

WHAT IS CLAIMED IS:

1. A method of sequencing target nucleic acids on an array, comprising:
 - (a) obtaining a first and a second population of beads, wherein:
 - the first population of beads comprise oligonucleotides comprising first capture probes, first barcodes, and barcode primer binding sites adjacent to the first barcodes, and
 - the second population of beads comprise oligonucleotides comprising second capture probes, second barcodes, and barcode primer binding sites adjacent to the second barcodes, wherein the barcode primer binding sites comprise the same sequence;
 - (b) obtaining a first and a second plurality of polynucleotides, wherein:
 - the first plurality of polynucleotides comprises first target nucleic acids, wherein the first plurality of polynucleotides is in solution, and
 - the second plurality of polynucleotides comprises second target nucleic acids, wherein the second plurality of polynucleotides is in solution;
 - (c) hybridizing the first target nucleic acids to the first capture probes to obtain hybridized first beads, and hybridizing the second target nucleic acids to the second capture probes to obtain hybridized second beads;
 - (d) randomly distributing the hybridized first beads and hybridized second bead on an array;
 - (e) decoding the locations of the first and second beads on the array by sequencing on the array the first and second barcodes; and
 - (f) extending the first and second capture probes to obtain nucleic acid sequence data for the first and second target nucleic acids.
2. The method of claim 1, wherein:
 - the first plurality of polynucleotides comprises first indexes, and index primer binding sites adjacent to the first indexes; and
 - the second plurality of polynucleotides comprises second target nucleic acids, second indexes, and index primer binding sites adjacent to the second indexes.
3. The method of claim 2, wherein the first or second plurality of polynucleotides is obtained by fragmenting a nucleic acid sample with a plurality of transposomes.

4. The method of claims 3, wherein the plurality of transposomes comprise the first or second indexes.
5. The method of claims 4, further comprising adding adaptors to the tagmented nucleic acid sample, wherein the adaptors comprise the first or second indexes.
6. The method of claims 5, further comprising amplifying the tagmented nucleic acid sample with primers comprising the first or second indexes.
7. The method of any one of claims 2-6, wherein extending the first and second capture probes incorporates sequences complementary to the first and second indexes and to the first and second index primer binding sites into the extended capture probes.
8. The method of claim 1, wherein:
the first population of beads comprise first indexes, and index primer binding sites adjacent to the first indexes, and
the second population of beads comprise second indexes, and index primer binding sites adjacent to the second indexes.
9. The method of claim 8, wherein the oligonucleotides of the first population of beads comprise the first indexes; and the oligonucleotides of the second population of beads comprise the second indexes.
10. The method of any one of claims 2-9, wherein the first indexes are indicative of a source of the first target nucleic acids, and the second indexes are indicative of a source of the second target nucleic acids.
11. The method of any one of claims 2-10, wherein the first indexes are the same as one another, and the second indexes are the same as one another.
12. The method of any one of claims 2-11, further comprising sequencing the first and second indexes.
13. The method of claim 12, wherein sequencing the first and second indexes comprises extending primers hybridized to the index primer binding sites.
14. The method of any one of claims 2-13, wherein the index binding primer sites are the same.
15. The method of any one of claims 1-14, wherein the first and second target nucleic acids are obtained from different nucleic acid samples.

16. The method of any one of claims 1-15, wherein the first and second target nucleic acids are obtained from genomic DNA.
17. The method of any one of claims 1-16, wherein the first and second barcodes are indicative of a nucleic acid sequence of the first or second capture probes.
18. The method of any one of claims 1-17, wherein the first barcodes are different from one another, and the second barcodes are different from one another.
19. The method of any one of claims 1-18, wherein sequencing the first and second barcodes comprises extending primers hybridized to the barcode primer binding sites.
20. The method of any one of claims 1-19, wherein extending the first and second capture probes comprises polymerase extension.
21. The method of claim 20, wherein extending the first and second capture probes comprises addition of a single nucleotide to a capture probe.
22. The method of claim 20 or 21, further comprising ligating locus specific oligonucleotides to the extended capture probes.
23. The method of any one of claims 20-22, wherein extending the first and second capture probes comprises ligating locus specific oligonucleotides to the capture probes.
24. The method of any one of claims 1-23, wherein step (c) is performed in solution.
25. The method of any one of claims 1-24, wherein the array is located on the surface of a flowcell.
26. The method of any one of claims 1-25, wherein the first and second beads are adapted to be attached to the array.
27. The method of claim 26, wherein the first and second beads comprise biotin, streptavidin, or a derivative thereof; and the array comprises biotin, streptavidin, or a derivative thereof.
28. The method of claim 26, wherein the first and second beads are magnetic.
29. A method of sequencing target nucleic acids on an array, comprising:
 - (a) obtaining a first and a second population of beads, wherein:
 - the first population of beads comprise oligonucleotides comprising first capture probes, first barcodes, and barcode primer binding sites adjacent to the first barcodes, and

