichment can include small amounts of tumor nucleic acids mixed with germline nucleic acids. Sequencing methods that increase sensitivity and specificity of detecting tumor nucleic acids, and, in particular, genetic sequence variants and copy number variation, can be useful in the methods of this invention. Such methods are described in, for example, in WO 2014/039556, WO 2014/149134 and WO 2015/100427, each of which is entirely incorporated herein by reference. These methods not only can detect molecules with a sensitivity of up to or greater than 0.1%, but also can distinguish these signals from noise typical in current sequencing methods. Increases in sensitivity and specificity from blood-based samples of cell-free nucleic acids can be achieved using various methods. One method includes high efficiency tagging of nucleic acid molecules in the sample, tagging at least any of 50%, 75% or 90% of the polynucleotides in a sample. This increases the likelihood that a low-abundance target molecule in a sample will be tagged and subsequently sequenced, and significantly increases sensitivity of detection of target molecules.

[00108] Another method involves molecular tracking, which identifies sequence reads that have been redundantly generated from an original parent molecule, and assigns the most likely identity of a base at each locus or position in the parent molecule. This significantly increases specificity of detection by reducing noise generated by amplification and sequencing errors, which reduces frequency of false positives.

[00109] Methods of the present disclosure can be used to detect genetic variation in non-uniquely tagged initial starting genetic material (rare nucleic acids) at a concentration that is less than 5%, 1%, 0.5%, 0.1%, 0.05%, or 0.01%, at a specificity of at least 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or 99.99999%. Sequence reads of tagged polynucleotides can be subsequently tracked to generate consensus sequences for polynucleotides with an error rate of no more than 2%, 1%, 0.1%, or 0.01%.

V. Gene Fusion Events and Breakpoint Regions

[00110] Gene fusion events are chromosomal rearrangements (inversion, deletion, and translocation) that bring together formerly separate portions of at least two different genes in a genome, resulting in a fusion gene. Fusion genes can be associated with and/or cause the formation of a neoplasm. A fusion gene can be a cancer fusion gene. A cancer fusion gene can be a fusion gene resulting from a somatic mutation that is present in a cancer. Non-limiting examples of pairs of genes that may form cancer fusion genes are found in **FIGs. 2A** and **2B**. Non-limiting examples of genes involved in fusion genes are listed in **FIG. 3**.

[00111] **FIG. 8** shows non-limiting examples of genomic regions of the ALK gene that may be targeted for deeper coverage. The genomic regions in **FIG. 8** may correspond to different

variants of the ALK gene. Such deep coverage may be quantified by the number of unique molecules obtained after sequencing and collapsing with molecular barcodes, e.g., about 2-3 thousand molecules for typical variants versus about 4 thousand molecules for the genomic regions of **FIG. 8**. A range of a few thousand unique molecules may corresponds to greater than 1000x, 2000x, 3000x, 4000x, 5000x, or 10,000x sequencing depth.

[00112] Typically, a fusion gene can result in an aberrant juxtaposition of two genes that can encode a fusion protein (BCR-ABLl), or the regulatory elements of one gene may drive the aberrant expression of an oncogene (TMPRSS2-ERG). Despite the recurrent nature of cancer fusion genes, the exact location of the breakpoint for each fusion gene can vary. A breakpoint region refers to a region of a gene that may be involved in gene fusions at which a breakpoint can occur. In some cases, the breakpoint region is at most within 500 nucleotides of a breakpoint. In some cases, the breakpoint region is within at most 200 nucleotides of a breakpoint, within at most 500 nucleotides of a breakpoint, within at most 750 nucleotides of a breakpoint, within at most 1 kilobase (kb) of a breakpoint, within at most 5 kb of a breakpoint, within at most 10 kb of a breakpoint, within at most 20 kb of a breakpoint, within at most 30 kb of a breakpoint, within at most 40 kb of a breakpoint, within at most 50 kb of a breakpoint, or within at most 100 kb of a breakpoint.

[00113] Exemplary, non-limiting breakpoints for given pairs of genes are provided in **FIG. 4A-4U** from the Catalogue of Somatic Mutations in Cancer (COSMIC; see Forbes et al., Nucleic Acids Research (2014) 43:D805-D811). For each gene pair, a specific mutation ID is provided in the first column that indicates a particular class of detected or inferred fusion construct from the literature. For example, **FIG. 4A** provides 29 classes of detected or inferred fusion constructs from the literature. For each mutation, the 5' and 3' fusion partner (5' and 3' are relative to the directionality of each gene's transcript) each provide the gene name, the last observed exon, the inferred breakpoint relative to the transcript, whether there and whether there is inserted sequence. For each mutation ID, a number of unique samples observed with the mutation and the percentage of gene fusions involving the two genes that have that particular mutation are also provided.

[00114] For example, the first row of **FIG. 4A** indicates that Mutation COSF463 is an EML4-ALK fusion, wherein the EML4 gene has fused upstream of the ALK gene. In this example, the last observed EML4 exon is exon 13, and the inferred breakpoint is at the genomic position corresponding to position 1751 of the EML4 gene transcript. The EML4 gene has fused such that the first ALK exon after the fusion junction is exon 20, and the inferred breakpoint position is the genomic position corresponding to position 4080 of the ALK gene transcript. There is no additional inserted sequence in either the 5' partner or 3' partner gene. The COSF463 fusion

gene has been detected in 170 unique samples, or 25% of all EML4-ALK fusion genes included in the COSMIC database. In some instances, such COSF488 (**FIG. 4A**, row 5), the inferred breakpoint includes a '+' followed by a number, denoting a genomic position that number of bases downstream (in an intron or UTR) of the transcript position indicated by the first number. If the number is in parentheses the position is approximate. In some instances, such COSF488 (**FIG. 4A**, row 5), the inferred breakpoint includes a '-' followed by a number, denoting a genomic position that number of bases upstream (in an intron or UTR) of the transcript position indicated by the first number. If the number is in parentheses the position is approximate. A '?' indicates that the precise breakpoint is unknown. For example, in COSF488, the breakpoint is 654 bases downstream of the genomic position corresponding to position 2318 of the EML4 gene transcript, which has fused to a position 172 bases upstream of the genomic position corresponding to position 4080 of the ALK gene transcript.

VI. High Affinity Polynucleotides

[00115] In some cases, the high affinity polynucleotide can be at least about 450 nucleotides in length, at least about 425 nucleotides in length, at least about 400 nucleotides in length, at least about 375 nucleotides in length, at least about 350 nucleotides in length, at least about 325 nucleotides in length, at least about 300 nucleotides in length, at least about 275 nucleotides in length, at least about 250 nucleotides in length, at least about 225 nucleotides in length, at least about 200 nucleotides in length, at least about 180 nucleotides in length, at least about 160 nucleotides in length, at least about 140 nucleotides in length, at least about 120 nucleotides in length, at least about 100 nucleotides in length, at least about 80 nucleotides in length, at least about 60 nucleotides in length, at least about 40 nucleotides in length, or at least about 20 nucleotides in length.

[00116] Furthermore, in some cases, the high affinity polynucleotide can be at most about 500 nucleotides in length, at most about 450 nucleotides in length, at most about 425 nucleotides in length, at most about 400 nucleotides in length, at most about 375 nucleotides in length, at most about 350 nucleotides in length, at most about 325 nucleotides in length, at most about 300 nucleotides in length, at most about 275 nucleotides in length, at most about 250 nucleotides in length, at most about 225 nucleotides in length, at most about 200 nucleotides in length, at most about 180 nucleotides in length, at most about 160 nucleotides in length, at most about 140 nucleotides in length, at most about 120 nucleotides in length, at most about 100 nucleotides in length, at most about 80 nucleotides in length, at most about 60 nucleotides in length, at most about 40 nucleotides in length, or at most about 20 nucleotides in length.

[00117] In particular, in some cases high affinity polynucleotides can be between about 20 and about 200 nucleotides in length. Furthermore, in some cases high affinity polynucleotides can be between about 80 and about 160 nucleotides in length.

[00118] In certain embodiments, high affinity polynucleotides of this invention have a sequence of at least 10, least 25, least 50, least 100 or at least 150 nucleotides perfectly complementary or substantially complementary to a target sequence of a fusion gene.

[00119] High affinity polynucleotides can contain one or more LNA nucleotides. In some cases, 100% of the nucleotides within the high affinity polynucleotide are LNA nucleotides. In some cases, at least 90%, at least 70%, at least 50%, at least 20%, at least 10%, at least 5%, or at least 1% of the nucleotides within the high affinity polynucleotides are LNA nucleotides. In some cases, at most 90%, at most 70%, at most 50%, at most 20%, at most 10%, at most 5%, or at most 1% of the nucleotides within the high affinity polynucleotide are LNA nucleotides.

[00120] If a high affinity polynucleotide contains more than one LNA nucleotide, in some cases the LNA nucleotides can be spaced no more than 30 nucleotides apart, no more than 20 nucleotides apart, no more than 15 nucleotides apart, no more than 10 nucleotides apart, or no more than 5 nucleotides apart. In other cases where the high affinity polynucleotide contains more than one LNA nucleotide, the LNA nucleotides can be spaced at least 30 nucleotides apart, at least 20 nucleotides apart, at least 15 nucleotides apart, at least 10 nucleotides apart, or at least 5 nucleotides apart.

[00121] For each LNA nucleotide inserted in place of a natural nucleotide in a high affinity polynucleotide, the melting temperature of the duplex of the high affinity polynucleotide and its complementary sequence comprising only natural nucleotides can increase at least 1 °C, at least 2 °C, at least 3 °C, 4 at least °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C, or at least 10 °C under stringent conditions. In particular, for each LNA nucleotide inserted in place of a natural nucleotide, the melting temperature can increase by between about 2 °C and about 8 °C.

[00122] In some cases, the melting temperature of a high affinity polynucleotide (comprising one or more LNA nucleotides) can be at least 0.5% higher, at least 1% higher, at least 2% higher, at least 3% higher, at least 4% higher, at least 5% higher, at least 10% higher, at least 15% higher, at least 20% higher, at least 25% higher, at least 30% higher, at least 35% higher, at least 40% higher, at least 45% higher, at least 50% higher, at least 55% higher, at least 60% higher, at least 65% higher, at least 70% higher, at least 75% higher, at least 80% higher, at least 85% higher, at least 90% higher, at least 95% higher, or at least 100% higher than the melting temperature of a polynucleotide comprising only natural nucleotides with the same sequence as the high affinity polynucleotide.

