

What is claimed is:

1. A sequencing probe comprising a target binding domain and a barcode domain;
wherein said target binding domain comprises at least four nucleotides and is capable of binding a target nucleic acid;
wherein said barcode domain comprises a synthetic backbone, said barcode domain comprising at least a first attachment region, said first attachment region comprising a nucleic acid sequence capable of being bound by a first complementary nucleic acid molecule and wherein said nucleic acid sequence of said first attachment region determines the position and identity of a first nucleotide in said target nucleic acid that is bound by a first nucleotide of said target binding domain.
2. The sequencing probe of claim 1, wherein said synthetic backbone comprises a polysaccharide, a polynucleotide, a peptide, a peptide nucleic acid, or a polypeptide.
3. The sequencing probe of claim 1 or claim 2, wherein said synthetic backbone comprises single stranded-stranded DNA.
4. The sequencing probe of any of claims 1 to 3, wherein said sequencing probe comprises a double-stranded DNA spacer between the target binding domain and the barcode domain.
5. The sequencing probe of any of claims 1 to 3 wherein said sequencing probe comprises a polymer-based spacer with similar mechanical properties as a double-stranded DNA between the target binding domain and the barcode domain.
6. The sequencing probe of any of claims 4 to 5, wherein said double-stranded DNA spacer has a length between 1 base-pair and 100 base-pair.
7. The sequencing probe of any of claims 4 to 6, wherein said double-stranded DNA spacer has length between 2 base-pair and 50 base-pair.

8. The sequencing probe of any of claims 1 to 7, wherein said first attachment region is adjacent to at least one flanking single-stranded polynucleotide.
9. The sequencing probe of any of claims 1 to 8, wherein the first complementary nucleic acid is RNA, DNA or PNA.
10. The sequencing probe of any of claims 1 to 9, wherein the first complementary nucleic molecule comprises a detectable label.
11. The sequencing probe of any of claims 1 to 9, wherein the first nucleotide in said target binding domain is a modified nucleotide or a nucleic acid analogue.
12. The sequencing probe of any of claims 1 to 11, wherein said barcode domain comprises at least a second attachment region, said second attachment region comprising a nucleic acid sequence capable of being bound by a second complementary nucleic acid molecule and wherein said nucleic acid sequence of said second attachment region determines the position and identity of a second nucleotide in said target nucleic acid that is bound by a second nucleotide of said target binding domain and
wherein the first complementary nucleic acid molecule is different from the second complementary nucleic acid molecule.
13. The sequencing probe of claim 12, wherein said second attachment region is adjacent to at least one flanking single-stranded polynucleotide.
14. The sequencing probe of claim 12, wherein the second complementary nucleic acid is RNA, DNA or PNA.
15. The sequencing probe of any of claims 14, wherein the second complementary nucleic molecule comprises a detectable label.
16. The sequencing probe of claim 12, wherein the second nucleotide in said target binding

domain is a modified nucleotide or a nucleic acid analogue.

17. The sequencing probe of claim 12, wherein the nucleic acid sequence of the first attachment region that determines the position and identity of the first nucleotide in the target nucleic acid and the nucleic acid sequence of the second attachment region that determines the position and identity of the second nucleotide in the target nucleic acid are different even when the first nucleotide in the target nucleic acid and the second nucleotide in the target nucleic acid are identical.

18. The sequencing probe of any of claims 1 to 17, wherein the number of nucleotides in a target binding domain equals the number of attachment regions in the barcode domain.

19. The sequencing probe of any of claims 1 to 17, wherein the number of nucleotides in a target binding domain is at least one more than the number of attachment regions in the barcode domain.

20. The sequencing probe of claim 19, wherein the number of nucleotides in a target binding domain is at least two more than the number of attachment regions in the barcode domain.

21. The sequencing probe of claim 20, wherein the number of nucleotides in a target binding domain is at least three more than the number of attachment regions in the barcode domain.

22. The sequencing probe of claim 21, wherein the number of nucleotides in a target binding domain is at least four more than the number of attachment regions in the barcode domain.

23. The sequencing probe of claim 22, wherein the number of nucleotides in a target binding domain is at least five more than the number of attachment regions in the barcode domain.

24. The sequencing probe of claim 23, wherein the number of nucleotides in a target binding domain is at least six more than the number of attachment regions in the barcode domain.

