CLAIMS

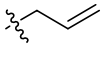
1. A method of determining the sequence of a plurality of different target polynucleotides in parallel, the method comprising:

(a) contacting a solid support with a solution comprising sequencing primers under hybridization conditions, wherein:

(i) the solid support comprises at least 5,000,000 spatially distinguishable sites/cm^2 that comprise multiple copies of target polynucleotides;

(ii) the solid support comprises a plurality of different target polynucleotides; and

(iii) the sequencing primers are complementary to at least a portion of the different target polynucleotides;

(b) contacting the solid support with an aqueous solution comprising DNA polymerase and nucleotides A, G, C and T or U under conditions suitable for DNA polymerase-mediated primer extension, wherein each nucleotide comprises a 2′ deoxyribose moiety with a 3′ allyl blocking group  attached to the 3' oxygen atom;

(c) imaging the solid support to determine the identity of incorporated nucleotides;

(d) contacting the solid support with an aqueous deblocking solution comprising a palladium catalyst and tris(hydroxyalkyl)phosphine under conditions suitable to chemically remove 3′ allyl blocking groups from incorporated nucleotides to expose a 3'-OH group for further nucleotide incorporation on the solid support;

(e) contacting said solid support with an aqueous wash solution; and

(f) repeating steps (b)-(e) to determine target polynucleotide sequences.

2. The method of claim 1, wherein the aqueous solution comprising DNA polymerase in step (b) further comprises a palladium scavenger.

3. The method of claim 2, wherein the palladium scavenger in step (b) is a Pd(0) scavenger.

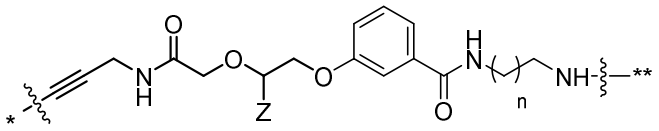
4. The method of claim 1, wherein the aqueous wash solution in step (e) further comprises a palladium scavenger.

5. The method of claim 4, wherein the palladium scavenger in step (e) is a Pd(II) scavenger.

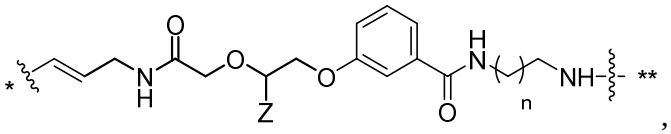
6. The method of claim 1, wherein the aqueous deblocking solution further comprises ascorbate.

7. The method of claim 1, wherein at least one type of nucleotide comprises a base attached to a detectable label via a cleavable linker.

8. The method of claim 7, wherein the detectable label is a fluorescent dye, and the cleavable linker is selected from the group consisting of:



and



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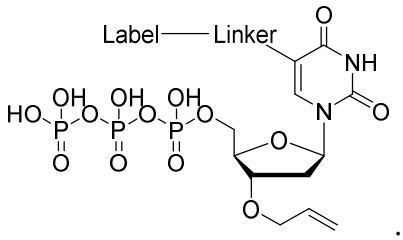
wherein Z is -O-CH_2-CH=CH_2; n is an integer of 1, 2, 3, 4 or 5; * indicates the attachment point of the cleavable linker to the base; and ** indicates the attachment point of the cleavable linker to the detectable label.

9. The method of claim 1, wherein the tris(hydroxyalkyl)phosphine is tris(hydroxypropyl)phosphine (THPP).

10. The method claim 1, wherein the solid support comprises at least 5,000,000 spatially distinguishable sites/cm^2 that comprise concatemers or clusters of immobilized nuclei acid molecules comprising said multiple copies of target polynucleotides.

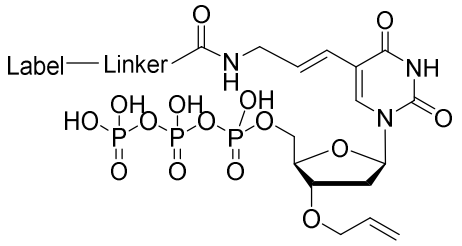
11. The method of claim 1, wherein the bases for the A and G nucleotides are deazapurines.