[00123] In one configuration, bound probes may be affinity purified using a combination of binding partners. In one example, probes may contain a binding partner such as biotin. The binding partner may then be used as bait for an additional binding partner, such as streptavidin, in an affinity purification step. In some cases, bound probes may be affinity purified from unbound probes. In other cases, sample polynucleotide strands, comprising a binding partner and bound probes may be affinity purified from unbound probes.

[00124] Generally, any chemical approach for capture of the bound probes may be suitable. In some cases, capture may be achieved through methods comprising biotin and streptavidin, or streptavidin derivatives. For example, one embodiment of the disclosure provides for capture of sequencing library fragments of fusion genes, wherein probes to the genes involved in the fusion gene, probes to the breakpoint region, and/or probes to the breakpoint are annealed to melted strands of the sequencing library and affinity purified away from other sequencing library fragments.

[00125] Magnetically attractable particles, such as beads, may be used for isolation. Any suitable bead isolation technique can be used with methods of the present disclosure. In some cases, Beads can be useful for isolation in that molecules of interest can be attached to the beads, and the beads can be washed to remove solution components not attached to the beads, allowing for enrichment, purification and/or isolation. The beads can be separated from other components in the solution based on properties such as size, density, or dielectric, ionic, and magnetic properties. In preferred embodiments, the particles are magnetically attractable. Magnetically attractable particles can be introduced, mixed, removed, and released into solution using magnetic fields. Processes utilizing magnetically attractable particles can also be automated. Magnetically attractable particles are supplied by a number of vendors including NEB, Dynal, Micromod, Turbobeads, and Spherotech. The particles can be functionalized using functionalization chemistry to provide a surface having the binding groups required for binding to polynucleotides.

[00126] In some cases, the probe and/or high affinity polynucleotide are configured to hybridize to a cancer fusion gene. For example, the probe and/or high affinity polynucleotide can be complementary to a portion of either gene that the fusion gene is derived from. In some cases, the cancer fusion gene can be one or more genes selected from the lists present in **FIGs. 2A-2B**.

[00127] In some cases, the probe and/or high affinity polynucleotide can be configured to hybridize to a breakpoint region. For example, in some cases the probe and/or high affinity polynucleotide can be complementary to a portion of a breakpoint region (the probe and/or high affinity polynucleotide can be complementary to a sequence within 500 nucleotides of a breakpoint). Furthermore, in some cases, the probe and/or high affinity polynucleotide can be

configured to hybridize across a breakpoint in a fusion gene (see **FIG. 6C**). For example, the probe and/or polynucleotide can be complementary to a portion of the sequence on each side of a breakpoint (see **FIG. 6D**).

VII. Sets of Probes and/or Polynucleotides

[00128] In some cases, sets of probes and/or polynucleotides are provided. In some cases, all of the probes and/or polynucleotides in the set comprise LNA nucleotides. In some cases, a subset of the probes and/or polynucleotides in the set comprises only natural nucleotides, referred to hereafter as a "standard affinity subset", and a second subset comprising one or more LNA nucleotides, referred to hereafter as a "high affinity subset."

[00129] In one embodiment, the probe set includes one or more probes directed to a nucleotide sequence in a breakpoint region of a fusion gene.

[00130] Probes and/or polynucleotides can be provided at a variety of coverage depths. For example, in some cases coverage depth can be at least 0.5x, wherein a set of probes or polynucleotides targets on average half of the bases in a region (see **FIG. 5A**).

[00131] In some cases, coverage depth can be at least 1x, wherein probes and/or polynucleotides are designed such that each base in a region is on average targeted by only one probes and/or polynucleotide sequence. In some cases, coverage depth can be at least 2x, wherein probes and/or polynucleotides are designed such that each base in a region is on average targeted by two probes and/or polynucleotide sequences. In some cases, coverage depth by a set of probes or polynucleotides can be at least 3x, at least 4x, or at least 5x. In some cases, probes and/or polynucleotides can be tiling, wherein a set of probes and/or polynucleotides are designed such that a contiguous target region is covered by the probes and/or polynucleotide sequences (see **FIG. 5B**).

[00132] In some cases, it may be preferable to use a standard affinity subset of probes and/or polynucleotides to enrich for some nucleic acid fragments of interest, and to use a high affinity subset of probes and/or polynucleotides to enrich for other nucleic acid fragments in the same sample. For example, in some cases a standard affinity subset of probes and/or polynucleotides can target exomes, oncogenes, or tumor suppressor genes, and a high affinity subset of probes and/or polynucleotides can target fusion genes, such as cancer fusion genes (e.g. the genes listed in **FIG. 3**). In another example, in some cases, a standard affinity subset targets with a first coverage depth a contiguous or non-contiguous portion of one or more genes involved in a gene fusion, including the breakpoint regions, and a high affinity subset targets with a second coverage depth the breakpoint region(s) (see **FIG. 6A**). In some cases, a standard affinity subset targets with a first coverage depth a contiguous or non-contiguous portion of each of the genes, excluding the breakpoint regions, and a high affinity subset targets with a second coverage depth

the breakpoint region(s) (see **FIG. 6B**). In some cases, a standard affinity subset targets with a first coverage depth a contiguous or non-contiguous portion of each of the genes, and a high affinity subset targets with a second coverage depth the breakpoints (see **FIG. 6C**). In some cases, a standard affinity subset targets with a first coverage depth a contiguous or non-contiguous portion of each of the genes, and a high affinity subset targets with a second coverage depth the sequence on either side of a breakpoint, but not the breakpoint itself (see **FIG. 6D**).

[00133] In some cases, a set of probes and/or polynucleotides is configured to target more than one gene in order to enrich for a panel of genes that may be involved in gene fusions (see, e.g., FIG. 7). Furthermore, in some cases, a set of probes and/or polynucleotides is configured to target more than one gene and their breakpoints or breakpoint regions.

[00134] In some cases, sets of probes and/or polynucleotides are configured to target a specific fusion gene. For example, the probes and/or polynucleotides can be designed to target one or both genes involved in the gene fusion. In some cases, a set of probes and/or polynucleotides comprises probes and/or polynucleotides that target a single gene and/or its breakpoints or breakpoint regions.

[00135] In some cases, the standard affinity probes and/or polynucleotides are mixed with the high affinity probes and/or polynucleotides. In some cases, the standard affinity probes and/or polynucleotides and the high affinity probes and/or polynucleotides are separate and employed sequentially. Furthermore, in some cases the sample is first contacted with the standard affinity probes, and then the uncaptured nucleic acid fragments are contacted with the high affinity probes.

[00136] In some cases, high affinity probe sets can include standard affinity polynucleotides doped with high affinity polynucleotides. In such a probe set, a target sequence can be targeted for hybridization by both standard and high affinity polynucleotides. In such a doped set, the high affinity polynucleotides can target only sequences at a breakpoint region.

VIII. Kits

[00137] The present disclosure provides kits for enriching samples for breakpoint fragments. The kits can comprise any of the probes and/or polynucleotides disclosed herein. In some cases, the kit can comprise a plurality of probe sets, wherein each probe set hybridizes to a different gene and at least one of the probe sets is configured to hybridize to a fusion gene and comprises one or more high affinity polynucleotides and/or probes.

IX. Methods of Use

[00138] The present disclosure provides methods for enriching for breakpoint fragments using any of the probes and/or polynucleotides disclosed herein. Such methods can comprise

contacting a probe set that hybridizes to a fusion gene, wherein one or more probes and/or polynucleotides is a high affinity polynucleotide and/or probe, with a mixture of polynucleotides to produce probe-captured polynucleotides. The probe-captured polynucleotides can then be isolated to produce a sample enriched for polynucleotides comprising breakpoint fragments of the fusion gene. In some cases, the polynucleotides are cell-free DNA. In some cases, the polynucleotides are fragmented genomic DNA. In some cases, the probe-captured polynucleotides are eluted to isolate the captured polynucleotides form the probes. In some cases, the eluted polynucleotides are directly sequenced or used to produce sequencing libraries.

[00139] Methods of detecting fusion genes are provided. In a method, at least one probe set comprising at least one high affinity polynucleotide is provided that is directed to a gene involved in a gene fusion. The probe set can include both standard affinity and high affinity polynucleotide probes. In some embodiments, the probe set comprises a plurality of probe subsets, each subset directed to sequences of a different gene of interest, one or more of which genes are involved in a gene fusion in cancer and, in some examples, at least of which genes is not involved in a gene fusion.

[00140] The probe set may be mixed with a sample comprising DNA, such as cfDNA, under stringent hybridization conditions, and the DNA may be allowed to hybridize to the probes. Because the probe set includes high affinity polynucleotide probes, the probability of capturing DNA fragments including a fusion gene break point is increased. Captured DNA may be isolated from the probe and sequenced. Sequences may be analyzed to detect DNA fragments having sequences that span a breakpoint, such as DNA fragments that include sequences from two different genes normally not fused. The presence of fusion genes may be correlated with a disease, such as cancer. Accordingly, this method is useful in the diagnosis of the disease, such as cancer.

Computer control systems

[00141] The present disclosure provides computer control systems that are programmed to implement methods of the disclosure. **FIG. 9** shows a computer system 901 that is programmed or otherwise configured to detect fusion genes and diagnose and/or provided a therapeutic intervention for a disease, such as cancer.

[00142] The computer system 901 includes a central processing unit (CPU, also "processor" and "computer processor" herein) 905, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system 901 also includes memory or memory location 910 (e.g., random-access memory, read-only memory, flash memory), electronic storage unit 915 (e.g., hard disk), communication interface 920 (e.g., network adapter) for communicating with one or more other systems, and peripheral devices 925, such as cache,

other memory, data storage and/or electronic display adapters. The memory 910, storage unit 915, interface 920 and peripheral devices 925 are in communication with the CPU 905 through a communication bus (solid lines), such as a motherboard. The storage unit 915 can be a data storage unit (or data repository) for storing data. The computer system 901 can be operatively coupled to a computer network ("network") 930 with the aid of the communication interface 920. The network 930 can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network 930 in some cases is a telecommunication and/or data network. The network 930 can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network 930, in some cases with the aid of the computer system 901, can implement a peer-to-peer network, which may enable devices coupled to the computer system 901 to behave as a client or a server.

