

CLAIMS

1. A method for identifying target ligands in an array comprising:

(a) obtaining a substrate having an array of beads distributed on a surface of the substrate, wherein the array of beads comprises a first and a second subpopulation of beads, comprising:

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

a capture probe which specifically binds to a target ligand,

a first polynucleotide comprising a barcode and a barcode primer binding site 3' of the barcode, wherein the barcode is indicative of the 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) specifically binding first target ligands to the capture probes of the first subpopulation of beads in a first reaction volume, and specifically binding second target ligands to the capture probes of the second subpopulation of beads in a second reaction volume; and

(iii) distributing the first and the second subpopulations of beads comprising the specifically bound first and second target ligands on the substrate, thereby obtaining the substrate having an array of beads;

(b) detecting in the array the specifically bound first and second target ligands;

(c) sequencing the barcodes of the first and the second subpopulations of beads by hybridizing a plurality of primers to the barcode primer binding sites of the first and the second subpopulations of beads; and extending the hybridized primers, thereby determining the location of the capture probes in the array;

(d) sequencing the indexes of the first and the second subpopulations of beads, thereby determining the locations of the first and the second subpopulations of beads in the array; and

(e) decoding the locations of beads of the array of beads comprising a detected target ligand in the array, thereby identifying target ligands of the first target ligands or of the second target ligands in the array.

2. The method of claim 1, wherein the first and second target ligands each comprise a nucleic acid.

3. The method of claim 2, wherein step (a) further comprises extending the capture probes specifically bound to the first and second target ligands.

4. The method of any one of claims 1-3, wherein the first polynucleotide comprises the capture probe.

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

6. The method of any one of claims 1-5, wherein step (b) comprises contacting the capture probes with a polymerase.

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

8. The method of any one of claims 1-7, wherein the first and second target ligands are obtained from different subjects.

9. The method of any one of claims 1-8, further comprising at least 10 different subpopulations of beads, each subpopulation comprising an index different from another subpopulation.