

CLAIMS

WHAT IS CLAIMED IS:

1. A method for nucleic acid analysis, comprising: (a) preparing a first single-stranded deoxynucleic acid (DNA) library from a first plurality of nucleic acid molecules, said first plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) preparing a second single-stranded DNA library from a second plurality of nucleic acid molecules, said second plurality of nucleic acid molecules comprising single-stranded DNA and double-stranded DNA derived from a control; (c) using said first single-stranded DNA library to measure a first size distribution for at least a subset of said first plurality of nucleic acid molecules; (d) using said second single-stranded DNA library to measure a second size distribution for at least a subset of said second plurality of nucleic acid molecules; (e) using said first size distribution and said second size distribution to determine a difference in size between said first size distribution and said second size distribution.
2. The method of claim 1, wherein (a) comprises denaturing said first plurality of nucleic acid molecules.
3. The method of claim 1 or claim 2, wherein (b) comprises denaturing said second plurality of nucleic acid molecules.
4. The method of any one of claims 1 to 3, wherein (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said first plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.
5. The method of any one of claims 1 to 4, wherein (b) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said second plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.
6. The method of any one of claims 1 to 5, wherein (a) comprises (i) circularizing individual single stranded DNA molecules of said first plurality of nucleic acid molecules to form a first plurality of circular nucleic acid molecules; and (ii) amplifying said first plurality of circular nucleic acid molecules to yield a first plurality of amplified nucleic acid molecules.
7. The method of any one of claims 1 to 6, wherein (b) comprises (i) circularizing individual single stranded DNA molecules of said second plurality of nucleic acid molecules to form a second plurality of circular nucleic acid molecules; and (ii) amplifying said second plurality of circular nucleic acid molecules to yield a second plurality of amplified nucleic acid molecules.
8. The method of claim 6 or claim 7, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.
9. The method of claim 6 or claim 7, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.
10. The method of claim 6 or claim 7, wherein said amplification is performed by a polymerase having strand displacement activity.

11. The method of claim 6 or claim 7, wherein said amplification is performed by a polymerase that does not have strand displacement activity.
12. The method of claim 6 or claim 7, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.
13. The method of claim 6 or claim 7, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers. The method of claim 1, further comprising enriching said first plurality of nucleic acid molecules or said second plurality of nucleic acid molecules for one or more target sequences.
14. The method of claim 13, wherein said enriching is performed with one or more primers or capture probes.
15. The method of claim 13, wherein said enriching is performed with one or more antibodies or fragments thereof.
16. The method of claim 1, wherein measuring said size distribution for said first single-stranded DNA library or said second single-stranded DNA library comprises sequencing said first single-stranded DNA library or said second single-stranded DNA library.
17. The method of claim 16, wherein sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid rolling circle sequencing (ROLONY), and ion torrent sequencing.
18. The method of claim 1, further comprising identifying an individual nucleic acid molecule of said single-stranded DNA library or said second single stranded library as having a genomic feature.
19. The method of claim 18, wherein said genomic feature comprises an epigenetic modification.
20. The method of claim 19, wherein said epigenetic modification is selected from the group consisting of methylation, phosphorylation, ubiquitination, sumoylation, acetylation, ribosylation, citrullination, and fragmentation.
21. The method of claim 18, said genomic feature comprises a copy number variation (CNV), a single nucleotide variant (SNV), an insertion, a deletion, a translocation, or a combination thereof.
22. The method of claim 1, wherein said first plurality of nucleic acid molecules or said second plurality of nucleic acid molecules is derived from a cell-free biological sample.
23. The method of claim 22, wherein said cell-free biological sample comprises a bodily fluid.
24. The method of claim 23, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.

25. The method of claim 1, wherein said first plurality of nucleic acids comprises tumor nucleic acids.

26. The method of claim 1, wherein said second plurality of nucleic acids is derived from a healthy control.

27. The method of claim 1, wherein said subject is determined to be at risk of or to have a disease when said difference is greater than a predetermined threshold.