[00143] The CPU 905 can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory 910. The instructions can be directed to the CPU 905, which can subsequently program or otherwise configure the CPU 905 to implement methods of the present disclosure. Examples of operations performed by the CPU 905 can include fetch, decode, execute, and writeback.

[00144] The CPU 905 can be part of a circuit, such as an integrated circuit. One or more other components of the system 901 can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00145] The storage unit 915 can store files, such as drivers, libraries and saved programs. The storage unit 915 can store user data, e.g., user preferences and user programs. The computer system 901 in some cases can include one or more additional data storage units that are external to the computer system 901, such as located on a remote server that is in communication with the computer system 901 through an intranet or the Internet.

[00146] The computer system 901 can communicate with one or more remote computer systems through the network 930. For instance, the computer system 901 can communicate with a remote computer system of a user (e.g., healthcare provider). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system 901 via the network 930.

[00147] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system 901, such as, for example, on the memory 910 or electronic storage unit 915. The machine

executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor 905. In some cases, the code can be retrieved from the storage unit 915 and stored on the memory 910 for ready access by the processor 905. In some situations, the electronic storage unit 915 can be precluded, and machine-executable instructions are stored on memory 910.

[00148] The code can be pre-compiled and configured for use with a machine having a processer adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[00149] Aspects of the systems and methods provided herein, such as the computer system 901, can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage" media, terms such as computer or machine "readable medium" refer to any medium that participates in providing instructions to a processor for execution.

[00150] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission

media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[00151] The computer system 901 can include or be in communication with an electronic display 935 that comprises a user interface (UI) 940 for providing, an output of a report, which may include a diagnosis of a subject or a therapeutic intervention for the subject. Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00152] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit 905. The algorithm can, for example, facilitate the enrichment, sequencing and/or detection of fusion genes.

EXAMPLES

Example 1: Enrichment and sequencing of cancer genes and cancer fusion genes

[00153] Circulating cell-free DNA is isolated from the plasma of a cancer patient using the QIAamp Circulating Nucleic Acid kit (Qiagen) per manufacturer's protocol, except that a double sided SPRI with AmpureXP beads (Beckman Coulter) is performed to removed fragments >500 bps and keep all lower molecular weight fragments. The resulting ~160-bp cfDNA fragments (5 to 30 ng) are then end-repaired and ligated to adapters with molecular barcode tags and sequences required for downstream next-generation sequencing (HiSeq2500, Illumina). The ligated cfDNA is amplified over 10 cycles using primers complementary to the ligated adapter sequences.

[00154] To enrich for regions of interest, including fusion genes, the resulting cfDNA libraries are denatured at 95 °C and then hybridized at 65 °C first to oligos that block the added sequences and then to 120-nt biotinylated RNA oligos (Agilent Technologies) and also 120-nt biotinylated RNA/LNA or DNA/LNA oligos (Exiqon) in stringent hybridization buffer for 16

hours. The hybridization reactions are captured using streptavidin beads (Invitrogen), washed to remove non-targeted cfDNA fragments, and eluted using sodium hydroxide. The resulting enriched libraries are amplified for another 12 cycles and sequenced on a HiSeq2500 (Illumina).

Example 2: Sequence Capture

[00155] Cell-free DNA is isolated from a cancer patient.

[00156] A probe set is provided that is configured to capture polynucleotides having sequences of 68 target genes, including four genes involved in gene rearrangements. The probe set comprises sub-sets each sub-set directed to one of the 68 genes in the panel. Each subset directed to a gene not involved in a gene rearrangement is standard affinity subset (includes only non-high affinity polynucleotides, polynucleotides with only natural nucleotides). Each subset directed to a gene involved in a gene rearrangement is a high affinity subset (includes at least one high affinity polynucleotide). The sets have 2X tiling across exons. In the high affinity subsets, high affinity polynucleotides are directed only to breakpoint regions of the gene. The high affinity subsets are doped with high affinity polynucleotides, so that both high affinity polynucleotides and standard affinity polynucleotides are directed to sequences in the breakpoint regions.

[00157] Cell-free DNA and the probe set are combined under stringent hybridization conditions and incubated overnight. The probe set with bound cfDNA is isolated from the mixture. Bound polynucleotides are separated from the probes and sequenced. Polynucleotides comprising sequences across a breakpoint are identified.

[00158] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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) providing a biological sample comprising cell-free nucleic acid molecules from a subject;

(b) contacting the cell-free nucleic acid molecules from the biological sample with a probe set 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 sequence complementary with the fusion gene and containing only unmodified nucleotides;

(c) isolating the probe-captured polynucleotides from the mixture, to produce a sample enriched with isolated 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) 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 one or more locked nucleic acid (LNA) nucleotides.

3. The method of claim 2, 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.

4. The method of claim 3, wherein the at least two of the LNA nucleotides are spaced no more than 15 apart.

5. 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 locked nucleic acid (LNA) nucleotides.

6. The method of claim 5, wherein at least 75% of the nucleotides of each of at least a subset of the plurality of polynucleotide probes are locked nucleic acid (LNA) nucleotides.

7. 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 complementary with the fusion gene and containing only unmodified nucleotides.

8. The method of claim 7, wherein the melting temperature is at least about 10 °C higher.

9. 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 complementary with the fusion gene and containing only unmodified nucleotides.

10. The method of claim 9, wherein the melting temperature is at least about 10% higher.

11. The method of claim 1, wherein the fusion gene is a cancer fusion gene.

12. 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.

13. 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.

14. 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.

15. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length less than about 500 nucleotides.

16. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length between about 20 and about 200 nucleotides.

17. The method of claim 1, wherein each of the plurality of polynucleotide probes has a length between about 80 and about 160 nucleotides.

18. The method of claim 1, wherein each of the breakpoint fragments has a length between about 140 nucleotides and 180 nucleotides.

19. The method of claim 1, wherein the plurality of polynucleotide probes is coupled to a solid support.

20. The method of claim 1, wherein the probe set comprises one or more natural polynucleotide probes.

21. 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.

22. The method of claim 1, wherein each of the plurality of polynucleotide probes provides at least 50% coverage of a breakpoint region of a nucleic acid sequence included in the fusion gene.

23. 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.

24. The method of claim 23, further comprising amplifying the tagged parent polynucleotides to produce tagged progeny polynucleotides.

25. The method of claim 24, 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.

26. The method of claim 25, 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.

27. A method for capturing a breakpoint fragment of a fusion gene, comprising:

(a) providing a biological sample containing or suspected of containing a cell-free nucleic acid molecule comprising the breakpoint fragment of the fusion gene; and

(b) contacting the biological sample with a polynucleotide probe 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 sequence complementary with the fusion gene 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.

28. The method of claim 27, wherein the polynucleotide probe comprises one or more locked nucleic acid (LNA) nucleotides.

29. The method of claim 28, 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.

30. The method of claim 29, wherein the at least two of the LNA nucleotides are spaced no more than 15 nucleotides apart.

31. A probe set comprising a plurality of polynucleotide probes, wherein each of the polynucleotide probes has (i) sequence complementarity with a fusion gene as part of a cell-free nucleic acid molecule and (ii) affinity for the fusion gene that is greater than a polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides.

32. The probe set of claim 31, wherein each of the plurality of polynucleotide probes comprises one or more locked nucleic acid nucleotides.

33. The probe set of claim 31, wherein further comprising one or more natural polynucleotide probes.

34. The probe set of claim 31, wherein each of 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.

35. The probe set of claim 31, wherein each of the plurality of polynucleotide probes provides at least 50% coverage of a breakpoint region of a nucleic acid sequence included in the fusion gene.

36. The probe set of claim 31, wherein the plurality of polynucleotide probes hybridize to portions of one or both of the different genes in the fusion gene.

37. The probe set of claim 31, further comprising a solid support, wherein the plurality of polynucleotide probes is coupled to the solid support.

38. The probe set of claim 31, 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 complementary with the fusion gene and containing only unmodified nucleotides.

39. The probe set of claim 38, wherein the melting temperature is at least about 10 °C higher.

40. The probe set of claim 31, wherein each of the plurality of polynucleotide probes has a melting temperature that is at least about 2 % higher than the polynucleotide having sequence complementary with the fusion gene and containing only unmodified nucleotides.

41. The probe set of claim 40, wherein the melting temperature is at least about 10% higher.

42. The probe set of claim 31, wherein the fusion gene is a cancer fusion gene.

43. The probe set of claim 31, 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.

44. A high affinity polynucleotide, comprising a sequence that is configured to specifically hybridize to a nucleic acid sequence associated with a fusion gene in a cell-free nucleic acid molecule.

45. A high affinity polynucleotide configured to specifically hybridize to a fusion gene.

46. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

47. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide has a melting temperature of at least 1 °C higher than a polynucleotide with the same sequence comprising only natural nucleotides.

48. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide has a melting temperature that is at least 2% higher than a polynucleotide with the same sequence comprising only natural nucleotides.

49. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide is configured to specifically hybridize to a cancer fusion gene.

50. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide is configured to specifically hybridize to a gene of a fusion gene pair of FIGs. 2A-2B or a fusion gene between two or more genes selected from FIG. 3.

51. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide is configured to hybridize within a breakpoint region no more than 500 nucleotides away from a breakpoint of the fusion gene.

52. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide is configured to hybridize across a breakpoint in the fusion gene.

53. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide has a length less than about 500 nucleotides.

54. The high affinity polynucleotide of claim 46, further comprising a plurality of locked nucleic acid (LNA) nucleotides, wherein at least two of the LNA nucleotides are spaced no more than 30 nucleotides apart.

55. The high affinity polynucleotide of claim 46, wherein at least 1% of the nucleotides in the polynucleotide are locked nucleic acid nucleotides.

56. The high affinity polynucleotide of claim 45, wherein the high affinity polynucleotide has a nucleotide sequence perfectly or substantially complementary to a nucleotide sequence of the fusion gene.

57. A high affinity polynucleotide probe comprising a high affinity polynucleotide configured to specifically hybridize to a fusion gene.

58. The probe of claim 57, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

59. The probe of claim 57, further comprising a functionality selected from a detectable label, a binding moiety or a solid support.

60. The probe of claim 57, wherein the high affinity polynucleotide is configured to hybridize to a breakpoint fragment of a fusion gene.

61. The probe of claim 60, wherein the breakpoint fragment has a length between about 140 nucleotides and about 180 nucleotides.

