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:

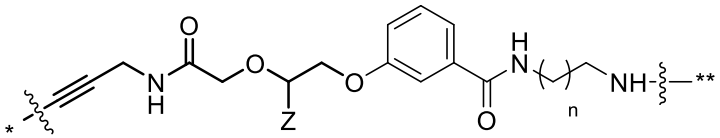
(i) the solid support comprises at least 5,000,000 spatially distinguishable sites/cm^2 that comprise multiple copies of target polynucleotides;

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

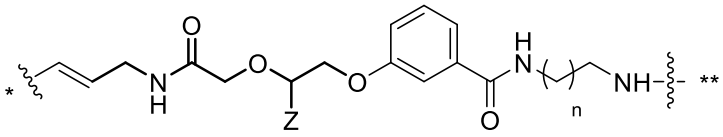
(iii) the sequencing primers are complementary to at least a portion of the different target polynucleotides;

(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:

(i) each of at least three of the nucleotides independently comprises a base that is attached to a detectable label via a cleavable linker, and the cleavable linker is selected from the group consisting of:

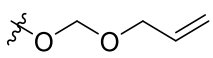


and



,

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;

(ii) each nucleotide comprises a 2′ deoxyribose moiety with a 3′ AOM group 

attached to the 3' carbon atom; and

(iii) 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);

(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, tris(hydroxypropyl)phosphine, and ascorbate under conditions suitable to chemically remove (i) 3′ AOM 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;

(e) contacting said solid support with an aqueous wash solution comprising a palladium scavenger; and

(f) repeating steps (b)-(e) at least about 100 times to determine target polynucleotide sequences,

wherein after about 100 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.25 and the phasing value is less than 0.25.

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

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

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

5. The method of claim 1, wherein at least one nucleotide comprises a base attached to a fluorescent dye via a cleavable linker.

6. The method of claim 1, wherein after about 100 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.18.

7. The method of claim 6, wherein after about 100 repeated cycles of steps (b)-(e) the phasing value is less than 0.18.

8. The method of claim 7, wherein after about 100 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.07.

9. 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:

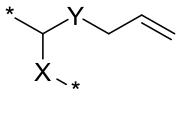
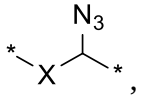
(i) the solid support comprises at least 1,000,000 spatially distinguishable sites/cm2 that comprise multiple copies of target polynucleotides;

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

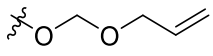
(iii) the sequencing primers are complementary to at least a portion of the different target polynucleotides;

(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:

(i) each of at least three of the nucleotides independently comprises a base that is attached to a detectable label via a cleavable linker, and the cleavable linker comprises a moiety selected from the group consisting of:

wherein the cleavable linker comprises  and ,

wherein X is O, Y is O, and * indicates where the moiety is connected to the remainder of the nucleotide;

(ii) each nucleotide comprises a 2′ deoxyribose moiety with a 3' AOM group  attached to the 3' carbon atom; and

(iii) the DNA polymerase is an altered family B archaeal DNA polymerase;

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

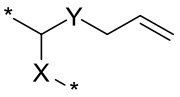
(d) contacting the solid support with an aqueous deblocking solution comprising a palladium catalyst and a water-soluble phosphine under conditions suitable to chemically remove

(i) 3′ AOM 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;

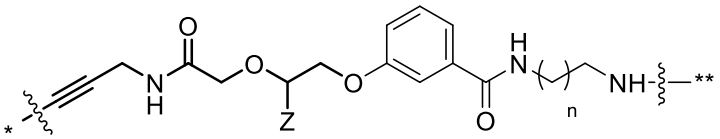
(e) contacting said solid support with an aqueous wash solution comprising a palladium scavenger; and

(f) repeating steps (b)-(e) at least about 50 times to determine target polynucleotide sequences,

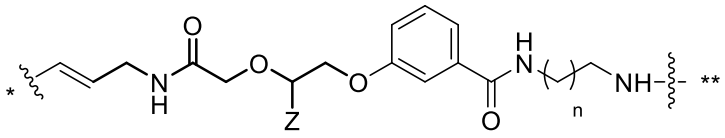
wherein after about 50 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.25 and the phasing value is less than 0.25.

