~~BRIEF~~ SUMMARY ~~OF THE DISCLOSURE~~

[0004] Applicant has developed an improved method of Sequencing-by-Synthesis utilizing magnetic sensor arrays and reagents incorporating detectable magnetic labels. Applicant has discovered that performing Sequencing-by-Synthesis using such magnetic sensor arrays and reagents including detectable magnetic labels dramatically increases sequencing throughput and reduces the cost of sequencing, while eliminating the need for high-power lasers and high-resolution optics in sequencing systems.

[0005] In ~~one aspect of~~ the present disclosure there is provided a method of sequencing a plurality of target polynucleotides arrayed on a solid support including: (a) 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 includes a different nucleobase and a different first reactive group; (b) 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 nascent nucleic acid copy strands, wherein the sequential formation of each different subset of nucleotide-conjugate complexes includes: (i) introducing a conjugate including a detectable label and which is orthogonally reactive with only one of the four different nucleotides incorporated into the nascent nucleic acid copy strands; (ii) detecting the formation of each nucleotide-conjugate complex within the subset by detecting the label of each introduced conjugate; (iii) determining a position within the solid support of each detected nucleotide-conjugate complex within the subset; and (iv) optionally cleaving at least the detectable label from each of the formed detectable nucleotide-conjugate complexes within the subset.

[0005a] In one aspect of the present disclosure there is provided a method of sequencing a plurality of target polynucleotides arrayed on a solid support including:

(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 include (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 includes 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 nascent nucleic acid copy strands, wherein the sequential formation of each different subset of nucleotide-conjugate complexes includes:

a. introducing a conjugate including a detectable label and which is orthogonally reactive with only one of the four 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;

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.

[0006] In some ~~embodiments~~examples, the different first reactive groups of each of the four different nucleotides are independently selected from a first member of a pair of reactive functional groups capable of participating in a "click chemistry" reaction, a first member of a pair of specifying binding entities, a first

oligonucleotide, and a first member of a pair of host / guest molecules. In some ~~embodiments~~examples, the introduced conjugate includes a second reactive group selected from a second member of the pair of reactive functional groups capable of participating in a "click chemistry" reaction, a second member of the pair of specifying binding entities, a second oligonucleotide capable of hybridizing with the first oligonucleotide, and a second member of the pair of host / guest molecules. In some ~~embodiments~~examples, the first and second reactive groups include first and second oligonucleotides capable of hybridizing with one another.

[0007] In some ~~embodiments~~examples, the different first reactive groups of each of the four different nucleotides comprise a different first oligonucleotide. In some ~~embodiments~~examples, the different first oligonucleotides may be selected from any one of SEQ ID NOS: 1 – 58. In some ~~embodiments~~examples, each different first oligonucleotide includes a first LNA-modified oligonucleotide. In some ~~embodiments~~examples, a first LNA-modified oligonucleotide includes a sequence selected from any one of SEQ ID NOS: 1 – 22 and 29 – 50.

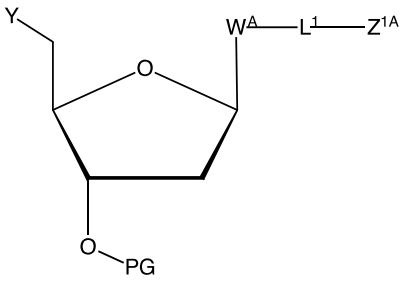
[0008] In some ~~embodiments~~examples, the second reactive group of the introduced conjugate includes a second oligonucleotide capable of hybridizing with the first oligonucleotide. In some ~~embodiments~~examples, the second oligonucleotide capable of hybridizing with the first oligonucleotide is selected from any one of SEQ ID NOS: 1 – 58. In some ~~embodiments~~examples, the second oligonucleotide capable of hybridizing with the first oligonucleotide includes a second LNA-modified oligonucleotide. In some ~~embodiments~~examples, the second LNA-modified oligonucleotide includes between 3 and 12 mer. In some ~~embodiments~~examples, the second LNA-modified oligonucleotide includes between 5 and 10 mer. In some ~~embodiments~~examples, the second LNA-modified oligonucleotide includes a sequence selected any one of SEQ ID NOS: 1 – 22 and 29 – 50. In some ~~embodiments~~examples, the second oligonucleotide capable of hybridizing with the first oligonucleotide includes an L-configured oligonucleotide. In some ~~embodiments~~examples, the second oligonucleotide capable of hybridizing with the first oligonucleotide includes a beta-L-LNA oligonucleotide.

[0009] In some ~~embodiments~~examples, the cleavable linker includes at least one cleavable group selected from the group consisting of a disulfide group, an alpha-azidoether, a nitrobenzyl-based group, and a phenacyl group. In some ~~embodiments~~examples,

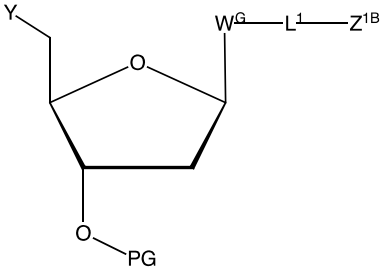
the incorporation of the different nucleotides into the nascent nucleic acid copy strands complementary to each of the plurality of target polynucleotides present on the solid support includes introducing a mixture of the four different nucleotides to the solid support. In some ~~embodiments~~examples, the sequential formation of the different subsets of nucleotide-conjugate complexes is performed three times such that three different subsets of detectable nucleotide-conjugate complexes are formed and detected.

[0010] In some ~~embodiments~~examples, the method further includes removing each of the 3'-hydroxyl protecting groups from the different nucleotides incorporated into the nascent nucleic acid copy strands complementary to each of the plurality of target polynucleotides. In some ~~embodiments~~examples, the 3'-hydroxyl protecting groups comprise azidomethyl groups. In some ~~embodiments~~examples, the method further includes introducing a mixture of the four different nucleotides to the solid support to extend each of the nascent nucleic acid strands complementary to each of the plurality of target polynucleotides on the solid support.