62. The probe of claim 60, wherein the breakpoint fragment is cell-free DNA or genomic DNA.

63. The probe of claim 57, wherein the high affinity polynucleotide is bound to a solid support.

64. A probe set comprising a plurality of polynucleotide probes, each probe configured to specifically hybridize to a fusion gene, wherein the probe set comprises one or more high affinity polynucleotide probes.

65. The probe set of claim 64, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

66. The probe set of claim 64, wherein the probe set comprises one or more natural polynucleotide probes.

67. The probe set of claim 64, further comprising at least one high affinity polynucleotide probe that specifically hybridizes to a breakpoint region of a gene involved in the fusion gene, and at least one natural polynucleotide probe that hybridizes to a non-breakpoint region of the gene involved in the fusion gene.

68. The probe set of claim 64, wherein the one or more high affinity polynucleotide probes in the probe set provide at least 50% (at least 0.5X to 5X) coverage of a breakpoint region of a gene involved in the fusion gene.

69. The probe set of claim 64, wherein the polynucleotide probes hybridize to portions of one or both of the different genes in the fusion gene.

70. The probe set of claim 64, wherein the probe set is configured as an oligonucleotide chip.

71. The probe set of claim 64, wherein a target sequence is targeted by both high affinity polynucleotide probes and standard affinity polynucleotide probes.

72. A kit comprising a plurality of probe sets, wherein each probe set specifically hybridizes to a different gene and at least one of the probe sets is a probe set of claim 64.

73. The kit of claim 72, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

74. A method for capturing a breakpoint fragment of a fusion gene comprising contacting the breakpoint fragment with a high affinity polynucleotide probe under stringent hybridization conditions and allowing hybridization, wherein the polynucleotide probe is bound to a solid support and wherein the polynucleotide probe has a nucleotide sequence that is substantially or perfectly complementary to a nucleotide sequence of the breakpoint fragment.

75. The method of claim 74, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

76. A method for enriching a sample for polynucleotides comprising a breakpoint of a fusion gene, comprising:

(a) contacting a probe set of claim 64 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.

77. The method of claim 76, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

78. The method of claim 76, wherein the polynucleotides comprise cell-free DNA or fragmented genomic DNA.

79. The method of claim 76, further comprising isolating captured polynucleotides from the probes.

80. The method of claim 76, further comprising sequencing the isolated polynucleotides.

81. A method of diagnosing cancer in a subject comprising:

(a) providing a sample comprising polynucleotides from a subject;

(b) contacting cell-free deoxyribonucleic acid from the sample with a probe set of claim 64 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.

82. The method of claim 81, wherein the high affinity polynucleotide comprises one or more locked nucleic acid nucleotides.

FIGURE 1

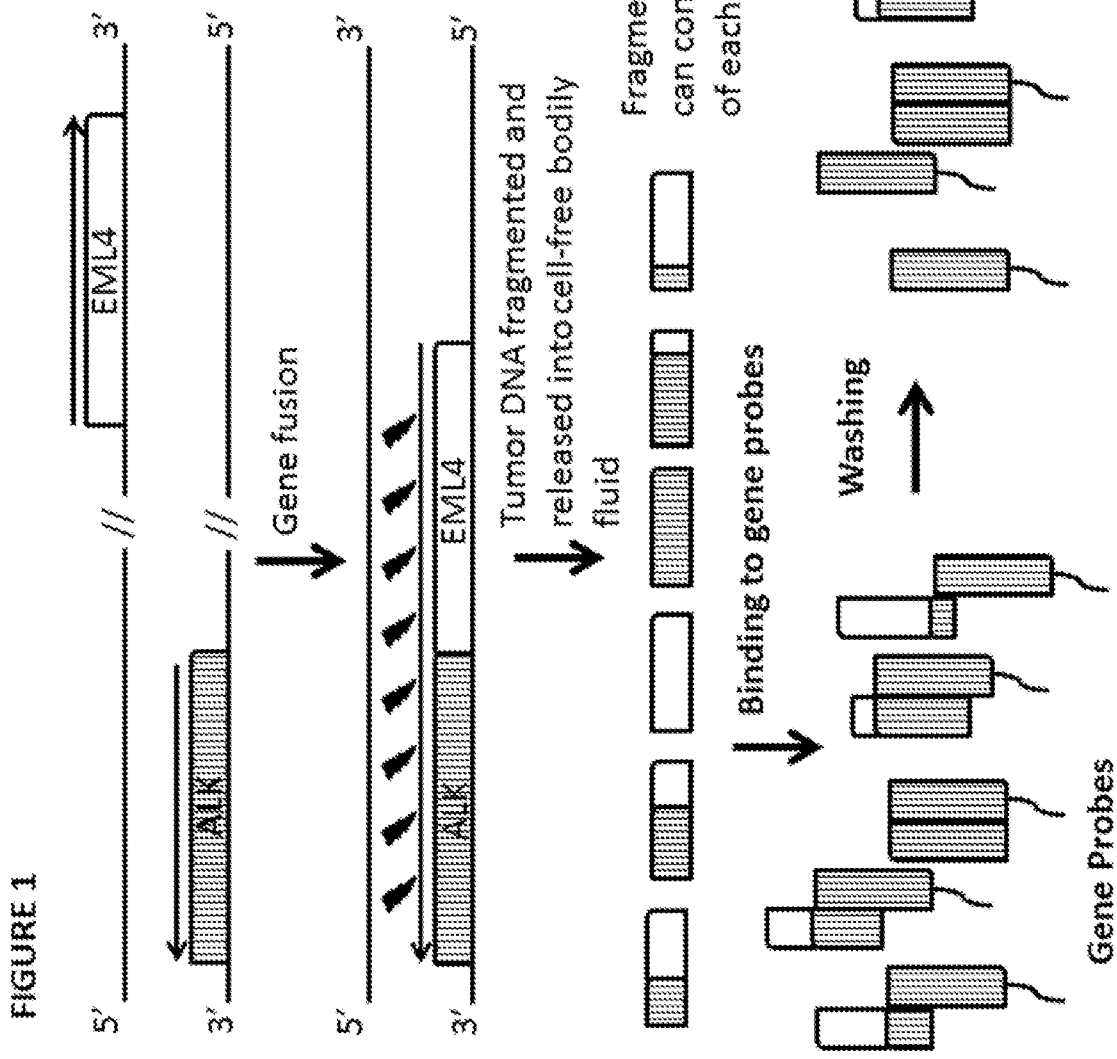

5'
3'
EML4
ALK
Gene fusion
Tumor DNA fragmented and released into cell-free bodily fluid
Fragments spanning breakpoints can contain different proportions of each gene
Binding to gene probes
Washing
Post washing, many breakpoint fragments are lost
Gene Probes

FIG. 2A

ACBD6/RRP15	KMT2A/ELL
ACSL3_ENST00000357430/ETV1	KMT2A/EP300
ACTB/GLI1	KMT2A/EPS15
AGPAT5/MCPH1	KMT2A/FOXO3
AGTRAP/BRAF	KMT2A/FOXO4
AKAP9_ENST00000356239/BRAF	KMT2A/FRYL
ARFIP1/FHDC1	KMT2A/GAS7
ARID1A/MAST2_ENST00000361297	KMT2A/GMPS
ASPSCR1/TFE3	KMT2A/GPHN
ATG4C/FBXO38	KMT2A/KIAA0284_ENST00000414716
ATIC/ALK	KMT2A/KIAA1524
BBS9/PKD1L1	KMT2A/LASP1
BCR/ABL1	KMT2A/LPP
BCR/JAK2	KMT2A/MAPRE1
BRD3/NUTM1	KMT2A/MLLT1
BRD4_ENST00000263377/NUTM1	KMT2A/MLLT10
C2orf44/ALK	KMT2A/MLLT11
CANT1/ETV4	KMT2A/MLLT3
CARS/ALK	KMT2A/MLLT4_ENST00000392108
CCDC6/RET	KMT2A/MLLT6
CD74_ENST00000009530/NRG1	KMT2A/MYO1F
CD74_ENST00000009530/ROS1	KMT2A/NCKIPSD
CDH11/USP6_ENST00000250066	KMT2A/NRIP3
CDKN2D/WDFY2	KMT2A/PDS5A
CEP89/BRAF	KMT2A/PICALM
CHCHD7/PLAG1	KMT2A/PRRC1

CIC/DUX4L1	KMT2A/SARNP
CIC/FOXO4	KMT2A/SEPT2
CLCN6/BRAF	KMT2A/SEPT5
CLIP1/ROS1	KMT2A/SEPT6
CLTC/ALK	KMT2A/SEPT9_ENST00000427177
CLTC/TFE3	KMT2A/SH3GL1
CNBP/USP6_ENST00000250066	KMT2A/SORBS2
COL1A1/PDGFB	KMT2A/TET1
COL1A1/USP6_ENST00000250066	KMT2A/TOP3A
COL1A2/PLAG1	KMT2A/ZFYVE19
CRTC1/MAML2	KTN1/RET
CRTC3/MAML2	LIFR_ENST00000263409/PLAG1
CTAGE5/SIP1	LMNA/NTRK1_ENST00000392302
CTNNB1/PLAG1	LRIG3/ROS1
DCTN1/ALK	LSM14A/BRAF
DDX5_ENST00000540698/ETV4	MARK4/ERCC2
DNAJB1/PRKACA	MBOAT2/PRKCE
EIF3E/RSPO2	MBTD1_ENST00000586178/CXorf67_ENST00000342995
EIF3K/CYP39A1	MEAF6/PHF1
EML4/ALK	MKRN1/BRAF
EPC1/PHF1	MSN/ALK
ERC1/RET	MYB_ENST00000341911/NFIB_ENST00000397581
ERC1/ROS1	MYO5A/ROS1
ERO1L/FERMT2	NAB2/STAT6
ESRP1/RAF1	NACC2/NTRK2
ETV6/ITPR2	NCOA4_ENST00000452682/RET
ETV6/JAK2	NDRG1/ERG
ETV6/NTRK3	NF1/ACCN1

FIG. 2A (continued)

