

to sequencing. Several target capture methodologies have been developed and integrated with high throughput sequencing systems, e.g., next-generation sequencing methods. Generally, targeted sequencing methods involve two separate steps, a target capture step and a library preparation step. Whole genome sequencing also generally involves a library preparation step. Preparation of a nucleic acid library for sequencing on a next-generation sequencing platform often involves ligation of two distinct adaptor oligonucleotides onto nucleic acid molecules, a multi-step process that has been predicted to result in about 1% of the nucleic acid molecules in a sample being correctly adapted. This major loss of nucleic acids, due to inefficient adaptor ligation, can result in zero representation of several genomic regions of interest in the resulting library. Furthermore, inefficient adaptor ligation can necessitate pre-amplification of library members to obtain enough material for sequencing to a desired read depth. This pre-amplification step has been shown to be a major source of bias in the final library, as inherent differences in PCR efficiency can result in over-representation of some genomic regions and underrepresentation of other genomic regions (see, e.g., Aird et al. Genome Biology 2011, 12:R18, hereby incorporated by reference in its entirety). Furthermore, pre-amplification of the nucleic acid library can introduce sequencing errors due to an intrinsic error rate of nucleic acid polymerases. The introduction of pre-amplification bias and errors resulting from pre-amplification can have deleterious consequences on diagnostic sequencing applications, in which the accurate detection of rare mutations from a limited starting sample is often desired.

SUMMARY OF THE INVENTION

[0017] Aspects of the invention relate to methods and kits for assessing cancer. Some aspects of the invention relate to methods and kits for preparing a sample library for sequencing. Some aspects of the invention relate to methods and kits for allele detection. Some aspects of the invention relate to high efficiency ligation methods and kits. Some aspects of the invention relate to sensitive detection of amplicons.

[0017a] In one aspect, there is provided a method comprising ligating single-stranded template nucleic acids to single-stranded adaptor molecules, wherein said single-stranded template nucleic acids have an average length of 150-10000 nucleotides, wherein an efficiency of said ligating said single-stranded template nucleic acids is at least 10%.

[0017b] In one aspect, there is provided a method of ligating single-stranded donor nucleic acid molecules and single-stranded acceptor nucleic acid molecules, said method comprising:

- a. transferring a nucleotide monophosphate (NMP) to single-stranded donor nucleic acid molecules in a reaction mixture, thereby generating single-stranded donor nucleic acid molecules comprising said NMP;
- b. after step a), adding acceptor nucleic acid molecules to said reaction mixture;
and
- c. ligating said single-stranded acceptor nucleic acid molecules to said single-stranded donor nucleic acid molecules comprising said NMP in said reaction mixture.

[0017c] In one aspect, there is provided a method of ligating single-stranded donor nucleic acids to single-stranded acceptor nucleic acids, said method comprising:

- a. transferring a nucleotide monophosphate (NMP) to single-stranded donor nucleic acid molecules, thereby generating single-stranded donor nucleic acid molecules comprising said NMP in a reaction mixture without single-stranded acceptor nucleic acid molecules;
- b. ligating single-stranded acceptor nucleic acid molecules to said single-stranded donor nucleic acid molecules comprising said NMP, wherein said single-stranded donor nucleic acid molecules or said single-stranded acceptor nucleic acid molecules are over 120 nucleotides, wherein said single-stranded donor nucleic acid molecules and said single-stranded acceptor nucleic acid molecules are different

[0018] In some instances, the invention provides a method of assessing cancer, comprising:

[0019] (a) determining the presence, absence, and/or amount of each of a subset of genes in a sample derived from a sample from a subject, wherein the subset is determined by

(i) performing targeted sequencing on a set of genes on a solid tissue sample from the subject wherein the solid tissue sample is known or suspected of comprising cancerous tissue;

(ii) determining a profile of genetic abnormalities for the set of genes based on the

sequencing; and (iii) selecting a subset of 2, 3, or 4, but no more than 4 genes of the set of

CLAIMS

WHAT IS CLAIMED IS:

1. A method of assessing cancer, comprising:
 - (a) determining a presence, absence, and/or amount of each of a subset of genes in a sample derived from a fluid sample in a subject, wherein the subset is determined by (i) performing targeted sequencing on a set of genes on a solid tissue sample from the subject wherein the solid tissue sample is known or suspected of comprising cancerous tissue; (ii) determining a profile of genetic abnormalities for said set of genes based on the targeted sequencing; and (iii) selecting a subset of 2, 3, 4, but no more than 4 genes of the set of genes based on said profile for said set, wherein said subset is specific to said subject; and
 - (b) from the results of step (a) determining the status of the cancer in the subject.
2. The method of claim 1, wherein said set of genes comprises at least 10 genes.
3. The method of claim 2, wherein said set of genes comprises at least 100 genes.
4. The method of claim 2, wherein said set of genes comprises at least 200 genes.
5. The method of claim 1, wherein the set of genes is selected from the group consisting of ABCA1, BRAF, CHD5, EP300, FLT1, ITPA, MYC, PIK3R1, SKP2, TP53, ABCA7, BRCA1, CHEK1, EPHA3, FLT3, JAK1, MYCL1, PIK3R2, SLC19A1, TP73, ABCB1, BRCA2, CHEK2, EPHA5, FLT4, JAK2, MYCN, PKHD1, SLC1A6, TPM3, ABCC2, BRIP1, CLTC, EPHA6, FN1, JAK3, MYH2, PLCB1, SLC22A2, TPMT, ABCC3, BUB1B, COL1A1, EPHA7, FOS, JUN, MYH9, PLCG1, SLC01B3, TPO, ABCC4, C1orf144, COPS5, EPHA8, FOXO1, KBTBD11, NAV3, PLCG2, SMAD2, TPR, ABCG2, CABLES1, CREB1, EPHB1, FOXO3, KDM6A, NBN, PML, SMAD3, TRIO, ABL1, CACNA2D1, CREBBP, EPHB4, FOXP4, KDR, NCOA2, PMS2, SMAD4, TRRAP, ABL2, CAMKV, CRKL, EPHB6, GAB1, KIT, NEK11, PPARG, SMARCA4, TSC1, ACVR1B, CARD11, CRLF2, EPO, GATA1, KLF6, NF1, PPARGC1A, SMARCB1, TSC2, ACVR2A, CARM1, CSF1R, ERBB2, GLI1, KLHDC4, NF2, PPP1R3A, SMO, TTK, ADCY9, CAV1, CSMD3, ERBB3, GLI3, KRAS, NKX2-1, PPP2R1A, SOCS1, TYK2, AGAP2, CBFA2T3, CSNK1G2, ERBB4, GNA11, LMO2, NOS2, PPP2R1B, SOD2, TYMS, AKT1, CBL, CTNNA1, ERCC1, GNAQ, LRP1B, NOS3, PRKAA2, SOS1, UGT1A1, AKT2, CCND1, CTNNA2, ERCC2, GNAS, LRP2, NOTCH1, PRKCA, SOX10, UMPS, AKT3, CCND2, CTNNB1, ERCC3, GPR124, LRP6, NOTCH2, PRKCZ, SOX2, USP9X, ALK, CCND3, CYFIP1, ERCC4, GPR133, LTK, NOTCH3, PRKDC, SP1, VEGF, ANAPC5, CCNE1, CYLD, ERCC5, GRB2, MAN1B1, NPM1, PTCH1, SPRY2, VEGFA, APC, CD40LG,

CYP19A1, ERCC6, GSK3B, MAP2K1, NQO1, PTCH2, SRC, VHL, APC2, CD44, CYP1B1, ERG, GSTP1, MAP2K2, NR3C1, PTEN, ST6GAL2, WRN, AR, CD79A, CYP2C19, ERN2, GUCY1A2, MAP2K4, NRAS, PTGS2, STAT1, WT1, ARAF, CD79B, CYP2C8, ESR1, HDAC1, MAP2K7, NRP2, PTPN11, STAT3, XPA, ARFRP1, CDC42, CYP2D6, ESR2, HDAC2, MAP3K1, NTRK1, PTPRB, STK11, XPC, ARID1A, CDC42BPB, CYP3A4, ETV4, HGF, MAPK1, NTRK2, PTPRD, SUFU, ZFY, ATM, CDC73, CYP3A5, EWSR1, HIF1A, MAPK3, NTRK3, RAD50, SULT1A1, ZNF521, ATP5A1, CDH1, DACH2, EXT1, HM13, MAPK8, OMA1, RAD51, SUZ12, ATR, CDH10, DCC, EZH2, HMGA1, MARK3, OR10R2, RAF1, TAF1, AURKA, CDH2, DCLK3, FANCA, HNF1A, MCL1, PAK3, RARA, TBX22, AURKB, CDH20, DDB2, FANCD2, HOXA3, MDM2, PARP1, RB1, TCF12, BAI3, CDH5, DDR2, FANCE, HOXA9, MDM4, PAX5, REM1, TCF3, BAP1, CDK2, DGKB, FANCF, HRAS, MECOM, PCDH15, RET, TCF4, BARD1, CDK4, DGKZ, FAS, HSP90AA1, MEN1, PCDH18, RICTOR, TEK, BAX, CDK6, DIRAS3, FBXW7, IDH1, MET, PCNA, RIPK1, TEP1, BCL11A, CDK7, DLG3, FCGR3A, IDH2, MITF, PDGFA, ROR1, TERT, BCL2, CDK8, DLL1, FES, IFNG, MLH1, PDGFB, ROR2, TET2, BCL2A1, CDKN1A, DNMT1, FGFR1, IGF1R, MLL, PDGFRA, ROS1, TGFB2, BCL2L1, CDKN1B, DNMT3A, FGFR2, IGF2R, MLL3, PDGFRB, RPS6KA2, THBS1, BCL2L2, CDKN2A, DNMT3B, FGFR3, IKBKE, MPL, PDZRN3, RPTOR, TNFAIP3, BCL3, CDKN2B, DOT1L, FGFR4, IKZF1, MRE11A, PHLPP2, RSPO2, TNKS, BCL6, CDKN2C, DPYD, FH, IL2RG, MSH2, PIK3C3, RSPO3, TNKS2, BCR, CDKN2D, E2F1, FHOD3, INHBA, MSH6, PIK3CA, RUNX1, TNN3K, BIRC5, CDX2, EED, FIGF, INSR, MTHFR, PIK3CB, SDHB, TNF, BIRC6, CEBPA, EGF, FLG2, IRS1, MTOR, PIK3CD, SF3B1, TOP1, BLM, CERK, EGFR, FLNC, IRS2, MUTYH, PIK3CG, SHC1, and TOP2A.

6. The method of claim 1, wherein the fluid sample is selected from the group consisting of blood, serum, plasma, urine, sweat, tears, saliva, or sputum.

7. The method of claim 1, wherein steps (a) and (b) are performed at a plurality of time points to monitor the status of the cancer over time.

8. The method of claim 7, wherein one time point is prior to a first administration of a cancer therapy and a subsequent time point is subsequent to said first administration.