10. The method of claim 9, wherein the cleavable linker comprises .

11. The method of claim 9, wherein the cleavable linker is selected from the group consisting of:



and



,

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.

12. 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:

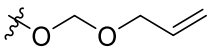
(i) the solid support comprises at least 1,000,000 spatially distinguishable sites/cm2 that comprise multiple copies of target polynucleotides;

(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:

(i) at least one of the nucleotides comprise a base that is attached to a detectable label via a cleavable linker; and

(ii) the nucleotides each comprises a 2′ deoxyribose moiety with a 3′ AOM group  attached to the 3' carbon 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 under conditions suitable to chemically remove (i) 3′ AOM 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;

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

(f) repeating steps (b)-(e) at least about 50 times to determine target polynucleotide sequences,

wherein after about 50 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.25 and the phasing value is less than 0.25.

13. The method of claim 12, wherein the solid support comprises at least 1,000,000 spatially distinguishable sites/cm^2 that comprise concatemers comprising said multiple copies of target polynucleotides.

14. The method of claim 12, wherein the solid support comprises at least 1,000,000 spatially distinguishable sites/cm^2 that comprise clusters of immobilized nucleic acid molecules comprising said multiple copies of target polynucleotides.

15. The method of claim 12, wherein the DNA polymerase is an altered family B archaeal DNA polymerase.

16. The method of claim 12, 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

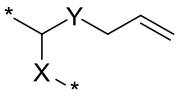
the 3-amino acid region is an amino acid selected from the group consisting of isoleucine (I), alanine (A), valine (V), and serine (S); the second amino acid of the 3-amino acid region is an amino acid selected from the group consisting of alanine (A) and glycine (G); and the third amino acid of the 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).

17. The method of claim 12, wherein the DNA polymerase is DNA polymerase 812 or DNA polymerase 1901.

18. The method of claim 12, wherein the bases for the A and G nucleotides are deazapurines.

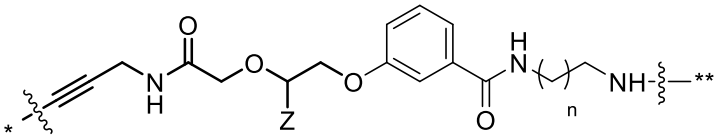
19. The method of claim 12, wherein at least one nucleotide comprises a base attached to a fluorescent dye via a cleavable linker.

20. The method of claim 12, wherein each detectable label is a fluorescent dye.

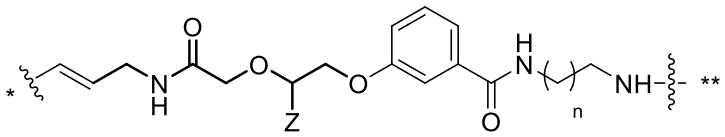
21. The method of claim 12, wherein the cleavable linker comprises 

, wherein X is O, Y is O, and * indicates where the moiety is connected to the remainder of the nucleotide.

22. The method of claim 12, wherein the cleavable linker is selected from the group consisting of:



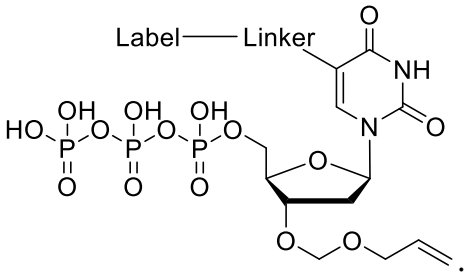
and



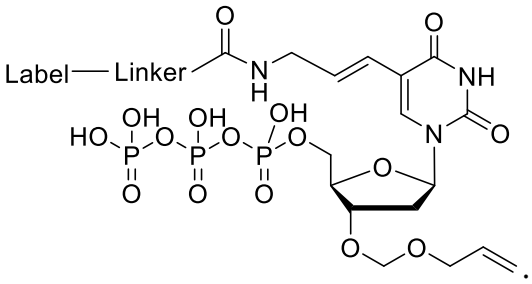
,

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.

