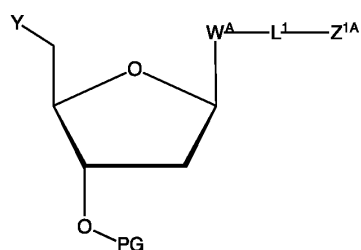


PATENT CLAIMS

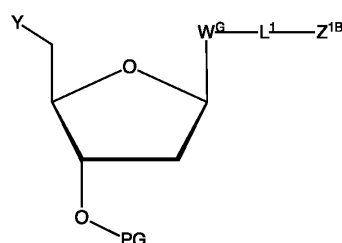
1. A method of sequencing a plurality of target polynucleotides arrayed on a solid support comprising:
- 5 (i) incorporating one of four different nucleotides into nascent nucleic acid copy strands complementary to each of the plurality of target polynucleotides, wherein each of the four different nucleotides comprise (i) a 3'-hydroxyl protecting group, and (ii) a first reactive group coupled to a nucleobase through a cleavable linker, and where each different nucleotide of the four different nucleotides comprises
- 10 a different nucleobase and a different first reactive group;
- (ii) sequentially forming different subsets of nucleotide-conjugate complexes, where each nucleotide-conjugate complex within each different subset of formed nucleotide-conjugate complexes is derived from only one of the different nucleotides incorporated into the
- 15 nascent nucleic acid copy strands, wherein the sequential formation of each different subset of nucleotide-conjugate complexes comprises:
- a. introducing a conjugate comprising a detectable label and which is orthogonally reactive with only one of the four
- 20 different nucleotides incorporated into the nascent nucleic acid strands;
- b. detecting the formation of each nucleotide-conjugate complex within the subset by detecting the label of each introduced conjugate;
- 25 c. determining a position within the solid support of each detected nucleotide-conjugate complex within the subset; and
- d. optionally cleaving at least a detectable label from each of the formed detectable nucleotide-conjugate complexes within the subset.

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2. The method of claim 1, wherein the four different nucleotides have the structures of Formulas (IC), (ID), (IE), and (IF):

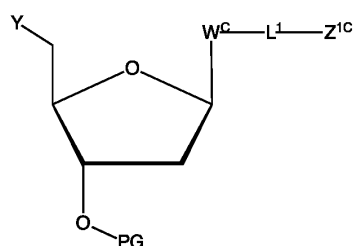


(IC),

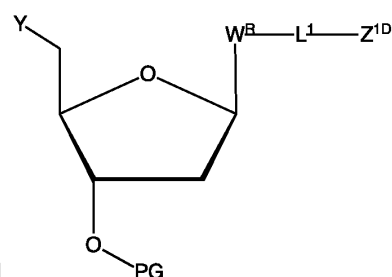


(ID),

5



(IE), and



(IF),

wherein

10 W^A is an adenine nucleobase, W^G is a guanine nucleobase; W^C is a cytosine nucleobase; W^R is one of a thymine nucleobase or an uracil nucleobase;

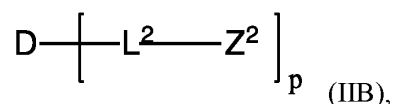
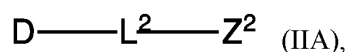
Z^{1A} , Z^{1B} , Z^{1C} , and Z^{1D} are each different first reactive groups;

Y is $-O-P(O)(OH)[-O-P(O)(OH)]_z-OH$, where z ranges from 2 to about 5;

15 PG is a protecting group; and

each L^1 is independently a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally substituted with one or more heteroatoms, provided that L^1 includes one or more cleavable groups.

3. The method of claim 1, wherein the introduced conjugate has the structure of any one of Formulas (IIA) or (IIB):



5 wherein

D is a detectable label or a conjugate including a detectable label;

L^2 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally comprising one or more heteroatoms;

10 Z^2 is a reactive group; and

p ranges from 2 to about 1000.