EWSR1/ATF1	NFIA/EHF
EWSR1/CREB1	NFIX_ENST00000360105/MAST1_ENST00000251472
EWSR1/DDIT3	NONO/TFE3
EWSR1/ERG	NOTCH1_ENST00000277541/GABBR2
EWSR1/ETV1	NPM1/ALK
EWSR1/ETV4	NTN1/ACLY
EWSR1/FEV	NUP107/LGR5
EWSR1/FLI1	OMD/USP6_ENST00000250066
EWSR1/NFATC1	PAX3/FOXO1
EWSR1/NFATC2	PAX3/NCOA1
EWSR1/NR4A3	PAX3/NCOA2
EWSR1/PATZ1	PAX5/JAK2
EWSR1/PBX1	PAX7/FOXO1
EWSR1/POU5F1	PAX8/PPARG
EWSR1/SMARCA5	PCM1/JAK2
EWSR1/SP3	PCM1/RET
EWSR1/WT1	PLA2R1/RBMS1
EWSR1/YY1	PLXND1/TMCC1
EWSR1/ZNF384	PPFIBP1/ALK
EWSR1/ZNF444_ENST00000337080	PPFIBP1/ROS1
EZR/ROS1	PRCC/TFE3
FAM131B_ENST00000443739/BRAF	PRKAR1A/RET
FBXL18/RNF216	PTPRK/RSPO3
FCHSD1/BRAF	PWWP2A/ROS1
FGFR1/ZNF703	QKI/NTRK2
FGFR1_ENST00000447712/PLAG1	RAF1/DAZL
FGFR1_ENST00000447712/TACC1	RANBP2/ALK
FGFR3/BAIAP2L1	RBM14/PACS1

FIG. 2A (continued)

FGFR3/TACC3	RGS22/SYCP1
FN1/ALK	RNF130/BRAF
FUS/ATF1	SDC4/ROS1
FUS/CREB3L1	SEC16A_NM_014866.1/NOTCH1_ENST00000277541
FUS/CREB3L2	SEC31A/ALK
FUS/DDIT3	SEC31A/JAK2
FUS/ERG	SEPT8/AFF4
FUS/FEV	SFPQ/TFE3
GATM/BRAF	SLC22A1/CUTA
GMDS/PDE8B	SLC26A6/PRKAR2A
GNAI1/BRAF	SLC34A2/ROS1
GOLGA5/RET	SLC45A3/BRAF
GOPC/ROS1	SLC45A3/ELK4
GPBP1L1/MAST2_ENST00000361297	SLC45A3/ERG
HACL1/RAF1	SLC45A3/ETV1
HAS2/PLAG1	SLC45A3/ETV5_ENST00000306376
HERPUD1/BRAF	SND1/BRAF
HEY1/NCOA2	SQSTM1/ALK
HIP1/ALK	SRGAP3/RAF1
HLA-A/ROS1	SS18/SSX1
HMGA2/ALDH2_ENST00000261733	SS18/SSX2
HMGA2/CCNB1IP1	SS18/SSX4
HMGA2/COX6C	SS18L1/SSX1
HMGA2/EBF1	SSBP2/JAK2
HMGA2/FHIT_ENST00000476844	SSH2/SUZ12
HMGA2/LHFP	STIL/TAL1
HMGA2/LPP	STRN/ALK
HMGA2/NFIB_ENST00000397581	SUSD1/ROD1

FIG. 2A (continued)

HMGA2/RAD51B	TADA2A_ENST00000394395/MAST1_ENST00000251472
HMGA2/WIF1_ENST00000286574	TAF15/NR4A3
HN1/USH1G	TCEA1_ENST00000521604/PLAG1
HNRNPA2B1/ETV1	TCF12/NR4A3
HOOK3/RET	TCF3/PBX1
IL6R/ATP8B2	TECTA/TBCEL
INTS4/GAB2	TFG/ALK
IRF2BP2/CDX1	TFG/NR4A3
JAZF1/PHF1	TFG/NTRK1_ENST00000392302
JAZF1/SUZ12	THRAP3/USP6_ENST00000250066
KIAA1549/BRAF	TMPRSS2/ERG
KIAA1598/ROS1	TMPRSS2/ETV1
KIF5B/ALK	TMPRSS2/ETV4
KIF5B/RET	TMPRSS2/ETV5_ENST00000306376
KLC1/ALK	TP53/NTRK1_ENST00000392302
KLK2/ETV1	TPM3/ALK
KLK2/ETV4	TPM3/NTRK1_ENST00000392302
KMT2A/ABI1	TPM3/ROS1
KMT2A/ABI2	TPM3_ENST00000368530/ROS1
KMT2A/ACTN4	TPM4/ALK
KMT2A/AFF1	TRIM24/RET
KMT2A/AFF3	TRIM27/RET
KMT2A/AFF4	TRIM33_ENST00000358465/RET
KMT2A/ARHGAP26	UBE2L3/KRAS
KMT2A/ARHGEF12	VCL/ALK
KMT2A/BTBD18	VTI1A/TCF7L2
KMT2A/CASC5	YWHAE_ENST00000264335/FAM22A_ENST00000381707
KMT2A/CASP8AP2	YWHAE_ENST00000264335/NUTM2B

FIG. 2A (continued)

KMT2A/CBL	ZC3H7B/BCOR_ENST00000378444
KMT2A/CREBBP	ZCCHC8/ROS1
KMT2A/CT45A2	ZNF700/MAST1_ENST00000251472
KMT2A/DAB2IP	ZSCAN30/BRAF
KMT2A/EEFSEC	

FIG. 2A (continued)

FIG. 2B

Fusion gene Pairs

No	Panel Gene	Partner Gene	No	Panel Gene	Partner Gene	No	Panel Gene	Partner Gene
1	ALK	ATIC	44	RET	ERC1	87	FGFR3	TACC3
2	ALK	C2orf44	45	RET	FKBP15	88	FGFR3	TNIP2
3	ALK	C2orf61	46	RET	GOLGA5	89	FGFR3	TPRG1
4	ALK	CAMKMT	47	RET	HOOK3	90	FGFR3	WHSC1
5	ALK	CARS	48	RET	KIF5B	91	ROS1	CCDC6
6	ALK	CENPF	49	RET	KTN1	92	ROS1	CD72
7	ALK	CLTC	50	RET	NCOA4	93	ROS1	CD74
8	ALK	CLIP4	51	RET	PCM1	94	ROS1	CEP85L
9	ALK	COL4A3	52	RET	PRKAR1A	95	ROS1	CLIP1
10	ALK	DCTN1	53	RET	SPECC1L	96	ROS1	CLTC
11	ALK	EIF2AK3	54	RET	SQSTM1	97	ROS1	ERC1
12	ALK	EML4	55	RET	TBL1XR1	98	ROS1	EZR
13	ALK	FANCL	56	RET	TRIM24	99	ROS1	HLA-A
14	ALK	FN1	57	RET	TRIM27	100	ROS1	KDELR2
15	ALK	GTF2IRD1	58	RET	TRIM33	101	ROS1	KIAA1598
16	ALK	HIP1	59	FGFR2	AFF3	102	ROS1	LRIG3

17	ALK	KCNQ5	60	FGFR2	AHCYL1	103	ROS1	MYO5A
18	ALK	KIF5B	61	FGFR2	BICC1	104	ROS1	PPFIBP1
19	ALK	KLC1	62	FGFR2	C10ORF68	105	ROS1	PWWP2A
20	ALK	MPRIP	63	FGFR2	CASP7	106	ROS1	RUNX1
21	ALK	MSN	64	FGFR2	CCAR2	107	ROS1	SDC4
22	ALK	MYH9	65	FGFR2	CCDC6	108	ROS1	SLC34A2
23	ALK	NPM1	66	FGFR2	CD44	109	ROS1	SLC35F1
24	ALK	PPFIBP1	67	FGFR2	CIT	110	ROS1	TFG
25	ALK	PPM1B	68	FGFR2	KIAA1598	111	ROS1	TMEM106B
26	ALK	PPP1CB	69	FGFR2	MGEA5	112	ROS1	TPM3
27	ALK	PRKAR1A	70	FGFR2	NCALD	113	ROS1	TTC28
28	ALK	PTPN3	71	FGFR2	OFD1	114	ROS1	YWHAE
29	ALK	RANBP2	72	FGFR2	PPAPDC1A	115	ROS1	ZCCHC8
30	ALK	RNF213	73	FGFR2	PPHLN1	116	NTRK1	CD74
31	ALK	SEC31A	74	FGFR2	RASAL2	117	NTRK1	IRF2BP2
32	ALK	SOCS5	75	FGFR2	SLC45A3	118	NTRK1	LMNA

FIG. 2B (continued)

33	ALK	SQSTM1	76	FGFR2	TACC2	119	NTRK1	MPRIP
34	ALK	STK32B	77	FGFR2	TACC3	120	NTRK1	NFASC
35	ALK	STRN	78	FGFR2	TXLNB	121	NTRK1	PEAR1
36	ALK	TFG	79	FGFR2	USP10	122	NTRK1	PLEKHA6
37	ALK	TPM1	80	FGFR2	VCL	123	NTRK1	QKI
38	ALK	TPM3	81	FGFR3	ADD1	124	NTRK1	RABGAP1L
39	ALK	TPM4	82	FGFR3	AES	125	NTRK1	SQSTM1
40	ALK	VCL	83	FGFR3	BAIAP2L1	126	NTRK1	SSBP2
41	RET	AFAP1	84	FGFR3	ELAVL3	127	NTRK1	TFG
42	RET	AKAP13	85	FGFR3	ETV6	128	NTRK1	TP53
43	RET	CCDC6	86	FGFR3	JAKMIP1	129	NTRK1	TPM3

FIG. 2B (continued)

FIG. 3

ACBD6
ACCN1
ACLY
ACSL3_ENST00000357430
ACTB
AFF4
AGPAT5
AGTRAP
AKAP9_ENST00000356239
ALDH2_ENST00000261733
ALK
ARFIP1
ARID1A
ASPSCR1
ATF1
ATG4C
ATIC
ATP8B2
BAIAP2L1
BBS9
BCOR_ENST00000378444
BCR
BRAF
BRD3
BRD4_ENST00000263377
C2orf44
CANT1
CARS
CCDC6

CCNB1IP1
CD74_ENST00000009530
CDH11
CDKN2D
CDX1
CEP89
CHCHD7
CIC
CLCN6
CLIP1
CLTC
CNBP
COL1A1
COL1A2
COX6C
CREB1
CREB3L1
CREB3L2
CRTC1
CRTC3
CTAGE5
CTNNB1
CUTA
CXorf67_ENST00000342995
CYP39A1
DAZL
DCTN1
DDIT3
DDX5_ENST00000540698
DUX4L1
EBF1
EHF

FIG. 3 (continued)