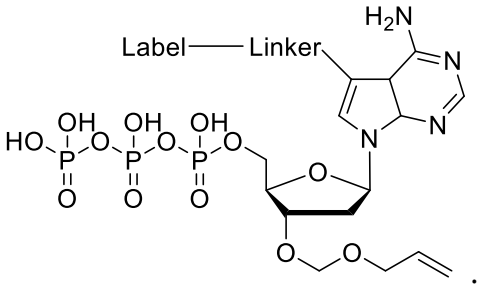
23. The method of claim 12, wherein the T nucleotide has the structure:



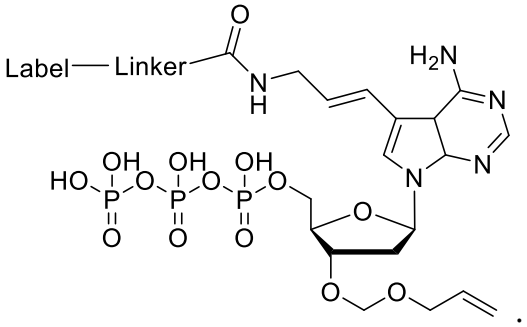
24. The method of claim 12, wherein the T nucleotide has the structure:



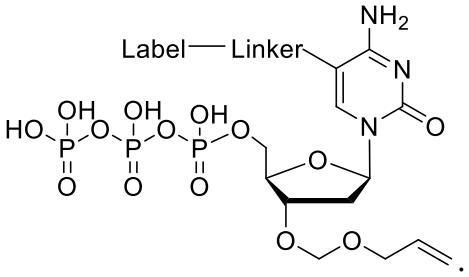
25. The method of claim 12, wherein the A nucleotide has the structure:



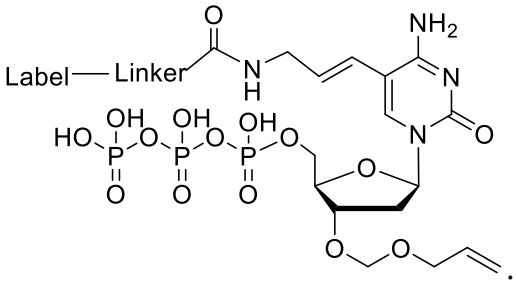
26. The method of claim 12, wherein the A nucleotide has the structure:



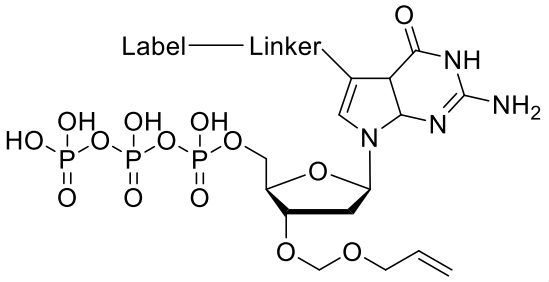
27. The method of claim 12, wherein the C nucleotide has the structure:



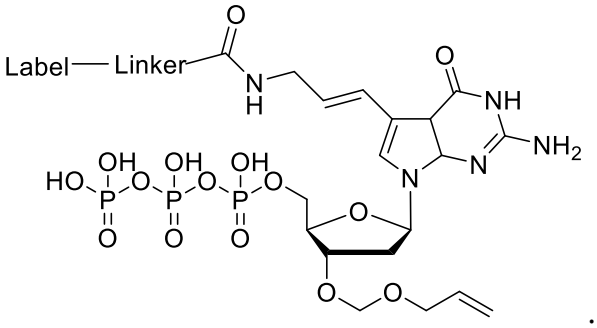
28. The method of claim 12, wherein the C nucleotide has the structure:



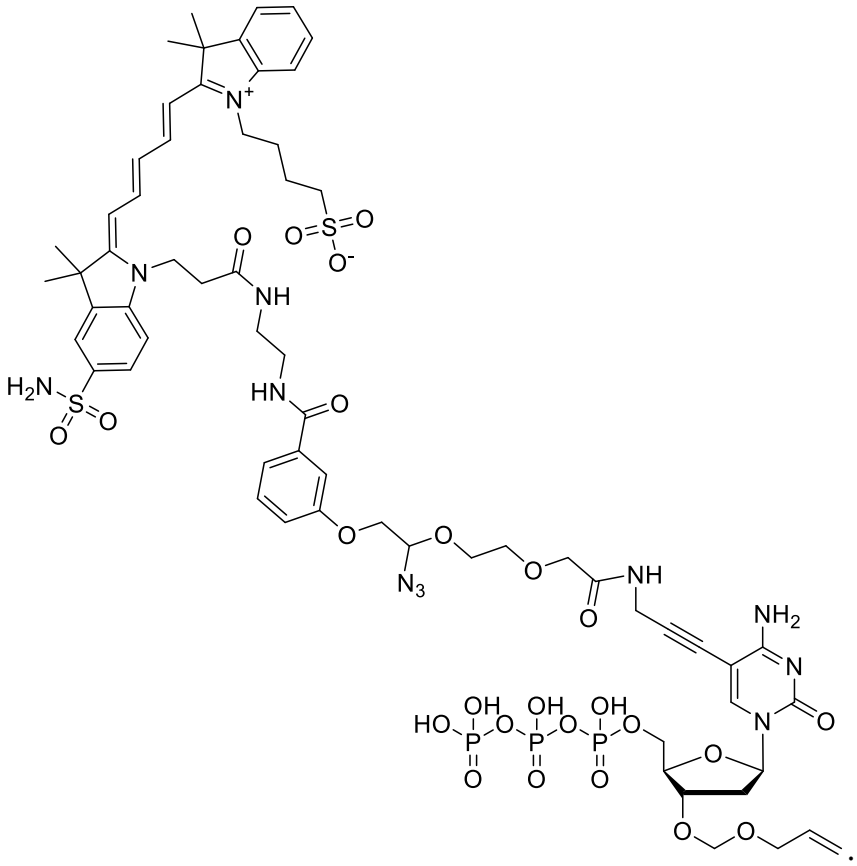
29. The method of claim 12, wherein the G nucleotide has the structure:



30. The method of claim 12, wherein the G nucleotide has the structure:



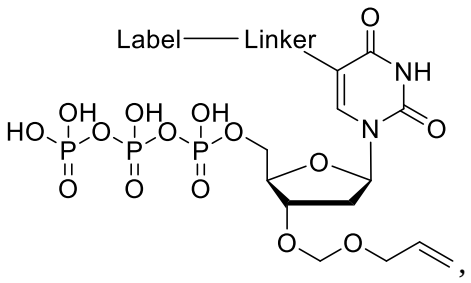
31. The method of claim 12, wherein one of the nucleotides has the structure:



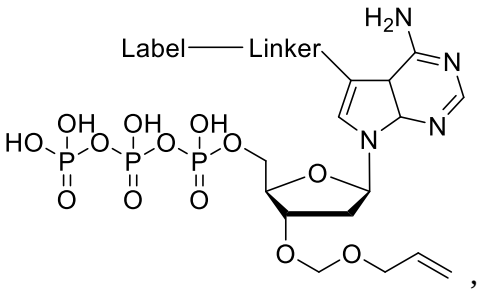
32. The method of claim 12, wherein at least three of the nucleotides comprise a base that is attached to a detectable label via a cleavable linker.

33. The method of claim 12, wherein at least three of A, G, C and T or at least three of A, G, C and U comprise a base that is attached to a detectable label via a cleavable linker.