25. The sequencing probe of claim 24, wherein the number of nucleotides in a target binding domain is at least seven more than the number of attachment regions in the barcode domain.
26. The sequencing probe of claim 17, wherein the target binding domain comprises at least seven nucleotides and is capable of binding the target nucleic acid.
27. The sequencing probe of claim 26, wherein the number of nucleotides in a target binding domain is at least one more than the number of attachment regions in the barcode domain.
28. The sequencing probe of claim 26, wherein the target binding domain comprises at least ten nucleotides and is capable of binding the target nucleic acid.
29. The sequencing probe of claim 28, wherein the number of nucleotides in a target binding domain is at least one more than the number of attachment regions in the barcode domain.
30. The sequencing probe of claim 28, wherein the target binding domain comprises ten nucleotides and the barcode domain comprises six attachment regions.
31. The sequencing probe of claim 1, wherein the barcode domain comprises at least two first attachment regions, wherein the at least two first attachment regions comprise an identical nucleic acid sequence that is capable of being bound by a first complementary nucleic acid molecule and that determines the position and identity of a first nucleotide in the target nucleic acid that is bound by a first nucleotide of said target binding domain.
32. The sequencing probe of claim 31, wherein each position in a barcode domain has the same number of attachment regions.
33. The sequencing probe of claim 1, wherein each position in a barcode domain has the same number of attachment regions.
34. The sequencing probe of claim 33, wherein each position in a barcode domain has one

attachment region.

35. The sequencing probe of claim 33, wherein each position in a barcode domain has more than one attachment region.

36. The sequencing probe of claim 1, wherein at least one position in a barcode domain has a greater number of attachment regions as another position.

37. The sequencing probe of any of claims 1 to 36, wherein the first attachment region is linked to a modified monomer in the synthetic backbone.

38. The sequencing probe of claim 37, wherein the modified monomer is a modified nucleotide.

39. The sequencing probe of any of claims 1 to 38, wherein the first attachment region branches from the synthetic backbone.

40. The sequencing probe of claim 12, wherein the second attachment region branches from the synthetic backbone.

41. The sequencing probe of claim 17, wherein each of the at least six attachment regions branches from the synthetic backbone.

42. The sequencing probe of claim of any of claims 1 to 41, wherein the target binding domain and the synthetic backbone are operably linked.

43. The sequencing probe of claim 12, wherein said barcode domain comprises at least a third attachment region, said third attachment region comprising a nucleic acid sequence capable of being bound by a third complementary nucleic acid molecule and wherein said nucleic acid sequence of said third attachment region determines the position and identity of a third nucleotide in said target nucleic acid that is bound by a third nucleotide of said target binding

domain and

wherein the third complementary nucleic acid molecule is different from the first and the second complementary nucleic acid molecules.

44. The sequencing probe of claim 43, wherein said third attachment region is adjacent to at least one flanking single-stranded polynucleotide.

45. The sequencing probe of claim 43, wherein said barcode domain comprises at least a fourth attachment region, said fourth attachment region comprising a nucleic acid sequence capable of being bound by a fourth complementary nucleic acid molecule and wherein said nucleic acid sequence of said fourth attachment region determines the position and identity of a fourth nucleotide in said target nucleic acid that is bound by a fourth nucleotide of said target binding domain and

wherein the fourth complementary nucleic acid molecule is different from the first, the second, and the third complementary nucleic acid molecules.

46. The sequencing probe of claim 45, wherein said fourth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

47. The sequencing probe of claim 45, wherein said barcode domain comprises at least a fifth attachment region, said fifth attachment region comprising a nucleic acid sequence capable of being bound by a fifth complementary nucleic acid molecule and wherein said nucleic acid sequence of said fifth attachment region determines the position and identity of a fifth nucleotide in said target nucleic acid that is bound by a fifth nucleotide of said target binding domain and

wherein the fifth complementary nucleic acid molecule is different from the first, the second, the third, and the fourth complementary nucleic acid molecules.

48. The sequencing probe of claim 47, wherein said fifth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

49. The sequencing probe of claim 47, wherein said barcode domain comprises at least a

sixth attachment region, said sixth attachment region comprising a nucleic acid sequence capable of being bound by a sixth complementary nucleic acid molecule and wherein said nucleic acid sequence of said sixth attachment region determines the position and identity of a sixth nucleotide in said target nucleic acid that is bound by a sixth nucleotide of said target binding domain and

wherein the sixth complementary nucleic acid molecule is different from the first, the second, the third, the fourth, and the fifth complementary nucleic acid molecules.

50. The sequencing probe of claim 49, wherein said sixth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

51. The sequencing probe of any of claims 1 to 50, wherein an attachment region comprises one to fifty copies of a nucleic acid sequence.

52. The sequencing probe of claim 51, wherein the attachment region comprises two to thirty copies of the nucleic acid sequence.

53. The sequencing probe of any of claim 1 to 52 comprising multiple copies of the target binding domain operably linked to a synthetic backbone.

54. The sequencing probe of any of claims 1 to 53, wherein each complementary nucleic acid molecule comprises a detectable label.

55. The sequencing probe of any of claims 1 to 54, wherein each complementary nucleic acid molecule is directly linked to a primary nucleic acid molecule.