12. The method of claim 1, wherein the T nucleotide has the structure:

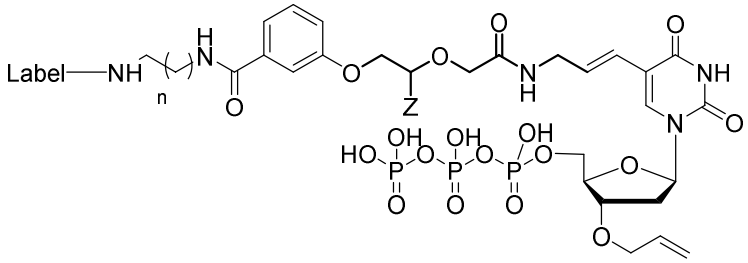


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13. The method of claim 12, wherein the T nucleotide has the structure:



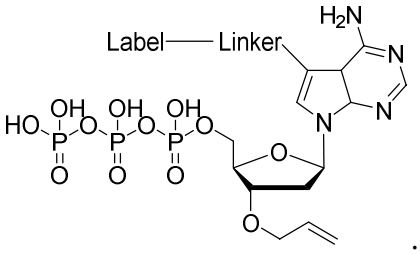
or



, wherein

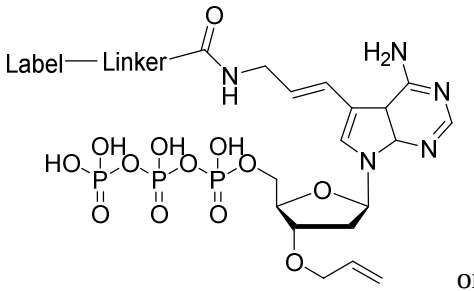
Z is -O-CH_2-CH=CH_2, and n is an integer of 1, 2, 3, 4 or 5.

14. The method of claim 1, wherein the A nucleotide has the structure:

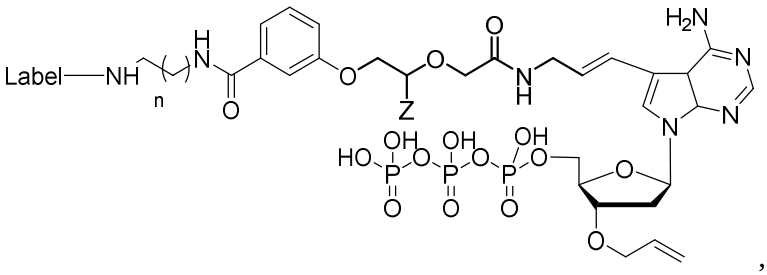


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15. The method of claim 14, wherein the A nucleotide has the structure:



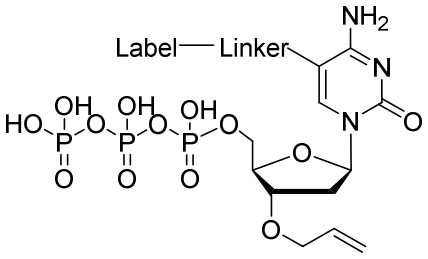
or



, wherein

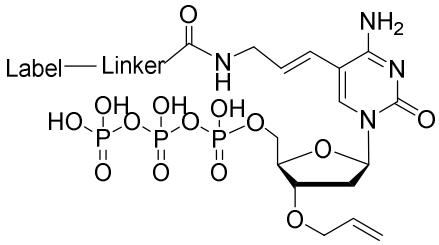
Z is -O-CH_2-CH=CH_2, and n is an integer of 1, 2, 3, 4 or 5.