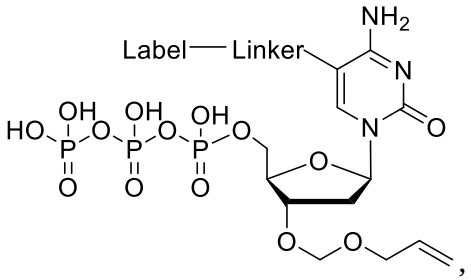
34. The method of claim 12, wherein at least three of the nucleotides have a structure selected from the group consisting of:



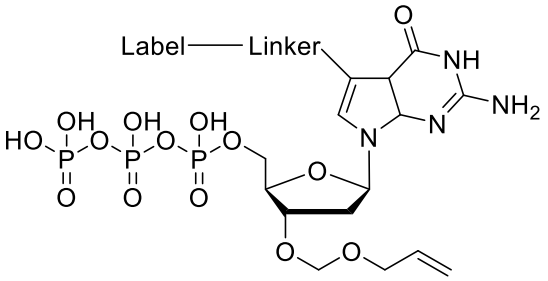
,



,

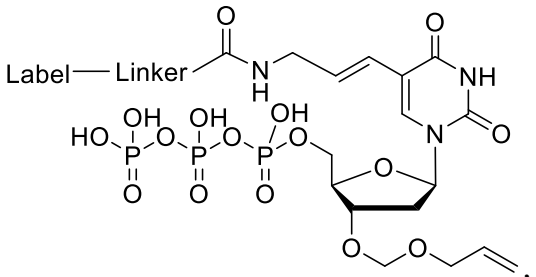


, and

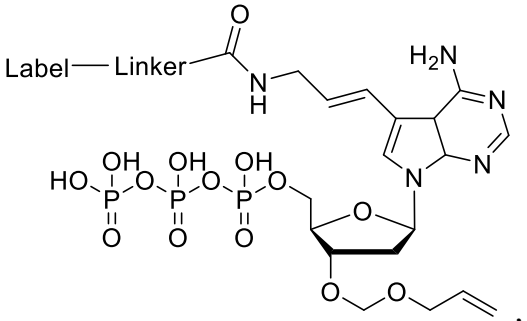


.

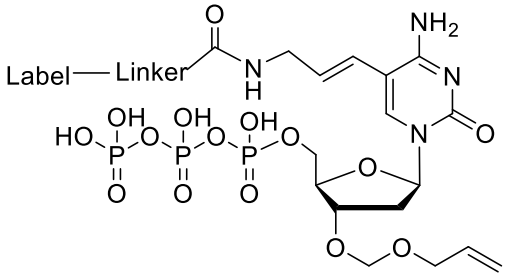
35. The method of claim 12, wherein at least three of the nucleotides have a structure selected from the group consisting of