the second population of beads comprise oligonucleotides comprising second capture probes, second barcodes, and barcode primer binding sites adjacent to the second barcodes, wherein the barcode primer binding sites comprise the same sequence;

(b) obtaining a first and a second plurality of polynucleotides, wherein:

the first plurality of polynucleotides comprises first target nucleic acids, first indexes, and index primer binding sites adjacent to the first indexes, wherein the first plurality of polynucleotides is in solution, and

the second plurality of polynucleotides comprises second target nucleic acids, second indexes, and second primer binding sites adjacent to the second indexes wherein the second plurality of polynucleotides is in solution;

(c) hybridizing the first target nucleic acids to the first capture probes to obtain hybridized first beads, and hybridizing the second target nucleic acids to the second capture probes to obtain hybridized second beads;

(d) randomly distributing the hybridized first beads and hybridized second bead on an array;

(e) decoding the locations of the first and second beads on the array by sequencing on the array the first and second barcodes;

(f) extending the first and second capture probes to obtain nucleic acid sequence data for the first and second target nucleic acids; and

(g) determining a source for the nucleic acid sequence data of the first and second target nucleic acids by sequencing the first and second indexes.

30. The method of claim 29, wherein the first or second plurality of polynucleotides is obtained by fragmenting a nucleic acid sample with a plurality of transposomes.

31. The method of claims 30, wherein the plurality of transposomes comprise the first or second indexes.

32. The method of claims 31, further comprising adding adaptors to the fragmented nucleic acid sample, wherein the adaptors comprise the first or second indexes.

33. The method of claims 32, further comprising amplifying the fragmented nucleic acid sample with primers comprising the first or second indexes.

34. The method of claim 33, wherein extending the first and second capture probes incorporates sequences complementary to the first and second indexes and to the first and second index primer binding sites into the extended capture probes.

35. A method of sequencing target nucleic acids on an array, comprising:

(a) obtaining a first and a second population of beads, wherein:

the first population of beads comprise oligonucleotides comprising first capture probes, first barcodes and barcode primer binding sites adjacent to the first barcodes, and first indexes and index primer binding sites adjacent to the first indexes, and

the second population of beads comprise oligonucleotides comprising second capture probes, second barcodes and barcode primer binding sites adjacent to the second barcodes, and second indexes and index primer binding sites adjacent to the second indexes, wherein the barcode primer binding sites comprise the same sequence;

(b) obtaining a first and a second plurality of polynucleotides, wherein:

the first plurality of polynucleotides comprises first target nucleic acids, wherein the first plurality of polynucleotides is in solution, and

the second plurality of polynucleotides comprises second target nucleic acids, wherein the second plurality of polynucleotides is in solution;

(c) hybridizing the first target nucleic acids to the first capture probes to obtain hybridized first beads, and hybridizing the second target nucleic acids to the second capture probes to obtain hybridized second beads;

(d) randomly distributing the hybridized first beads and hybridized second bead on an array;

(e) decoding the locations of the first and second beads on the array by sequencing on the array the first and second barcodes; and

(f) extending the first and second capture probes to obtain nucleic acid sequence data for the first and second target nucleic acids.

36. The method of any one of claims 29-35, wherein the first indexes are indicative of a source of the first target nucleic acids, and the second indexes are indicative of a source of the second target nucleic acids.