EIF3E
EIF3K
ELK4
EML4
EPC1
ERC1
ERCC2
ERG
ERO1L
ESRP1
ETV1
ETV4
ETV5_ENST00000306376
ETV6
EWSR1
EZR
FAM131B_ENST00000443739
FAM22A_ENST00000381707
FBXL18
FBXO38
FCHSD1
FERMT2
FEV
FGFR1
FGFR1_ENST00000447712
FGFR3
FHDC1
FHIT_ENST00000476844
FLI1
FN1
FOXO1
FOXO4

FIG. 3 (continued)

FUS
GAB2
GABBR2
GATM
GLI1
GMDS
GNAI1
GOLGA5
GOPC
GPBP1L1
HACL1
HAS2
HERPUD1
HEY1
HIP1
HLA-A
HMGA2
HN1
HNRNPA2B1
HOOK3
IL6R
INTS4
IRF2BP2
ITPR2
JAK2
JAZF1
KIAA1549
KIAA1598
KIF5B
KLC1
KLK2
KRAS

FIG. 3 (continued)

KTN1
LGR5
LHFP
LIFR_ENST00000263409
LMNA
LPP
LRIG3
LSM14A
MAML2
MARK4
MAST1_ENST00000251472
MAST2_ENST00000361297
MBOAT2
MBTD1_ENST00000586178
MCPH1
MEAF6
MKRN1
MSN
MYB_ENST00000341911
MYO5A
NAB2
NACC2
NCOA1
NCOA2
NCOA4_ENST00000452682
NDRG1
NF1
NFATC1
NFATC2
NFIA
NFIB_ENST00000397581
NFIX_ENST00000360105

FIG. 3 (continued)

NONO
NOTCH1_ENST00000277541
NPM1
NR4A3
NRG1
NTN1
NTRK1_ENST00000392302
NTRK2
NTRK3
NUP107
NUTM1
NUTM2B
OMD
PACS1
PATZ1
PAX3
PAX5
PAX7
PAX8
PBX1
PCM1
PDE8B
PDGFB
PHF1
PKD1L1
PLA2R1
PLAG1
PLXND1
POU5F1
PPARG
PPFIBP1
PRCC

FIG. 3 (continued)

PRKAR1A
PRKAR2A
PRKCE
PTPRK
PWWP2A
QKI
RAD51B
RAF1
RANBP2
RBM14
RBMS1
RET
RGS22
RNF130
RNF216
ROD1
ROS1
RRP15
RSPO2
RSPO3
SDC4
SEC16A_NM_014866.1
SEC31A
SEPT8
SFPQ
SIP1
SLC22A1
SLC26A6
SLC34A2
SLC45A3
SMARCA5
SND1

FIG. 3 (continued)

SP3
SQSTM1
SRGAP3
SS18
SS18L1
SSBP2
SSH2
SSX1
SSX2
SSX4
STAT6
STRN
SUSD1
SUZ12
SYCP1
TACC1
TACC3
TADA2A_ENST00000394395
TAF15
TBCEL
TCEA1_ENST00000521604
TCF12
TCF3
TCF7L2
TECTA
TFE3
TFG
THRAP3
TMCC1
TMPRSS2
TP53
TPM3

FIG. 3 (continued)

TPM3_ENST00000368530
TPM4
TRIM24
TRIM27
TRIM33_ENST00000358465
UBE2L3
USH1G
USP6_ENST00000250066
VCL
VTI1A
WDFY2
WIF1_ENST00000286574
WT1
YWHAE_ENST00000264335
YY1
ZC3H7B
ZCCHC8
ZNF384
ZNF444_ENST00000337080
ZNF700
ZNF703
ZSCAN30

FIG. 3 (continued)

Figure 4A

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF463	EML4	13	1751	-	ALK	20	4080	-	170	25%
COSF474	EML4	6	929+220	-	ALK	20	4080	-	93	13.68%
COSF465	EML4	20	2504	-	ALK	20	4080	-	48	7.06%
COSF734	EML4	6	929	-	ALK	20	4080	-	31	4.56%
COSF488	EML4	18	2318+654	y	ALK	20	4080-172	-	4	.59%
COSF1545	EML4	6	929	-	ALK	20	4080-18	-	2	.29%
COSF1376	EML4	18	2318	-	ALK	20	4080	-	2	.29%
COSF480	EML4	2	470	-	ALK	20	4080-117	-	2	.29%
COSF475	EML4	15	2029	-	ALK	20	4080	-	2	.29%
COSF733	EML4	17	2229	y	ALK	20	4080	-	1	.15%
COSF1063	EML4	13	1751	-	ALK	20	4080-69	-	1	.15%
COSF1065	EML4	14	1903	-	ALK	20	4080	-	1	.15%
COSF1128	EML4	14	1903	-	ALK	20	4080	-	1	.15%
COSF1297	EML4	6	929	-	ALK	20	3975	-	1	.15%
COSF1368	EML4	17	2229+2522	-	ALK	20	4126	-	1	.15%
COSF1540	EML4	13	1751	-	ALK	20	4080-90	-	1	.15%
COSF1542	EML4	14	1903	-	ALK	20	4080-123	-	1	.15%
COSF1543	EML4	2	470	-	ALK	20	4080	-	1	.15%
COSF731	EML4	20	2504	-	ALK	20	4080-18	-	1	.15%
COSF493	EML4	6	929+805	y	ALK	20	4080-115	-	1	.15%
COSF491	EML4	14	1903	y	ALK	20	4129	-	1	.15%
COSF490	EML4	20	2504+182	-	ALK	20	4080-67	-	1	.15%

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF489	EML4	13	1751+447	-	ALK	20	4080-161	-	1	.15%
COSF476	EML4	6	929+(7320)	-	ALK	20	4080	-	1	.15%
COSF464	EML4	20	2504+545	-	ALK	20	4080-232	-	1	.15%
COSF462	EML4	13	1751+(3600)	-	ALK	20	4080-297	-	1	.15%
COSF410	EML4	13	1751+1485	-	ALK	20	4080-1254	-	1	.15%
COSF414	EML4	13	1751+2575	-	ALK	20	4080-203	-	1	.15%
COSF473	EML4	?	?	-	ALK	?	?	-	307	45%
Total Mutations									**680**	**100%**

FIG. 4A (continued)

Figure 4B

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1272	CCDC6	1	535	-	RET	12	2369	-	583	98.65%
COSF1516	CCDC6	2	685	-	RET	12	2369	-	3	.51%
COSF1532	CCDC6	1	535+1111	-	RET	12	4080-232	-	1	.17%
COSF1533	CCDC6	1	535+1054	-	RET	12	2369-?	-	1	.17%
COSF1518	CCDC6	8	1462+848	-	RET	11	2237	-	1	.17%
COSF1480	CCDC6	?	?	-	RET	?	?	-	2	0%
Total Mutations									591	100%

Figure 4C

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF125	TMPRSS2	1(utr)	79	-	ERG	4	312	-	476	20.82%
COSF128	TMPRSS2	2	150	-	ERG	4	312	-	38	1.66%
COSF123	TMPRSS2	1(utr)	79	-	ERG	2(utr)	124	-	27	1.18%
COSF126	TMPRSS2	1(utr)	79	-	ERG	5	530	-	21	.92%
COSF127	TMPRSS2	2	150	-	ERG	2(utr)	124	-	7	.31%
COSF736	TMPRSS2	2	150	-	ERG	3	226	-	4	.17%
COSF130	TMPRSS2	3	373	-	ERG	4	312	-	3	.13%
COSF135	TMPRSS2	3	373	-	ERG	2(utr)	124	-	3	.13%
COSF124	TMPRSS2	1(utr)	79	-	ERG	3	226	-	3	.13%
COSF116	TMPRSS2	5	580	-	ERG	4	312	-	3	.13%
COSF129	TMPRSS2	2	150	-	ERG	5	530	-	3	.13%
COSF139	TMPRSS2	1(utr)	79+?	-	ERG	4	312	-	1	.04%
COSF138	TMPRSS2	1(utr)	79	-	ERG	4	312	-	1	.04%
COSF118	TMPRSS2	4	460	-	ERG	4	312	-	1	.04%
COSF121	TMPRSS2	?	?	-	ERG	?	?	-	1695	74%
Total Mutations									**2286**	**100%**

Figure 4D

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1492	NCOA4_ENST00000452682	8	1014	-	RET	12	2369	-	351	97.23%
COSF1341	NCOA4_ENST00000452682	7	870+196	-	RET	12	2369-1570	-	3	.83%
COSF1531	NCOA4_ENST00000452682	8	1014+21	-	RET	12	2369-1591	-	1	.28%
COSF1499	NCOA4_ENST00000452682	8	996	-	RET	12	2369-7	-	1	.28%
COSF1501	NCOA4_ENST00000452682	8	1014+1186	-	RET	11	2273	-	1	.28%
COSF1502	RET	11	2271	-	NCOA4_ENST00000452682	9	1015-490	-	1	.28%
COSF1495	RET	11	2368+?	-	NCOA4_ENST00000452682	8	871-7	-	1	.28%
COSF1493	NCOA4_ENST00000452682	7	870+42	-	RET	12	2369-1673	-	1	.28%
COSF1497	RET	11	2368+?	-	NCOA4_ENST00000452682	9	1015	-	1	.28%
Total Mutations									**361**	**100%**

Figure 4E

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1233	KIF5B	15	2183	-	RET	12	2327	-	36	59.02%
COSF1231	KIF5B	16	2372+476	-	RET	12	2327-436	-	7	11.48%
COSF1610	KIF5B	16	2372	-	RET	12	2327	-	3	4.92%
COSF1254	KIF5B	22	2897	-	RET	12	2327	-	3	4.92%
COSF1235	KIF5B	23	3002	-	RET	12	2327	-	2	3.28%
COSF1242	KIF5B	24	3219+372	-	RET	8	1713-416	-	1	1.64%
COSF1256	KIF5B	15	2183	-	RET	11	2177	-	1	1.64%
COSF1263	KIF5B	24	3219	-	RET	11	2070	-	1	1.64%
COSF1242	KIF5B	24	3219+372	-	RET	7	1317-416	-	1	1.64%
COSF1241	KIF5B	23	3002+152	y	RET	12	2327-12	-	1	1.64%
COSF1239	KIF5B	15	2183+2335	-	RET	12	2327-1785	-	1	1.64%
COSF1238	KIF5B	15	2183+550	y	RET	12	2327-741	-	1	1.64%
COSF1237	KIF5B	15	2183+2204	-	RET	12	2327-318	-	1	1.64%
COSF1240	KIF5B	16	2372+334	-	RET	12	2327-450	-	1	1.64%
COSF1252	KIF5B	?	?	-	RET	?	?	-	2	3%
Total Mutations									**61**	**100%**