28. The method of claim 1, wherein said subject is determined to be at risk of or to have a disease when an average of said first size distribution is less than an average of said second size distribution.

29. The method of claim 27 or claim 28, wherein said disease is cancer.

30. The method of claim 29, wherein said cancer is selected from the group consisting of colon cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, and brain cancer.

31. The method of any one of claims 27 to 30, further comprising administering a therapeutic to said subject.

32. The method of any one of claims 27 to 31, further comprising recommending additional cancer monitoring to said subject.

33. The method of any one of claims 27 to 32, further comprising using said difference to monitor said subject for a progression or a regression of said disease.

34. A method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature; (c) measuring a size for said individual nucleic acid molecule; (d) associating said genomic feature with a disease based on said size of said individual nucleic acid molecule.

35. The method of claim 34, wherein (a) comprises denaturing said plurality of nucleic acid molecules.

36. The method of claim 34 or claim 35, wherein (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.

37. The method of any one of claims 34 to 36, wherein (a) comprises (i) circularizing individual single stranded DNA molecules of said plurality of nucleic acid molecules to form a plurality of circular nucleic acid molecules; and (ii) amplifying said plurality of circular nucleic acid molecules to yield a plurality of amplified nucleic acid molecules.

38. The method of claim 37, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.

39. The method of claim 37, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.

40. The method of claim 37, wherein said amplification is performed by a polymerase having strand displacement activity.

41. The method of claim 37, wherein said amplification is performed by a polymerase that does not have strand displacement activity.

42. The method of claim 37, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.

43. The method of claim 37, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers.

44. The method of claim 34, further comprising enriching said plurality of nucleic acid molecules for one or more target sequences.

45. The method of claim 44, wherein said enriching is performed with one or more primers or capture probes.

46. The method of claim 44, wherein said enriching is performed with one or more antibodies or fragments thereof.

47. The method of claim 34, wherein (b) comprises sequencing said single-stranded DNA library.

48. The method of claim 34, wherein (c) comprises sequencing said single-stranded DNA library.

49. The method of claim 47 or claim 48, wherein sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid rolling circle sequencing (ROLONY), and ion torrent sequencing.

50. The method of claim 34, wherein said genomic feature comprises an epigenetic modification.

51. The method of claim 50, wherein said epigenetic modification is selected from the group consisting of methylation, phosphorylation, ubiquitination, sumoylation, acetylation, ribosylation, citrullination, and fragmentation.

52. The method of claim 34, wherein said genomic feature comprises a copy number variation (CNV), a single nucleotide variant (SNV), an insertion, a deletion, a translocation, or a combination thereof.

53. The method of claim 34, wherein said plurality of nucleic acid molecules is derived from a cell-free biological sample.

54. The method of claim 53, wherein said cell-free biological sample comprises a bodily fluid.

55. The method of claim 54, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.

56. The method of claim 34, wherein said plurality of nucleic acids comprises tumor nucleic acids.

57. The method of claim 34, wherein said individual nucleic acid molecule having said genomic feature is determined to be associated with a disease when said individual nucleic acid molecule has an average size below a predetermined threshold.

58. The method of claim 34, wherein said individual nucleic acid molecule having said genomic feature is determined to be associated with disease when said individual nucleic acid molecule having said genomic feature has an average size smaller than a nucleic acid molecule without said genomic feature.

59. The method of claim 34, wherein said subject is determined to be at risk of or to have a disease when said individual nucleic acid molecule having said genomic feature has a size below a predetermined threshold.

60. The method of claim 34 or any one of claims 57 to 59, wherein said disease is cancer.

61. The method of claim 60, wherein said cancer is selected from the group consisting of colon cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, and brain cancer.

62. The method of any one of claims 57 to 61, further comprising administering a therapeutic to said subject.

63. The method of any one of claims 57 to 62, further comprising recommending additional cancer monitoring to said subject.

64. The method of any one of claims 57 to 63, further comprising using said genomic feature or said size to monitor said subject for a progression or a regression of said disease.