9. The method of claim 1, further comprising generating a report communicating the profile of genetic abnormalities for the set of genes and communicating said report to a caregiver.

10. The method of claim 9, wherein the report comprises a list of one or more therapeutic candidates based on said profile.

11. The method of claim 9, wherein said report is generated 1 week from collection of the solid tissue sample.
12. The method of claim 9, wherein the report comprises copy number alterations of said set of genes.
13. The method of claim 9, wherein the report comprises a description of a therapeutic agent targeting each of said genetic abnormalities (from the tumor).
14. The method of claim 1, further comprising generating a report communicating the profile of the subset of genes at each of the plurality of time points.
15. The method of claim 1, wherein said determining comprises the step of diluting said nucleic acid molecules from said sample into discrete reaction volumes, wherein said discrete reaction volumes contain between 1 to 10 molecules of said nucleic acid molecule from said sample.
16. The method of claim 15, wherein said discrete reaction volumes are droplets in an emulsion.
17. The method of claim 15, wherein said discrete reaction volumes further comprise primers for allelic discrimination of said genetic abnormalities in the subset of genes
18. The method of claim 1, wherein determining the status comprises quantifying the number of nucleic acids harboring said genetic abnormalities in said subset of genes.
19. The method of claim 1, wherein the step of targeted sequencing comprises preparing a DNA library from said solid tissue sample wherein said preparation can be completed in in less than a number of hours selected from the group consisting of: 4 hours, 5 hours, 6 hours, and 7 hours.
20. The method of claim 19, wherein said preparing does not require exponential PCR amplification prior to sequencing of said library.
21. The method of claim 19, wherein said preparing comprises a linear amplification step.
22. The method of claim 1, wherein the step of targeted sequencing comprises a targeted library preparation step, wherein the targeted library preparation step comprises:
 - (a) contacting a single-stranded DNA fragment from said solid tissue sample with a target-specific oligonucleotide comprising (i) a region specific for a region of a cancer-related gene and (ii) an adaptor sequence specific for coupling to a sequencing platform;

(b) performing a hybridization reaction to join said target specific oligonucleotides to a single-stranded DNA fragment containing a region of complementarity to said target-specific oligonucleotide;

(c) performing an extension reaction to create an extension product comprising said region and comprising said adaptor; and

(d) sequencing said extension product.

23. The method of claim 22, wherein said contacting occurs with the target-specific oligonucleotide attached to a sequencing platform.

24. The method of claim 22, wherein said contacting occurs with the target-specific oligonucleotide free in a solution.

25. A method, comprising:

(a) hybridizing a target-selective oligonucleotide (TSO) to a single-stranded DNA (ssDNA) fragment in an ssDNA library to create a hybridization product; and

(b) extending said hybridization product to create a double stranded extension product, wherein said TSO comprises (i) a sequence that is complementary to a single target region and (ii) a first single-stranded adaptor sequence located at a first end of said TSO but not to both ends of said TSO, and wherein said ssDNA fragment comprises a second single-stranded adaptor sequence but does not comprise said first single-stranded adaptor sequence, and wherein said ssDNA fragment is ligated to a second single-stranded adaptor sequence by a ligation method comprising over 10%, 50%, 70%, or 90% ligation efficiency.

26. A method, comprising:

(a) hybridizing a target-selective oligonucleotide (TSO) to a single-stranded DNA (ssDNA) fragment in an ssDNA library to create a hybridization product; and

(b) extending said hybridization product to create a double stranded extension product, wherein said TSO comprises (i) a sequence that is complementary to a single target region and (ii) a first single-stranded adaptor sequence located at a first end of said TSO but not to both ends of said TSO, and wherein said ssDNA fragment comprises a second single-stranded adaptor sequence but does not comprise said first single-stranded adaptor sequence, and wherein said ssDNA fragment is ligated to a second single-stranded adaptor sequence by a single-stranded ligation method.

27. The method of claim 25 or 26, wherein said second single-stranded adaptor sequence is located at a first end of said ssDNA fragment but not at both ends of said ssDNA fragment.

28. The method of claim 27, wherein said first end of said ssDNA fragment is a 5' end.
29. The method of claim 25 or 26, wherein said first or second adaptor sequence comprises a barcode sequence.
30. The method of claim 25 or 26, wherein said first end of said TSO is a 5' end.
31. The method of claim 25 or 26, wherein said first or second adaptor sequence comprises a sequence that is at least 70% identical to a support-bound oligonucleotide conjugated to a solid support.
32. The method of claim 31, wherein said solid support is coupled to a sequencing platform.
33. The method of claim 25 or 26, wherein said first or second adaptor sequence comprises a binding site for a sequencing primer.
34. The method of claim 31, further comprising annealing said extension products to said support-bound oligonucleotides.
35. The method of claim 34, further comprising amplifying said annealed extension products.
36. The method of claim 35, further comprising sequencing said annealed extension products.
37. The method of claim 25 or 26, wherein said ssDNA library comprises genomic DNA fragments.
38. The method of claim 25 or 26, wherein said ssDNA library comprises cDNA fragments.
39. The method of claim 25 or 26, further comprising removing unhybridized TSOs and unhybridized ssDNA library members.
40. The method of claim 25 or 26, wherein steps a) and b) are performed when said ssDNA library members and said TSOs are free-floating in a solution.
41. The method of claim 25 or 26, wherein said single target region flanks a genomic region.
42. The method of claim 41, wherein said genomic region comprises a portion of an exon region from a cancer-related gene.
43. The method of claim 42, wherein said cancer-related gene is selected from the group consisting of ABCA1, BRAF, CHD5, EP300, FLT1, ITPA, MYC, PIK3R1, SKP2, TP53, ABCA7, BRCA1, CHEK1, EPHA3, FLT3, JAK1, MYCL1, PIK3R2, SLC19A1, TP73, ABCB1, BRCA2, CHEK2, EPHA5, FLT4, JAK2, MYCN, PKHD1, SLC1A6, TPM3,

ABCC2, BRIP1, CLTC, EPHA6, FN1, JAK3, MYH2*, PLCB1, SLC22A2, , TPMT, ABCC3, BUB1B, COL1A1, EPHA7, FOS, JUN, MYH9, PLCG1, SLC01B3, , TPO, ABCC4, C1orf144, COPS5, EPHA8, FOXO1, KBTBD11, NAV3, PLCG2, SMAD2, TPR, ABCG2, CABLES1, CREB1, EPHB1, FOXO3, KDM6A, NBN, PML, SMAD3, TRIO, ABL1, CACNA2D1, CREBBP, EPHB4, FOXP4, KDR, NCOA2, PMS2, SMAD4, TRRAP, ABL2, CAMKV, CRKL, EPHB6, GAB1, KIT, NEK11, PPARG, SMARCA4, TSC1, ACVR1B, CARD11, CRLF2, EPO, GATA1, KLF6, NF1, PPARGC1A, SMARCB1, TSC2, ACVR2A, CARM1, CSF1R, ERBB2, GLI1, KLHDC4, NF2, PPP1R3A, SMO, TTK, ADCY9, CAV1, CSMD3, ERBB3, GLI3, KRAS, NKX2-1, PPP2R1A, SOCS1, TYK2, AGAP2, CBFA2T3, CSNK1G2, ERBB4, GNA11, LMO2, NOS2, PPP2R1B, SOD2, TYMS, AKT1, CBL, CTNNA1, ERCC1, GNAQ, LRP1B, NOS3, PRKAA2, SOS1, UGT1A1, AKT2, CCND1, CTNNA2, ERCC2, GNAS, LRP2, NOTCH1, PRKCA, SOX10, UMPS, AKT3, CCND2, CTNNB1, ERCC3, GPR124, LRP6, NOTCH2, PRKCZ, SOX2, USP9X, ALK, CCND3, CYFIP1, ERCC4, GPR133, LTK, NOTCH3, PRKDC, SP1, VEGF, ANAPC5, CCNE1, CYLD, ERCC5, GRB2, MAN1B1, NPM1, PTCH1, SPRY2, VEGFA, APC, CD40LG, CYP19A1, ERCC6, GSK3B, MAP2K1, NQO1, PTCH2, SRC, VHL, APC2, CD44, CYP1B1, ERG, GSTP1, MAP2K2, NR3C1, PTEN, ST6GAL2, WRN, AR, CD79A, CYP2C19, , ERN2, GUCY1A2, MAP2K4, NRAS, PTGS2, STAT1, WT1, ARAF, CD79B, CYP2C8, , ESR1, HDAC1, MAP2K7, NRP2, PTPN11, STAT3, XPA, ARFRP1, CDC42, CYP2D6, ESR2, HDAC2, MAP3K1, NTRK1, PTPRB, STK11, XPC, ARID1A, CDC42BPB, CYP3A4, ETV4, HGF, MAPK1, NTRK2, PTPRD, SUFU, ZFY, ATM, CDC73, CYP3A5, EWSR1, HIF1A, MAPK3, NTRK3, RAD50, SULT1A1, ZNF521, ATP5A1, CDH1, DACH2, EXT1, HM13, MAPK8, OMA1, RAD51, SUZ12, ATR, CDH10, DCC, EZH2, HMGA1, MARK3, OR10R2, RAF1, TAF1, AURKA, CDH2, DCLK3, FANCA, HNF1A, MCL1, PAK3, RARA, TBX22, AURKB, CDH20, DDB2, FANCD2, HOXA3, MDM2, PARP1, RB1, TCF12, BAI3, CDH5, DDR2, FANCE, HOXA9, MDM4, PAX5, REM1, TCF3, BAP1, CDK2, DGKB, FANCF, HRAS, MECOM, PCDH15, RET, TCF4, BARD1, CDK4, DGKZ, FAS, HSP90AA1, MEX1, PCDH18, RICTOR, TEK, BAX, CDK6, DIRAS3, FBXW7, IDH1, MET, PCNA, RIPK1, TEP1, BCL11A, CDK7, DLG3, FCGR3A, IDH2, MITF, PDGFA, ROR1, TERT, BCL2, CDK8, DLL1, FES, IFNG, MLH1, PDGFB, ROR2, TET2, BCL2A1, CDKN1A, DNMT1, FGFR1, IGF1R, MLL, PDGFRA, ROS1, TGFB2, BCL2L1, CDKN1B, DNMT3A, FGFR2, IGF2R, MLL3, PDGFRB, RPS6KA2, THBS1, BCL2L2, CDKN2A, DNMT3B, FGFR3, IKBKE, MPL, PDZRN3, RPTOR, TNFAIP3, BCL3, CDKN2B, DOT1L, FGFR4, IKZF1, MRE11A, PHLPP2, RSPO2, TNKS, BCL6, CDKN2C,

DPYD, FH, IL2RG, MSH2, PIK3C3, RSPO3, TNKS2, BCR, CDKN2D, E2F1, FHOD3, INHBA, MSH6, PIK3CA, RUNX1, TNNI3K, BIRC5, CDX2, EED, FIGF, INSR, MTHFR, PIK3CB, SDHB, TNF, BIRC6, CEBPA, EGF, FLG2, IRS1, MTOR, PIK3CD, SF3B1, TOP1, BLM, CERK, EGFR, FLNC, IRS2, MUTYH, PIK3CG, SHC1, and TOP2A.

