

CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of determining whether a test nucleotide is the next correct nucleotide comprising a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid, comprising the steps of:

(a) contacting the primed template nucleic acid with a first reaction mixture that comprises a mutant Family A DNA polymerase and the test nucleotide, wherein, if the test nucleotide is the next correct nucleotide, there is formed a complex comprising the primed template nucleic acid, the mutant Family A DNA polymerase and the test nucleotide,

wherein the mutant Family A DNA polymerase comprises either a polypeptide sequence comprising SEQ ID NO:12 in Motif A, or a polypeptide sequence comprising SEQ ID NO:14 in Motif C, and

wherein the mutant Family A DNA polymerase is substantially incapable of magnesium-catalyzed phosphodiester bond formation;

(b) measuring binding of the primed template nucleic acid to the mutant Family A DNA polymerase in the presence of the test nucleotide, without chemical incorporation of the test nucleotide into the primer of the primed template nucleic acid; and

(c) determining from the results of step (b) whether the test nucleotide is the next correct nucleotide.

2. The method of claim 1, wherein the mutant Family A DNA polymerase catalyzes formation of phosphodiester bonds in the presence of divalent manganese ions, and wherein the first reaction mixture does not contain a concentration of divalent manganese ions that promotes formation of phosphodiester bonds.

3. The method of any one of claims 1 or 2, wherein the test nucleotide comprises an exogenous label.

4. The method of claim 2, wherein the exogenous label of the test nucleotide comprises a fluorescent moiety, and wherein step (b) comprises measuring a fluorescent signal produced by the fluorescent moiety of the test nucleotide.

5. The method of any one of claims 1-4, wherein the mutant Family A DNA polymerase comprises an exogenous label, and wherein step (b) comprises detecting the exogenous label of the mutant Family A DNA polymerase.
6. The method of claim 5, wherein the exogenous label of the mutant Family A DNA polymerase comprises a fluorescent moiety, and wherein step (b) comprises measuring a fluorescent signal produced by the fluorescent moiety of the mutant Family A DNA polymerase.
7. The method of any one of claims 1-6, wherein the primer comprises a free 3' hydroxyl moiety.
8. The method of any one of claims 1-7, further comprising, after step (b), the step of replacing the first reaction mixture with a second reaction mixture that comprises a second polymerase and a second type of nucleotide, and then incorporating the second type of nucleotide into the primer of the primed template nucleic acid.
9. The method of claim 8, wherein the second type of nucleotide is a reversible terminator nucleotide that comprises a reversible terminator moiety, and wherein incorporation of the reversible terminator nucleotide produces a blocked primed template nucleic acid molecule.
10. The method of claim 9, further comprising the step of removing the reversible terminator moiety from the blocked primed template nucleic acid molecule to regenerate the primed template nucleic acid molecule.
11. The method of claim 9, further comprising repeating steps (a)-(c) using the blocked primed template nucleic acid molecule in place of the primed template nucleic acid.
12. The method of claim 10, further comprising repeating steps (a)-(c).

13. The method of claim 1, wherein the polypeptide sequence of the mutant Family A DNA polymerase is either SEQ ID NO:1 with the exception of having amino acid position 381 substituted by glutamate, or SEQ ID NO:1 with the exception of having amino acid position 558 substituted by glutamate.

14. The method of claim 1, wherein the polypeptide sequence of the mutant Family A DNA polymerase is either SEQ ID NO:2 with the exception of having amino acid position 364 substituted by glutamate, or SEQ ID NO:2 with the exception of having amino acid position 541 substituted by glutamate.

15. The method of claim 1, wherein the polypeptide sequence of the mutant Family A DNA polymerase is either SEQ ID NO:3 with the exception of having amino acid position 355 substituted by glutamate, or SEQ ID NO:3 with the exception of having amino acid position 532 substituted by glutamate.

16. The method of claim 1, wherein the first reaction mixture comprises divalent magnesium ion.

17. The method of any one of claims 1-16, wherein the first reaction mixture comprises Mg^{2+} ions, and wherein the primer comprises a 3' hydroxyl moiety.