37. The method of any one of claims 29-36, wherein the first indexes are the same as one another, and the second indexes are the same as one another.
38. The method of any one of claims 29-37, further comprising sequencing the first and second indexes.
39. The method of claim 38, wherein sequencing the first and second indexes comprises extending primers hybridized to the index primer binding sites.
40. The method of any one of claims 29-39, wherein the index binding primer sites are the same.
41. The method of any one of claims 29-40, wherein the first and second target nucleic acids are obtained from different nucleic acid samples.
42. The method of any one of claims 29-41, wherein the first and second target nucleic acids are obtained from genomic DNA.
43. The method of any one of claims 29-44, wherein the first and second barcodes are indicative of a nucleic acid sequence of the first or second capture probes.
44. The method of any one of claims 29-43, wherein the first barcodes are different from one another, and the second barcodes are different from one another.
45. The method of any one of claims 29-44, wherein sequencing the first and second barcodes comprises extending primers hybridized to the barcode primer binding sites.
46. The method of any one of claims 29-45, wherein extending the first and second capture probes comprises polymerase extension.
47. The method of claim 46, wherein extending the first and second capture probes comprises addition of a single nucleotide to a capture probe.
48. The method of claim 46 or 47, further comprising ligating locus specific oligonucleotides to the extended capture probes.
49. The method of any one of claims 46-48, wherein extending the first and second capture probes comprises ligating locus specific oligonucleotides to the capture probes.
50. The method of any one of claims 29-49, wherein step (c) is performed in solution.
51. The method of any one of claims 29-50, wherein a flowcell comprises the array.
52. The method of any one of claims 29-51, wherein the array comprises a plurality of wells.

53. The method of any one of claims 29-52, wherein the first and second beads are adapted to be attached to the array.

54. The method of claim 53, wherein the first and second beads comprise biotin, streptavidin, or a derivative thereof; and the array comprises biotin, streptavidin, or a derivative thereof.

55. The method of claim 53, wherein the first and second beads are magnetic.

56. A kit comprising: a plurality of populations of beads comprising oligonucleotides attached to the beads, the oligonucleotides comprising indexes, index primer binding sites adjacent to the indexes, capture probes, barcodes, and barcode primer binding sites adjacent to the barcodes, wherein the indexes are different between the populations of beads.

57. The kit of claim 56, wherein the index primer binding sites are the same in the plurality of populations.

58. The kit of claim 56 or 57, wherein the barcodes are indicative of a nucleic acid sequence of the capture probes.

59. The kit of any one of claims 56-58, wherein the barcodes are different in a population of beads.

60. The kit of any one of claims 56-59, wherein the barcode primer binding sites are the same in the plurality of populations.

61. The kit of any one of claims 56-60, further comprising a reagent selected from:
a locus specific oligonucleotide;
a transposome for tagmenting a nucleic acid sample;
a transposome comprising an index and an index primer binding site;
an adaptor comprising an index and an index primer binding site;
a primer capable of hybridizing to an index primer binding site or complement thereof; and
a primer capable of hybridizing to a barcode primer binding site or complement thereof.

62. The kit of any one of claims 56-61, further comprising a flowcell.

63. A method of preparing an indexed population of beads, comprising:

(a) obtaining a population of beads, wherein each bead comprises an adaptor, a capture probe, and a first polynucleotide comprising a barcode, and a barcode primer binding site;

(b) obtaining a plurality of index polynucleotides, wherein each index polynucleotide comprises an index, and an index primer binding site; and

(c) attaching the plurality of index polynucleotides to the population of beads via the adaptors, thereby obtaining an indexed population of beads.

64. The method of claim 63, wherein (c) comprises extending the adaptors by polymerase extension.

65. The method of claim 64, wherein each index polynucleotide comprises an adaptor binding site, and the attaching comprises hybridizing the adaptor binding sites to the adaptors.

66. The method of claim 63, wherein (c) comprises ligating the index polynucleotides to the adaptors.

67. The method of claim 66, wherein the attaching comprises hybridizing a splint polynucleotide to the adaptor and to the index polynucleotide.

68. The method of claim 63, wherein (c) comprises attaching the plurality of index polynucleotides to the adaptors of the population of beads via a chemically reactive moiety.

69. The method of claim 68, wherein the attaching comprises a click chemistry reaction.

70. The method of any one of claims 63-69, wherein the first polynucleotides of the population of beads comprise capture probes different from one another.