4. A method of determining a sequence of a plurality of target polynucleotides arrayed on a solid support comprising:

15 (i) incorporating one of four different nucleotides into nascent nucleic acid strands complementary to each of the plurality of target polynucleotides, wherein each of the four different nucleotides comprises a first reactive group coupled to a nucleobase through a cleavable linker and a 3'-hydroxyl protecting group, and where each
20 different nucleotide of the four different nucleotides comprises a different nucleobase and a different first reactive group;

(ii) sequentially labeling each one of the four different nucleotides incorporated into the nascent nucleic acid strands, wherein the sequential labeling comprises:

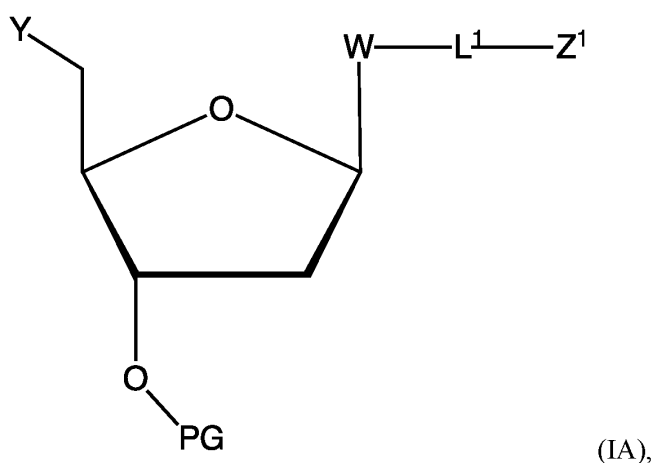
- 25 a. introducing a conjugate comprising a detectable label and which is orthogonally reactive with only one of the four different nucleotides incorporated into the nascent nucleic acid strands to provide one or more labeled nucleotides;
- b. detecting the label of the one or more labeled nucleotides;

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- c. based on the detected labels, identifying a position within the solid support of the one or more labeled nucleotides; and
- d. optionally cleaving at least a detectable label from the one or more labeled nucleotides incorporated into the nascent nucleic acid strands.

5

5. A compound or any salt thereof having a structure defined by Formula (IA):



wherein

Y is $-O-P(O)(OH)[-O-P(O)(OH)]_z-OH$ or $-O-P(O)(OH)-$ oligonucleotide, where z is an integer ranging from 2 - about 5;

10

PG is a protecting group;

W is a nucleobase;

L¹ is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally comprising one or more heteroatoms, provided that L¹ includes one or more cleavable groups; and

15

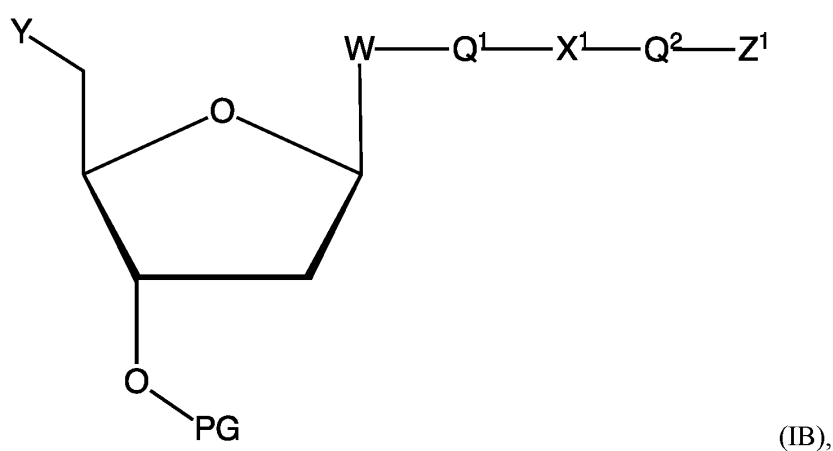
Z¹ is an oligonucleotide.

6. The compound of claim 5, wherein Z¹ comprises an LNA- or PNA-modified oligonucleotide.

20

7. The compound of claim 5, wherein Z¹ comprises L-configured monomers.

8. The compound of claim 5, wherein the Z^1 oligonucleotide comprises a sequence selected from any of SEQ ID NOS: 1 – 22 or 29 – 50.
9. The compound of claim 5, wherein the at least one cleavable group is selected from the group consisting of a chemically cleavable group, an enzymatically cleavable group, and a photocleavable group.
10. The compound of claim 5, wherein the compound or salt thereof has the structure of Formula (IB):



wherein

- 10 Y is $-O-P(O)(OH)[-O-P(O)(OH)-]_z-OH$ or $-O-P(O)(OH)-$ oligonucleotide, where z ranges from 2 – about 5;

PG is a protecting group;

W is a nucleobase;

- 15 Q^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated aliphatic moiety having between 1 and about 25 carbon atoms and optionally comprising one or more heteroatoms;

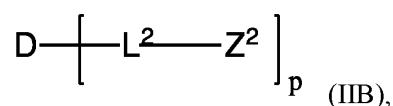
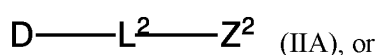
Q^2 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and 45 carbon atoms and optionally comprising one or more heteroatoms;

- 20 X^1 is a cleavable group; and

Z^1 is an oligonucleotide.