44. The method of claim 25, wherein said ligation method is a single-stranded ligation method.

45. The method of claim 26 or 44, wherein said single-stranded ligation method comprises use of an RNA ligase.

46. The method of claim 45, wherein said RNA ligase is CircLigase or CircLigase II.

47. A method of preparing a single-stranded DNA library, comprising:

- (a) denaturing a double stranded DNA fragment into single stranded DNA (ssDNA) fragments;
- (b) removing 5' phosphates from said ssDNA fragments;
- (c) ligating single-stranded primer docking oligonucleotides (pdo's) to 3' ends of said ssDNA fragments, wherein said pdo's are conjugated to a capture moiety capable of binding to an immobilized capturing reagent;
- (d) hybridizing primers to said pdo's, wherein said primers comprise a sequence complementary to said adaptor oligonucleotide sequence and comprise a first adaptor sequence that is at least 70% identical to a support-bound oligonucleotide coupled to a sequencing platform;
- (e) extending said hybridized primers to create duplexes, wherein each duplex comprises an ss fragment and an extended primer strand;
- (f) immobilizing said duplexes to said immobilized capturing reagent;

and

- (g) denaturing said double-stranded extension product, wherein said denaturing results in release of said extended primer strands from said immobilized capturing reagent and retention of said ssDNA fragments on said immobilized capturing reagent; and
- (h) collecting said extended primer strands,

wherein said extended primer strands comprise said ss DNA library.

48. The method of claim 47, wherein step (c) results in ligation of at least 50% of said ssDNA fragments to said pdo's.

49. The method of claim 47, wherein said ligating is performed using an ATP-dependent ligase.

50. The method of claim 49, wherein said ATP-dependent ligase is an RNA ligase.
51. The method of claim 50, wherein said RNA ligase is CircLigase or CircLigase II.
52. The method of claim 47, wherein said pdo's are adenylated.
53. The method of claim 47, wherein said extending is performed using a proofreading DNA polymerase.
54. A method of preparing a single-stranded DNA library, comprising:
- (a) denaturing a double stranded DNA fragment into single stranded DNA (ssDNA) fragments;
 - (b) ligating a first single-stranded adaptor sequence to a first end of said ssDNA fragments; and
 - (c) ligating a second single-stranded adaptor sequence to a second end of said ssDNA fragments.
55. A kit, comprising:
- (a) a primer docking oligonucleotide (pdo), wherein said pdo is conjugated to a capture moiety capable of binding to an immobilized capturing reagent;
 - (b) a primer, wherein said primer comprises a sequence that is at least 70% complementary to said pdo sequence and further comprises a first adaptor sequence that is at least 70% identical to a first support-bound oligonucleotide coupled to a sequencing platform; and
 - (c) instructions for use.
56. The kit of claim 55, further comprising an ATP-dependent ligase.
57. The kit of claim 56, wherein said ATP-dependent ligase is an RNA ligase.
58. The kit of claim 57, wherein said RNA ligase is CircLigase or CircLigase II.
59. The kit of claim 55, further comprising a proofreading DNA polymerase.
60. The kit of claim 55, further comprising said immobilized capturing reagent.
61. The kit of claim 55, wherein said first adaptor sequence comprises a sequence that is at least 70% complementary to a first sequencing primer.
62. The kit of claim 55, wherein said first adaptor sequence comprises a barcode sequence.
63. The kit of claim 55, further comprising a target-selective oligonucleotide (TSO).

64. The kit of claim 63, wherein said TSO further comprises a second adaptor sequence located at a first end but not a second end.
65. The kit of claim 64, wherein said first end of said TSO is a 5' end.
66. The kit of claim 64, wherein said second adaptor sequence comprises a sequence that is at least 70% identical to a second support-bound oligonucleotide coupled to a sequencing platform.
67. The kit of claim 64, wherein said second adaptor sequence comprises a binding site for a sequencing primer.
68. A kit, comprising:
- (a) a first adaptor oligonucleotide, wherein said first adaptor comprises a sequence that is at least 70% complementary to a first support-bound oligonucleotide coupled to a sequencing platform;
 - (b) a second adaptor oligonucleotide, wherein said second adaptor comprises a sequence that is distinct from said first adaptor oligonucleotide;
 - (c) an RNA ligase; and
 - (d) instructions for use.
69. The kit of claim 68, wherein said second adaptor comprises a sequence that is at least 70% complementary to a sequencing primer.
70. The kit of claim 68, wherein said second adaptor comprises a sequence that is at least 70% complementary to a second support-bound oligonucleotide coupled to a sequencing platform.
71. The kit of claim 68, wherein said first adaptor comprises a sequence that is at least 70% complementary to a sequencing primer.
72. The kit of claim 68, wherein one of said first or second adaptor comprises a barcode sequence.
73. The kit of claim 68, wherein said first adaptor comprises a 3' terminal blocking group that prevents the formation of a covalent bond between the 3' terminal base and another nucleotide.
74. The kit of claim 73, wherein said 3' terminal blocking group is dideoxy-dNTP, biotin, o alkyl, amino-alkyl, or fluorophore dideoxygenin.
75. The kit of claim 68, wherein said first adaptor comprises a 5' polyadenylation sequence.
76. The kit of claim 68, wherein said RNA ligase is truncated or mutated ligase 2 from T4 or Mth.

77. The kit of claim 68, further comprising a second RNA ligase.
78. The kit of claim 77, wherein said second RNA ligase is CircLigase or CircLigase II.
79. An oligonucleotide primer, comprising:
- (a) a probe-binding region; and
 - (b) a template binding region that is at least 50% complementary to a template nucleic acid suspected of harboring a mutation, wherein a portion of said template binding region at least partially overlays a locus of said suspected mutation;
 - (c) wherein said oligonucleotide primer upon hybridization to said template nucleic acid is extendable by a polymerase if said mutation is present but is not extendable by said polymerase if said mutation is not present.
80. The oligonucleotide primer of claim 79, wherein said mutation is a single nucleotide polymorphism (SNP).
81. The oligonucleotide primer of claim 80, wherein said template binding region comprises a 3' terminal region that overlays said SNP locus.
82. The oligonucleotide primer of claim 81, wherein said 3' terminal region is complementary to a mutant allele of said SNP locus.
83. The oligonucleotide primer of claim 81, wherein said 3' terminal region is complementary to a wild-type allele of said SNP locus.
84. The oligonucleotide primer of claim 79, wherein said probe-binding region does not hybridize to any genomic sequence from said subject.
85. The oligonucleotide primer of claim 79, wherein said polymerase is a DNA polymerase lacking 3' to 5' exonuclease activity.
86. A kit, comprising:
- (a) An oligonucleotide primer, wherein said oligonucleotide primer comprises (i) a probe-binding region, and (ii) a template binding region that is at least 70% complementary to a template nucleic acid suspected of harboring a mutation, wherein a portion of said template binding region at least partially overlays locus of said suspected mutation, wherein said oligonucleotide primer upon hybridization to said template nucleic acid is extendable by a polymerase if said mutation is present but is not extendable by said polymerase if said mutation is not present; and
 - (b) instructions for use.
87. The kit of claim 86, wherein said mutation is a single nucleotide polymorphism (SNP).

88. The kit of claim 87, wherein said template binding region comprises a 3' terminal base that overlays said SNP locus.
89. The kit of claim 88, wherein said 3' terminal base is complementary to a mutant allele of said SNP locus.
90. The kit of claim 88, wherein said 3' terminal base is complementary to a wild-type allele of said SNP locus.
91. The kit of claim 86, wherein said probe-binding region does not hybridize to any genomic sequence from said subject.
92. The kit of claim 86, further comprising a reporter probe that is at least 70% complementary to said probe binding region.
93. The kit of claim 92, wherein said reporter probe comprises a detectable moiety and a quencher moiety, wherein said quencher moiety suppresses detection of said detectable moiety when said reporter probe is intact.
94. The kit of claim 92, wherein a hybridization product consisting of said oligonucleotide primer and reporter probe has a T_m that is at least 10 degrees higher than a T_m of a hybridization product consisting of said oligonucleotide primer and said template nucleic acid.
95. The kit of claim 86, further comprising a reverse primer that is at least 70% complementary to a reverse complement sequence downstream of said locus.
96. The kit of claim 86, further comprising said polymerase.
97. The kit of claim 96, wherein said polymerase is a thermostable polymerase having a 5' to 3' exonuclease activity and not having a 3' to 5' exonuclease activity.
98. The kit of claim 86, further comprising (i) one or more alternative oligonucleotide primers, wherein said one or more alternative oligonucleotide primers each comprises a distinct probe binding region and a template binding region that is at least 70% complementary to said template nucleic acid, wherein a portion of said template binding region at least partially overlays said locus, wherein said alternative oligonucleotide primer upon hybridization to said template nucleic acid is extendable by a polymerase if an alternative allele is present but is not extendable by said polymerase if said alternative allele is not present.
99. The kit of claim 98, further comprising one or more alternative reporter probes, wherein each of said alternative reporter probes is at least 70% complementary to one of said distinct probe binding regions but not to any other probe binding region of said kit.

100. The kit of claim 99, wherein each of said alternative reporter probes comprises an alternative detectable moiety and a quencher moiety, wherein each of said detectable moieties of said kit is detectably distinct from any other detectable moiety of said kit.

101. A method of detecting a mutation in a target polynucleotide region, comprising:

- (a) selectively hybridizing an oligonucleotide primer to said target polynucleotide region, wherein said oligonucleotide primer comprises (i) a probe-binding region, and (ii) a template binding region that is at least 70% complementary to a template nucleic acid suspected of harboring a mutation, wherein a portion of said template binding region at least partially overlays a locus of said suspected mutation, and wherein said oligonucleotide primer upon hybridization to said template nucleic acid is extendable by a polymerase if said mutation is present but is not extendable by said polymerase if said mutation is not present;
- (b) extending said hybridized oligonucleotide primer to form an extension product; and
- (c) detecting said extension product, whereby wherein said detecting indicates the presence of said mutation.

102. The method of claim 101, wherein said extending comprises extending with a DNA polymerase that does not comprise 3' to 5' exonuclease activity.

103. The method of claim 101, wherein said detecting comprises selectively hybridizing a reporter probe to said probe binding region.

104. The method of claim 103, wherein said reporter probe comprises a detectable moiety and a quencher moiety, wherein said quencher moiety suppresses detection of said detectable moiety when said reporter probe is intact.

105. The method of claim 104, wherein said detecting further comprises separating said detectable moiety from said quencher moiety of said hybridized reporter probe.

106. The method of claim 103, further comprising amplifying said extension product with a reverse primer that is capable of hybridizing to a region of said extension product downstream of said locus.

107. The method of claim 106, wherein said amplifying comprises amplifying with a DNA polymerase that comprises 5' to 3' exonuclease activity.