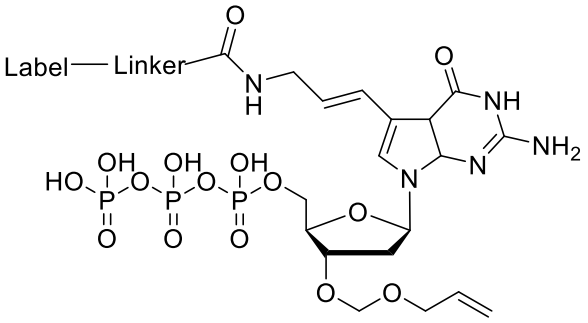
,



,



, and

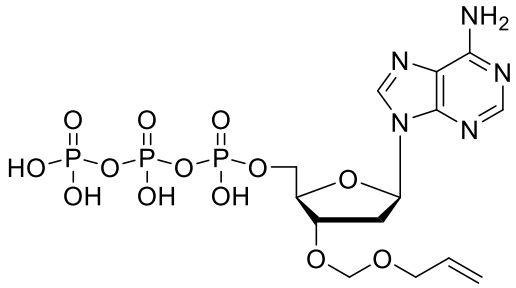
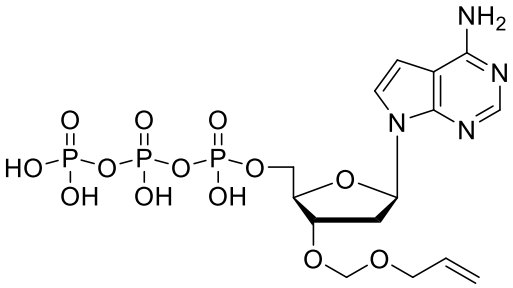
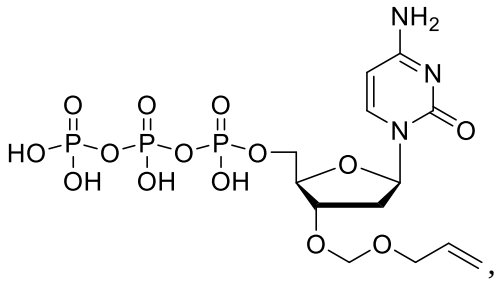
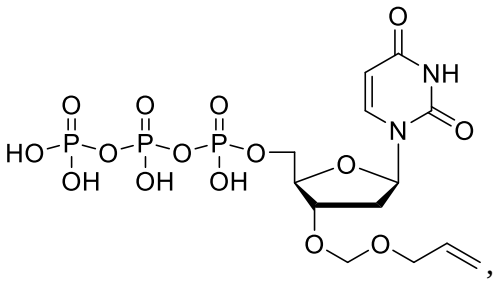
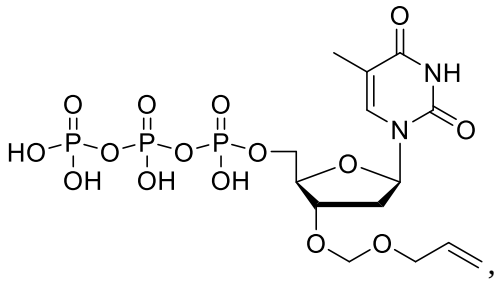
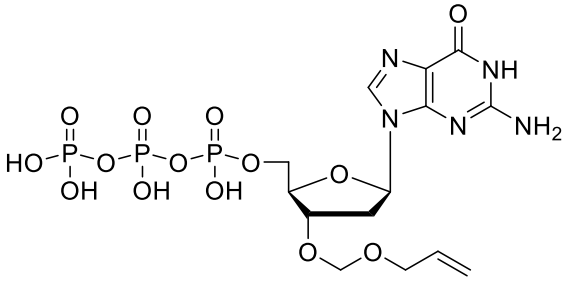
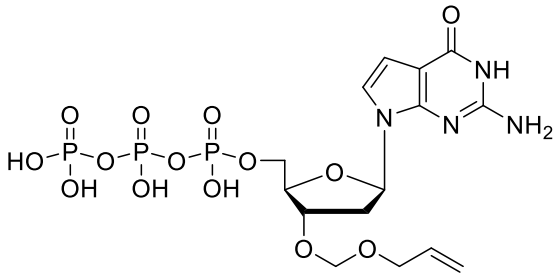


.

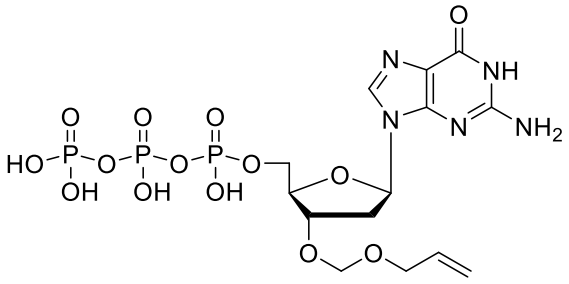
36. The method of claim 12, wherein at least one of the nucleotides comprises a base that is not attached to a detectable label.

37. The method of claim 12, wherein exactly one of the nucleotides comprises a base that is not attached to a detectable label.

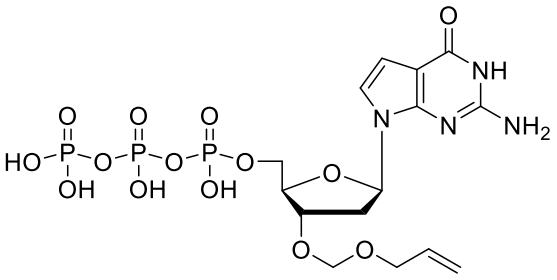
38. The method of claim 12, wherein at least one nucleotide has a structure selected from the group consisting of:


,

,

,

,

,

, and

.