56. The sequencing probe of any of claims 1 to 54, wherein each complementary nucleic acid molecule is indirectly linked to a primary nucleic acid molecule via a nucleic acid spacer.

57. The sequencing probe of claim 55 or claim 56, wherein each complementary nucleic acid molecule comprises between about 8 nucleotides and about 20 nucleotides.

58. The sequencing probe of claim 57, wherein each complementary nucleic acid molecule comprises about 10 nucleotides.
59. The sequencing probe of claim 58, wherein each complementary nucleic acid molecule comprises about 12 nucleotides.
60. The sequencing probe of claim 59, wherein each complementary nucleic acid molecule comprises about 14 nucleotides.
61. The sequencing probe of any of claims 55 to 60, wherein each primary nucleic acid molecule is hybridized to at least one secondary nucleic acid molecule.
62. The sequencing probe of claim 61, wherein each primary nucleic acid molecule is hybridized to at least two secondary nucleic acid molecules.
63. The sequencing probe of claim 62, wherein each primary nucleic acid molecule is hybridized to at least three secondary nucleic acid molecules.
64. The sequencing probe of claim 63, wherein each primary nucleic acid molecule is hybridized to at least four secondary nucleic acid molecules.
65. The sequencing probe of claim 64, wherein each primary nucleic acid molecule is hybridized to at least five secondary nucleic acid molecules.
66. The sequencing probe of any of claims 61 to 65, wherein the secondary nucleic acid molecule or molecules comprise at least one detectable label.
67. The sequencing probe of any of claims 61 to 65, wherein each secondary nucleic acid molecule is hybridized to at least one tertiary nucleic acid molecule comprising at least one detectable label.

68. The sequencing probe of claim 67 wherein each secondary nucleic acid molecule is hybridized to at least two tertiary nucleic acid molecules comprising at least one detectable label.
69. The sequencing probe of claim 68, wherein each secondary nucleic acid molecule is hybridized to at least three tertiary nucleic acid molecules comprising at least one detectable label.
70. The sequencing probe of claim 69, wherein each secondary nucleic acid molecule is hybridized to at least four tertiary nucleic acid molecules comprising at least one detectable label.
71. The sequencing probe of claim 70, wherein each secondary nucleic acid molecule is hybridized to at least five tertiary nucleic acid molecules comprising at least one detectable label.
72. The sequencing probe of claim 71, wherein each secondary nucleic acid molecule is hybridized to at least six tertiary nucleic acid molecules comprising at least one detectable label.
73. The sequencing probe of claim 71, wherein each secondary nucleic acid molecule is hybridized to at least seven tertiary nucleic acid molecules comprising at least one detectable label.
74. The sequencing probe of any of claims 61 to 71, wherein at least one secondary nucleic acid molecule comprises a region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule.
75. The sequencing probe of claim 74, wherein each secondary nucleic acid molecule comprises a region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule.
76. The sequencing probe of claim 74 or claim 75, wherein the region that does not hybridize

to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises the nucleotide sequence of the complementary nucleic acid molecule that is linked to the primary nucleic acid molecule.

77. The sequencing probe of any of claims 74 to 76, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule is located at a terminus of the secondary nucleic acid molecule.

78. The sequencing probe of any of claims 74 to 77, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises between about 8 nucleotides and about 20 nucleotides.

79. The sequencing probe of claim 78, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises about 10 nucleotides.

80. The sequencing probe of claim 79, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises about 12 nucleotides

81. The sequencing probe of claim 80, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises about 14 nucleotides

82. A method for sequencing a nucleic acid comprising steps of:

(1) hybridizing at least one sequencing probe to a target nucleic acid that is immobilized to a substrate; wherein said sequencing probe comprises:

a target binding domain and a barcode domain;

wherein said target binding domain comprises at least four nucleotides and is capable of binding the immobilized target nucleic acid;

wherein said barcode domain comprises a synthetic backbone, said barcode

domain comprising at least a first attachment region, said first attachment region comprising a nucleic acid sequence capable of being bound by a first complementary nucleic acid molecule and wherein said nucleic acid sequence of said first attachment region determines the position and identity of a first nucleotide in said immobilized target nucleic acid that is bound by a first nucleotide of said target binding domain and

(2) binding to the first attachment region a first complementary nucleic acid molecule comprising a detectable label or a first complementary nucleic acid molecule of a first reporter complex comprising a detectable label;

(3) detecting the detectable label of the bound first complementary nucleic acid molecule or the detectable label of the bound first complementary nucleic acid molecule of the first reporter complex; and

(4) identifying the position and identity of the first nucleotide in the immobilized target nucleic acid.