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11. The compound of claim 10, wherein Q^1 comprises a $C_1 - C_{10}$ straight chain or branched, substituted or unsubstituted alkyl or heteroalkyl group.
12. The compound of claim 10, wherein Q^2 comprises a $C_1 - C_{20}$ straight chain or branched, substituted or unsubstituted alkyl or heteroalkyl group.
- 5 13. The compound of claim 10, wherein X^1 is selected from the group consisting of a photocleavable group, an enzymatically cleavable group, a chemically cleavable, and a pH sensitive group.
14. A nucleotide comprising (i) a 3'-hydroxyl protecting group, and (ii) a reactive group coupled to a nucleobase through a cleavable linker, wherein the
10 reactive group comprises an LNA-modified oligonucleotide or a beta-L-LNA modified oligonucleotide,
wherein the LNA-modified oligonucleotide or a beta-L-LNA modified oligonucleotide comprises a sequence having any of SEQ ID NOS: 1 – 22 or 29 – 50.
- 15 15. A kit comprising: (i) the compounds or salts thereof of any one of claims 10 – 13, or the nucleotide or salt thereof of claim 14; and (ii) a conjugate having the structure of any of Formulas (IIA) or (IIB):



20

wherein

D is a detectable label or a conjugate including a detectable label;

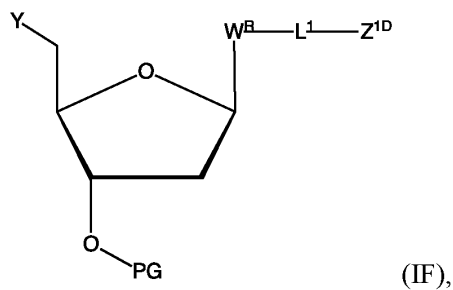
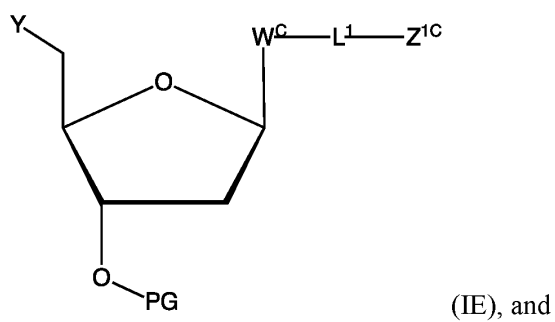
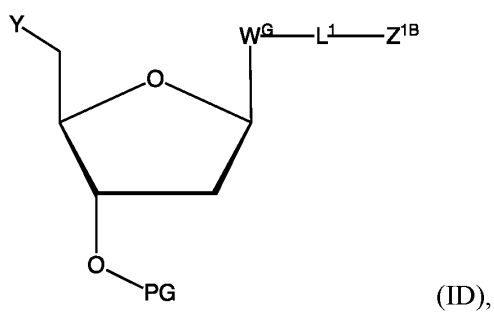
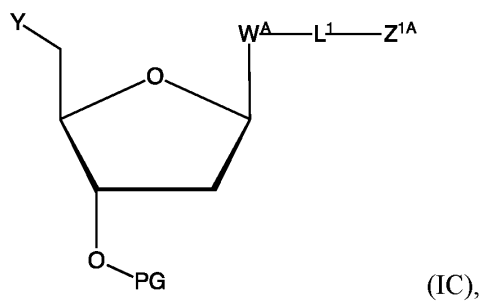
L^2 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon
25 atoms and optionally comprising one or more heteroatoms;

Z^2 is an oligonucleotide which is complementary to the oligonucleotide of Z^1 ; and

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p is an integer ranging from 2 to about 1000.