65. A method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature; (c) identifying at least a 5' end or a 3' end for said individual nucleic acid molecule; (d) associating said genomic feature with a disease based on said 5' end or said 3' end of said individual nucleic acid molecule.

66. The method of claim 65, wherein (a) comprises denaturing said plurality of nucleic acid molecules.

67. The method of claim 65 or claim 66, wherein (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.

68. The method of any one of claims 65 to 67, wherein (a) comprises (i) circularizing individual single stranded DNA molecules of said plurality of nucleic acid molecules to form a plurality of circular nucleic acid molecules; and (ii) amplifying said plurality of circular nucleic acid molecules to yield a plurality of amplified nucleic acid molecules.

69. The method of claim 68, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.

70. The method of claim 68, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.

71. The method of claim 68, wherein said amplification is performed by a polymerase having strand displacement activity.

72. The method of claim 68, wherein said amplification is performed by a polymerase that does not have strand displacement activity.

73. The method of claim 68, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.

74. The method of claim 68, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers.

75. The method of claim 65, further comprising enriching said plurality of nucleic acid molecules for one or more target sequences.

76. The method of claim 75, wherein said enriching is performed with one or more primers or capture probes.

77. The method of claim 65, wherein said enriching is performed with one or more antibodies or fragments thereof.

78. The method of claim 65, wherein (b) comprises sequencing said single-stranded DNA library.

79. The method of claim 65, wherein (c) comprises sequencing said single-stranded DNA library.

80. The method of claim 78 or claim 79, wherein sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid rolling circle sequencing (ROLONY), and ion torrent sequencing.

81. The method of claim 65, wherein said genomic feature comprises an epigenetic modification.

82. The method of claim 81, wherein said epigenetic modification is selected from the group consisting of methylation, phosphorylation, ubiquitination, sumoylation, acetylation, ribosylation, citrullination, and fragmentation.

83. The method of claim 65, wherein said genomic feature comprises a copy number variation (CNV), a single nucleotide variant (SNV), an insertion, a deletion, or a combination thereof.

84. The method of claim 65, wherein said plurality of nucleic acid molecules is derived from a cell-free biological sample.

85. The method of claim 84, wherein said cell-free biological sample comprises a bodily fluid.

86. The method of claim 85, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.

87. The method of claim 65, wherein said plurality of nucleic acids comprises tumor nucleic acids.

88. The method of claim 65, wherein said subject is determined to be at risk of or to have a disease when said 5' end or said 3' end of said individual nucleic acid molecule having said genomic feature has a predetermined sequence.

89. The method of claim 65 or claim 88, wherein said disease is cancer.

90. The method of claim 89, wherein said cancer is selected from the group consisting of colon cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, and brain cancer.

91. The method of any one of claims 88 to 90, further comprising administering a therapeutic to said subject.

92. The method of any one of claims 88 to 91, further comprising recommending additional cancer monitoring to said subject.

93. The method of any one of claims 88 to 92, further comprising using said genomic feature or said 5' end or 3' end to monitor said subject for a progression or a regression of said disease.

94. A method for preparing a library enriched for cancer-derived nucleic acids, said method comprising: subjecting a population of nucleic acid molecules from a cell-free biological sample to enrichment nucleic acid molecules having sizes less than a predetermined threshold, thereby creating a library enriched for cancer-derived nucleic acids.

95. The method of claim 94, wherein said enrichment comprises size selection of said population of nucleic acid molecules.

96. The method of claim 95, wherein size selection comprises a bead purification or a gel purification of said population of nucleic acid molecules.

97. The method of claim 96, wherein said enrichment comprises (a) ligating adapters to said population of nucleic acid molecules and (b) contacting said population of nucleic acid molecules with a cleaving agent.

98. The method of claim 97, wherein said cleaving agent comprises a nuclease.

99. The method of claim 94, wherein said enrichment comprises circularizing individual nucleic acid molecules of said population of nucleic acid molecules using an enzyme that favors small fragments.

100. The method of claim 94, wherein said enrichment comprises (a) circularizing individual nucleic acid molecules of said population of nucleic acid molecules; and (b) contacting said population of nucleic acid molecules with a cleaving agent.

