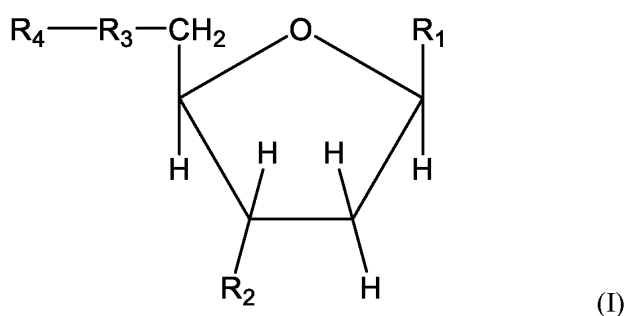


WHAT IS CLAIMED:

1. A method comprising:

a) contacting a polymerase with a template polynucleotide and a plurality of free nucleotides, wherein the template polynucleotide is hybridized to a complementary polynucleotide comprising a 3' end overhung by a 5' terminal fragment of the template polynucleotide, and the plurality of free nucleotides comprise a compound of Formula (I):



wherein R_1 comprises a nitrogenous base selected from adenine, guanine, cytosine, thymine and uracil; R_2 consists of $-O-R_2$ wherein R_2 is H or Z wherein Z is a removable protecting group comprising an azido group; R_3 comprises a linker comprising three or more phosphate groups; and R_4 comprises a fluorescent label;

wherein said contacting occurs under a complexation condition, the complexation condition effective to form a complex but not effective to form polymerization, wherein the complex comprises the polymerase, the template polynucleotide, the complementary polynucleotide, and one of the plurality of free nucleotides that is complementary to a first nucleotide of the 5' terminal fragment of the template polynucleotide;

b) detecting a signal from the fluorescent label; and

c) exposing the complex to a polymerization condition.

2. The method of claim 1, wherein R_2 consists of $-O-R_2$ wherein R_2 is Z wherein Z is a removable protecting group comprising an azido group.

3. The method of claim 1, wherein the template polynucleotide is one of a plurality of template polynucleotides attached to a substrate.

4. The method of claim 3, wherein the plurality of template polynucleotides attached to the substrate comprise a cluster of copies of a library polynucleotide.
5. The method of claim 1, further comprising:
repeating steps a) through c) one or more times.
6. The method of claim 1, wherein the polymerization condition comprises a concentration of Mg^{2+} ions, wherein the concentration of Mg^{2+} ions is in a range of about 0.1 mM to about 10 mM, or a concentration of Mn^{2+} ions, wherein the concentration of Mn^{2+} ions is in a range of about 0.1 mM to about 10 mM.
7. The method of claim 1, wherein the complexation condition comprises a non-catalytic metal cation.
8. The method of claim 7, wherein the non-catalytic metal cation is selected from the group consisting of one or more of Ca^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Eu^{2+} , Sr^{2+} , Ba^{2+} , Fe^{2+} , and Eu^{2+} .
9. The method of claim 7, wherein the concentration of the non-catalytic metal cation is less than or equal to about 10 mM.
10. The method of claim 1, wherein the complexation condition comprises a chelating agent.
11. The method of claim 10, wherein the chelating agent is selected from the group consisting of ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), nitriloacetic acid, tetrasodium iminodisuccinate, ethylene glycol tetraacetic acid, polyaspartic acid, ethylenediamine-N,N'-disuccinic acid (EDDS), methylglycindiacytic acid (MGDA), and a combination thereof.

12. The method of claim 10, wherein the complexation condition further comprises an inhibitor selected from the group consisting of a non-competitive inhibitor, a competitive inhibitor, and a combination thereof.

13. The method of claim 1, wherein the complexation condition comprises a pH that is less than about 6.

14. The method of claim 1, wherein the polymerization condition comprises a pH that is greater than or equal to about 6.

15. The method of claim 1, wherein the complexation condition comprises a non-competitive inhibitor.

16. The method of claim 15, wherein the non-competitive inhibitor is selected from the group consisting of an aminoglycoside, a pyrophosphate analog, a melanin, a phosphonoacetate, a hypophosphate, a rifamycin, and a combination thereof.

17. The method of claim 1, wherein the complexation condition comprises a competitive inhibitor.

18. The method of claim 17, wherein the competitive inhibitor is selected from the group consisting of aphidicolin, beta-D-arabinofuranosyl-CTP, amiloride, dehydroaltenusin, and a combination thereof.

19. The method of claim 1, wherein the complexation condition comprises a solvent additive.

20. The method of claim 19, wherein the solvent additive is selected from the group consisting of ethanol, methanol, tetrahydrofuran, dioxane, dimethylamine, dimethylformamide, dimethyl sulfoxide, lithium, L-cysteine, and a combination thereof.

21. The method of claim 1, wherein the complexation condition comprises deuterium.
22. The method of claim 2, wherein the 3'-hydroxy blocking group comprises a reversible terminator.
23. The method of claim 22, wherein the reversible terminator comprises an azidomethyl group or an acetal group.
24. The method of claim 22, further comprising:
removing the reversible terminator after the 3' end of the complementary polynucleotide is covalently bonded to a phosphate group of the linker.
25. The method of claim 1, wherein the free nucleotide further comprises a non-bridging thiol or a bridging nitrogen.
26. The method of claim 1, wherein the polymerase comprises a mutation.
27. The method of claim 26, wherein the mutation modifies speed of one or more of steps a) through c).