83. The method of 82, further comprising steps of:

(5) contacting the first attachment region with a first hybridizing nucleic acid molecule lacking a detectable label thereby unbinding the first complementary nucleic acid molecule and binding to the first attachment region the first hybridizing nucleic acid molecule lacking a detectable label;

(6) binding to the second attachment region a second complementary nucleic acid molecule comprising a detectable label or a second complementary nucleic acid molecule of a second reporter complex comprising a detectable label, said second attachment region comprising a nucleic acid sequence that determines the position and identity of a second nucleotide in the immobilized target nucleic acid that is bound by a second nucleotide of the target binding domain;

(7) detecting the detectable label of the bound second complementary nucleic acid molecule or the detectable label of the bound second complementary nucleic acid molecule of the second reporter complex; and

(8) identifying the position and identity of the second nucleotide in the immobilized target nucleic acid.

84. The method of claim 82 or claim 83, wherein steps (5) and (6) occur sequentially or concurrently.

85. The method of any of claims 82 to 84, wherein steps (5) to (8) are repeated until each attachment region in the barcode domain has been sequentially bound by a complementary nucleic acid molecule comprising a detectable label or a complementary nucleic acid molecule of a reporter complex comprising a detectable label, and the detectable label of the sequentially bound complementary nucleic acid molecule or the detectable label of the sequentially bound complementary nucleic acid molecule of a reporter complex has been detected,

thereby identifying the linear order of nucleotides for a region of the immobilized target nucleic acid that was hybridized by the target binding domain of the sequencing probe.

86. The method of any of claims 82 to 85, wherein the target nucleic acid is first immobilized to a substrate by at least binding a first position of the target nucleic acid with a first capture probe that comprises a first affinity tag that selectively binds to a substrate.

87. The method of claim 86, wherein the target nucleic acid is elongated by applying a force sufficient to extend the target nucleic acid that is immobilized to a substrate at a first position.

88. The method of claim 87, wherein the force is gravity, hydrodynamic force, electromagnetic force, flow-stretching, a receding meniscus technique, or combinations thereof.

89. The method of any of claims 86 to 88, wherein the target nucleic acid is further immobilized to a substrate by binding an at least second position of the target nucleic acid with an at least second capture probe that comprises an affinity tag that selectively binds to the substrate.

90. The method of claim 89, wherein the target nucleic acid is immobilized to a substrate at about three to about ten position.

91. The method of claim 89, wherein the force can be removed once the second position of

the target nucleic acid is immobilized to the substrate.

92. The method of claim 82, wherein said target nucleic acid is immobilized to a substrate at one or more positions.

93. The method of any of claims 82 to 92, wherein said immobilized target nucleic acid is elongated.

94. The method of any of claims 82 to 93, wherein said synthetic backbone comprises a polysaccharide, a polynucleotide, a peptide, a peptide nucleic acid, or a polypeptide.

95. The method of any of claims 82 to 94, wherein said synthetic backbone comprises single stranded-stranded DNA or single-stranded RNA or single-stranded PNA.

96. The method of any of claims 82 to 95, wherein said sequencing probe comprises a double-stranded DNA spacer between the target binding domain and the barcode domain.

97. The method of any of claims 82 to 96, wherein said first attachment region is adjacent to at least one flanking single-stranded polynucleotide.

98. The method of any of claims 82 to 97, wherein the first complementary nucleic acid is RNA, DNA or PNA or other polynucleotide analogue.

99. The method of any of claims 82 to 98, wherein the first nucleotide in said target binding domain is a modified nucleotide or a nucleic acid analogue.

100. The method of claim 82, wherein said barcode domain comprises at least a second attachment region, said second attachment region comprising a nucleic acid sequence capable of being bound by a second complementary nucleic acid molecule and wherein said nucleic acid sequence of said second attachment region determines the position and identity of a second nucleotide in said immobilized target nucleic acid that is bound by a second nucleotide of said

target binding domain and

wherein the first complementary nucleic acid molecule is different from the second complementary nucleic acid molecule.

101. The method of claim 100, wherein said second attachment region is adjacent to at least one flanking single-stranded polynucleotide or polynucleotide analogue.

102. The method of claim 100, wherein the second complementary nucleic acid is RNA, DNA or PNA.

103. The method of claim 100, wherein the second nucleotide in said target binding domain is a modified nucleotide or a nucleic acid analogue.

104. The method of any of claims 83 to 103, wherein the first complementary nucleic acid molecule and the first hybridizing nucleic acid molecule lacking a detectable label comprise the same nucleic acid sequence.

105. The method of any of claims 82 to 104, wherein the first hybridizing nucleic acid molecule lacking a detectable label comprises a nucleic acid sequence complementary to a flanking single-stranded polynucleotide adjacent to said first attachment region.