16. A kit comprising one nucleotide of Formula (IC), one nucleotide of Formula (ID), one nucleotide of Formula (IE), and one nucleotide of Formula (IF):



wherein

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Y is $-\text{O}-\text{P}(\text{O})(\text{OH})[-\text{O}-\text{P}(\text{O})(\text{OH})]_z-\text{OH}$, where z ranges from 2 to about 5;

PG is a protecting group;

W^{A} is an adenine nucleobase;

5 W^{G} is a guanine nucleobase;

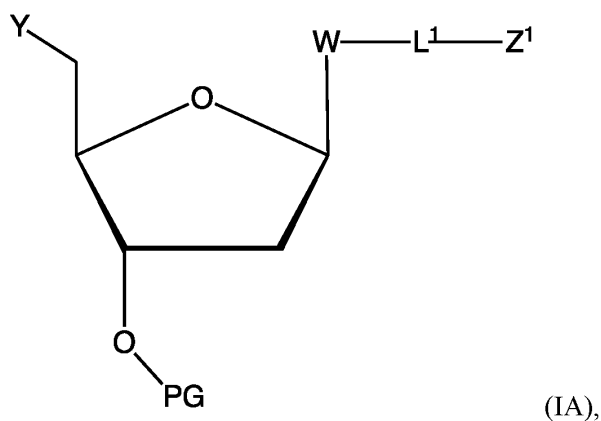
W^{C} is a cytosine nucleobase;

W^{R} is either a thymine nucleobase or an uracil nucleobase;

10 L^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally comprising one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

$\text{Z}^{1\text{A}}$, $\text{Z}^{1\text{B}}$, $\text{Z}^{1\text{C}}$, and $\text{Z}^{1\text{D}}$ each comprise a different first reactive group.

15 17. A nucleotide-conjugate complex, wherein the nucleotide-conjugate complex is produced by a process comprising: reacting (i) a nucleotide having Formula (IA):



wherein

20 Y is $-\text{O}-\text{P}(\text{O})(\text{OH})[-\text{O}-\text{P}(\text{O})(\text{OH})]_z-\text{OH}$; where z ranges from 2 to about 5;

PG is a protecting group;

W is a nucleobase;

L^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between

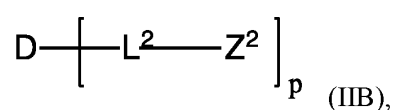
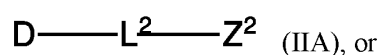
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1 and about 60 carbon atoms and optionally substituted with one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

Z^1 is a first reactive group;

5

with (ii) a conjugate having any one of Formulas (IIA) or (IIB):



wherein

10

D is a detectable label or a conjugate including a detectable label;

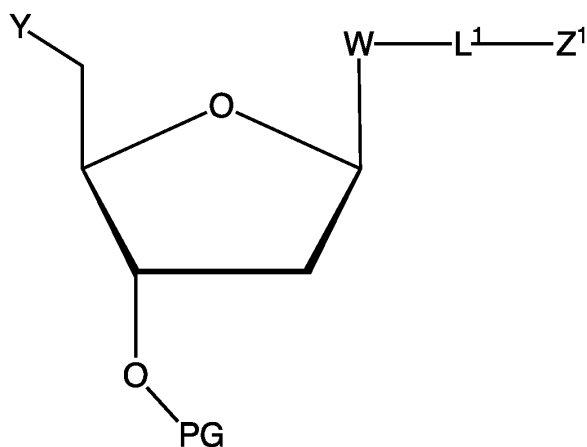
L^2 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally comprising one or more heteroatoms;

Z^2 is a second reactive group which is orthogonally reactive with the first reactive group Z^1 ; and

15

p is an integer ranging from 2 to about 1000.

18. A solid support comprising a plurality of nascent nucleic acid copy strands indirectly coupled thereto, wherein the plurality of nascent nucleic acid copy strands each comprise an incorporated nucleotide having Formula (IA):



20

(IA),

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wherein

Y is $\text{--O--P(O)(OH)--O--}$ oligonucleotide, where

PG is a protecting group;

W is a nucleobase;

- 5 L^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally comprising one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and
- Z^1 is a first oligonucleotide.

- 10 19. An assembly comprising a solid support of claim 18 and a magnetic sensor array suitable for sequentially detecting different formed subsets of nucleotide-conjugate complexes comprising a magnetic nanoparticle.