Figure 4F

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1059	KIF5B	24	3219	-	ALK	20	4080	-	4	40%
COSF1382	KIF5B	15	2183	-	ALK	20	4080	-	2	20%
COSF1258	KIF5B	17	2490	-	ALK	20	4080	-	2	20%
COSF1061	KIF5B	15	2183+2477	-	ALK	19	4005	-	1	10%
COSF1485	KIF5B	?	?	-	ALK	?	?	-	1	10%
Total Mutations									10	100%

Figure 4G

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF166	EWSR1	7	1112	-	FLI1	6	920	-	736	54.04%
COSF168	EWSR1	7	1112	-	FLI1	5	854	-	345	25.33%
COSF170	EWSR1	10	1364	-	FLI1	6	920	-	41	3.01%
COSF172	EWSR1	10	1364	-	FLI1	5	854	-	33	2.42%
COSF177	EWSR1	7	1112	-	FLI1	8	1046	-	15	1.1%
COSF181	EWSR1	7	1112	-	FLI1	7	986	-	9	.66%
COSF185	EWSR1	10	1364	-	FLI1	8	1046	-	4	.29%
COSF178	EWSR1	9	1331	-	FLI1	4	650	-	4	.29%
COSF184	EWSR1	9	1331	-	FLI1	7	986	-	3	.22%
COSF228	EWSR1	7	1112	-	FLI1	9	1094	-	3	.22%
COSF1303	EWSR1	10	1364	-	FLI1	7	986	-	2	.15%
COSF183	EWSR1	8	1293	-	FLI1	7	986	-	1	.07%
COSF205	EWSR1	8	1293	-	FLI1	6	920	-	1	.07%
COSF179	EWSR1	?	?	-	FLI1	?	?	-	165	12%
Total Mutations									**1362**	**100%**

Figure 4H

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF155	EWSR1	7	1112	-	ERG	8	967	-	27	22.13%
COSF154	EWSR1	7	1112	-	ERG	11	1141	-	15	12.3%
COSF161	EWSR1	7	1112	-	ERG	9	1036	-	5	4.1%
COSF162	EWSR1	10	1364	-	ERG	8	967	-	4	3.28%
COSF156	EWSR1	?	1112	-	ERG	10	1093	-	3	2.46%
COSF159	EWSR1	?	?	-	ERG	?	?	-	68	56%
Total Mutations									**122**	**100%**

Figure 4I

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1201	CD74_ENST00000009530	6	627	-	ROS1	34	5757	-	32	84.21%
COSF1203	CD74_ENST00000009530	6	627	-	ROS1	32	5448	-	3	7.89%
COSF1619	CD74_ENST00000009530	?	?	-	ROS1	?	?	-	3	8%
Total Mutations									**38**	**100%**

Figure 4J

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1220	PAX8	10	1355	-	PPARG	2	216	-	22	25.58%
COSF1224	PAX8	8	1064	-	PPARG	2	216	-	7	8.14%
COSF1218	PAX8	9	1253	-	PPARG	2	216	-	4	4.65%
COSF1225	PAX8	7	943	-	PPARG	2	216	-	1	1.16%
COSF1216	PAX8	?	?	-	PPARG	?	?	-	52	60%
Total Mutations									86	100%

Figure 4K

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF199	NPM1	5	487	-	ALK	20	4080	-	301	94.36%
COSF448	NPM1	5	487+192	-	ALK	20	4080-616	-	1	.31%
COSF449	NPM1	5	487+201	-	ALK	20	4080-24	-	1	.31%
COSF450	NPM1	5	487+230	-	ALK	20	4080-1134	-	1	.31%
COSF451	NPM1	5	487+295	-	ALK	20	4080-954	-	1	.31%
COSF458	NPM1	5	487+897	-	ALK	20	4080-1511	-	1	.31%
COSF453	NPM1	5	487+443	-	ALK	20	4080-1040	-	1	.31%
COSF454	NPM1	5	487+546	-	ALK	20	4080-583	-	1	.31%
COSF455	NPM1	5	487+726	-	ALK	20	4080-660	-	1	.31%
COSF456	NPM1	5	487+778	-	ALK	20	4080-208	-	1	.31%
COSF457	NPM1	5	487+865	-	ALK	20	4080-629	-	1	.31%
COSF201	NPM1	5	487	-	ALK	20	4080	-	1	.31%
COSF452	NPM1	5	487+358	-	ALK	20	4080-1128	-	1	.31%
COSF420	NPM1	?	?	-	ALK	?	?	-	6	2%
Total Mutations									**319**	**100%**

Figure 4L

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF482	KIAA1549	15	5097	-	BRAF	9	1202	-	318	53.45%
COSF484	KIAA1549	14	4779	-	BRAF	9	1202	-	105	17.65%
COSF486	KIAA1549	15	5097	-	BRAF	11	1376	-	39	6.55%
COSF1229	KIAA1549	17	5302	-	BRAF	10	1239	-	4	.67%
COSF1227	KIAA1549	14	4779	-	BRAF	11	1376	-	3	.5%
COSF1477	KIAA1549	14	4779+34	-	BRAF	10	1239	-	1	.17%
COSF510	KIAA1549	17	5302	-	BRAF	10	1239	-	1	.17%
COSF1284	KIAA1549	15	5097	-	BRAF	10	1239	-	1	.17%
COSF1472	KIAA1549	18	5448	-	BRAF	9	1202	-	1	.17%
COSF1475	KIAA1549	12	4401	-	BRAF	9	1202	-	1	.17%
COSF512	KIAA1549	18	5448	-	BRAF	9	1202	-	1	.17%
COSF588	KIAA1549	?	?	-	BRAF	?	?	-	120	20%
Total Mutations									**595**	**100%**

Figure 4M

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF501	SS18	10	1286	-	SSX2	6	439	-	334	93.3%
COSF587	SS18	10	1286+?	-	SSX2	6	439-?	-	5	1.4%
COSF530	SS18	10	1286+9829	-	SSX2	6	439	-	2	.56%
COSF567	SS18	10	1286	y	SSX2	6	439-1995	-	1	.28%
COSF579	SS18	10	1286+1984	-	SSX2	6	439-684	-	1	.28%
COSF580	SSX2	5	438+280	-	SS18	11	1287-12012	-	1	.28%
COSF583	SS18	10	1286+1455	-	SSX2	6	439-1412	-	1	.28%
COSF584	SS18	10	1286+4538	-	SSX2	6	439-606	-	1	.28%
COSF585	SSX2	5	438+420	-	SS18	11	1287-12550	-	1	.28%
COSF586	SSX2	5	438+1318	-	SS18	11	1287-9312	-	1	.28%
COSF524	SSX2	4	388+1711	-	SS18	11	1287-12732	-	1	.28%
COSF523	SS18	10	1286+701	-	SSX2	5	389-6	-	1	.28%
COSF517	SS18	10	1286	-	SSX2	4	313	-	1	.28%
COSF574	SS18	10	1286+10808	-	SSX2	4	293	-	1	.28%
COSF576	SSX2	?	?	-	SS18	?	?	-	6	2%
Total Mutation									358	100%

Figure 4N

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF504	SS18	10	1286	-	SSX1	6	467	-	569	95.95%
COSF514	SS18	10	1286	-	SSX1	5	417	-	1	.17%
COSF519	SS18	10	1286	-	SSX1	6	467-103	-	1	.17%
COSF581	SS18	10	1286+8793	-	SSX1	6	467-7	-	1	.17%
COSF528	SS18	9	1152	-	SSX1	4	321	-	1	.17%
COSF577	SS18	10	1286+1941	-	SSX1	6	467-1313	-	1	.17%
COSF578	SSX1	5	466+575	-	SS18	11	1287-12019	-	1	.17%
COSF506	SS18	9	1152	-	SSX1	5	417	-	1	.17%
COSF521	SS18	10	1286	y	SSX1	4	321	-	1	.17%
COSF575	SSX1	?	?	-	SS18	?	?	-	15	3%
COSF515	SS18	?	?	-	SSX1	?	?	-	1	0%
Total Mutations									**593**	**100%**

Figure 4O

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1197	SLC34A2	4	429	-	ROS1	32	5448	-	8	66.6%
COSF1260	SLC34A2	13	2076	-	ROS1	32	5448	-	1	8.33%
COSF1618	SLC34A2	?	?	-	ROS1	?	?	-	3	25%
Total Mutations									**12**	**100%**

Figure 4P

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF248	PAX3	7	1539	-	FOXO1	2	1016	-	357	92.49%
COSF906	FOXO1	1	1015	-	PAX3	8	1540	-	6	1.55%
COSF343	PAX3	?	?	-	FOXO1	?	?	-	23	6%
Total Mutations									**386**	**100%**

Figure 4Q

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1512	PRKARIA	7	825	-	RET	12	2369	-	30	100%
Total Mutations									**30**	**100%**

Figure 4R

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF1268	EZR	10	1259	-	ROS1	34	5757	-	6	85.71%
COSF1396	EZR	10	1259+207	-	ROS1	34	1259+210	-	1	14.29%
Total Mutation									**7**	**100%**

Figure 4S

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF115	TMPRSS2	2	150	-	ETV1	6	850	-	2	20%
COSF134	TMPRSS2	2	150	-	ETV1	7	904	-	1	10%
COSF122	TMPRSS2	?	?	-	ETV1	?	?	-	7	70%
Total Mutations									**10**	**100%**

Figure 4T

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF572	ETV6	5	1283	-	NTRK3	15	1908	-	118	81.38%
COSF1535	ETV6	4	737	-	NTRK3	14	1719	-	15	10.34%
COSF1537	ETV6	5	1283	-	NTRK3	14	1719	-	1	.69%
COSF824	ETV6	4	737	-	NTRK3	15	1908	-	1	.69%
COSF889	NTRK3	14	1907	-	ETV6	6	1284	-	1	.69%
COSF495	ETV6	?	?	-	NTRK3	?	?	-	9	6%
Total Mutations									**145**	**100%**

Figure 4U

Mutation ID	5' Partner Gene				3' Partner Gene				No. of Mutations	Mutation Frequency
	Gene Name	Last Observed Exon	Inferred Breakpoint	Inserted Sequence	Gene Name	First Observed Exon	Inferred Breakpoint	Inserted Sequence		
COSF288	PAX7	?	1753	-	FOXO1	2	1016	-	98	98.99%
COSF344	PAX7	?	?	-	FOXO1	?	?	-	1	1%
Total Mutations									**99**	**100%**

FIG. 7

68 Gene Panel

Genes with Complete Exon and Partial Intron Coverage

APC	***AR***	*ARID1A*	***BRAF***	*BRCA1*	*BRCA2*	*CCND1*	*CCND2*	***CCNE1***
CDK4	***CDK6***	*CDKN2A*	*CDKN2B*	***EGFR****	***ERBB2***	***FGFR1***	***FGFR2***	*HRAS*
KIT	***KRAS***	***MET***	***MYC***	*NF1*	*NRAS*	***PDGFRA***	***PIK3CA***	*PTEN*
RAF1	*TP53*							

Genes in bold are those that are also analyzed for copy number variations (CMVs).

