

AMENDED CLAIMS

1. A method for decoding the locations of first polynucleotides in an array comprising:

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

(i) obtaining first and second subpopulations of beads, wherein each bead comprises:

the first capture probe which specifically binds to a target nucleic acid,
the first polynucleotide, wherein the barcode is indicative of the first capture probe, and

a second polynucleotide comprising an index and an index primer binding site 3' of the index, wherein the indexes of the first subpopulation are different from the indexes of the second subpopulation;

(ii) contacting first target nucleic acids to the first capture probes of the first subpopulation of beads, and contacting second target nucleic acids to the first capture probes of the second subpopulation of beads;

(iii) distributing the first and second subpopulations of beads comprising the specifically bound first and second target nucleic acids on the substrate; and

(iv) extending the first capture probe specifically bound to the first and second target nucleic acids and detecting the extended first capture probes;

(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 first polynucleotide in the array.

~~2. The method of claim 1, wherein step (a) comprises:~~

~~(i) obtaining first and second subpopulations of beads, wherein each bead comprises:~~

~~the first capture probe which specifically binds to a target nucleic acid,~~

~~the first polynucleotide, wherein the barcode is indicative of the first capture probe, and~~

~~a second polynucleotide comprising an index and an index primer binding site 3' of the index, wherein the indexes of the first subpopulation are different from the indexes of the second subpopulation;~~

~~(ii) contacting first target nucleic acids to the first capture probes of the first subpopulation of beads, and contacting second target nucleic acids to the first capture probes of the second subpopulation of beads;~~

~~(iii) distributing the first and second subpopulations of beads comprising the specifically bound first and second target nucleic acids on the substrate; and~~

~~(iv) extending the first capture probe specifically bound to the first and second target nucleic acids and detecting the extended first capture probes.~~

~~3.2.~~ The method of claim ~~2~~1, wherein step (c) comprises decoding the locations of beads comprising the detected first capture probes on the substrate.

~~4.3.~~ The method of claim ~~2 or 3~~1 or 2, wherein the first polynucleotide comprises the first capture probe.

~~5.4.~~ The method of any one of claims ~~2-4~~1-3, wherein the first capture probes of the first and the second subpopulations of beads each comprise different nucleotide sequences from one another.

~~6.5.~~ The method of any one of claims ~~1-4~~2-5, wherein step (iv) comprises contacting the first capture probes with a polymerase.

~~7.6.~~ The method of any one of claims ~~1-5~~2-6, wherein the contacting first target nucleic acids to the first capture probes of the first subpopulation of beads, and the contacting second target nucleic acids to the first capture probes of the second subpopulation of beads are performed in different reaction volumes.

~~8.7.~~ The method of any one of claims ~~1-6~~2-7, wherein the first subpopulation of beads is distributed on the substrate before the second subpopulation of beads is distributed on the substrate.

~~9.8.~~ The method of any one of claims ~~1-7~~2-8, wherein step (iv) is performed prior to step (iii).

~~10.9.~~ The method of any one of claims ~~1-82-9~~, wherein step (iv) further comprises determining the location of the detected capture probes on the substrate by at least one cycle of sequencing by synthesis.

~~11.10.~~ The method of any one of claims ~~1-92-10~~, wherein step (c) comprises decoding the location of the indexes of the beads comprising ~~a-the~~ detected first capture probe by sequencing the indexes on the substrate.

~~12.11.~~ The method of any one of claims ~~1-102-11~~, wherein step (c) comprises decoding the location of the barcodes of the beads comprising ~~a-the~~ detected first capture probe by sequencing the barcodes on the substrate.

~~13.12.~~ The method of any one of claims ~~1-112-12~~, wherein the first and second target ~~ligands-nucleic acids~~ are obtained from different subjects.

~~14.13.~~ The method of any one of claims ~~1-132-14~~, further comprising at least 10 different subpopulations of beads, each subpopulation comprising an index different from another subpopulation.

~~15.14.~~ A method for decoding the locations of first polynucleotides in an array comprising:~~The method of claim 1, wherein step (a) comprises:~~

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

(i) obtaining a target nucleic acid comprising a first portion capable of hybridizing to the first capture probe, and a second portion capable of hybridizing to a second capture probe;

(ii) obtaining a population of beads, each bead comprising:

the first capture probe,

the second capture probe, wherein the second capture probe is attached to the bead via a cleavable linker, and wherein the second capture probe comprises a detectable label, and

the first polynucleotide, wherein the barcode is indicative of the first or second capture probe;

(iii) ligating the first capture probe to the second capture probe, comprising:

hybridizing the target nucleic acid to the first and second capture probes of a bead of the population of beads to generate a double-stranded nucleic acid comprising a single-stranded gap between the first and second capture probes, and

filing-in the gap between the first and second capture probes;

(iv) cleaving the cleavable linker to generate a detectable polynucleotide comprising the detectable label; and

(v) distributing the population of beads on a substrate;

(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 first polynucleotide in the array.

~~16.15.~~ The method of claim ~~14~~5, wherein (c) comprises decoding the location of the bead comprising the first capture probe comprising the detectable label on the substrate.

~~17.16.~~ The method of claim ~~15~~6, wherein the first capture probe comprises the first polynucleotide.

~~18.17.~~ The method of claim ~~15~~6 or ~~16~~7, wherein step (v) is performed before step (iv).

~~19.18.~~ The method of any one of claims ~~15-17~~16-18, wherein each bead comprises a second polynucleotide comprising an index indicative of the source of the target nucleic acid, and an index primer binding site 3' of the index.

~~20.19.~~ The method of any one of claims ~~15-18~~16-19, wherein step (c) comprises determining the location of the detectable polynucleotide comprising the detectable label on the substrate, and decoding the location of the barcode of the bead comprising the detectable polynucleotide comprising the detectable label on the substrate by sequencing the barcode on the substrate.

~~21.20.~~ The method of any one of claims ~~15-19~~16-20, wherein the population of beads comprises first and second subpopulations of beads, each bead comprising a second polynucleotide comprising an index and an index primer binding site 3' of the index, wherein the indexes of the first subpopulation are different from the indexes of the second subpopulation.

~~22.~~21. The method of ~~any one of claims 16-21~~claim 18 or 20, wherein step (c) comprises sequencing the indexes on the substrate.

- ~~23.~~—A kit comprising:
- ~~a first and a second subpopulation of beads, wherein each bead comprises:~~
 - ~~a capture probe which specifically binds to a target ligand,~~
 - ~~a first polynucleotide comprising a barcode indicative of the capture probe, and a barcode primer binding site 3' of the barcode, and~~
 - ~~a second polynucleotide comprising an index and an index primer binding site 3' of the index,~~
 - ~~wherein the indexes of the first subpopulation are different from the indexes of the second subpopulation,~~
 - ~~wherein a first volume comprises the first subpopulation of beads, and a second volume comprises the second subpopulation of beads.~~
- ~~24.~~—The kit of claim 23, wherein the capture probe is selected from a nucleic acid, an antibody or antigen binding fragment thereof.
- ~~25.~~—The kit of claim 23 or 24, wherein the first polynucleotide comprises the capture probe.
- ~~26.~~—The kit of any one of claims 23-25, wherein the capture probes of the first and the second subpopulations of beads each specifically bind to different target ligands from one another.
- ~~27.~~—The kit of claim 26, wherein the different capture probes of the first and second subpopulations of beads specifically bind to the same target ligands.