108. The method of claim 103, wherein a hybridization product of said oligonucleotide primer and reporter probe has a T_m that is at least 10 degrees higher than a T_m of a hybridization product between said oligonucleotide primer and target polynucleotide.

109. The method of claim 103, wherein a concentration of said reporter probe is at least 10X a concentration of said forward primer.

110. The method of claim 101, further comprising selectively hybridizing one or more alternative oligonucleotide primers to said target polynucleotide region, wherein said one or more alternative oligonucleotide primers each comprises a distinct probe binding region and a template binding region that is at least 70% complementary to said template nucleic acid, wherein a portion of said template binding region at least partially overlays said locus, wherein said alternative oligonucleotide primer upon hybridization to said template nucleic acid is extendable by a polymerase if an alternative allele is present but is not extendable by said polymerase if said alternative allele is not present.

111. The method of claim 110, wherein said detecting further comprises selectively hybridizing one or more alternative reporter probes to said one or more alternative oligonucleotide primers, wherein each of said alternative reporter probes is at least 70% complementary to one of said distinct probe binding regions but not to any other of said probe binding regions.

112. The method of claim 111, wherein each of said alternative reporter probes comprises an alternative detectable moiety and a quencher moiety, wherein each of said alternative detectable moieties is detectably distinct from any other of said detectable moieties.

113. The method of claim 101, wherein said mutation is a single nucleotide polymorphism (SNP).

114. The method of claim 113, wherein said template binding region comprises a 3' terminal region having a base that overlays said SNP locus.

115. The method of claim 114, wherein said base is complementary to a mutant allele of said SNP locus.

116. The method of claim 114, wherein said base is complementary to a wild-type allele of said SNP locus.

117. The method of claim 101, wherein said probe-binding region does not hybridize to said target polynucleotide region.

118. The method of claim 101, wherein said nucleic acid sample is subdivided into a plurality of discrete reaction volumes prior to steps b-c.

119. The method of claim 118, further comprising detection of said detectable moiety in each of said reaction volumes.

120. The method of claim 119, further comprising counting a number of said reaction volumes wherein said detectable moiety is detected.
121. The method of claim 118, wherein said nucleic acid sample is subdivided such that said plurality of discrete reaction volumes contain an average of <1 template nucleic acid molecule.
122. The method of claim 101, further comprising providing a conclusion and transmitting the conclusion over a network.
123. A composition, comprising:
- (a) an oligonucleotide primer hybridized to a template nucleic acid, wherein said template nucleic acid comprises a wild-type allele at a locus, wherein the 3' terminal region of said oligonucleotide primer overlays said locus and is not complementary to said wild-type allele; and
 - (b) an intact reporter probe comprising a detectable and quencher moiety, wherein said intact reporter probe is hybridized to said oligonucleotide primer.
124. A method of conducting a high-efficiency ligation reaction, comprising ligating a plurality of acceptor nucleic acid molecules to a first end of at least 10% of a plurality of donor nucleic acid molecules, wherein one of said donor or acceptor nucleic acid molecules is >120 nt long.
125. A method of conducting a high-efficiency ligation reaction, comprising ligating a plurality of donor nucleic acid molecules to a first end of at least 10% of a plurality of acceptor nucleic acid molecules, wherein one of said donor or acceptor nucleic acid molecules is >120 nt long.
126. The method of claim 124 or 125, wherein said acceptor nucleic acid molecules are said donor nucleic acid molecules.
127. The method of claim 124 or 125, comprising:
- (a) transferring a nucleoside monophosphate (NMP) to an amount of donor nucleic acid molecules in a reaction mixture for a time sufficient to effect an accumulation of NMP-carrying donor nucleic acid molecules; and
 - (b) effecting formation of a covalent bond between an NMP-carrying donor nucleic acid molecules and an acceptor nucleic acid molecule,
- wherein steps (a) and (b) are carried out sequentially in said reaction mixture.
128. The method of claim 127, wherein said transferring results in transfer of an NMP to at least 10% of said donor nucleic acid molecules.

129. The method of claim 128, wherein said transferring results in transfer of an NMP to at least 50% of said donor nucleic acid molecules.
130. The method of claim 128, wherein said transferring results in transfer of an NMP to at least 70% of said donor nucleic acid molecules.
131. The method of claim 128, wherein said transferring results in transfer of an NMP to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of said donor nucleic acid molecules.
132. The method of any of claims 127-131, wherein said NMP is AMP.
133. The method of any of claims 127-131, wherein said NMP is GMP.
134. The method of claim 127, wherein a 3' terminal region of at least one member of said donor nucleic acid molecules is an unmodified 3' terminal region.
135. The method of claim 127, wherein said reaction mixture comprises
- (a) an amount of a nucleoside triphosphate (NTP)-dependent ligase that is at least equimolar to said amount of donor nucleic acid molecules; and
 - (b) NTP that is present in an amount that is at least 10-fold higher than a Michaelis constant (K_m) of said ATP-dependent ligase.
136. The method of claim 135, wherein said NTP-dependent ligase is an RNA ligase.
137. The method of claim 135, wherein said RNA ligase is a thermophilic RNA ligase.
138. The method of claim 135, wherein said NTP-dependent ligase is an ATP-dependent RNA ligase.
139. The method of claim 138, wherein said ATP-dependent RNA ligase is MthRnl, T4 RNA ligase, CircLigase, or CircLigase II.
140. The method of claim 139, wherein said ATP-dependent ligase is CircLigase or CircLigase II.
141. The method of claim 135, wherein said NTP-dependent ligase is a GTP-dependent RNA ligase.
142. The method of claim 141, wherein said GTP-dependent RNA ligase is RtcB.
143. The method of claim 135, wherein a 3' terminal region of a donor nucleic acid molecule is modified with a 3' terminal blocking group.

144. The method of claim 135, wherein said effecting formation of a covalent bond comprises adding to said reaction mixture:

- (a) said acceptor nucleic acid molecule; and
- (b) Mn^{2+} .

145. The method of claim 144, wherein said Mn^{2+} is present in an amount that is at least 2.5 mM.

146. The method of claim 144, further comprising reducing concentration of said NTP in said reaction mixture.

147. The method of claim 146, wherein said reducing concentration comprises reducing concentration of said NTP at least 10-fold.

148. The method of claim 146, wherein said reducing concentration comprises adding to said reaction mixture an amount of liquid sufficient to dilute said NTP at least 10-fold.

149. The method of any of claims 127-148, wherein said donor nucleic acid molecules comprises nucleic acid molecules isolated from a biological source and wherein said acceptor nucleic acid molecules comprises an adaptor sequence.

150. The method of any of claims 127-149 wherein said acceptor nucleic acid molecules comprises nucleic acid isolated from a biological subject and wherein said donor nucleic acid molecules comprises an adaptor sequence.

151. The method of any of claims 127-148, wherein said acceptor nucleic acid molecules comprises nucleic acid isolated from a biological subject and wherein said donor nucleic acid molecules comprises a barcode sequence.

152. The method of any of claims 127-148, wherein said donor nucleic acid molecules comprises nucleic acid isolated from a biological subject and wherein said acceptor nucleic acid molecules comprises a barcode sequence.

153. The method of any of claims 127-148, wherein said acceptor nucleic acid molecules or donor nucleic acid molecules comprise a detectable tag.

154. A method of preparing a nucleic acid library, comprising ligating an oligonucleotide sequence to a first end of at least 10% of a plurality of template nucleic acid molecules to create said nucleic acid library, wherein one of said template nucleic acid molecules is >120 nt long.

155. The method of claim 154, comprising ligating an oligonucleotide sequence to a first end of at least 50% of said plurality of template nucleic acid molecules.

156. The method of claim 154, comprising ligating an oligonucleotide sequence to a first end of at least 70% of said plurality of template nucleic acid molecules.
157. The method of claim 154, comprising ligating an oligonucleotide sequence to a first end of at least 90% of said plurality of template nucleic acid molecules.
158. The method of claim 154, wherein said oligonucleotide sequence is an adaptor sequence.
159. The method of claim 158, further comprising sequencing said nucleic acid library.
160. The method of claim 154, wherein said oligonucleotide sequence comprises a detectable label.
161. The method of claim 160, further comprising analyzing said nucleic acid library by array hybridization.
162. A method of preparing a nucleic acid library, comprising
- (a) ligating an adaptor sequence to a first end of at least 10% of a plurality of template nucleic acid molecules to create said nucleic acid library; and
 - (b) sequencing said nucleic acid library.
163. The method of claim 159 or 162, wherein said sequencing is performed without pre-amplification of said nucleic acid library.
164. The method of claim 154 or 162, wherein said plurality of template nucleic acid molecules comprises genomic DNA (gDNA).
165. The method of claim 164, wherein said gDNA is isolated from a solid tissue sample.
166. The method of claim 164, wherein said gDNA is isolated from plasma, serum, sputum, saliva, urine, or sweat.
167. The method of claim 154 or 162, wherein said plurality of template nucleic acid molecules comprises single-stranded nucleic acid fragments.
168. The method of claim 154 or 162, comprising ligating an adaptor sequence to a first end of at least 50% of said plurality of template nucleic acid molecules.
169. The method of claim 154 or 162, comprising ligating an adaptor sequence to a first end of at least 70% of said plurality of template nucleic acid molecules.
170. The method of claim 154 or 162, comprising ligating an adaptor sequence to a first end of at least 90% of said plurality of template nucleic acid molecules.
171. The method of claim 154 or 162, comprising ligating an adaptor sequence to a first end of at least 95% of said plurality of template nucleic acid molecules.

172. The method of claim 154 or 162, wherein said ligating comprises the steps of:

(a) transferring a nucleoside monophosphate (NMP) to an amount of a first population of nucleic acids (reactant 1) in a first reaction mixture for a time sufficient to effect an accumulation of NMP-carrying reactant 1; and

(b) effecting formation of a covalent bond between said NMP-carrying reactant 1 and a second population of nucleic acids (reactant 2)

wherein said reactant 1 is either (i) said plurality of template nucleic acids or (ii) said oligonucleotide or adaptor sequence, wherein said reactant 2 is the other of (i) said plurality of template nucleic acids or (ii) said oligonucleotide or adaptor sequence, and wherein said adenylated reactant 1 is not purified prior to said effecting formation of a covalent bond.

173. The method of claim 172, wherein said transferring results in transfer of an NMP to at least 10% of said of reactant 1.

174. The method of claim 173, wherein said transferring results in transfer of an NMP to at least 50% of said of reactant 1.

175. The method of claim 173, wherein said transferring results in transfer of an NMP to at least 70% of said of reactant 1.

176. The method of claim 173, wherein said transferring results in transfer of an NMP to at least 90% of said of reactant 1.

177. The method of claim 172, wherein a 3' terminal region of at least one member of said reactant 1 is an unmodified 3' terminal region.

178. The method of claim 172, wherein said first reaction mixture comprises

(a) an amount of an NTP-dependent ligase that is at least equimolar to said amount of reactant 1; and

(b) NTP that is present in an amount that is at least 10-fold higher than a Michaelis constant (Km) of said NTP-dependent ligase.