71. The method of any one of claims 63-70, wherein the index of each index polynucleotide is the same.

72. The method of any one of claims 63-71, wherein the first polynucleotide comprises the capture probe.

73. The method of any one of claims 63-72, further comprising contacting the indexed population of beads with a plurality of nucleic acids comprising a target nucleic acid.

74. The method of claim 73, further comprising mixing the indexed population of beads contacted with a plurality of nucleic acids comprising a target nucleic with an additional indexed population of beads, wherein the additional indexed population of beads comprises an

index polynucleotide comprising an index different from the index of the indexed population of beads contacted with a plurality of nucleic acids.

75. The method of any one of claims 63-71, wherein the capture probe comprises a protein.

76. The method of any one of claims 63-75, performed on a flow cell.

~~77. A method for detecting a target ligand, comprising:~~

~~(a) obtaining a population of beads, wherein each bead comprises a capture probe, a first polynucleotide comprising a barcode, and a barcode primer binding site;~~

~~(b) obtaining an index polynucleotide comprising an index, an index primer binding site, and an adaptor capable of binding to the barcode primer binding site;~~

~~(c) specifically binding a target ligand to the capture probe;~~

~~(d) hybridizing the index polynucleotide to the first polynucleotide via the adaptor;~~

~~(e) detecting the target ligand on an array; and~~

~~(f) determining the index and the barcode of the first polynucleotide.~~

~~78. The method of claim 77, wherein (e) comprises distributing the population of beads on an array.~~

~~79. The method of claim 77 or 78, wherein (f) comprises hybridizing an index primer to the index primer binding site, and determining the sequence of the index.~~

~~80. The method of claim 79, further comprising dehybridizing the index polynucleotide from the first polynucleotide; hybridizing a barcode primer to the barcode primer binding site; and extending the barcode primer to determine the sequence of the barcode.~~

~~81. The method of claim 77 or 78, wherein the index polynucleotide further comprises a cleavable linker located between the adaptor and the index, and (f) comprises:~~

~~(i) cleaving the cleavable linker; and~~

~~(ii) extending the adaptor to determine the sequence of the barcode.~~

~~82. The method of any one of claims 77-81, wherein the capture probe comprises a protein.~~

~~83. The method of any one of claims 77-81, wherein the target ligand comprises a target nucleic acid.~~

84. — ~~The method of claim 83, wherein the first polynucleotide comprises the capture probe.~~

85. — ~~The method of claim 83 or 84, wherein (c) comprises extending the first polynucleotide hybridized to the target nucleic acid.~~

86. — ~~The method of claim 85, wherein the extension comprises adding a detectable dideoxynucleotide.~~

87. — ~~The method of any one of claims 77-86, performed on a flow cell.~~

88. — ~~A method for detecting a target ligand, comprising:~~

~~(a) obtaining a population of beads, wherein each bead comprises a capture probe, a first polynucleotide comprising a barcode, and a barcode primer binding site, and a second polynucleotide;~~

~~(b) obtaining an index polynucleotide comprising an index, an index primer binding site, and an adaptor;~~

~~(c) specifically binding a target ligand to the capture probe;~~

~~(d) attaching the index polynucleotide to the second polynucleotide via the adaptor;~~

~~(e) detecting the target ligand on an array; and~~

~~(f) determining the index and the barcode of the first polynucleotide.~~

89. — ~~The method of claim 88, wherein the second polynucleotide comprises a barcode, and a barcode primer binding site.~~

90. — ~~The method of 88 or 89, wherein (d) comprises adding a reactive moiety to the second polynucleotide, wherein the adaptor is capable of attaching to the reactive moiety.~~

91. — ~~The method of claim 90, wherein the adding a reactive moiety comprises a click chemistry reaction.~~

92. — ~~The method of any one of claims 88-91, wherein (e) comprises distributing the population of beads on an array.~~

93. — ~~The method of any one of claims 88-92, wherein (f) comprises hybridizing an index primer to the index primer binding site, and determining the sequence of the index.~~

94. — ~~The method of any one of claims 88-93, wherein (f) comprises hybridizing a barcode primer to the barcode primer binding site, and determining the sequence of the barcode.~~