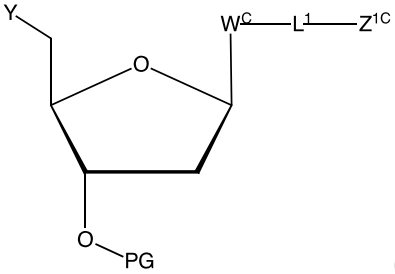
[0011] In some ~~embodiments~~examples, the four different nucleotides have the structures of any one of Formulas (IC), (ID), (IE), and (IF):



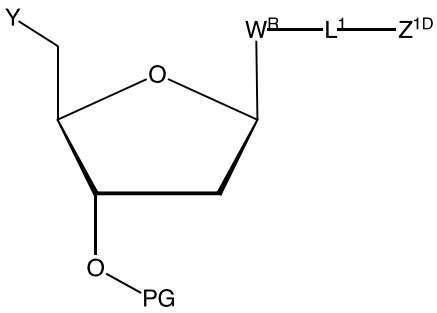
(IC),



(ID),



(IE), and



(IF),

[0012] wherein

[0013] 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;

[0014] Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} are each different first reactive groups;

[0015] Y is –O–P(O)(OH)[–O–P(O)(OH)]$_z$–OH, wherein z ranges from 2 to about 5;

[0016] PG is a protecting group; and

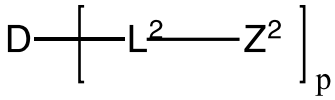
[0017] each L^1 is independently a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally substituted with one or more heteroatoms, provided that L^1 includes one or more cleavable groups.

[0018] In some ~~embodiments~~examples, z is 2.

[0019] In some ~~embodiments~~examples, the detectable label of each introduced conjugate includes a magnetic nanoparticle. In some ~~embodiments~~examples, the magnetic nanoparticle includes a material selected from the group consisting of FeO, Fe_3O_4, FePt, FePd, and CoPt. In some ~~embodiments~~examples, the magnetic nanoparticles are detected using a magnetic sensor array.

[0020] In some ~~embodiments~~examples, the introduced conjugate has the structure of any one of Formulas (IIA) and (IIB):


(IIA),


(IIB),

[0021] wherein

[0022] D is a detectable label or a conjugate including a detectable label;

[0023] 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 including one or more heteroatoms;

[0024] Z^2 is a second reactive group; and

[0025] p ranges from 2 to about 1000.

[0026] In some ~~embodiments~~examples, L^2 includes a cleavable group. In some ~~embodiments~~examples, L^2 includes a moiety derived from biotin.

[0027] In some ~~embodiments~~examples, the method further includes removing non-incorporated nucleotides prior to the sequential formation of the different subsets of nucleotide-conjugate complexes.

[0028] In another aspect of the present disclosure there is provided a method of determining a sequence of a plurality of target polynucleotides arrayed on a solid support including: (~~a~~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 includes a first reactive group coupled to a nucleobase through a cleavable linker and a 3'-hydroxyl protecting group, and where each different nucleotide of the four different nucleotides includes a different nucleobase and a different first reactive group; (~~b~~ii) sequentially labeling each one of the four different nucleotides incorporated into the nascent nucleic acid strands, wherein the sequential labeling includes: ~~(i)~~a. introducing a conjugate including 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; ~~(ii)~~b. detecting the label of the one or more labeled nucleotides; ~~(iii)~~c. based on the detected labels, identifying a position within the solid support of the one or more labeled nucleotides; and ~~(iv)~~d. optionally cleaving at least a detectable label from the one or more labeled nucleotides incorporated into the nascent nucleic acid strands.

[0029] In some ~~embodiments~~examples, the different first reactive groups of each of the four different nucleotides are independently selected from the group consisting of a first member of a pair of reactive functional groups capable of participating in a "click chemistry" reaction, a first member of a pair of specifying binding entities, a first oligonucleotide, and a first member of a pair of host / guest molecules. In some ~~embodiments~~examples, the different first reactive groups of each of the four different nucleotides comprise a different first oligonucleotide. In some ~~embodiments~~examples, each of the introduced conjugates comprise a second oligonucleotide complementary to one of the first oligonucleotides. In some ~~embodiments~~examples, the second oligonucleotide complementary to one of the first oligonucleotides includes an L-configured oligonucleotide. In some ~~embodiments~~examples, the second oligonucleotide complementary to one of the first oligonucleotides includes a beta-L-LNA oligonucleotide. In some

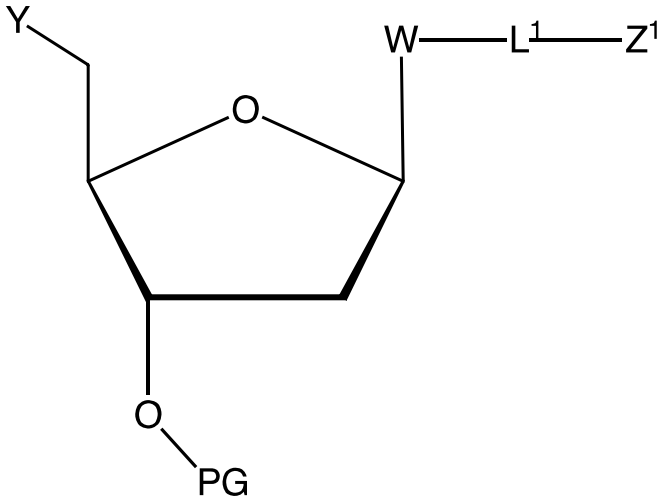
~~embodiments~~examples, the second oligonucleotide complementary to one of the first oligonucleotides includes one or more LNA monomers. In some ~~embodiments~~examples, the second oligonucleotide has a sequence selected from the group consisting of any one of SEQ ID NOS: 1 – 22 and 29 – 50. In some ~~embodiments~~examples, the second oligonucleotide complementary to one of the first oligonucleotides includes one or more PNA monomers.