179. The method of claim 178, wherein said NTP-dependent ligase is an RNA ligase.

180. The method of claim 179, wherein said RNA ligase is a thermophilic RNA ligase.

181. The method of claim 179, wherein said NTP-dependent ligase is an ATP-dependent RNA ligase.

182. The method of claim 181, wherein said ATP-dependent RNA ligase is MthRnl, T4 RNA ligase, CircLigase, or CircLigase II.

183. The method of claim 182, wherein said ATP-dependent ligase is CircLigase or CircLigase II.

184. The method of claim 179, wherein said NTP-dependent ligase is a GTP-dependent RNA ligase.

185. The method of claim 184, wherein said GTP-dependent RNA ligase is RtcB.

186. The method of claim 172, wherein a 3' terminal region of at least one member of reactant 1 is modified with a 3' terminal blocking group.

187. The method of claim 178, wherein said effecting formation of a covalent bond comprises adding to said first reaction mixture:

- (a) a cation;
- (b) said reactant 2; and
- (c) a liquid in an amount sufficient to dilute said NTP at least 10-fold.

188. The method of claim 187, wherein said cation is Mn^{2+} .

189. The method of claim 154 or 162, further comprising ligating a second adaptor sequence to a second end of at least 10% of said plurality of template nucleic acid molecules.

190. The method of claim 154 or 162, further comprising ligating a second adaptor sequence to a second end of at least 50% of said plurality of template nucleic acid molecules.

191. The method of claim 154 or 162, further comprising ligating a second adaptor sequence to a second end of at least 70% of said plurality of template nucleic acid molecules.

192. The method of claim 154 or 162, further comprising ligating a second adaptor sequence to a second end of at least 90% of said plurality of template nucleic acid molecules.

193. The method of claim 154 or 162, further comprising:

- (a) hybridizing a target-selective oligonucleotide (tso) to a member of said DNA library, wherein the target-selective oligonucleotide comprises (i) a sequence specific for a region of gDNA and (ii) a second adaptor sequence; and
- (b) extending said hybridized tso to create a double-stranded library member comprising said first and second adaptor.

194. The method of claim 193, wherein said tso comprises a sequence having at least 70% identity or complementarity to a region of a cancer-related gene.

195. The method of any claim 159 or 162, wherein said sequencing comprises massively parallel sequencing.

196. The method of claim 154 or 162, wherein said ligating is performed using a reaction protocol that can be performed in less than 3 hours.

197. A kit, comprising:
- (a) an NTP-dependent ligase;
 - (b) a cation;
 - (c) NTP; and
 - (d) instructions for carrying out a method of any of claims ____.
198. The kit of claim 197, wherein said NTP is ATP.
199. The kit of claim 198, wherein said NTP-dependent ligase is an ATP-dependent ligase.
200. The kit of claim 199, wherein said ATP-dependent ligase is an ATP-dependent RNA ligase.
201. The kit of claim 200, wherein said ATP-dependent RNA ligase is MthRnl, T4 RNA ligase, CircLigase, or CircLigase II.
202. The kit of claim 201, wherein said ATP-dependent ligase is CircLigase or CircLigase II.
203. The kit of claim 197, wherein said NTP is GTP.
204. The kit of claim 203, wherein said NTP-dependent ligase is a GTP-dependent ligase.
205. The kit of claim 204, wherein said GTP-dependent ligase is a GTP-dependent RNA ligase.
206. The kit of claim 205, wherein said GTP-dependent RNA ligase is RtcB.
207. A method of tracking tumor-specific mutations using tumor genomic DNA (gDNA) isolated from a subject's tumor and normal gDNA isolated from non-tumor tissue from said subject; comprising:
- (a) sequencing a DNA library prepared from said tumor gDNA without pre-amplification to produce a first dataset;
 - (b) sequencing a DNA library prepared from said normal gDNA without pre-amplification to produce a second dataset;
 - (c) analyzing said first and second dataset to identify one or more tumor-specific mutations in said subject; and
 - (d) detecting the presence or absence of said tumor-specific mutations in cell-free DNA isolated from a liquid sample from said subject.
208. The method of claim 207, wherein said liquid sample is selected from the group consisting of plasma, serum, sputum, saliva, urine, and sweat.

209. The method of claim 207, wherein said DNA library of step (a) or (b) is prepared using the method of claim 154 or 162.

210. The method of claim 207, wherein said sequencing comprises sequencing at least 200 cancer-related genes.

211. The method of claim 210, wherein said cancer-related genes are selected from the group consisting of ABCA1, BRAF, CHD5, EP300, FLT1, ITPA, MYC, PIK3R1, SKP2, TP53, ABCA7, BRCA1, CHEK1, EPHA3, FLT3, JAK1, MYCL1, PIK3R2, SLC19A1, TP73, ABCB1, BRCA2, CHEK2, EPHA5, FLT4, JAK2, MYCN, PKHD1, SLC1A6, TPM3, ABCC2, BRIP1, CLTC, EPHA6, FN1, JAK3, MYH2, PLCB1, SLC22A2, TPMT, ABCC3, BUB1B, COL1A1, EPHA7, FOS, JUN, MYH9, PLCG1, SLC01B3, TPO, ABCC4, C1orf144, COPS5, EPHA8, FOXO1, KBTBD11, NAV3, PLCG2, SMAD2, TPR, ABCG2, CABLES1, CREB1, EPHB1, FOXO3, KDM6A, NBN, PML, SMAD3, TRIO, ABL1, CACNA2D1, CREBBP, EPHB4, FOXP4, KDR, NCOA2, PMS2, SMAD4, TRRAP, ABL2, CAMKV, CRKL, EPHB6, GAB1, KIT, NEK11, PPARG, SMARCA4, TSC1, ACVR1B, CARD11, CRLF2, EPO, GATA1, KLF6, NF1, PPARGC1A, SMARCB1, TSC2, ACVR2A, CARM1, CSF1R, ERBB2, GLI1, KLHDC4, NF2, PPP1R3A, SMO, TTK, ADCY9, CAV1, CSMD3, ERBB3, GLI3, KRAS, NKX2-1, PPP2R1A, SOCS1, TYK2, AGAP2, CBFA2T3, CSNK1G2, ERBB4, GNA11, LMO2, NOS2, PPP2R1B, SOD2, TYMS, AKT1, CBL, CTNNA1, ERCC1, GNAQ, LRP1B, NOS3, PRKAA2, SOS1, UGT1A1, AKT2, CCND1, CTNNA2, ERCC2, GNAS, LRP2, NOTCH1, PRKCA, SOX10, UMPS, AKT3, CCND2, CTNNB1, ERCC3, GPR124, LRP6, NOTCH2, PRKCZ, SOX2, USP9X, ALK, CCND3, CYFIP1, ERCC4, GPR133, LTK, NOTCH3, PRKDC, SP1, VEGF, ANAPC5, CCNE1, CYLD, ERCC5, GRB2, MAN1B1, NPM1, PTCH1, SPRY2, VEGFA, APC, CD40LG, CYP19A1, ERCC6, GSK3B, MAP2K1, NQO1, PTCH2, SRC, VHL, APC2, CD44, CYP1B1, ERG, GSTP1, MAP2K2, NR3C1, PTEN, ST6GAL2, WRN, AR, CD79A, CYP2C19, ERN2, GUCY1A2, MAP2K4, NRAS, PTGS2, STAT1, WT1, ARAF, CD79B, CYP2C8, ESR1, HDAC1, MAP2K7, NRP2, PTPN11, STAT3, XPA, ARFRP1, CDC42, CYP2D6, ESR2, HDAC2, MAP3K1, NTRK1, PTPRB, STK11, XPC, ARID1A, CDC42BPB, CYP3A4, ETV4, HGF, MAPK1, NTRK2, PTPRD, SUFU, ZFY, ATM, CDC73, CYP3A5, EWSR1, HIF1A, MAPK3, NTRK3, RAD50, SULT1A1, ZNF521, ATP5A1, CDH1, DACH2, EXT1, HM13, MAPK8, OMA1, RAD51, SUZ12, ATR, CDH10, DCC, EZH2, HMGA1, MARK3, OR10R2, RAF1, TAF1, AURKA, CDH2, DCLK3, FANCA, HNF1A, MCL1, PAK3, RARA, TBX22, AURKB, CDH20, DDB2, FANCD2, HOXA3, MDM2, PARP1, RB1, TCF12, BAI3, CDH5, DDR2, FANCE, HOXA9, MDM4, PAX5, REM1, TCF3, BAP1, CDK2,

DGKB, FANCF, HRAS, MECOM, PCDH15, RET, TCF4, BARD1, CDK4, DGKZ, FAS, HSP90AA1, MEN1, PCDH18, RICTOR, TEK, BAX, CDK6, DIRAS3, FBXW7, IDH1, MET, PCNA, RIPK1, TEP1, BCL11A, CDK7, DLG3, FCGR3A, IDH2, MITF, PDGFA, ROR1, TERT, BCL2, CDK8, DLL1, FES, IFNG, MLH1, PDGFB, ROR2, TET2, BCL2A1, CDKN1A, DNMT1, FGFR1, IGF1R, MLL, PDGFRA, ROS1, TGFB2, BCL2L1, CDKN1B, DNMT3A, FGFR2, IGF2R, MLL3, PDGFRB, RPS6KA2, THBS1, BCL2L2, CDKN2A, DNMT3B, FGFR3, IKBKE, MPL, PDZRN3, RPTOR, TNFAIP3, BCL3, CDKN2B, DOT1L, FGFR4, IKZF1, MRE11A, PHLPP2, RSPO2, TNKS, BCL6, CDKN2C, DPYD, FH, IL2RG, MSH2, PIK3C3, RSPO3, TNKS2, BCR, CDKN2D, E2F1, FHOD3, INHBA, MSH6, PIK3CA, RUNX1, TNNI3K, BIRC5, CDX2, EED, FIGF, INSR, MTHFR, PIK3CB, SDHB, TNF, BIRC6, CEBPA, EGF, FLG2, IRS1, MTOR, PIK3CD, SF3B1, TOP1, BLM, CERK, EGFR, FLNC, IRS2, MUTYH, PIK3CG, SHC1, and TOP2A.

212. The method of claim 207, further comprising generating a report communicating a profile of said tumor-specific mutations.

213. The method of claim 207, wherein step (d) is performed at a plurality of time points.

214. The method of claim 213, wherein one time point is prior to a first administration of a cancer therapy and a second time point is subsequent to said first administration.

215. The method of claim 213, further comprising generating a report communicating the profile of tumor-specific mutations at said plurality of time points.

216. The method of claim 212 or 215, wherein said report comprises a list of one or more therapeutic candidates targeting a gene that harbors one of said tumor-specific mutations.