101. The method of claim 99 or claim 100, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.

102. The method of claim 99 or claim 100, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.

103. The method of claim 94, further comprising amplifying said nucleic acid molecules having sizes less than said predetermined threshold.

104. The method of claim 103, wherein said amplifying is performed by a polymerase having strand displacement activity.

105. The method of claim 103, wherein said amplifying is performed by a polymerase that does not have strand displacement activity.

106. The method of claim 103, wherein said amplifying comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.

107. The method of claim 103, wherein said amplifying comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers.

108. The method of claim 94, further comprising enriching said nucleic acid molecules having sizes less than said predetermined threshold for one or more target sequences.

109. The method of claim 108, wherein said enriching is performed with one or more primers or capture probes.

110. The method of claim 108, wherein said enriching is performed with one or more antibodies or fragments thereof.

111. The method of claim 94, wherein said cell-free biological sample comprises a bodily fluid.

112. The method of claim 111, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.

113. A method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature; (c) measuring a size for said individual nucleic acid molecule; (d) identifying a plurality of nucleic acid molecules of said single-stranded DNA library without said genomic feature; (e) measuring a size for said plurality of nucleic acid molecules; (f) determining a size difference between said nucleic acid molecule having a genomic feature and said plurality of nucleic acid molecules of said single-stranded DNA library without said genomic feature; (g) associating said genomic feature with a disease based on said size difference.

114. The method of claim 113, wherein said individual nucleic acid molecule and said plurality of nucleic acid molecules are derived from a single genomic locus.

115. The method of claim 113 or claim 114, wherein (a) comprises denaturing said plurality of nucleic acid molecules.

116. The method of any one of claims 113 to 115, wherein (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.

117. The method of any one of claims 113 to 116, wherein (a) comprises (i) circularizing individual single stranded DNA molecules of said plurality of nucleic acid molecules to form a plurality of circular nucleic acid molecules; and (ii) amplifying said plurality of circular nucleic acid molecules to yield a plurality of amplified nucleic acid molecules.

118. The method of claim 117, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.

119. The method of claim 117, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.

120. The method of claim 117, wherein said amplification is performed by a polymerase having strand displacement activity.

121. The method of claim 117, wherein said amplification is performed by a polymerase that does not have strand displacement activity.

122. The method of claim 117, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.

123. The method of claim 117, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers.

124. The method of claim 113, further comprising enriching said plurality of nucleic acid molecules for one or more target sequences.

125. The method of claim 124, wherein said enriching is performed with one or more primers or capture probes.
126. The method of claim 113, wherein said enriching is performed with one or more antibodies or fragments thereof.
127. The method of claim 113, wherein (b) comprises sequencing said single-stranded DNA library.
128. The method of claim 113, wherein (c) comprises sequencing said single-stranded DNA library.
129. The method of claim 113, wherein (d) comprises sequencing said single-stranded DNA library.
130. The method of claim 113, wherein (e) comprises sequencing said single-stranded DNA library.
131. The method of any one of claims 127 to 130, wherein sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid rolling circle sequencing (ROLONY), and ion torrent sequencing.
132. The method of claim 113, wherein said genomic feature comprises an epigenetic modification.
133. The method of claim 132, wherein said epigenetic modification is selected from the group consisting of methylation, phosphorylation, ubiquitination, sumoylation, acetylation, ribosylation, citrullination, and fragmentation.
134. The method of claim 113, wherein said genomic feature comprises a copy number variation (CNV), a single nucleotide variant (SNV), an insertion, a deletion, or a combination thereof.
135. The method of claim 113, wherein said plurality of nucleic acid molecules is derived from a cell-free biological sample.
136. The method of claim 135, wherein said cell-free biological sample comprises a bodily fluid.
137. The method of claim 136, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.
138. The method of claim 113, wherein said plurality of nucleic acids comprises tumor nucleic acids.
139. The method of claim 113, wherein said disease is cancer.
140. The method of claim 139, wherein said cancer is selected from the group consisting of colon cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, and brain cancer.