106. The method of claim 105, wherein said target binding domain comprises at least three nucleotides and wherein the barcode domain comprises at least a third attachment region, said third attachment region comprising a nucleic acid sequence capable of being bound by a third complementary nucleic acid molecule and wherein said nucleic acid sequence of said third attachment region determines the position and identity of a third nucleotide in said target nucleic acid that is bound by a third nucleotide of said target binding domain.

107. The method of claim 106, wherein said third attachment region is adjacent to at least one flanking single-stranded polynucleotide or polynucleotide analogue.

108. The method of claim 106 or claim 107, wherein said target binding domain comprises at least four nucleotides and wherein the barcode domain comprises at least a fourth attachment region, said fourth attachment region comprising a nucleic acid sequence capable of being bound by a fourth complementary nucleic acid molecule and wherein said nucleic acid sequence of said fourth attachment region determines the position and identity of a fourth nucleotide in said target nucleic acid that is bound by a fourth nucleotide of said target binding domain.

109. The method of claim 108, wherein said fourth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

110. The method of claim 108 or claim 109, wherein said target binding domain comprises at least five nucleotides and wherein the barcode domain comprises at least a fifth attachment region, said fifth attachment region comprising a nucleic acid sequence capable of being bound by a fifth complementary nucleic acid molecule and wherein said nucleic acid sequence of said fifth attachment region determines the position and identity of a fifth nucleotide in said target nucleic acid that is bound by a fifth nucleotide of said target binding domain.

111. The method of claim 110, wherein said fifth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

112. The method of claim 110 or claim 111, wherein said target binding domain comprises at least six nucleotides and the barcode domain comprises at least a sixth attachment region, said sixth attachment region comprising a nucleic acid sequence capable of being bound by a sixth complementary nucleic acid molecule and wherein said nucleic acid sequence of said sixth attachment region determines the position and identity of a sixth nucleotide in said target nucleic acid that is bound by a sixth nucleotide of said target binding domain.

113. The method of claim 112, wherein said sixth attachment region is adjacent to at least one flanking single-stranded polynucleotide.

114. The method of any of claims 82 to 113, wherein the number of nucleotides in a target

binding domain equals the number of attachment regions in the barcode domain.

115. The method of any of claims 82 to 113, wherein the number of nucleotides in a target binding domain is at least one more than the number of attachment regions in the barcode domain.

116. The method of any of claims 82 to 113, wherein at least the first attachment region branches from the synthetic backbone.

117. The method of claim 116, wherein the second attachment region branches from the synthetic backbone.

118. The method of claim 117, wherein each of the at least a six attachment regions branches from the synthetic backbone.

119. The method of any of claims 82 to 118, wherein the barcode domain comprises at least two first attachment regions, wherein the at least two first attachment regions comprise an identical nucleic acid sequence that is capable of being bound by a first complementary nucleic acid molecule and that determines the position and identity of a first nucleotide in the target nucleic acid that is bound by a first nucleotide of said target binding domain.

120. The method of claim 119, wherein each position in a barcode domain has the same number of attachment regions.

121. The method of claim 82, wherein each position in a barcode domain has the same number of attachment regions.

122. The method of claim 121, wherein each position in a barcode domain has one attachment region.

123. The method of claim 121, wherein each position in a barcode domain has more than one

attachment region.

124. The method of claim 82, wherein at least one position in a barcode domain has a greater number of attachment regions as another position.

125. The method of any of claims 82 to 124, wherein an attachment region comprises one to fifty copies of a nucleic acid sequence.

126. The method of claim 125, wherein the attachment region comprises two to thirty copies of the nucleic acid sequence.

127. The method of any of claim 82 to 126, wherein the sequencing probe comprises multiple copies of the target binding domain operably linked to a synthetic backbone.

128. The method of any of claims 82 to 127, wherein each reporter complex comprising a detectable label comprises a complementary nucleic acid molecule directly linked to a primary nucleic acid molecule.

129. The method of any of claims 80 to 128, wherein each reporter complex comprising a detectable label comprises a complementary nucleic acid molecule indirectly linked to a primary nucleic acid molecule via a nucleic acid spacer.

130. The method of any of claims 82 to 129, wherein each reporter complex comprising a detectable label comprises a complementary nucleic acid molecule indirectly linked to a primary nucleic acid molecule via a polymeric spacer with a similar mechanical properties as nucleic acid spacer.

131. The method of any one of claims 82 to 130, wherein each complementary nucleic acid molecule comprises between about 8 nucleotides and about 20 nucleotides.

132. The method of any one of claims 82 to 131, wherein each complementary nucleic acid

molecule comprises about 10 nucleotides.

133. The method of any one of claims 82 to 132, wherein each complementary nucleic acid molecule comprises about 12 nucleotides.

134. The method of any one of claims 82 to 133, wherein each complementary nucleic acid molecule comprises about 14 nucleotides.