217. The method of claim 212, wherein said report is generated 1 week from isolating said gDNA.

218. The method of claim 207, wherein said mutations comprise copy number variation.

219. The method of claim 207, wherein said detecting comprises sequencing said cell-free DNA.

220. The method of claim 219, comprising sequencing at least 10 cancer-related genes present in said cell-free DNA, wherein one of said at least 10 cancer-related genes is identified in step c) of claim 207 as harboring a tumor-specific mutation.

221. The method of claim 219, comprising sequencing at least 100 cancer-related genes present in said cell-free DNA, wherein one of said at least 100 cancer-related genes is identified in step c) of claim 207 as harboring a tumor-specific mutation.

222. The method of claim 219, wherein said sequencing comprises the method of claim 159 or 162.

223. An oligonucleotide probe for sensitive detection of amplicons, comprising:

- (a) a detectable moiety;
- (b) a quencher moiety;
- (c) a melting temperature (T_m) below 50°C.

224. The oligonucleotide probe of claim 223, wherein said probe has a length of 8-30 nucleotides.

225. The oligonucleotide probe of any of the preceding claims, wherein the detectable moiety is quenched at a temperature of 55°C or higher

226. The oligonucleotide probe of any of the preceding claims, wherein the probe does not hybridize to a complementary template nucleic acid at an ambient temperature above 55°C.

227. The oligonucleotide probe of any of the preceding claims, wherein the quencher moiety quenches the detectable moiety if the probe is not hybridized to a template strand.

228. The oligonucleotide probe of any of the preceding claims, wherein the T_m is between 30-45°C.

229. The oligonucleotide probe of any of the preceding claims, wherein the fluorophore moiety and quencher moiety are spaced at least seven nucleotides apart.

230. The oligonucleotide probe of any of the preceding claims, comprising a T_m -enhancing base nucleotide.

231. The oligonucleotide probe of claim 230, wherein the T_m -enhancing base nucleotide is a locked or bridge nucleotide.

232. The oligonucleotide probe of any of the preceding claims, wherein the detectable moiety comprises a fluorophore.

233. The oligonucleotide probe of any of the preceding claims, having a length of at least 15 nucleotides.

234. The oligonucleotide probe of any of the preceding claims, having a GC content of at least 40%.

235. The oligonucleotide probe of any of the preceding claims, having a GC content that is less than 80%.

236. The oligonucleotide probe of any of the preceding claims, having a GC content that is less than 50%.

237. The oligonucleotide probe of any of the preceding claims, having a GC content that is less than 40%.

238. The oligonucleotide probe of any of the preceding claims, having a length of less than 15 nucleotides.

239. The oligonucleotide probe of any of the preceding claims, having a GC content of less than 40%.

240. The oligonucleotide probe of any of the preceding claims, having a GC content that is at least 40%.

241. The oligonucleotide probe of any of the preceding claims, having a GC content that is between 40-80%.

242. The oligonucleotide probe of any of the preceding claims, having a GC content of less than 40%, and further comprising a locked or bridged nucleotide.

243. The oligonucleotide probe of any of the preceding claims, comprising a sequence having at least 70% complementarity or identity to a nucleotide sequence of at least 10 contiguous nucleotides contained in a gene selected from the group consisting of ABCA1, BRAF, CHD5, EP300, FLT1, ITPA, MYC, PIK3R1, SKP2, TP53, ABCA7, BRCA1, CHEK1, EPHA3, FLT3, JAK1, MYCL1, PIK3R2, SLC19A1, TP73, ABCB1, BRCA2, CHEK2, EPHA5, FLT4, JAK2, MYCN, PKHD1, SLC1A6, TPM3, ABCC2, BRIP1, CLTC, EPHA6, FN1, JAK3, MYH2, PLCB1, SLC22A2, TPMT, ABCC3, BUB1B, COL1A1, EPHA7, FOS, JUN, MYH9, PLCG1, SLC01B3, TPO, ABCC4, C1orf144, COPS5, EPHA8, FOXO1, KBTBD11, NAV3, PLCG2, SMAD2, TPR, ABCG2, CABLES1, CREB1, EPHB1, FOXO3, KDM6A, NBN, PML, SMAD3, TRIO, ABL1, CACNA2D1, CREBBP, EPHB4, FOXP4, KDR, NCOA2, PMS2, SMAD4, TRRAP, ABL2, CAMKV, CRKL, EPHB6, GAB1, KIT, NEK11, PPARG, SMARCA4, TSC1, ACVR1B, CARD11, CRLF2, EPO, GATA1, KLF6, NF1, PPARGC1A, SMARCB1, TSC2, ACVR2A, CARM1, CSF1R, ERBB2, GLI1, KLHDC4, NF2, PPP1R3A, SMO, TTK, ADCY9, CAV1, CSMD3, ERBB3, GLI3, KRAS, NKX2-1, PPP2R1A, SOCS1, TYK2, AGAP2, CBFA2T3, CSNK1G2, ERBB4, GNA11, LMO2, NOS2, PPP2R1B, SOD2, TYMS, AKT1, CBL, CTNNA1, ERCC1, GNAQ, LRP1B, NOS3, PRKAA2, SOS1, UGT1A1, AKT2, CCND1, CTNNA2, ERCC2, GNAS, LRP2, NOTCH1, PRKCA, SOX10, UMPS, AKT3, CCND2, CTNNB1, ERCC3, GPR124, LRP6,

NOTCH2, PRKCZ, SOX2, USP9X, ALK, CCND3, CYFIP1, ERCC4, GPR133, LTK, NOTCH3, PRKDC, SP1, VEGF, ANAPC5, CCNE1, CYLD, ERCC5, GRB2, MAN1B1, NPM1, PTCH1, SPRY2, VEGFA, APC, CD40LG, CYP19A1, ERCC6, GSK3B, MAP2K1, NQO1, PTCH2, SRC, VHL, APC2, CD44, CYP1B1, ERG, GSTP1, MAP2K2, NR3C1, PTEN, ST6GAL2, WRN, AR, CD79A, CYP2C19, ERN2, GUCY1A2, MAP2K4, NRAS, PTGS2, STAT1, WT1, ARAF, CD79B, CYP2C8, ESR1, HDAC1, MAP2K7, NRP2, PTPN11, STAT3, XPA, ARFRP1, CDC42, CYP2D6, ESR2, HDAC2, MAP3K1, NTRK1, PTPRB, STK11, XPC, ARID1A, CDC42BPB, CYP3A4, ETV4, HGF, MAPK1, NTRK2, PTPRD, SUFU, ZFY, ATM, CDC73, CYP3A5, EWSR1, HIF1A, MAPK3, NTRK3, RAD50, SULT1A1, ZNF521, ATP5A1, CDH1, DACH2, EXT1, HM13, MAPK8, OMA1, RAD51, SUZ12, ATR, CDH10, DCC, EZH2, HMGA1, MARK3, OR10R2, RAF1, TAF1, AURKA, CDH2, DCLK3, FANCA, HNF1A, MCL1, PAK3, RARA, TBX22, AURKB, CDH20, DDB2, FANCD2, HOXA3, MDM2, PARP1, RB1, TCF12, BAI3, CDH5, DDR2, FANCE, HOXA9, MDM4, PAX5, REM1, TCF3, BAP1, CDK2, DGKB, FANCF, HRAS, MECOM, PCDH15, RET, TCF4, BARD1, CDK4, DGKZ, FAS, HSP90AA1, MEN1, PCDH18, RICTOR, TEK, BAX, CDK6, DIRAS3, FBXW7, IDH1, MET, PCNA, RIPK1, TEP1, BCL11A, CDK7, DLG3, FCGR3A, IDH2, MITF, PDGFA, ROR1, TERT, BCL2, CDK8, DLL1, FES, IFNG, MLH1, PDGFB, ROR2, TET2, BCL2A1, CDKN1A, DNMT1, FGFR1, IGF1R, MLL, PDGFRA, ROS1, TGFB2, BCL2L1, CDKN1B, DNMT3A, FGFR2, IGF2R, MLL3, PDGFRB, RPS6KA2, THBS1, BCL2L2, CDKN2A, DNMT3B, FGFR3, IKBKE, MPL, PDZRN3, RPTOR, TNFAIP3, BCL3, CDKN2B, DOT1L, FGFR4, IKZF1, MRE11A, PHLPP2, RSPO2, TNKS, BCL6, CDKN2C, DPYD, FH, IL2RG, MSH2, PIK3C3, RSPO3, TNKS2, BCR, CDKN2D, E2F1, FHOD3, INHBA, MSH6, PIK3CA, RUNX1, TNNI3K, BIRC5, CDX2, EED, FIGF, INSR, MTHFR, HADH, RPP30, ZFP3, PIK3CB, SDHB, TNR, BIRC6, CEBPA, EGF, FLG2, IRS1, MTOR, PIK3CD, SF3B1, TOP1, BLM, CERK, EGFR, FLNC, IRS2, MUTYH, PIK3CG, SHC1, and TOP2A.

244. A reaction mixture comprising at least one primer/probe set, wherein the primer/probe set comprises:

- (a) a forward primer designed to hybridize to a genomic region at a first location; and
- (b) an oligonucleotide probe of any of claims 1-243.

245. The reaction mixture of claim 244, further comprising a reverse primer designed to hybridize to said genomic region at a second location.

246. The reaction mixture of any of the preceding claims, wherein said oligonucleotide probe has a T_m that is at least 15 °C lower than the T_m of said forward primer.
247. The reaction mixture of any of the preceding claims, wherein said oligonucleotide probe has a T_m that is at least 15 °C lower than an average of the T_m of the first primer and the T_m of the second primer.
248. The reaction mixture of any of the preceding claims, wherein said oligonucleotide probe is designed to hybridize to said genomic region at a third location located between said first and second location.
249. The reaction mixture of any of the preceding claims, wherein the reverse primer is present in an amount that is at least 2 to 10-fold less than an amount of the forward primer.
250. The reaction mixture of any of the preceding claims, wherein the reverse primer is present in an amount that is no more than 2-fold different than an amount of the forward primer.
251. The reaction mixture of any of the preceding claims, further comprising a nucleic acid sample isolated from a biological sample.
252. The reaction mixture of any of the preceding claims, wherein said biological sample is a sample isolated from a subject.
253. The reaction mixture of any of the preceding claims, wherein said subject is a human subject.
254. The reaction mixture of any of the preceding claims, wherein said human subject is diagnosed, suspected of having, or suspected of being at increased risk for a disease.
255. The reaction mixture of any of the preceding claims, wherein said disease is cancer.
256. The reaction mixture of any of the preceding claims, wherein said template nucleic acid comprises a genomic region.
257. The reaction mixture of any of the preceding claims, wherein said template nucleic acid comprises DNA, RNA, or cDNA.
258. The reaction mixture of any of the preceding claims, further comprising a polymerase.
259. The reaction mixture of any of the preceding claims, wherein said polymerase is a DNA polymerase.