[0030] In some ~~embodiments~~examples, the cleavable linker includes at least one cleavable group selected from the group consisting of a disulfide group, an alpha-azidoether, a nitrobenzyl-based group, and a phenacyl group. In some ~~embodiments~~examples, the incorporation of the different nucleotides into the nascent nucleic acid strands complementary to each of the plurality of target polynucleotides present on the solid support includes introducing a mixture of the four different nucleotides to the solid support.

[0031] In some ~~embodiments~~examples, the sequential labeling is performed three times. In some ~~embodiments~~examples, the sequential labeling is performed four times. In some ~~embodiments~~examples, the method further includes removing each of the 3'-hydroxyl protecting groups from the different nucleotides incorporated into the nascent nucleic acid strands complementary to each of the plurality of target polynucleotides. In some ~~embodiments~~examples, the 3'-hydroxyl protecting groups comprise azidomethyl groups.

[0032] In some ~~embodiments~~examples, the method further includes introducing a mixture of the four different nucleotides to the solid support to extend each of the nascent nucleic acid strands complementary to each of the plurality of target polynucleotides on the solid support. In some ~~embodiments~~examples, the method further includes removing non-incorporated nucleotides prior to the sequential formation of the different subsets of nucleotide-conjugate complexes. In some ~~embodiments~~examples, the method further includes removing any unreacted conjugates between each sequential labeling.

[0033] In ~~another aspect of~~ the present disclosure ~~is~~ there is provided a compound or a salt thereof having a structure defined by Formula (IA):



(IA),

[0034] wherein

[0035] Y is –O–P(O)(OH)[–O–P(O)(OH)]$_z$–OH or –O–P(O)(OH)–oligonucleotide; where z ranges from between 2 to about 5;

[0036] PG is a protecting group;

[0037] W is a nucleobase;

[0038] 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 including one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

[0039] Z^1 is an oligonucleotide.

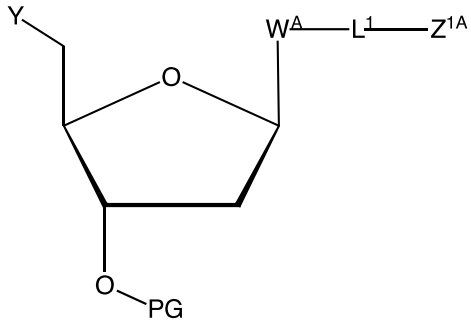
[0040] In some ~~embodiments~~examples, z is 2.

[0041] In some ~~embodiments~~examples, Z^1 includes an LNA-modified oligonucleotide. In some ~~embodiments~~examples, Z^1 includes a PNA-modified oligonucleotide. In some ~~embodiments~~examples, Z^1 includes L-configured monomers, e.g. at least one L-configured monomer. In some ~~embodiments~~examples, the L-configured monomers are L-configured LNA monomers. In some ~~embodiments~~examples, the L-configured LNA monomers are beta-L-LNA monomers. In some ~~embodiments~~examples, the oligonucleotide includes between 4 and 12 mer. In some ~~embodiments~~examples, the oligonucleotide includes between 5 and 10 mer. In some ~~embodiments~~examples, the oligonucleotide includes a sequence selected from any one of SEQ ID NOS: 1 – 22

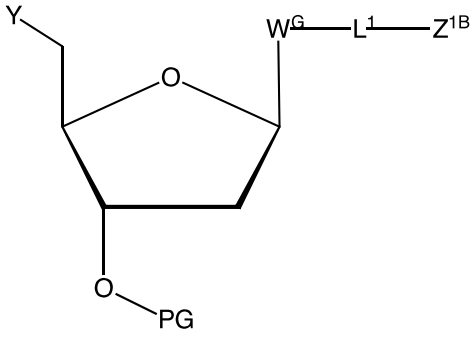
or 29 - 50. In some ~~embodiments~~examples, the at least one cleavable group is selected from the group consisting of a chemically cleavable group, an enzymatically cleavable group, and a photocleavable group.

[0042] In ~~another aspect of~~ the present disclosure ~~is~~ there is provided a nucleotide including (i) a 3'-hydroxyl protecting group, and (ii) a reactive group coupled to a nucleobase through a cleavable linker, wherein the reactive group includes an LNA-modified oligonucleotide or a beta-L-LNA modified oligonucleotide. In some ~~embodiments~~examples, the LNA-modified oligonucleotide or the beta-L-LNA modified oligonucleotide includes a sequence having any of SEQ ID NOS: 1 – 22 or 29 - 50.