95. — ~~The method of any one of claims 88-94, wherein the capture probe comprises a protein.~~

96. — ~~The method of any one of claims 88-94, wherein the target ligand comprises a target nucleic acid.~~

97. — ~~The method of claim 96, wherein the first polynucleotide comprises the capture probe.~~

98. — ~~The method of claim 96 or 97, wherein (c) comprises extending the first polynucleotide hybridized to the target nucleic acid.~~

99. — ~~The method of claim 98, wherein the extension comprises adding a detectable dideoxynucleotide.~~

100. — ~~The method of any one of claims 88-99, performed on a flow cell.~~

101. — ~~A method of detecting a target ligand on an array, comprising:~~

~~(a) obtaining a first and a second population of beads, wherein each bead comprises:~~

~~a capture probe, wherein the capture probe is capable of specifically binding to a target ligand,~~

~~a nucleic acid encoding a barcode and a barcode primer binding site, wherein the barcode is indicative of the capture probe, and~~

~~a nucleic acid encoding an index and an index primer binding site, wherein the index is indicative of the source of the bead from the first population or the second population, and~~

~~(b) contacting the first population of beads with a first sample comprising a first target ligand, wherein the first target ligand specifically binds to a capture probe of the first population of beads, and thereby obtaining a target-bound first population of beads;~~

~~(c) contacting the second population of beads with a second sample comprising a second target ligand, wherein the second target ligand specifically binds to a capture probe of the second population of beads, and thereby obtaining a target-bound second population of beads;~~

~~(d) randomly distributing the target-bound first population of beads and the target-bound second population of beads on an array;~~

~~(e) detecting the location of the beads comprising the first target ligand and the second target ligand on the array; and~~

~~(f) determining the sequence of the index and of the barcode of the beads comprising the first target ligand and the second target ligand on the array.~~

~~102.—The method of claim 101, wherein the capture probe comprises a polynucleotide.~~

~~103.—The method of claim 101 or 102, wherein the target ligand comprises a nucleic acid.~~

~~104.—The method of any one of claims 101–103, wherein detecting the location of the beads comprising the first target ligand and the second target ligand on the array comprises extending the capture probe by polymerase extension or by ligation.~~

~~105.—The method of claim 101, wherein the capture probe comprises a protein.~~

~~106.—The method of any one of claims 101–105, wherein step (e) is performed after step (f).~~

~~107.—The method of any one of claims 101–106, wherein the barcodes of the first population of beads comprise barcodes different from one another, and the barcodes of the second population of beads comprise barcodes different from one another.~~

~~108.—The method of any one of claims 101–107, wherein the indexes of the first population of beads are the same as one another, and the indexes of the second population of beads are the same as one another.~~

~~109.—The method of any one of claims 101–108, wherein the array is located on the surface of a flowcell.~~

~~110.—The method of any one of claims 101–109, wherein the first and a second population of beads are adapted to be attached to the array.~~

~~111.—The method of claim 110, wherein the first and a second population of beads comprise biotin, streptavidin, or a derivative thereof; and the array comprises biotin, streptavidin, or a derivative thereof.~~

~~112.—The method of claim 110, wherein the first and a second population of beads are magnetic.~~

~~113.—A method for decoding the locations of polynucleotides in an array comprising:~~

~~(a) obtaining a substrate having an array of polynucleotides distributed on a surface of the substrate, wherein each polynucleotide comprises a primer binding site 3' of a barcode, wherein each polynucleotide is linked to a capture probe;~~

~~(b) hybridizing a plurality of primers to the primer binding sites; and~~

~~(c) determining the sequences of the barcodes by extending the hybridized primers, wherein the sequence of each barcode is indicative of the location of a polynucleotide in the array.~~

~~114.—The method of claim 113, wherein each polynucleotide is linked to a capture probe via a bead.~~

~~115.—The method of claim 113, wherein each polynucleotide comprises the capture probe.~~

~~116.—A method of sequencing a target nucleic acid comprising:~~

~~(a) decoding the locations of polynucleotides in an array according to the method of any one of claims 113-115;~~

~~(b) hybridizing the target nucleic acid to the capture probes;~~

~~(c) extending the capture probes hybridized to the target nucleic acid; and~~

~~(d) detecting the location of the extended capture probes.~~