260. The reaction mixture of any of the preceding claims, comprising:
- (a) a first template nucleic acid;
 - (b) an amount of forward primer;
 - (c) a second amount of reverse primer, wherein said second amount of reverse primer is at least 2 to 10-fold less than said amount of the forward primer; and
 - (d) an oligonucleotide probe.
261. The reaction mixture any of the preceding claims, comprising a plurality of primer/probe sets.
262. The reaction mixture of any of the preceding claims, wherein each primer/probe set of said plurality is specific for a different region of genomic DNA.
263. The reaction mixture of any of the preceding claims, wherein said genomic region is associated with a disease-related mutation.
264. The reaction mixture of any of the preceding claims, wherein said mutation comprises a copy number variation.
265. The reaction mixture of any of the preceding claims, wherein said mutation comprises a single nucleotide polymorphism (SNP), insertion, deletion, or inversion.
266. The reaction mixture of any of the preceding claims, wherein one of said forward or reverse primers overlays said SNP, insertion, deletion, or inversion.
267. The reaction of any of the preceding claims, wherein said oligonucleotide probe overlays said SNP, insertion, deletion, or inversion.
268. The reaction mixture of any of the preceding claims, wherein said disease is a cancer.
269. The reaction mixture of any of the preceding claims, wherein said primer/probe set comprises a plurality of oligonucleotide probes, wherein each oligonucleotide probe is an allele-specific probe designed to bind with greater avidity to a sequence comprising one specific allele of said genomic region as compared to a sequence comprising any other allele of said genomic region, wherein each allele-specific probe is specific for a different allele.
270. The reaction mixture of any of the preceding claims, wherein each of said allele-specific probes each comprise a spectrally distinct fluorophore.
271. The reaction mixture of any of the preceding claims, wherein the difference in binding energy of an allele specific probe to said one specific allele as compared to a binding energy of said allele specific probe to any other allele is more than 1% of the overall binding energy of said probe to said genomic region.

272. The reaction mixture of any of the preceding claims, wherein said oligonucleotide probe is a beacon probe.

273. The reaction mixture of any of the preceding claims, wherein said oligonucleotide probe is a Pleiades probe.

274. A method, comprising:

(a) partitioning a reaction mixture of any of the preceding claims into a plurality of reaction volumes; and

(b) performing, in at least one of said reaction volumes, a PCR amplification reaction comprising multiple rounds of thermal cycling, wherein said oligonucleotide probe does not affect efficiency of said PCR amplification reaction.

275. The method of any of the preceding claims, wherein said oligonucleotide probe does not hybridize to a template nucleic acid or PCR reaction product during an annealing phase or extension phase of said PCR amplification reaction.

276. The method of any of the preceding claims, further comprising cooling at least one of said reaction volumes to below 50°C, wherein said cooling enables hybridization of said oligonucleotide probe to a nucleic acid comprising a sequence having at least 70% complementarity to said oligonucleotide probe.

277. The method of any of the preceding claims, comprising cooling at least one of said reaction volumes to below 37°C, wherein said cooling enables hybridization of at least 70% of an amount of oligonucleotide probes to nucleic acids comprising a sequence having at least 70% complementarity to said oligonucleotide probe.

278. The method of any of the preceding claims, wherein said partitioning results in each reaction volume containing on average <1 molecule of template nucleic acid.

279. The method of any of the preceding claims, comprising performing an exponential PCR amplification reaction and a linear PCR amplification reaction in at least one of said reaction volumes.

280. The method of any of the preceding claims, wherein said exponential PCR amplification and said linear PCR amplification reaction occurs sequentially without adding or removing components from said reaction volumes.

281. The method of any of the preceding claims, wherein said PCR amplification reaction results in at least 50% of the amplification products being single-stranded amplification products.

282. The method of any of the preceding claims, wherein said reaction volumes are droplets.

283. The method of any of the preceding claims, wherein said hybridization results in emission of fluorescence from said oligonucleotide probe.

284. The method of any of the preceding claims, further comprising detecting the presence or absence of said fluorescence in at least one of said reaction volumes.

285. The method of any of the preceding claims, comprising measuring intensity of said fluorescence in said reaction volumes.

286. The method of any of the preceding claims, further comprising determining a number and/or fraction of fluorescence-positive reaction volumes.

287. The method of any of the preceding claims, comprising determining the presence, absence, or amount of one or more mutations in said sample based on said number and/or fraction of fluorescence-positive reaction volumes.

288. The method of any of the preceding claims, wherein said one or more mutations comprises a SNP, deletion, insertion, or inversion.

289. The method of any of the preceding claims, wherein said one or more mutations comprises a copy number variation of a gene.

290. The method of any of the preceding claims, wherein said one or more mutations comprises a disease-related mutation.

291. The method of any of the preceding claims, wherein said disease is cancer.

292. The method of any of the preceding claims, wherein said one or more mutations comprises a mutation of one or more genes selected from the group consisting of ABCA1, BRAF, CHD5, EP300, FLT1, ITPA, MYC, PIK3R1, SKP2, TP53, ABCA7, BRCA1, CHEK1, EPHA3, FLT3, JAK1, MYCL1, PIK3R2, SLC19A1, TP73, ABCB1, BRCA2, CHEK2, EPHA5, FLT4, JAK2, MYCN, PKHD1, SLC1A6, TPM3, ABCC2, BRIP1, CLTC, EPHA6, FN1, JAK3, MYH2, PLCB1, SLC22A2, TPMT, ABCC3, BUB1B, COL1A1, EPHA7, FOS, JUN, MYH9, PLCG1, SLC01B3, TPO, ABCC4, C1orf144, COPS5, EPHA8, FOXO1, KBTBD11, NAV3, PLCG2, SMAD2, TPR, ABCG2, CABLES1, CREB1, EPHB1, FOXO3, KDM6A, NBN, PML, SMAD3, TRIO, ABL1, CACNA2D1, CREBBP, EPHB4, FOXP4, KDR, NCOA2, PMS2, SMAD4, TRRAP, ABL2, CAMKV, CRKL, EPHB6, GAB1, KIT, NEK11, PPARG, SMARCA4, TSC1, ACVR1B, CARD11, CRLF2, EPO, GATA1, KLF6, NF1, PPARGC1A, SMARCB1, TSC2, ACVR2A, CARM1, CSF1R, ERBB2, GLI1, KLHDC4, NF2, PPP1R3A, SMO, TTK, ADCY9, CAV1, CSMD3, ERBB3, GLI3, KRAS, NKX2-1, PPP2R1A, SOCS1, TYK2, AGAP2, CBFA2T3, CSNK1G2, ERBB4, GNA11, LMO2, NOS2, PPP2R1B, SOD2, TYMS, AKT1, CBL, CTNNA1, ERCC1, GNAQ, LRP1B, NOS3, PRKAA2, SOS1, UGT1A1, AKT2, CCND1,

CTNNA2, ERCC2, GNAS, LRP2, NOTCH1, PRKCA, SOX10, UMPS, AKT3, CCND2, CTNNB1, ERCC3, GPR124, LRP6, NOTCH2, PRKCZ, SOX2, USP9X, ALK, CCND3, CYFIP1, ERCC4, GPR133, LTK, NOTCH3, PRKDC, SP1, VEGF, ANAPC5, CCNE1, CYLD, ERCC5, GRB2, MAN1B1, NPM1, PTCH1, SPRY2, VEGFA, APC, CD40LG, CYP19A1, ERCC6, GSK3B, MAP2K1, NQO1, PTCH2, SRC, VHL, APC2, CD44, CYP1B1, ERG, GSTP1, MAP2K2, NR3C1, PTEN, ST6GAL2, WRN, AR, CD79A, CYP2C19, ERN2, GUCY1A2, MAP2K4, NRAS, PTGS2, STAT1, WT1, ARAF, CD79B, CYP2C8, ESR1, HDAC1, MAP2K7, NRP2, PTPN11, STAT3, XPA, ARFRP1, CDC42, CYP2D6, ESR2, HDAC2, MAP3K1, NTRK1, PTPRB, STK11, XPC, ARID1A, CDC42BPB, CYP3A4, ETV4, HGF, MAPK1, NTRK2, PTPRD, SUFU, ZFY, ATM, CDC73, CYP3A5, EWSR1, HIF1A, MAPK3, NTRK3, RAD50, SULT1A1, ZNF521, ATP5A1, CDH1, DACH2, EXT1, HM13, MAPK8, OMA1, RAD51, SUZ12, ATR, CDH10, DCC, EZH2, HMGA1, MARK3, OR10R2, RAF1, TAF1, AURKA, CDH2, DCLK3, FANCA, HNF1A, MCL1, PAK3, RARA, TBX22, AURKB, CDH20, DDB2, FANCD2, HOXA3, MDM2, PARP1, RB1, TCF12, BAI3, CDH5, DDR2, FANCE, HOXA9, MDM4, PAX5, REM1, TCF3, BAP1, CDK2, DGKB, FANCF, HRAS, MECOM, PCDH15, RET, TCF4, BARD1, CDK4, DGKZ, FAS, HSP90AA1, MEN1, PCDH18, RICTOR, TEK, BAX, CDK6, DIRAS3, FBXW7, IDH1, MET, PCNA, RIPK1, TEP1, BCL11A, CDK7, DLG3, FCGR3A, IDH2, MITF, PDGFA, ROR1, TERT, BCL2, CDK8, DLL1, FES, IFNG, MLH1, PDGFB, ROR2, TET2, BCL2A1, CDKN1A, DNMT1, FGFR1, IGF1R, MLL, PDGFRA, ROS1, TGFB2, BCL2L1, CDKN1B, DNMT3A, FGFR2, IGF2R, MLL3, PDGFRB, RPS6KA2, THBS1, BCL2L2, CDKN2A, DNMT3B, FGFR3, IKBKE, MPL, PDZRN3, RPTOR, TNFAIP3, BCL3, CDKN2B, DOT1L, FGFR4, IKZF1, MRE11A, PHLPP2, RSPO2, TNKS, BCL6, CDKN2C, DPYD, FH, IL2RG, MSH2, PIK3C3, RSPO3, TNKS2, BCR, CDKN2D, E2F1, FHOD3, INHBA, MSH6, PIK3CA, RUNX1, TNNI3K, BIRC5, CDX2, EED, FIGF, INSR, MTHFR, HADH, RPP30, ZFP3, PIK3CB, SDHB, TNF, BIRC6, CEBPA, EGF, FLG2, IRS1, MTOR, PIK3CD, SF3B1, TOP1, BLM, CERK, EGFR, FLNC, IRS2, MUTYH, PIK3CG, SHC1, and TOP2A.

293. The method of any of the preceding claims, wherein said one or more mutations comprises a mutation of one or more genes selected from the group consisting of DDR2, EGFR, AURKA, VEGFA, FGFR1, CDK4, EFBB2, CDK6, JAK2, MET, BRAF, ERBB3, SRC, HADH, RPP30, and ZFP3.

294. The method of any of the preceding claims, comprising generating a report communicating a profile of the presence, absence, and/or level of said mutation in said sample.

295. The method of any of the preceding claims, further comprising a description of a therapeutic agent targeting said mutation.