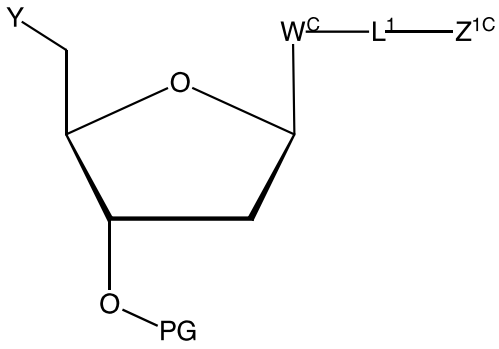
[0043] In ~~another aspect of~~ the present disclosure ~~is~~ there is provided a kit including one nucleotide of Formula (IC), one nucleotide of Formula (ID), one nucleotide of Formula (IE), and one nucleotide of Formula (IF):



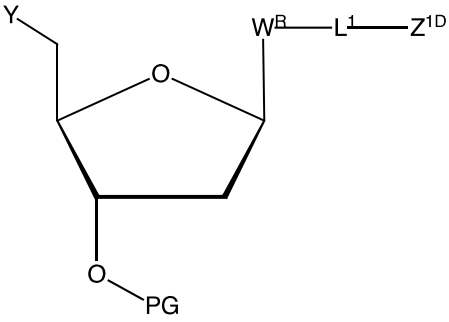
(IC),



(ID),



(IE), and



(IF),

[0044] wherein

[0045] Y is –O–P(O)(OH)[–O–P(O)(OH)]$_z$–OH, where z is na integer ranging from2 to about 5;

[0046] PG is a protecting group;

[0047] W^A is an adenine nucleobase;

[0048] W^G is a guanine nucleobase;

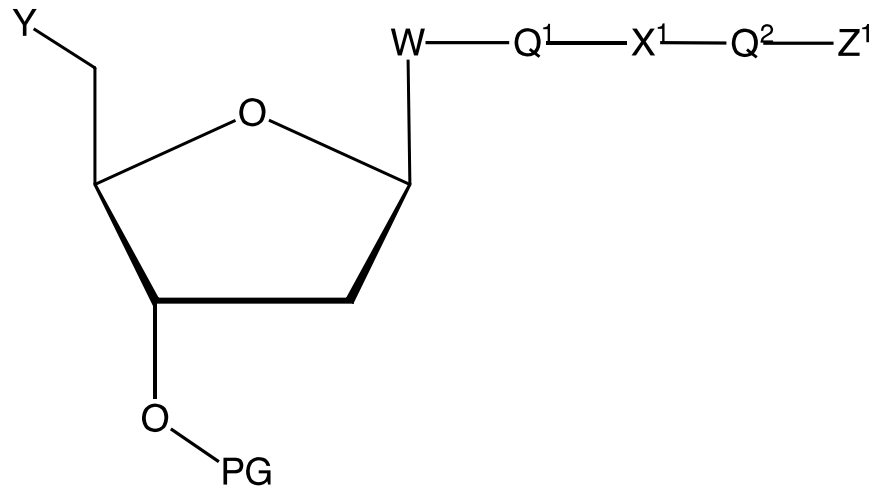
[0049] W^C is a cytosine nucleobase;

[0050] W^R is one of a thymine nucleobase or an uracil nucleobase;

[0051] 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 including one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

[0052] Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} each comprise a different first reactive group.

[0052a] In another aspect of the present disclosure there is provided a kit including: (i) a compound or salt thereof which has the structure of Formula (IB):



(IB),

wherein

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;

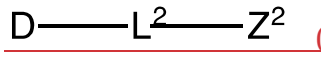
W is a nucleobase;

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 including one or more heteroatoms;

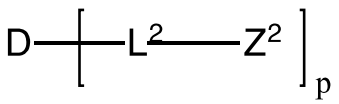
Q^2 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and 45 carbon atoms and optionally including one or more heteroatoms;

X^1 is a cleavable group; and Z^1 is an oligonucleotide; and

(ii) a conjugate having the structure of any of Formulas (IIA) or (IIB):



(IIA), or



(IIB),

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 including one or more heteroatoms;

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

p is an integer ranging from 2 to about 1000.

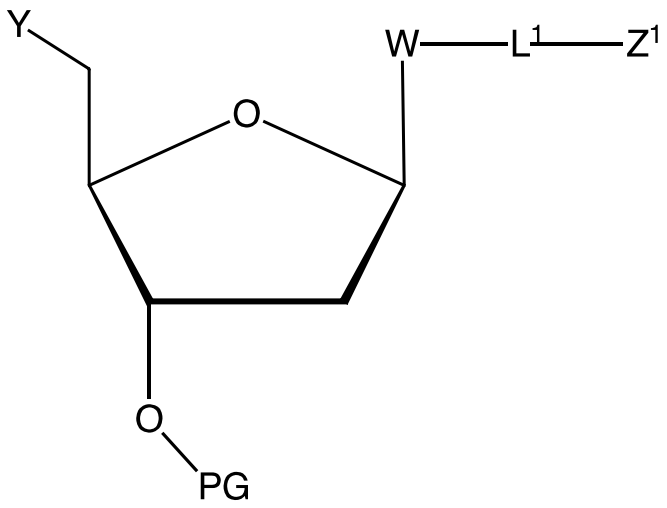
[0053] In some ~~embodiments~~examples, z is 2.

[0054] In some ~~embodiments~~examples, the Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} first reactive groups are independently selected from the group consisting of a first member of a pair of reactive functional groups capable of participating in a "click chemistry" reaction, a first member of a pair of specifying binding entities, a first oligonucleotide, and a first member of a pair of host / guest molecules. In some ~~embodiments~~examples, the Z^{1A}, Z^{1B},

Z^{1C}, and Z^{1D} first reactive groups each comprise a different first oligonucleotide. In some ~~embodiments~~examples, the Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} first reactive groups each comprise a different LNA-modified first oligonucleotide. In some ~~embodiments~~examples, the Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} first reactive groups each comprise a different beta-L-LNA-modified first oligonucleotide.

[0055] In some ~~embodiments~~examples, the kit further includes at least three different conjugates, where each of the at least three different conjugates includes a different second reactive group which reacts orthogonally with one of Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D}. In some ~~embodiments~~examples, each different second reactive group reacts orthogonally with one of the Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} first reactive groups. In some ~~embodiments~~examples, each different second reactive group includes a different second oligonucleotide. In some ~~embodiments~~examples, each different second oligonucleotide includes at least one LNA monomer. In some ~~embodiments~~examples, each different second oligonucleotide includes at least one beta-L-LNA monomer.