141. The method of claim 113, wherein the method does not comprise a 3' end fill step or an exonuclease treatment step.

142. A method for nucleic acid analysis, comprising: (a) preparing a single-stranded deoxynucleic acid (DNA) library from a plurality of nucleic acid molecules said plurality comprising single-stranded DNA and double-stranded DNA derived from a subject; (b) identifying at least a 5' end or a 3' end for said individual nucleic acid molecule; (c) associating said 5' end or said 3' end of said individual nucleic acid molecule with a disease.

143. The method of claim 142, wherein (a) comprises denaturing said plurality of nucleic acid molecules.

144. The method of claim 142 or claim 143, wherein (a) comprises ligating an adapter to 5' ends, 3' ends or 5' and 3' ends of individual single stranded DNA molecules of said plurality of nucleic acid molecules using a DNA ligase specific for single stranded DNA.

145. The method of any one of claims 142 to 144, wherein (a) comprises (i) circularizing individual single stranded DNA molecules of said plurality of nucleic acid molecules to form a plurality of circular nucleic acid molecules; and (ii) amplifying said plurality of circular nucleic acid molecules to yield a plurality of amplified nucleic acid molecules.

146. The method of claim 145, wherein circularizing comprises ligating 5' ends of said individual nucleic acid molecules to 3' ends of said individual nucleic acid molecules.

147. The method of claim 145, wherein circularizing comprises coupling adapters to 3' ends, 5' ends, or both 5' ends and 3' ends of said individual nucleic acid molecules.

148. The method of claim 145, wherein said amplification is performed by a polymerase having strand displacement activity.

149. The method of claim 145, wherein said amplification is performed by a polymerase that does not have strand displacement activity.

150. The method of claim 145, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising random primers.

151. The method of claim 145, wherein said amplification comprises contacting said individual nucleic acid molecules to an amplification reaction mixture comprising target-specific primers.

152. The method of claim 142, further comprising enriching said plurality of nucleic acid molecules for one or more target sequences.

153. The method of claim 152, wherein said enriching is performed with one or more primers or capture probes.

154. The method of claim 152, wherein said enriching is performed with one or more antibodies or fragments thereof.

155. The method of claim 145, wherein (b) comprises sequencing said single-stranded DNA library.

156. The method of claim 155, wherein sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid rolling circle sequencing (ROLONY), and ion torrent sequencing.

157. The method of claim 142, further comprising identifying an individual nucleic acid molecule of said single-stranded DNA library as having a genomic feature.

158. The method of claim 157, wherein said genomic feature comprises an epigenetic modification.

159. The method of claim 158, wherein said epigenetic modification is selected from the group consisting of methylation, phosphorylation, ubiquitination, sumoylation, acetylation, ribosylation, citrullination, and fragmentation.

160. The method of claim 157, wherein said genomic feature comprises a copy number variation (CNV), a single nucleotide variant (SNV), an insertion, a deletion, or a combination thereof.

161. The method of claim 142, wherein said plurality of nucleic acid molecules is derived from a cell-free biological sample.

162. The method of claim 161, wherein said cell-free biological sample comprises a bodily fluid.

163. The method of claim 162, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.

164. The method of claim 142, wherein said plurality of nucleic acids comprises tumor nucleic acids.

165. The method of claim 142, wherein said subject is determined to be at risk of or to have a disease when said 5' end or said 3' end of said individual nucleic acid molecule has a predetermined sequence.

166. The method of claim 142 or claim 165, wherein said disease is cancer.

167. The method of claim 166, wherein said cancer is selected from the group consisting of colon cancer, non-small cell lung cancer, small cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, and brain cancer.

168. The method of any one of claims 165 to 167, further comprising administering a therapeutic to said subject.

169. The method of any one of claims 165 to 168, further comprising recommending additional cancer monitoring to said subject.

170. The method of any one of claims 165 to 169, further comprising using said 5' end or 3' end to monitor said subject for a progression or a regression of said disease.

171. The method of claim 142, wherein (a) does not comprise end repair.