296. A method of treating cancer in a subject in need thereof, comprising:

- (a) obtaining a biological sample from the subject;
- (b) from a nucleic acid sample isolated from the biological sample,

determining a presence or absence of a copy number variation (CNV) in at least five genes selected from the group consisting of MET, FGFR1, FGFR2, FLT3, HER3, EGFR, mTOR, CDK4, HER2, RET, HADH, ZFP3, DDR2, AURKA, VEGFA, CDK6, JAK2, BRAF, and SRC;

- (c) based on the determining, generating a subject-specific CNV profile;

and

- (d) based on the subject-specific CNV profile, selecting a cancer therapy for the subject.

297. The method of claim 296, wherein the determining a presence or absence of a CNV comprises use of a method of any of claims 274-289.

298. The method of claim 296, wherein the digital PCR assay comprises use of an oligonucleotide probe of any of the preceding claims.

299. The method of claim 298, wherein the oligonucleotide probe comprises a nucleotide sequence of any of SEQ ID NOS: 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, or 118.

300. The method of claim 296, wherein the digital PCR assay comprises use of an primer of any of the preceding claims.

301. The method of claim 300, wherein the primer comprises a nucleotide sequence of any of SEQ ID NOS. 59, 60, 62, 63, 65, 66, 68, 69, 71, 72, 74, 75, 77, 78, 80, 81, 83, 84, 86, 87, 89, 90, 92, 93, 95, 96, 98, 99, 101, 102, 104, 105, 107, 108, 110, 111, 113, 114, 116, or 117.

302. The method of claim 296, comprising determining of presence or absence of a CNV in at least 10, 12, or 18 genes.

303. The method of claim 296, wherein the biological sample is suspected of harboring nucleic acids originating from the cancer.

304. The method of claim 296, wherein the biological sample is a solid tissue sample.

305. The method of claim 296, wherein the solid tissue sample is a formalin fixed, paraffin embedded sample.

306. The method of claim 296, wherein the biological sample is a liquid biological sample.

307. The method of claim 296, wherein the liquid biological sample is selected from the group consisting of blood, serum, plasma, urine, sweat, tears, saliva, and sputum.

308. A computer system, comprising:

- (a) a memory unit configured to receive data from a sample, wherein said data is generated by the method of any of the preceding claims;
- (b) computer executable instructions for analysis of said data; and
- (c) computer executable instructions to determine the presence, absence, or amount of a mutation in said sample based on said analysis.

309. The computer system of any of the preceding claims, further comprising computer executable instructions to generate a report of said presence, absence, or amount of a mutation in said sample.

310. The computer system of any of the preceding claims, further comprising computer executable instructions to generate a report of therapeutic options based on said presence, absence, or amount of a mutation in said sample.

311. The computer system of any of the preceding claims, further comprising a user interface configured to communicate or display said report to a user.

312. A kit, comprising:

- (a) at least one primer/probe set, wherein the primer/probe set comprises (i) a forward primer designed to hybridize to a genomic region at a first location, (ii) a reverse primer designed to hybridize to said genomic region at a second location, and (iii) an oligonucleotide probe of any of the preceding claims, wherein said oligonucleotide probe is designed to hybridize to said genomic region at a third location located between said first and second location; and
- (b) instructions for use.

313. An oligonucleotide probe as set forth in any of SEQ ID NO: 4-21, 23, 24, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, or 118.

314. A target-selective oligonucleotide as set forth in any of SEQ. ID. NOS: 1948-5593.

315. An oligonucleotide primer having a sequence as set forth in SEQ ID NO: 25 or 26.

316. An oligonucleotide primer having a sequence as set forth in any of SEQ ID NOS. 1-3, 22, 27-58, 59, 60, 62, 63, 65, 66, 68, 69, 71, 72, 74, 75, 77, 78, 80, 81, 83, 84, 86, 87, 89, 90, 92, 93, 95, 96, 98, 99, 101, 102, 104, 105, 107, 108, 110, 111, 113, 114, 116, or 117.

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising ligating single-stranded template nucleic acids to single-stranded adaptor molecules, wherein said single-stranded template nucleic acids have an average length of 150-10000 nucleotides, wherein an efficiency of said ligating said single-stranded template nucleic acids is at least 10%.
2. The method of claim 1, wherein said efficiency of said ligating said single-stranded template nucleic acids is at least 30%.
3. The method of claim 1, wherein said efficiency of said ligating said single-stranded template nucleic acids is at least 50%.
4. The method of claim 1, wherein said efficiency of said ligating said single-stranded template nucleic acids is at least 60%.
5. The method of claim 1, wherein said single-stranded template nucleic acids comprises a 5' nucleotide monophosphate (NMP).
6. The method of claim 5, wherein said 5' nucleotide monophosphate comprises adenosine monophosphate (AMP).
7. The method of claim 1, wherein said single-stranded template nucleic acids comprise DNA.
8. The method of claim 1, wherein said single-stranded template nucleic acids comprise RNA.
9. The method of claim 1, wherein said single-stranded adaptor molecules comprise DNA.
10. The method of claim 1, wherein said single-stranded template nucleic acids have an average length of 150-250 nucleotides.
11. The method of claim 1, wherein said single-stranded template nucleic acids have an average length of 300-700 nucleotides.
12. The method of claim 1, wherein said single-stranded template nucleic acids have an average length of 500-1000 nucleotides.
13. The method of claim 1, wherein said single-stranded template nucleic acid molecules comprise a 3' terminal blocking group.
14. The method of claim 13, wherein said terminal blocking group comprises a dideoxy-NTP.

15. The method of claim 1, further comprising adding a 3' terminal blocking group to said single-stranded template nucleic acid molecules using a terminal nucleotidyl transferase (TdT).
16. The method of claim 1, wherein said ligating is effected without separating said adenylated single-stranded template nucleic acid molecules from said first reaction mixture.
17. The method of claim 5, wherein an NMP is transferred to at least 10% of said single-stranded template nucleic acid molecules.
18. The method of claim 17, wherein said NMP is transferred to at least 50% of said single-stranded template nucleic acid molecules.
19. The method of claim 1, further comprising phosphorylating a 5' end of said single-stranded template nucleic acid molecules.
20. The method of claim 19, wherein said phosphorylating comprises use of T4 polynucleotide kinase (PNK).
21. A method of ligating single-stranded donor nucleic acid molecules and single-stranded acceptor nucleic acid molecules, said method comprising:
 - a. transferring a nucleotide monophosphate (NMP) to single-stranded donor nucleic acid molecules in a reaction mixture, thereby generating single-stranded donor nucleic acid molecules comprising said NMP;
 - b. after step a), adding acceptor nucleic acid molecules to said reaction mixture; and
 - c. ligating said single-stranded acceptor nucleic acid molecules to said single-stranded donor nucleic acid molecules comprising said NMP in said reaction mixture.
22. The method of claim 21, wherein step b) comprises adding Mn^{2+} to said reaction mixture and wherein a final concentration of Mn^{2+} in said reaction mixture after said adding is from 2.5 mM-7.5 mM.
23. The method of claim 21, wherein step b) comprises reducing concentration of an NTP in said reaction mixture of step a) at least 10-fold.
24. The method of claim 21, wherein said single-stranded donor nucleic acid molecules are over 120 nucleotides.

25. The method of claim 21, wherein said single-stranded acceptor nucleic acid molecules are over 120 nucleotides.
26. The method of claim 21, wherein said single-stranded donor nucleic acid molecules comprise DNA.
27. The method of claim 21, wherein said single-stranded donor nucleic acid molecules comprise RNA.
28. The method of claim 21, wherein said single-stranded acceptor nucleic acid molecules comprise DNA.
29. The method of claim 21, wherein said single-stranded acceptor nucleic acid molecules comprise RNA.
30. The method of claim 21, wherein an efficiency of said ligating said single-stranded donor nucleic acid molecules is over 10%.
31. The method of claim 21, wherein an efficiency of said ligating said single-stranded donor nucleic acid molecules is over 50%.
32. The method of claim 21, wherein an efficiency of said ligating said single-stranded acceptor nucleic acid molecules is over 10%.
33. The method of claim 21, wherein said NMP is adenosine monophosphate (AMP).
34. The method of claim 21, wherein said transferring comprises transferring said NMP to a 5' end of said single-stranded donor nucleic acid molecules.
35. The method of claim 21, wherein said single-stranded donor nucleic acid molecules and said single-stranded acceptor nucleic acid molecules are over 120 nucleotides.
36. The method of claim 21, wherein said ligating comprises use of an ATP-dependent ligase.
37. The method of claim 21, wherein said transferring comprises use of an ATP-dependent ligase.
38. The method of claim 36 or 37, wherein said ATP-dependent ligase comprises an RNA ligase.
39. The method of claim 38, wherein said ATP-dependent ligase comprises an archaeal RNA ligase.
40. The method of claim 39, wherein said archaeal RNA ligase is from a thermophilic archaeon.

41. The method of claim 40, wherein said thermophilic archaeon comprises *Methanobacterium thermoautotrophicum*.
42. The method of claim 38, wherein said RNA ligase comprises MthRnl.
43. The method of claim 38, wherein said RNA ligase comprises an Rn1 1 family ligase.
44. The method of claim 43, wherein said RNA ligase comprises T4 RNA ligase.
45. The method of claim 43, wherein said RNA ligase comprises a ligase from *Thermus scotoductus* bacteriophage TS2126.
46. The method of claim 38, wherein said RNA ligase comprises CircLigase.
47. The method of claim 38, wherein said RNA ligase comprise CircLigase II.
48. The method of claim 38, wherein said RNA ligase comprises a thermophilic RNA ligase.
49. The method of claim 21, wherein said ligating comprises ligating a 5' end of said single-stranded donor nucleic acid molecules comprising said NMP to a 3' end of said single-stranded acceptor nucleic acid molecules.
50. A method of ligating single-stranded donor nucleic acids to single-stranded acceptor nucleic acids, said method comprising:
 - a. transferring a nucleotide monophosphate (NMP) to single-stranded donor nucleic acid molecules, thereby generating single-stranded donor nucleic acid molecules comprising said NMP in a reaction mixture without single-stranded acceptor nucleic acid molecules;
 - b. ligating single-stranded acceptor nucleic acid molecules to said single-stranded donor nucleic acid molecules comprising said NMP, wherein said single-stranded donor nucleic acid molecules or said single-stranded acceptor nucleic acid molecules are over 120 nucleotides, wherein said single-stranded donor nucleic acid molecules and said single-stranded acceptor nucleic acid molecules are different.
51. The method of claim 50, wherein an efficiency of said ligating said donor nucleic acid molecules is over 10%.
52. The method of claim 51, wherein an efficiency of said ligating said donor nucleic acid molecules is over 50%.

53. The method of claim 50, wherein an amount of ligase in said reaction mixture is at least equimolar to an amount of single-stranded donor nucleic acid molecules in said reaction mixture.
54. The method of claim 50, wherein said transferring and said ligating are carried out sequentially in the reaction mixture.
55. The method of claim 50, wherein said ligating and said transferring occur in the reaction mixture, and wherein said ligating is effected without separating said single-stranded donor nucleic acid molecules from said reaction mixture.