[0056] In ~~another aspect of~~ the present disclosure there is provided a nucleotide-conjugate complex, wherein the nucleotide-conjugate complex is produced by a process including: reacting (i) a nucleotide having the structure of Formula (IA):



(IA),

[0057] wherein

[0058] Y is –O–P(O)(OH)–oligonucleotide;

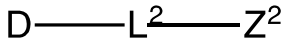
[0059] PG is a protecting group;

[0060] W is a nucleobase;

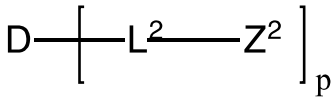
[0061] L^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally substituted with one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

[0062] Z^1 is a first reactive group;

[0063] with (ii) a conjugate having the structure of any one of Formulas (IIA) or (IIB):



(IIA),



(IIB),

[0064] wherein

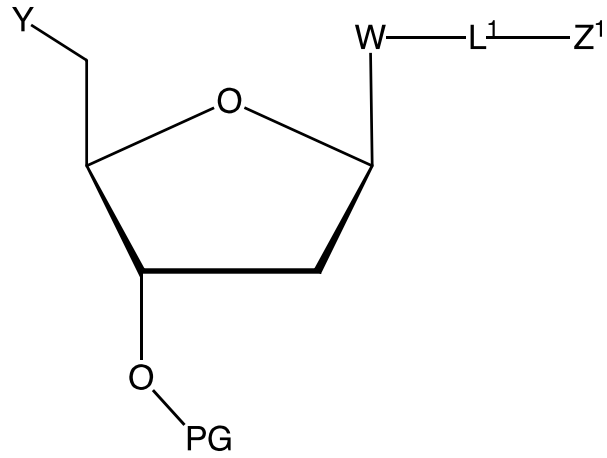
[0065] D is a detectable label or a conjugate including a detectable label;

[0066] 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 including one or more heteroatoms;

[0067] Z^2 is a second reactive group which is orthogonally reactive with the first reactive group Z^1; and

[0068] p is an integer ranging from 2 to about 1000.

[0068a] In another aspect of the present disclosure there is provided a nucleotide-conjugate complex, wherein the nucleotide-conjugate complex is produced by a process including: reacting (i) a nucleotide having Formula (IA):



(IA),

wherein

Y is –O–P(O)(OH)[–O–P(O)(OH)]z–OH; where z ranges from 2 to about 5;

PG is a protecting group;

W is a nucleobase;

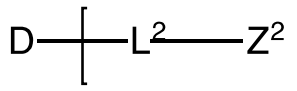
L^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated, aliphatic or aromatic moiety having between 1 and about 60 carbon atoms and optionally substituted with one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

Z^1 is a first reactive group;

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



(IIA), or



(IIB),

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 including one or more heteroatoms;

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

p is an integer ranging from 2 to about 1000.

[0069] In some ~~embodiments~~examples, L^2 includes biotin or a moiety derived from biotin.

[0070] In some ~~embodiments~~examples, the conjugate has the structure of Formula (IIA). In some ~~embodiments~~examples, the conjugate has the structure of Formula (IIB), and where p ranges from 2 to about 100.

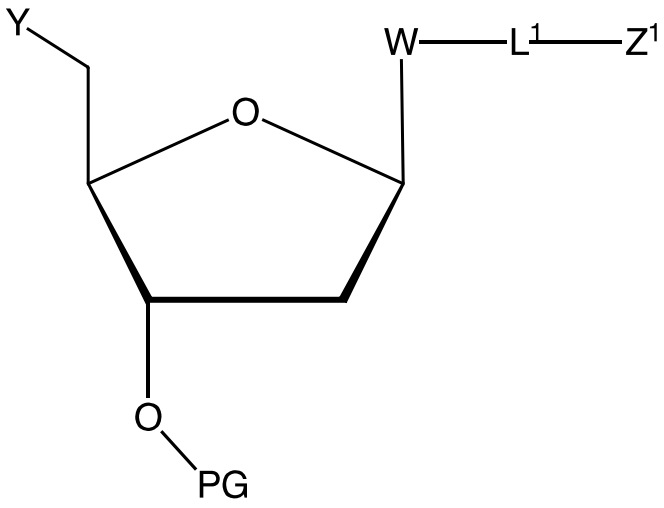
[0071] In some ~~embodiments~~examples, the first reactive group includes a first member of a pair of reactive functional groups capable of participating in a "click chemistry" reaction. In some ~~embodiments~~examples, the first reactive group includes a first member of a pair of specific binding entities. In some ~~embodiments~~examples, the first member of the pair of specific binding entities is a hapten. In some ~~embodiments~~examples, the first reactive group includes a first member of a pair of host / guest molecules. In some ~~embodiments~~examples, the first reactive group includes a first oligonucleotide. In some ~~embodiments~~examples, the first oligonucleotide is an LNA-modified first oligonucleotide. In some

~~embodiments~~examples, the LNA-modified first oligonucleotide includes at least one L-configured LNA monomer. In some ~~embodiments~~examples, the LNA-modified first oligonucleotide includes a sequence having any one of SEQ ID NOS: 1 – 22 or 29 - 50.

[0072] In some ~~embodiments~~examples, the second reactive group includes a second oligonucleotide which is complementary to the first oligonucleotide. In some ~~embodiments~~examples, the second oligonucleotide is an LNA-modified second oligonucleotide or a beta-L-LNA-modified second oligonucleotide. In some ~~embodiments~~examples, the LNA-modified second oligonucleotide includes a sequence having any one of SEQ ID NOS: 1 – 22 or 29 – 50.