16. The method of claim 1, wherein the C nucleotide has the structure:

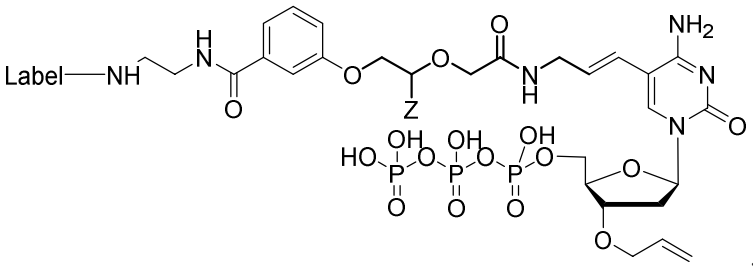


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17. The method of claim 16, wherein the C nucleotide has the structure:



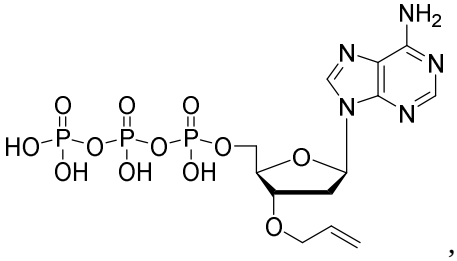
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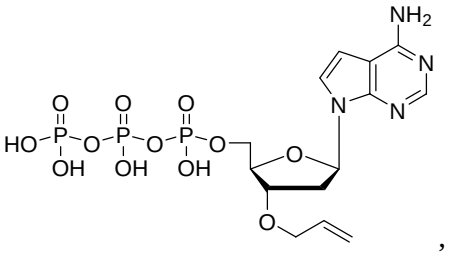
, wherein

Z is -O-CH_2-CH=CH_2, and n is an integer of 1, 2, 3, 4 or 5.

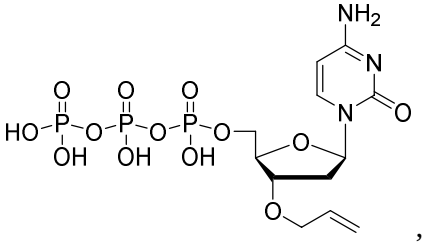
18. The method of claim 1, wherein at least one type of nucleotide has a structure selected from the group consisting of:



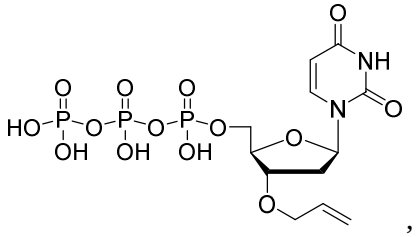
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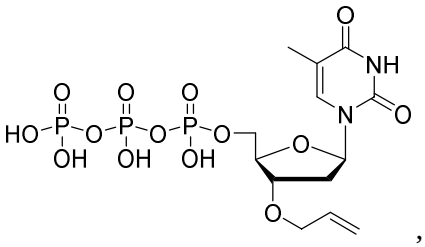
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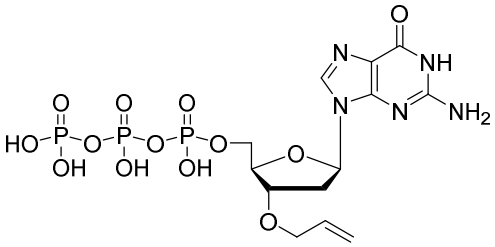
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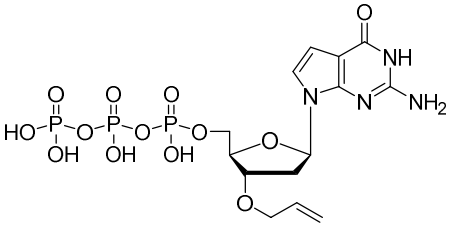
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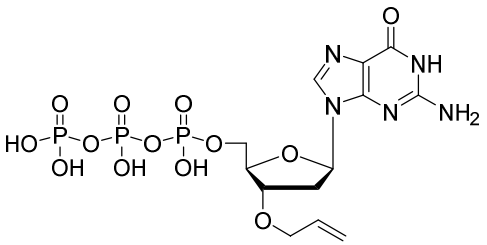


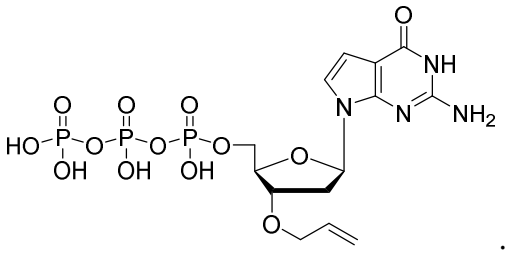
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19. The method of claim 18, wherein at least one type of nucleotide has a structure selected from the group consisting of:



and


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20. A method of determining the sequence of a plurality of different target polynucleotides in parallel, the method comprising:

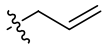
(a) contacting a solid support with a solution comprising sequencing primers under hybridization conditions, wherein:

(i) the solid support comprises at least 5,000,000 spatially distinguishable sites/cm^2 that comprise multiple copies of target polynucleotides;

(ii) the solid support comprises a plurality of different target polynucleotides; and

(iii) the sequencing primers are complementary to at least a portion of the different target polynucleotides;

(b) contacting the solid support with an aqueous incorporation mixture comprising DNA polymerase and one or more of four types of nucleotides A, G, C, and T or U under conditions suitable for DNA polymerase-mediated primer extension, wherein:

(i) the nucleotides each comprises a 2′ deoxyribose moiety with a 3′ allyl blocking group  attached to the 3' oxygen atom;

(ii) at least two types of nucleotides are unlabeled; and

(iii) the first type of unlabeled nucleotides comprises a first functional moiety;

(c) contacting the extended copy polynucleotides with an aqueous labeling mixture comprising a first labeling reagent, wherein the first labeling reagent comprises one or more first detectable labels and a first binding moiety that is capable of specific binding to the first functional moiety of the first type of unlabeled nucleotide;

(d) imaging the solid support and performing one or more fluorescent measurements to determine the identity of incorporated nucleotides;

(e) contacting the solid support with an aqueous deblocking solution comprising a palladium catalyst and tris(hydroxyalkyl)phosphine under conditions suitable to chemically remove (i) 3′ allyl groups from incorporated nucleotides to expose a 3’-OH group for further nucleotide incorporation on the solid support;

(f) contacting said solid support with an aqueous wash solution; and

(g) repeating steps (b)-(f) to determine target polynucleotide sequences.

21. The method of claim 1, wherein sequencing cycles are repeated at least about 20 times, 30 times, 50 times, 100 times, 150 times, 200 times, 250 times, 300 times, 350 times, 400 times, 450 times or 500 times.

22. The method of claim 21, wherein after about 50 repeated cycles the pre-phasing value or the phasing value is less than 0.18.

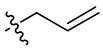
23. The method of claim 21, wherein after about 150 repeated sequencing cycles the pre-phasing value is less than 0.25 and the phasing value is less than 0.25.

24. The method of claim 1, wherein the deblocking solution further comprises one or more buffer reagents selected from the group consisting of a primary amine, a secondary amine, a tertiary amine, a carbonate salt, a phosphate salt, and a borate salt, and combinations thereof.

25. The method of claim 1, wherein the DNA polymerase is an altered family B archaeal DNA polymerase comprising a 3-amino acid region that is functionally equivalent or homologous to amino acids 408-410 in 9° N DNA polymerase, wherein the first amino acid of the 3-amino acid region is an amino acid selected from the group consisting of isoleucine (I), alanine (A), valine (V), and serine (S); the second amino acid of the 3-amino acid region is an amino acid selected from the group consisting of alanine (A) and glycine (G); and the third amino acid of the 3-amino acid region is an amino acid selected from the group consisting of alanine (A), isoleucine (I), valine (V), leucine (L), threonine (T), and proline (P).

26. A sequencing kit comprising:

(a) an incorporation mixture comprising DNA polymerase and nucleotides A, G, C, and T or U, wherein:

(i) the nucleotides comprise a 2’ deoxyribose moiety with a 3’ allyl group  attached to the 3' carbon atom; and

(ii) the DNA polymerase is an altered archaeal DNA polymerase;

(b) an aqueous deblocking solution comprising a palladium catalyst, tris(hydroxyalkyl)phosphine, and one or more buffer reagents that is suitable to chemically remove (i) 3′ allyl groups from incorporated nucleotides to expose a 3’-OH group for further nucleotide incorporation on the solid support, and (ii) detectable labels attached via cleavable linkers; and

(c) an aqueous wash solution comprising a Pd(II) scavenger;

wherein said kit is configured for performing at least about 100 cycles of sequencing-by-synthesis.