135. The method of any of claims 82 to 134, wherein each primary nucleic acid molecule is hybridized to at least one secondary nucleic acid molecule.

136. The method of claim 135, wherein each primary nucleic acid molecule is hybridized to at least two secondary nucleic acid molecules.

137. The method of claim 136, wherein each primary nucleic acid molecule is hybridized to at least three secondary nucleic acid molecules.

138. The method of claim 137, wherein each primary nucleic acid molecule is hybridized to at least four secondary nucleic acid molecules.

139. The method of claim 138, wherein each primary nucleic acid molecule is hybridized to at least five secondary nucleic acid molecules.

140. The sequencing probe of any of claims 135 to 139, wherein the secondary nucleic acid molecule or molecules comprise at least one detectable label.

141. The method of any of claims 135 to 139, wherein each secondary nucleic acid molecule is hybridized to at least one tertiary nucleic acid molecule comprising at least one detectable label.

142. The method of claim 141, wherein each secondary nucleic acid molecule is hybridized to

at least two tertiary nucleic acid molecules comprising at least one detectable label.

143. The method of claim 142, wherein each secondary nucleic acid molecule is hybridized to at least three tertiary nucleic acid molecules comprising at least one detectable label.

144. The method of claim 143, wherein each secondary nucleic acid molecule is hybridized to at least four tertiary nucleic acid molecules comprising at least one detectable label.

145. The method of claim 144, wherein each secondary nucleic acid molecule is hybridized to at least five tertiary nucleic acid molecules comprising at least one detectable label.

146. The method of claim 145, wherein each secondary nucleic acid molecule is hybridized to at least six tertiary nucleic acid molecules comprising at least one detectable label.

147. The method of claim 146, wherein each secondary nucleic acid molecule is hybridized to at least seven tertiary nucleic acid molecules comprising at least one detectable label.

148. The method of any of claims 135 to 147, wherein at least one secondary nucleic acid molecule comprises a region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule.

149. The method of claim 148, wherein each secondary nucleic acid molecule comprises a region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule.

150. The method of claim 148 or claim 149, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises the nucleotide sequence of the complementary nucleic acid molecule that is directly linked to the primary nucleic acid molecule.

151. The method of any of claims 148 to 150, wherein the region that does not hybridize to a

primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule is located at a terminus of the secondary nucleic acid molecule.

152. The method of any of claims 148 to 151, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises between about 8 nucleotides and about 20 nucleotides.

153. The method of claim 152, wherein the region that does not hybridize to a primary nucleic acid molecule and does not hybridize to a tertiary nucleic acid molecule comprises about 12 nucleotides.

154. A method for sequencing a nucleic acid comprising steps of:

(1) hybridizing a first population of sequencing probes to a target nucleic acid that is immobilized to a substrate, wherein each sequencing probe in the first population comprises:

a target binding domain and a barcode domain;

wherein said target binding domain comprises at least four nucleotides and is capable of binding a target nucleic acid;

wherein said barcode domain comprises a synthetic backbone, said barcode domain comprising a first attachment region, said first attachment region comprising a nucleic acid sequence capable of being bound by a first complementary nucleic acid molecule and wherein said nucleic acid sequence of said first attachment region determines the position and identity of a first nucleotide in said target nucleic acid that is bound by a first nucleotide of said target binding domain and

said barcode domain further comprising at least a second attachment region, said second attachment region comprising a nucleic acid sequence capable of being bound by a second complementary nucleic acid molecule and wherein said nucleic acid sequence of said second attachment region determines the position and identity of a second nucleotide in said target nucleic acid that is bound by a second nucleotide of said target binding domain and

wherein the first complementary nucleic acid molecule is different from the second complementary nucleic acid molecule;

wherein each sequencing probe in the first population de-hybridizes from the immobilized target nucleic acid under about the same conditions;

(2) binding to a first attachment region in each sequencing probe in the first population a plurality of first complementary nucleic acid molecules each comprising a detectable label or a plurality of first complementary nucleic acid molecules of a plurality of first reporter complexes each complex comprising a detectable label;

(3) detecting the detectable label of each bound first complementary nucleic acid molecule or of each first complementary nucleic acid molecule of each first reporter complex,

(4) identifying the position and identity of a plurality of first nucleotides in the immobilized target nucleic acid hybridized by sequencing probes in the first population;

(5) contacting each first attachment region of each sequencing probe of the first population with a plurality first hybridizing nucleic acid molecules each lacking a detectable label thereby unbinding the first complementary nucleic acid molecules comprising a detectable label or the first complementary nucleic acid molecules of each first reporter complex and binding to each first attachment region a first hybridizing nucleic acid molecule lacking a detectable label;

(6) binding to a second attachment region in each sequencing probe in the first population a plurality of second complementary nucleic acid molecules each comprising a detectable label or a plurality of second complementary nucleic acid molecules of a plurality of second reporter complexes each complex comprising a detectable label;

(7) detecting the detectable label of each bound second complementary nucleic acid molecule or of each second complementary nucleic acid molecule of each second reporter complex,

(8) identifying the position and identity of a plurality of second nucleotides in the immobilized target nucleic acid hybridized by sequencing probes in the first population; and

(9) repeating steps (5) to (8) until each nucleotide in the immobilized target nucleic acid corresponding to the target binding domain of each sequencing probe in the first population has been identified.