[0073] In some ~~embodiments~~examples, the detectable label includes a fluorescent molecule. In some ~~embodiments~~examples, the detectable label includes a magnetic nanoparticle. In some ~~embodiments~~examples, the magnetic nanoparticle includes a ferromagnetic material. In some ~~embodiments~~examples, the magnetic nanoparticle includes a material selected from the group consisting of FeO, Fe_3O_4, FePt, FePd, and CoPt.

[0074] In ~~another aspect of~~ the present disclosure there is provided a solid support including a plurality of nascent nucleic acid copy strands indirectly coupled thereto, wherein the plurality of nascent nucleic acid copy strands each include an incorporated nucleotide having Formula (IA):



(IA),

[0075] wherein

[0076] Y is–O–P(O)(OH)–O–oligonucleotide, where

[0077] PG is a protecting group;

[0078] W is a nucleobase;

[0079] 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 including one or more heteroatoms, provided that L^1 includes one or more cleavable groups; and

[0080] Z^1 is a first oligonucleotide.

[0081] In some ~~embodiments~~examples, the first oligonucleotide is an LNA-modified first oligonucleotide. In some ~~embodiments~~examples, the LNA-modified first oligonucleotide includes at least one L-configured LNA monomer. In some ~~embodiments~~examples, the LNA-modified first oligonucleotide includes between 3 mer and 12 mer. In some ~~embodiments~~examples, the LNA-first modified oligonucleotide includes a sequence having any of SEQ ID NOS: 1 – 22 or 29 - 50.

BRIEF DESCRIPTION OF THE FIGURES

[0082] For a general understanding of the features of the disclosure, reference is made to the drawings. In the drawings, like reference numerals have been used throughout to identify identical elements.

[0083] FIG. 1A provides a flowchart setting forth a method of sequencing in accordance with one embodiment of the present disclosure.

[0084] FIG. 1B provides a flowchart illustrating a method of sequentially forming different subsets of nucleotide-conjugate complexes in accordance with one embodiment of the present disclosure.

[0085] FIG. 2A provides a flowchart setting forth a method of sequencing in accordance with one embodiment of the present disclosure.

[0086] FIG. 2B provides a flowchart illustrating a method of sequentially labeling nucleotides incorporated into a nucleic acid strand in accordance with one embodiment of the present disclosure.

[0087] FIG. 3 provides a flowchart depicting a method of sequencing in accordance with one embodiment of the present disclosure.

[0088] FIG. 4A illustrates a method of incorporating four different nucleotides of the present disclosure into four different nucleic acid strands, and then

PATENT CLAIMS

1. A method of sequencing a plurality of target polynucleotides arrayed on a solid support comprising:

   (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 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 nascent nucleic acid copy strands, wherein the sequential formation of each different subset of nucleotide-conjugate complexes comprises:

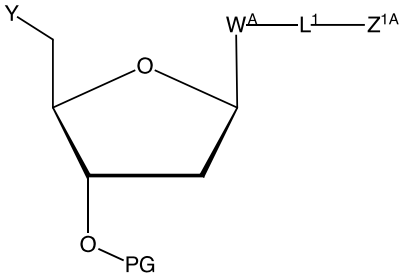
      a. introducing a conjugate comprising a detectable label and which is orthogonally reactive with only one of the four different nucleotides incorporated into the nascent nucleic acid strands;

      b. detecting the formation of each nucleotide-conjugate complex within the subset by detecting the label of each introduced conjugate;

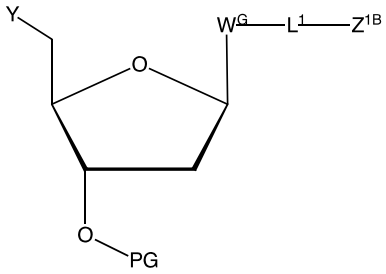
      c. determining a position within the solid support of each detected nucleotide-conjugate complex within the subset; and

      d. optionally cleaving at least a detectable label from each of the formed detectable nucleotide-conjugate complexes within the subset.

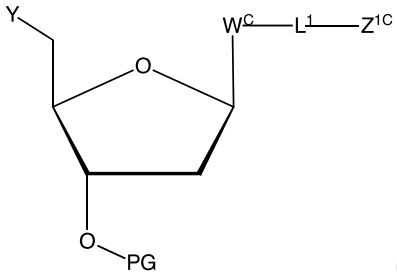
2. The method of claim 1, wherein the four different nucleotides have the structures of Formulas (IC), (ID), (IE), and (IF):



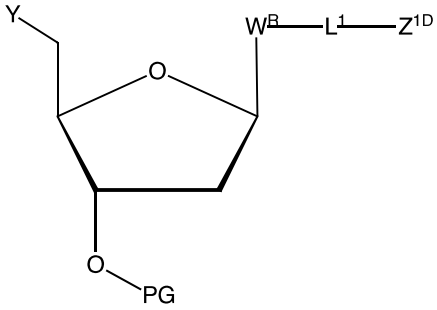
(IC),



(ID),



(IE), and



(IF),

wherein

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;

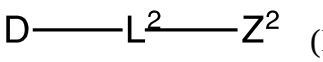
Z^{1A}, Z^{1B}, Z^{1C}, and Z^{1D} are each different first reactive groups;

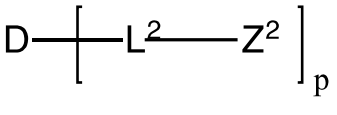
Y is –O–P(O)(OH)[–O–P(O)(OH)]$_z$–OH, where z ranges from 2 to about 5;

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):


(IIA),


(IIB),

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;

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:

(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 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:

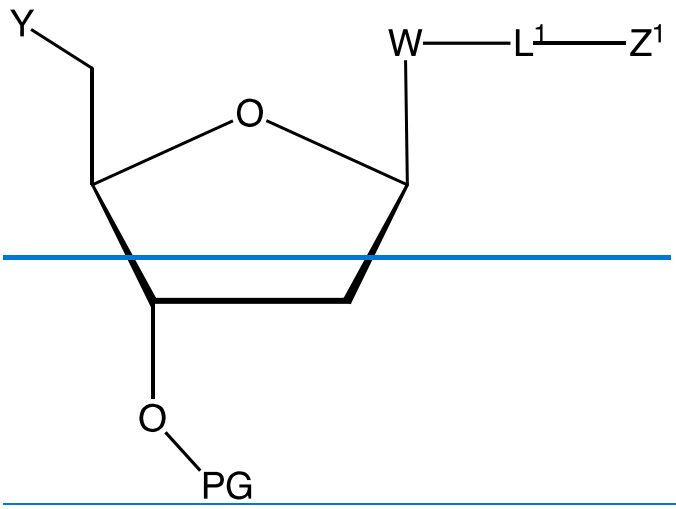
a. introducing a conjugate comprising a detectable label and which is orthogonally reactive with only one of the four different nucleotides incorporated into the nascent nucleic acid strands to provide one or more labeled nucleotides;

b. detecting the label of the one or more labeled nucleotides;

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



~~(IA),~~

~~wherein~~

~~Y is O P(O)(OH)[O P(O)(OH)]z OH or O P(O)(OH) oligonucleotide, where z is na integer ranging from 2 - 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 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 an oligonucleotide.~~

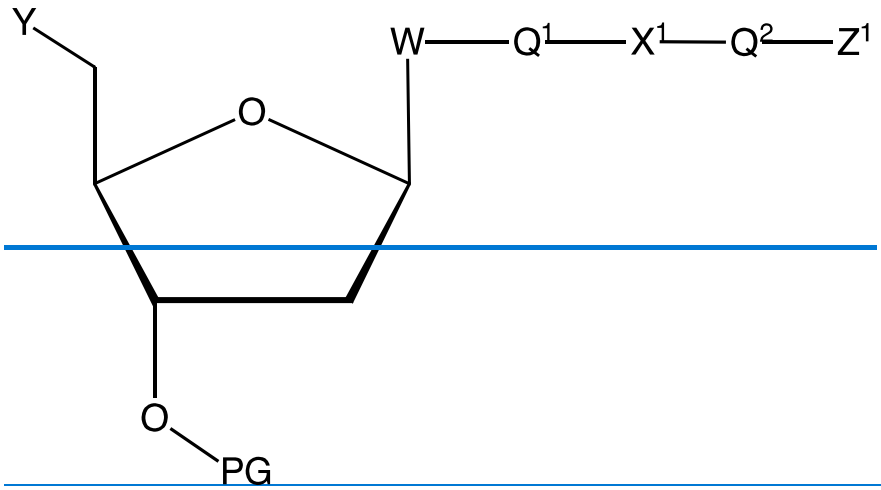
~~6. The compound of claim 5, wherein Z^1 comprises an LNA- or PNA-modified oligonucleotide.~~

~~7. The compound of claim 5, wherein Z^1 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):



(IB),

wherein

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;

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;

X^1 is a cleavable group; and

Z^1 is an oligonucleotide.

~~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.~~

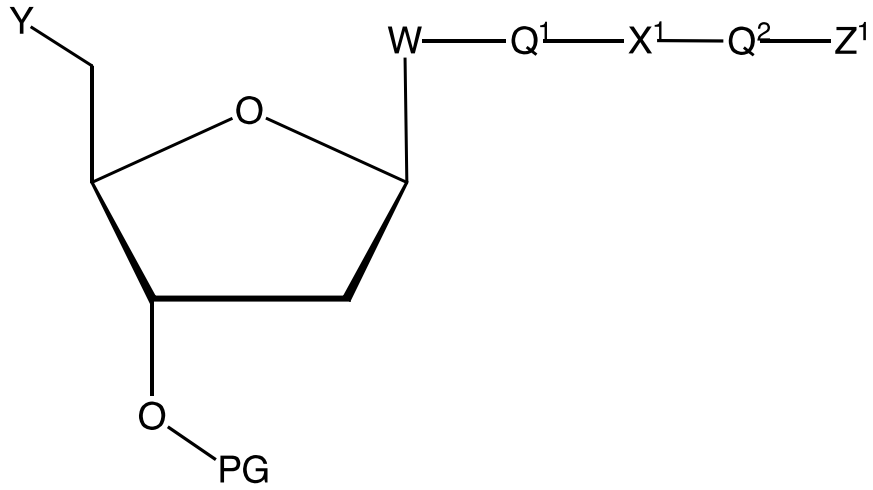
~~12. The compound of claim 10, wherein Q^2 comprises a C_1 – C_{20} straight chain or branched, substituted or unsubstituted alkyl or heteroalkyl group.~~

~~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 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.~~5. A kit comprising: (i) a compound or salt thereof which has the structure of Formula (IB):



(IB),

wherein

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;

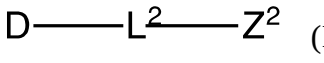
W is a nucleobase;

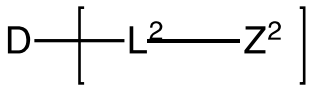
Q^1 is a straight chain or branched, substituted or unsubstituted, saturated or unsaturated aliphatic moiety having between 1 and about 25 carbon atoms and optionally comprising one or more heteroatoms;

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;

X^1 is a cleavable group; and Z^1 is an oligonucleotide~~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):