*Includes indels in Exon 19 and 20

Genes with Critical Exon Coverage

AKT1	*ALK*	*ARAF*	*ATM*	*CDH1*	*CTNNB1*	*ESR1*	*EZH2*	*FBXW7*
FGFR3	*GATA3*	*GNA11*	*GNAQ*	*GNAS*	*HNF1A*	*IDH1*	*IHD2*	*JAK2*
JAK3	*MAP2K1*	*MAP2K2*	*MLH1*	*MPL*	*NFE2L2*	*NOTCH1*	*NPM1*	*NTRK1*
PTPN11	*RET*	*RHEB*	*RHOA*	*RIT1*	*ROS1*	*SMAD4*	*SMO*	*SRC*
STK11	*TERT*	*VHL*						

*Includes promoter region

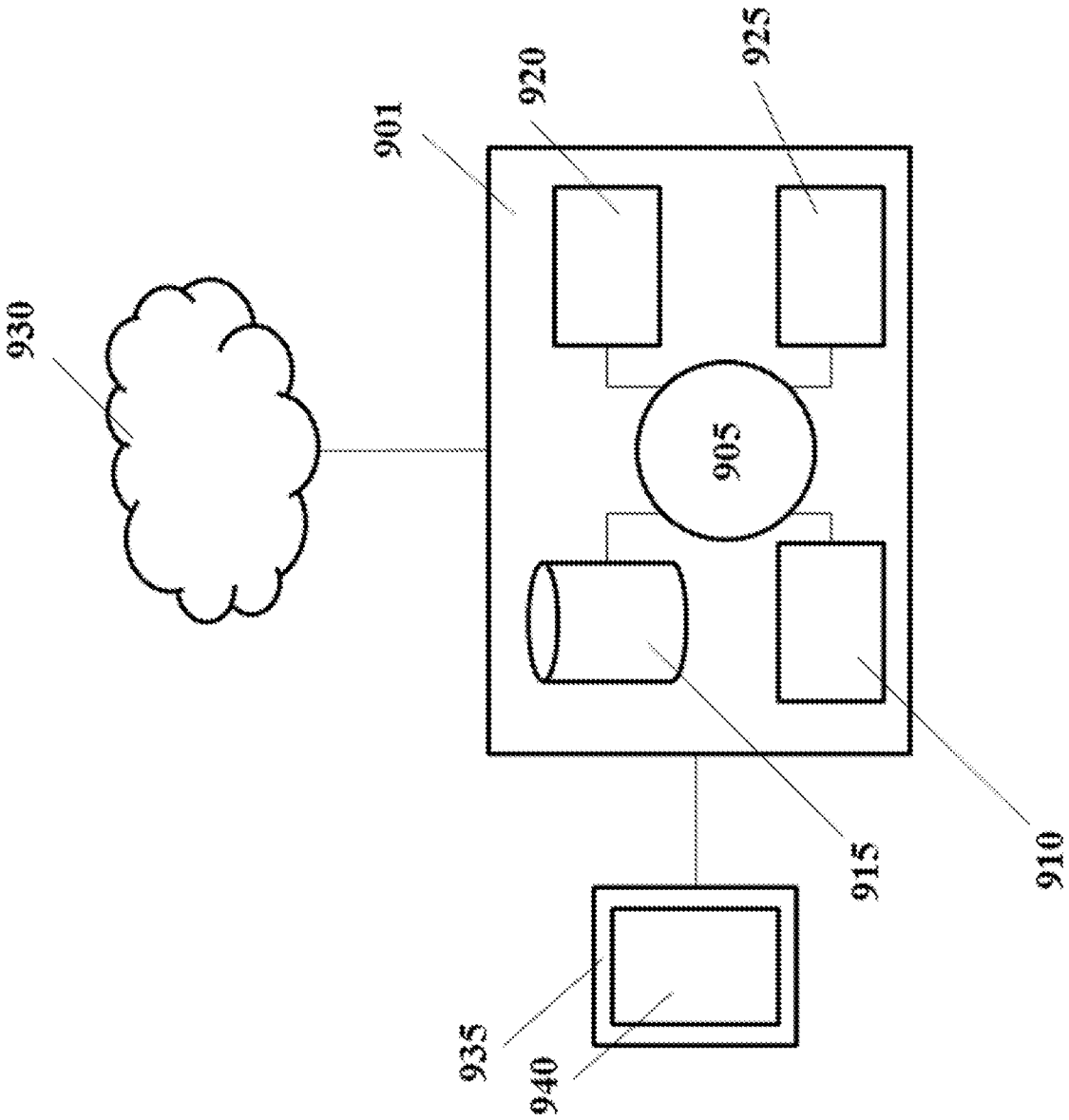
Rearrangements

ALK	*NTRK1*	*RET*	*ROS1*

FIG. 8

Gene	chromosome	start	stop	length (bps)	Exons Covered
ALK	chr2	29446405	29446655	250	intron 19
ALK	chr2	29446062	29446197	135	intron 20
ALK	chr2	29446198	29446404	206	20
ALK	chr2	29447353	29447473	120	intron 19
ALK	chr2	29447614	29448316	702	intron 19
ALK	chr2	29448317	29448441	124	19
ALK	chr2	29449366	29449777	411	intron 18
ALK	chr2	29449778	29449950	172	18

FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43430

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C40B 40/06, 30/04; C12P 19/34 (2016.01)
CPC - C12Q 1/6878, 1/6886, 1/6869, 1/6837, 1/6827, 2600/156, 2310/3231
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/68, C40B 40/06, 30/04; C12P 19/34 (2016.01)
CPC: C12Q 1/6878, 1/6886, 1/6869, 1/6837, 1/6827, 2600/156, 2310/3231

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C12Q 1/6878, 1/6886, 1/6869, 1/6837, 1/6827, 2600/156, 2310/3231 (text search)
USPC: 506/9, 506/16; 435/91.1; 536/23.41 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Patents; Google Scholar
Search terms: Fusion junction, breakpoint, cancer, detection, microarray, probe, locked nucleic acid (LNA), capture, amplification, NGS sequencing, cell-free nucleic acid sample

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0279890 A1 (LOTHE et al.) 04 November 2010 (04.11.2010). Especially para [0015], [0016], [0027], [0038], [0074], [0092], [0097], [0098], [0102], [0106], [0108].	27-30, 74-77, 79, 80 --- 1-26, 78, 81, 82
Y	WO 2015/100427 A1 (Guardant Health, Inc.) 02 July 2015 (02.07.2015). Especially para [0019], [0034], [0095].	1-26, 78, 81, 82
Y	YOU et al. Design of LNA probes that improve mismatch discrimination. Nucleic Acids Res 2 May 2006 Vol 34 No 8 Pages e60 1-11. Especially pg 9 table 4.	7-10

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 November 2016	15 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43430

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
----Go to Extra Sheet for continuation-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-30, 74-82

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43430

-----continuation of Box III (Lack of Unity of Invention)--------

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-30, 74-82 drawn to a method of capturing a breakpoint fragment of a fusion gene

Group II: Claims 31-73, drawn to a probe set of high affinity oligonucleotide probes configures to hybridize to a fusion gene.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of a method of capturing a breakpoint fragment of a fusion gene, not required by Group II.

Group II has the special technical feature of a composition comprising high affinity oligonucleotide probes, not required by Group I.

Common Technical Feature:

1. a plurality of polynucleotide probes, each probe configured to specifically hybridize to a fusion gene

2. a cell-free nucleic acid sample.

Group I claims 76 and 81 depends from Group II claim 64.

However, said common technical features do not represent a contribution over the prior art, and is obvious over US 2010/0279890 A1 to LOTHE et al. (hereinafter "Lothe"), in view of WO 2015/100427 A1 to Guardant Health Inc. (hereinafter "Guardant")[published 2 July 2015].

As to common technical feature #1 and claim 64, Lothe teaches a probe set comprising a plurality of polynucleotide probes, each probe configured to specifically hybridize to a fusion gene (para [0028]; "Preferably, the microarray of the invention comprises intragenic probes targeting fusion gene partners of more than one fusion gene. For example the microarray of the present invention may comprise intragenic probes for at least 2 fusion genes, such as at least 5 fusion genes or at least 10 fusion genes"; para [0038]; "A chimeric probe as used herein is a nucleic acid or a nucleic acid analogue, capable of sequence-specific base pairing, which comprises a first sequence corresponding to an exon of a first gene and a second sequence corresponding to an exon of a second gene"), wherein the probe set comprises one or more high affinity polynucleotide probes (para [0038] "The chimeric probe may consist of or comprise non-natural nucleotides such as LNA monomers (locked nucleic acid monomers)").

As to common technical feature #2, although Lothe teaches a nucleic acid sample (para [0092]; "The sample may be any biological material, such as e.g. blood or bone marrow from a patient or person suspected having a cancer"), but does not specifically list cell-free nucleic acid. However, using cell-free nucleic acid for diagnostic purposes was well--known in the art, for example, as taught by Guardant (para [0095]; A polynucleotide can also be cell-free DNA (cfDNA). For example, the polynucleotide can be circulating DNA. The circulating DNA can comprise circulating tumor DNA (ctDNA)"). It would have been obvious to one of ordinary skill in the art to have detected the presence of fusion gene in a sample comprising cell-free tumor DNA of Guardant, because Guardant teaches that circulating tumor DNA can be used for tumor detection.

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.