155. The method of claim 154, wherein conditions that de-hybridize each sequencing probe in the first population from the immobilized target nucleic acid comprise one or more of addition of

a chaotropic agent, a reducing agent, a change in pH, a change in salt concentration, a change of temperature, or a hydrodynamic force.

156. The method of claim 155, wherein the chaotropic agent is selected from the group consisting of butanol, ethanol, guanidinium chloride, lithium acetate, lithium perchlorate, magnesium chloride, phenol, propanol, sodium dodecyl sulfate, lithium dodecyl sulfate, formamide, thiourea, and urea.

157. The method of claim 155, wherein the reducing agent is selected from the group consisting of TCEP (tris(2-carboxyethyl)phosphine), DTT (dithiothreitol) and β -mercaptoethanol.

158. The method of claim 155, wherein the change in temperature is an increase in temperature.

159. The method of 154, wherein steps (5) and (6) occur sequentially or concurrently.

160. The method of claim 154 or claim 159, further comprising steps of:

(10) de-hybridizing each sequencing probe of the first population of sequencing probes from the nucleic acid;

(11) removing each de-hybridized sequencing probe of the first population;

(12) hybridizing at least a second population of sequencing probes to the immobilized target nucleic acid, wherein each sequencing probe in the second population comprises:

a target binding domain and a barcode domain;

wherein said target binding domain comprises at least four nucleotides and is capable of binding a target nucleic acid;

wherein said barcode domain comprises a synthetic backbone, said barcode domain comprising a first attachment region, said first attachment region comprising a nucleic acid sequence capable of being bound by a first RNA molecule and wherein said nucleic acid sequence of said first attachment region determines the position and identity of a first nucleotide in said target nucleic acid that is bound by a first nucleotide of said

target binding domain and

said barcode domain comprising at least a second attachment region, said second attachment region comprising a nucleic acid sequence capable of being bound by a second complementary nucleic acid molecule and wherein said nucleic acid sequence of said second attachment region determines the position and identity of a second nucleotide in said target nucleic acid that is bound by a second nucleotide of said target binding domain and wherein the first complementary nucleic acid molecule is different from the second complementary nucleic acid molecule;

wherein each sequencing probe in the second population de-hybridizes from the immobilized target nucleic acid under about the same conditions; and de-hybridizes from the immobilized target nucleic acid under different conditions than the sequencing probes in the first population;

(13) binding to a first attachment region in each sequencing probe in the second population a plurality of first complementary nucleic acid molecules each comprising a detectable label or a plurality of first complementary nucleic acid molecules of a plurality of first reporter complexes each complex comprising a detectable label;

(14) detecting the detectable label of each bound first complementary nucleic acid molecule or of each first complementary nucleic acid molecule of each first reporter complex,

(15) identifying the position and identity of a plurality of first nucleotides in the immobilized target nucleic acid hybridized by sequencing probes in the second population;

(16) contacting each first attachment region of each sequencing probe of the second population with a plurality first hybridizing nucleic acid molecules lacking a detectable label thereby unbinding the first complementary nucleic acid molecules comprising a detectable label or the first complementary nucleic acid molecules of each first reporter complex and binding to each first attachment region a first hybridizing nucleic acid molecule lacking a detectable label;

(17) binding to a second attachment region in each sequencing probe in the second population a plurality of second complementary nucleic acid molecules each comprising a detectable label or a plurality of second complementary nucleic acid molecules of a plurality of second reporter complexes each complex comprising a detectable label;

(18) detecting the detectable label of each bound second complementary nucleic acid molecule or of each second complementary nucleic acid molecule of each second reporter

complex;

(19) identifying the position and identity of a plurality of second nucleotides in the immobilized target nucleic acid hybridized by sequencing probes in the second population; and

(20) repeating steps (16) to (20) until each nucleotide in the immobilized target nucleic acid and corresponding to the target binding domain of each sequencing probe in the second population has been identified.

161. The method of 160, wherein steps (16) and (17) occur sequentially or concurrently.

162. The method of claim 160 or claim 161, wherein conditions that de-hybridize each sequencing probe in the second population from the immobilized target nucleic acid comprise one or more of addition of a chaotropic agent, a reducing agent, a change in pH, a change in salt concentration, a change of temperature, or a hydrodynamic force.