(IIA), or


(IIB),

wherein

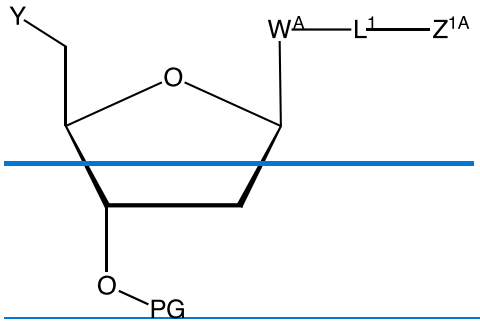
D is a detectable label or a conjugate including a detectable label;

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;

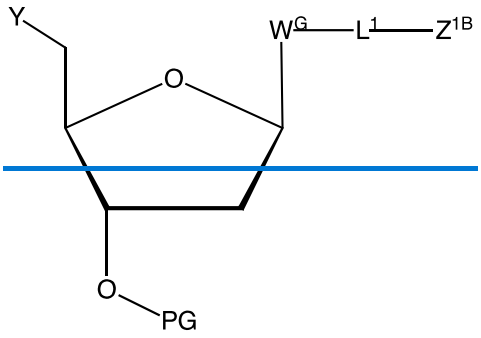
Z^2 is an oligonucleotide which is complementary to the oligonucleotide of Z^1; and

p is an integer ranging from 2 to about 1000.

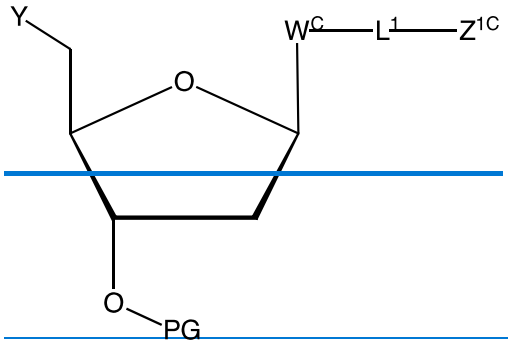
~~16. A kit comprising one nucleotide of Formula (IC), one nucleotide of Formula (ID), one nucleotide of Formula (IE), and one nucleotide of Formula (IF):~~



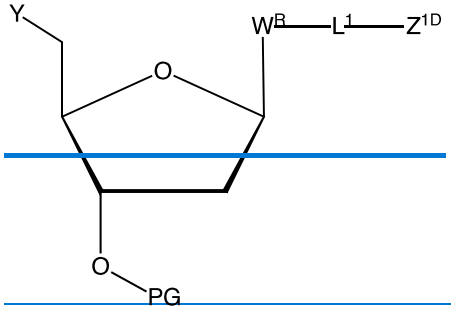
(IC),



(ID),



(IE), and



(IF),

wherein

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

PG is a protecting group;

W^A is an adenine nucleobase;

~~W^G is a guanine nucleobase;~~

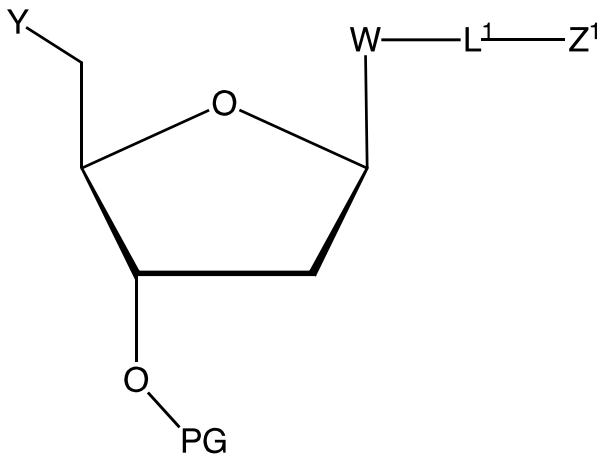
~~W^C is a cytosine nucleobase;~~

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

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

~~17.~~6. A nucleotide-conjugate complex, wherein the nucleotide-conjugate complex is produced by a process comprising: reacting (i) a nucleotide having Formula (IA):



(IA),

wherein

Y is –O–P(O)(OH)[–O–P(O)(OH)]z–OH; where z ranges from 2 to about 5;

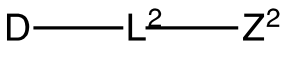
PG is a protecting group;

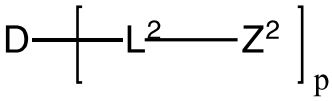
W is a nucleobase;

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

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


(IIA), or


(IIB),

wherein

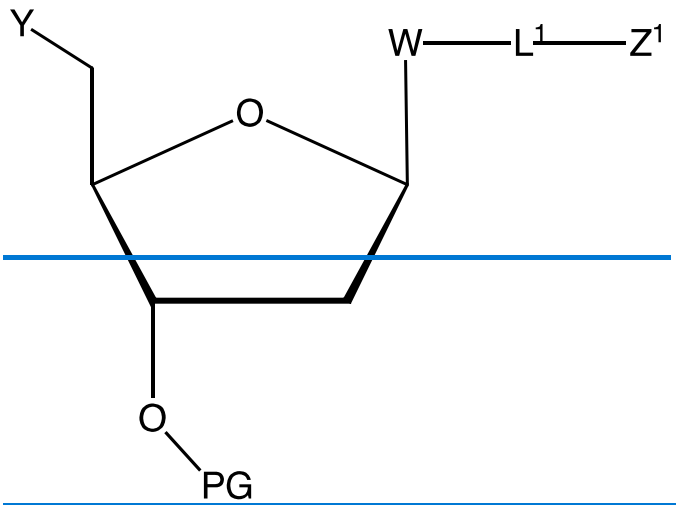
D is a detectable label or a conjugate including a detectable label;

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;

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

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):~~


~~(IA),~~

~~wherein~~

~~Y is –O–P(O)(OH)–O–oligonucleotide, where~~

~~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 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.~~

~~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.~~