

CLAIMS

What is claimed is:

1. A method of determining the identification of a first nucleotide at a detection position of a target sequence comprising a plurality of detection positions, said method comprising:

a) providing a plurality of nucleic acid constructs, wherein each nucleic acid construct comprises a plurality of monomers and each monomer comprises:

i) a first target domain of said target sequence comprising a first set of target detection positions; and

ii) a first adaptor comprising an anchor site;

b) hybridizing a first anchor probe to said anchor site;

c) hybridizing a second anchor probe to a sequence adjacent to and contiguous with the anchor site, wherein the second anchor probe is partly or entirely degenerate and the hybridized second anchor probe can be ligated to the hybridized first anchor probe;

d) hybridizing one, two, or three additional anchor probes to the target sequence between the first target domain and the site of hybridization of the second anchor probe;

wherein said additional anchor probe(s) are fully degenerate;

e) hybridizing at least a first sequencing probe to said first target domain, wherein said first sequencing probe comprises:

i) a first probe domain complementary to said target domain;

ii) a unique nucleotide at a first interrogation position; and

iii) a label;

under conditions wherein said sequencing probe hybridizes to said target domain if said unique nucleotide is complementary to a first nucleotide in said first set of target detection positions; wherein said hybridized first anchor probe, second anchor probe, additional anchor probe(s) and first sequencing probe are hybridized to adjacent sequences and can be ligated together to form a ligation product; and

f) ligating said anchor probes and said sequencing probe to form a probe ligation product; and then

g) detecting said probe ligation product thereby identifying said first nucleotide.

CLAIMS

1. A method for identifying a nucleotide at a target detection position of a target sequence comprising a plurality of detection positions, said method comprising:

(a) providing a surface comprising a plurality of concatemers immobilized thereon, wherein said surface comprises a plurality of spatially distinct regions comprising said immobilized concatemers, wherein each concatemer comprises a plurality of monomers, and wherein each monomer comprises:

i) a target domain of said target sequence comprising a first set of target detection positions;

ii) a first adaptor comprising an anchor site;

b) hybridizing a first anchor probe to said anchor site;

c) hybridizing a second anchor probe to a region of the target sequence that is between the first anchor site and the target domain, and contiguous with both, wherein the second anchor probe is fully degenerate;

d) hybridizing a sequencing probe to said target domain, wherein said sequencing probe comprises:

i) a sequence complementary to said target domain, wherein said sequence includes a nucleotide at an interrogation position, wherein the said nucleotide is complementary to a nucleotide at a detection position; and

ii) a label that identifies the nucleotide at the interrogation position;

e) ligating said anchor probes and said sequencing probe to form a probe ligation product; and then

f) detecting said probe ligation product thereby identifying said nucleotide.

2. The method according to claim 1 wherein a set of sequencing probes is contacted with said surface, each sequencing probe comprising:

a) a sequence complementary to a target domain of a monomer;

b) a unique nucleotide at a first interrogation position; and

c) a label;

wherein each label of said set corresponds to said unique nucleotide.

3. The method according to claim 1 wherein each of said monomers comprises a plurality of adaptors.

4. The method according to claim 3 wherein at least one of said adaptors comprises at least one Type IIs endonuclease recognition site.

5. The method according to claim 1 wherein steps b)-e) are repeated for multiple cycles to identify nucleotides at additional target detection positions in said first set of target detection positions, wherein in different cycles different sequencing probes are used, wherein said different sequencing probes differ by having a unique nucleotide at different interrogation positions.

6. The method according to claim 1, wherein said second anchor probe comprises at least one terminus that is selectively activatable for ligation.

7. The method according to claim 1 wherein said surface is functionalized.

8. The method according to claim 7 wherein said functionalized surface comprises functional moieties selected from the group consisting of amines, silanes, and hydroxyls.

9. The method according to claim 1 wherein said concatemers are immobilized on said surface using capture probes.

10. The method according to claim 1 further comprising fragmenting genomic nucleic acid to form target sequences.

11. The method according to claim 1 wherein said target sequence is a genomic nucleic acid sequence.
12. The method according to claim 12 wherein said genomic nucleic acid sequences are human.
13. The method of claim 1 wherein
 - a) the second anchor probe is phosphorylated at the 5' and 3' termini; or
 - b) the first anchor probe is phosphorylated at its 5' terminus and the second anchor probe is not phosphorylated at its 5' and 3' termini.
14. The method of claim 1 wherein the first and second anchor probes are ligated together in first step to form an extended anchor, and in a separate subsequent step the sequencing probe is ligated to the extended anchor to form the probe ligation product.