163. The method of claim 162, wherein the chaotropic agent is selected from the group consisting of butanol, ethanol, guanidinium chloride, lithium acetate, lithium perchlorate, magnesium chloride, phenol, propanol, sodium dodecyl sulfate, thiourea, and urea.

164. The method of claim 162, wherein the reducing agent is selected from the group consisting of TCEP (tris(2-carboxyethyl)phosphine), DTT (dithiothreitol) and β -mercaptoethanol.

165. The method of claim 162, wherein the change in temperature is an increase in temperature.

166. The method of any of claims 160 to 165, wherein each sequencing probe in the second population de-hybridizes from the immobilized target nucleic acid at a higher temperature than the average temperature that the sequencing probes in the first population de-hybridize from the target nucleic acid.

167. The method of claim 166, wherein steps (10) to (20) are repeated with one or more additional populations of probes.

168. The method of claim 167, further comprising steps of assembling each identified linear order of nucleotides for each region of the immobilized target nucleic acid, thereby identifying a sequence for the immobilized target nucleic acid.

169. The method of claim 168, wherein steps of assembling comprise a non-transitory computer-readable storage medium with an executable program stored thereon, wherein the program instructs a microprocessor to arrange each identified linear order of nucleotides for each region of the target nucleic acid, thereby obtaining the sequence of the nucleic acid.

170. The method of any of claims 154 to 169, wherein a population of sequencing probes comprises additional sequencing probes directed to a specific region of interest in the target nucleic acid.

171. The method of claim 170, wherein the region of interest comprises a mutation or a SNP allele.

172. The method of claim 170, wherein the region of interest does not comprises of a known mutation or a SNP allele.

173. The method of any of claims 154 to 172, wherein a population of sequencing probes comprises fewer sequencing probes directed to a specific region not of interest in the target nucleic acid.

174. The method of any of claims 154 to 173, wherein the lengths of target binding domains in a population of sequencing probes is reduced to increase coverage of probes in a specific region of a target nucleic acid.

175. The method of any of claims 154 to 174, wherein the lengths of target binding domains in a population of sequencing probes is increased to decrease coverage of probes in a specific region of a target nucleic acid.
176. The method of any of claims 154 to 175, wherein a population of sequencing probes is compartmentalized into discrete smaller pools of sequencing probes.
177. The method of claim 176, wherein the compartmentalization is based on predicted melting temperature of the target binding domain in the sequencing probes.
178. The method of claim 176, wherein the compartmentalization is based on sequence motif of the target binding domain in the sequencing probes.
179. The method of claim 176, wherein the compartmentalization is based on empirically-derived rules.
180. The method of any of claims 176 to 179, wherein the different pools of sequencing probes can be reacted with the target nucleic acid using different reaction conditions.
181. The method of claim 180, wherein the reaction condition is based on temperature.
182. The method of claim 180, wherein the reaction condition is based on salt concentration.
183. The method of claim 180, wherein the reaction condition is based on buffer content.
184. The method of any of claims 176 to 183, wherein a compartmentalization is performed to cover target nucleic acid with uniform coverage.
185. The method of any of claims 176 to 183, wherein a compartmentalization is performed to cover target nucleic acid with known coverage profile.

186. The method of any of claims 154 to 185, wherein the target nucleic acid is between about 4 and 1,000,000 nucleotides in length up to the length of an intact chromosome or a fragment thereof.
187. The method of any of claims 154 to 186, wherein an attachment region comprises one to fifty copies of a nucleic acid sequence.
188. The method of claim 187, wherein the attachment region comprises two to thirty copies of the nucleic acid sequence.
189. The method of any of claim 154 to 188, wherein the sequencing probe comprises multiple copies of the target binding domain operably linked to a synthetic backbone.
190. The method of any of claim 82 to 189, wherein the rate at which a complementary nucleic acid molecule is unbound from a sequencing probe is accelerated *via* contact of the sequencing probe with hybridizing nucleic acid molecule lacking a detectable label.
191. The method of any of claims 154 to 190, wherein when a first aliquot of a population of probes is de-hybridized from the target nucleic acid and a second aliquot of the population of probes is hybridized to the target nucleic acid, the second aliquot of the population of probes has not previously been hybridized to the target nucleic acid.
192. An apparatus for performing the method of any of claims 82 to 191.
193. The apparatus of claim 192 comprising a consumable sequencing card as shown in Figure 24.
194. A kit comprising a substrate, a plurality of sequencing probes of any of claims 1 to 81, at least one capture probe, at least one complementary nucleic acid molecule comprising a detectable label, at least one complementary nucleic acid molecule which lacks a detectable label, and instructions for use.

195. The kit of claim 193, further comprising a consumable sequencing card as shown in Figure 24.