39. The method of claim 12, wherein at least one nucleotide has a structure selected from the group consisting of:



and



.

40. The method of claim 12, wherein nucleotides A, G, C, and T or U comprise a base that is attached to a detectable label via a cleavable linker.

41. The method of claim 12, wherein the aqueous deblocking solution comprises a palladium catalyst, a water-soluble phosphine, and a reducing agent.

42. The method of claim 12, wherein the aqueous deblocking solution comprises a palladium catalyst, tris(hydroxypropyl)phosphine, and ascorbate.

43. The method of claim 12, wherein the palladium deblocking reagent is in situ generated Pd(0).

44. The method of claim 12, wherein said contacting the solid support with a deblocking solution is performed for 4-5 seconds.

45. The method of claim 12, wherein said contacting the solid support with a deblocking solution is performed via continuous flow without pausing to incubate.

46. The method of claim 12, wherein said contacting the solid support with an aqueous deblocking solution causes chemical removal of the 3′ AOM groups from incorporated nucleotides with at least 100% improved deblocking rate relative to an azidomethyl deblocking rate.

47. The method of claim 12, wherein the wash solution comprises a palladium scavenger.

48. The method of claim 12, wherein steps (b)-(e) are repeated at least about 150 times.

49. The method of claim 12, wherein steps (b)-(e) are repeated at least about 250 times.

50. The method of claim 12, wherein steps (b)-(e) are repeated at least about 300 times.

51. The method of claim 12, wherein after about 50 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.18.

52. The method of claim 51, wherein after about 50 repeated cycles of steps (b)-(e) the phasing value is less than 0.18.

53. The method of claim 52, wherein after about 50 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.07.

54. The method of claim 12, wherein after about 100 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.20 and the phasing value is less than 0.20.

55. The method of claim 12, wherein after about 150 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.25 and the phasing value is less than 0.25.

56. The method of claim 12, wherein after about 150 repeated cycles of steps (b)-(e) the pre-phasing value is less than 0.20 and the phasing value is less than 0.20.

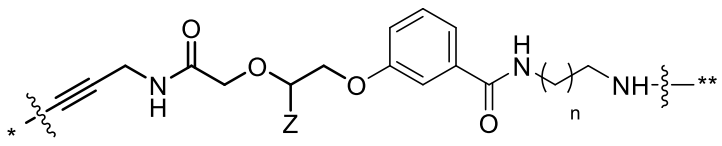
57. The method of claim 12, wherein the deblocking solution further comprises one or more buffer reagents selected from the group consisting of a primary amine, a secondary amine, a tertiary amine, a carbonate salt, a phosphate salt, and a borate salt, and combinations thereof.

58. The method of claim 57, wherein the buffer reagents are selected from the group consisting of ethanolamine (EA), tris(hydroxymethyl)aminomethane (Tris), glycine, a carbonate salt, a phosphate salt, a borate salt, 2-dimethylaminoethanol (DMEA), 2-diethylaminoethanol (DEEA), N,N,N′,N′-tetramethylethylenediamine (TEMED), and N,N,N′,N′-tetraethylethylenediamine (TEEDA), and combinations thereof.

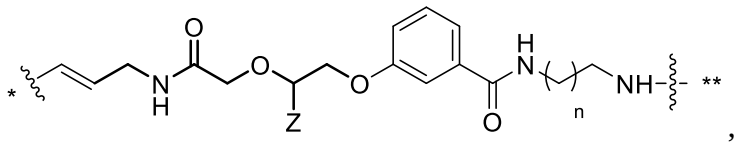
59. A sequencing kit comprising:

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

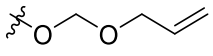
(i) each of at least three of the nucleotides independently comprises a base that is attached to a detectable label via a cleavable linker, and the cleavable linker is selected from the group consisting of:



and



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;

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

(iii) 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);

(b) an aqueous deblocking solution comprising a palladium catalyst, tris(hydroxypropyl)phosphine, and ascorbate that is suitable to chemically remove (i) 3′ AOM 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 palladium scavenger,

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