18. A kit for identifying a next correct nucleotide to be incorporated into a primed template nucleic acid molecule, the kit comprising in packaged combination of one or more containers:

- (a) a mutant Family A DNA polymerase that forms a ternary complex with the primed template nucleic acid and the next correct nucleotide, but which is substantially incapable of magnesium-catalyzed phosphodiester bond formation, wherein the mutant Family A DNA polymerase comprises either a polypeptide sequence comprising SEQ ID NO:12 in Motif A, or a polypeptide sequence comprising SEQ ID NO:14 in Motif C;
- (b) four types of deoxyribonucleotide triphosphate molecules; and
- (c) four types of reversible terminator nucleotide.

19. The kit of claim 18, wherein the four reversible terminator nucleotide are four non-fluorescent reversible terminator nucleotides.
20. The kit of claim 18, wherein the mutant Family A DNA polymerase comprises a detectable label.
21. The kit of claim 18, wherein at least one of the four types of deoxyribonucleotide triphosphate molecules comprises a detectable label.
22. The kit of claim 18, wherein the mutant Family A DNA polymerase catalyzes phosphodiester bond formation in the presence of manganese ions.
23. The kit of claim 18, further comprising a chemical reagent that removes reversible terminator moieties from the four types of reversible terminator nucleotide.
24. The kit of claim 18, wherein each of the four different types of deoxyribonucleotide triphosphate molecules is incorporable by a DNA polymerase comprising magnesium-dependent polymerase activity.
25. The kit of claim 18, further comprising a second DNA polymerase that incorporates the next correct nucleotide into the primed template nucleic acid molecule.
26. The kit of claim 25, wherein the second DNA polymerase incorporates one of the four types of reversible terminator nucleotide as the next correct nucleotide.
27. The kit of claim 18, further comprising a flow cell.
28. The kit of claim 18, wherein the four types of deoxyribonucleotide triphosphate molecules comprise dATP, dGTP, dCTP, and either dTTP or dUTP; and wherein the four types of reversible terminator nucleotides comprise analogs of dATP, dGTP, dCTP, and either dTTP or dUTP, each analog comprising a 3'-ONH₂ reversible terminator moiety.

29. The kit of claim 18, wherein the polypeptide sequence comprising SEQ ID NO:12 comprises SEQ ID NO:3 with the exception of having amino acid position 355 substituted by glutamate.

30. The kit of claim 18, wherein the polypeptide sequence comprising SEQ ID NO:14 comprises SEQ ID NO:3 with the exception of having amino acid position 532 substituted by glutamate.

31. A mutant Family A DNA polymerase comprising a polypeptide sequence, said polypeptide sequence comprising SEQ ID NO:12 in Motif A,
wherein said mutant Family A DNA polymerase forms ternary complexes with primed template nucleic acid molecules and cognate nucleotides, and
wherein said mutant Family A DNA polymerase is substantially incapable of magnesium-catalyzed phosphodiester bond formation.

32. The mutant Family A DNA polymerase of claim 31, wherein the mutant Family A DNA polymerase catalyzes formation of phosphodiester bonds in the presence of divalent manganese ions.

33. The mutant Family A DNA polymerase of claim 31, wherein the polypeptide sequence of the mutant Family A DNA polymerase is selected from the group consisting of:
SEQ ID NO:1 with the exception of having amino acid position 381 substituted by glutamate,
SEQ ID NO:2 with the exception of having amino acid position 364 substituted by glutamate, and
SEQ ID NO:3 with the exception of having amino acid position 355 substituted by glutamate.

34. The mutant Family A DNA polymerase of claim 33, further comprising a reporter moiety attached thereto.

35. A mutant Family A DNA polymerase comprising a polypeptide sequence, said polypeptide sequence comprising SEQ NO:14 in Motif C,

wherein said mutant Family A DNA polymerase forms ternary complexes with primed template nucleic acid molecules and cognate nucleotides, and

wherein said mutant Family A DNA polymerase is substantially incapable of magnesium-catalyzed phosphodiester bond formation.

36. The mutant Family A DNA polymerase of claim 35, wherein the polypeptide sequence of the mutant Family A DNA polymerase is selected from the group consisting of:

SEQ ID NO:1 with the exception of having amino acid position 558 substituted by glutamate,

SEQ ID NO:2 with the exception of having amino acid position 541 substituted by glutamate, and

SEQ ID NO:3 with the exception of having amino acid position 532 substituted by glutamate.

37. The mutant Family A DNA polymerase of claim 36, further comprising a reporter moiety attached thereto.