CLAIMS

1. A polynucleotide sequencing method comprising:

(a) introducing a first chain extending enzyme and a mixture of blocked, labeled nucleotides to a flow cell comprising a site at which multiple template polynucleotide strands having the same nucleotide sequence are bound to a surface of the flow cell, wherein the first chain extending enzyme is configured to incorporate an appropriate one of the blocked, labeled nucleotides into copy polynucleotide strands, based on the sequence of the template strands to which the copy polynucleotide strands correspond;

(b) washing unincorporated blocked, labeled nucleotides away from the flow cell;

(c) introducing a composition comprising a mixture of blocked, unlabeled nucleotides to the flow cell during or after the washing away of the unincorporated blocked, labeled nucleotides from the flow cell, wherein a blocked, unlabeled nucleotide of the mixture of the blocked, unlabeled nucleotides is available for incorporation into the copy polynucleotide strands, based on a sequence of the template strands to which the copy polynucleotide strands correspond, provided that the previously incorporated nucleotide in the copy strand, if any, is not blocked; and

(d) detecting the identity of the one blocked, labeled nucleotide incorporated into the copy strands, if any, while the mixture of blocked, unlabeled nucleotides is incubated with the flow cell;

(e) removing a label and blocking moiety from any blocked, labeled nucleotide incorporated into the copy strands, and removing a blocking moiety from any blocked, unlabeled nucleotide incorporated into the copy strands; and

(f) repeating steps (a)-(e),

wherein the composition comprising the mixture of blocked unlabeled nucleotides has a pH of about 9 to about 9.5 and comprises a buffer, a detergent, magnesium sulfate, EDTA, and an antioxidant,

wherein the error rate of detected nucleotide incorporation is less than 0.4% over 80 cycles when step (a) is performed for 7.5 seconds in each cycle,

wherein the first chain extending enzyme comprises a recombinant DNA polymerase comprising an amino acid sequence that is at least 80% identical to a 9°N DNA polymerase amino acid sequence of SEQ ID NO:1, wherein the recombinant DNA polymerase comprises an amino acid substitution mutation at a position functionally equivalent to Tyr497 and at least on amino acid substitution mutation at a position functionally equivalent to Phe 152, Val278, Met329, Val471, Thr514, Leu631, or Glu734 in the 9°N DNA polymerase amino acid sequence.

2. The method of claim 1, wherein the composition comprising the mixture of blocked, unlabeled nucleotides is introduced to the flow cell after the washing away of the unincorporated blocked, labeled nucleotides from the flow cell.

3. The method of claim 1, wherein the washing the unincorporated blocked, labeled nucleotides away from the flow cell comprises the introducing the composition comprising the mixture of blocked, unlabeled nucleotides to the flow cell.

4. The method of claim 1, wherein the blocked, unlabeled nucleotide of the mixture of the blocked, unlabeled nucleotides is available for incorporation into the copy polynucleotide strands when the identity of the blocked, labeled nucleotide incorporated into the copy strands is being detected.

5. The method of claim 1, wherein detecting the identity of the blocked, labeled nucleotide incorporated into the copy strands comprises imaging the flow cell.

6. The method of claim 1, wherein the detecting the identity of the blocked, labeled nucleotide incorporated into the copy strands occurs over a period of time of 120 seconds or less.

7. The method of claim 1, wherein the detecting the identity of the blocked, labeled nucleotide incorporated into the copy strands occurs over a period of time of 10 seconds or more.

8. The method of claim 1, wherein the first chain extending enzyme and the mixture of blocked, labeled nucleotides are incubated with the flow cell for 15 seconds or less prior to the washing the unincorporated blocked, labeled nucleotides away from the flow cell.

9. The method of claim 1, wherein the first chain extending enzyme and the mixture of blocked, labeled nucleotides are incubated with the flow cell for 10 seconds or less prior to the washing the unincorporated blocked, labeled nucleotides away from the flow cell.

10. The method of claim 1, wherein the first chain extending enzyme and the mixture of blocked, labeled nucleotides are incubated with the flow cell for 7.5 seconds or less prior to the washing the unincorporated blocked, labeled nucleotides away from the flow cell.

11. The method of claim 1, wherein the composition comprising the mixture of blocked, unlabeled nucleotides comprises a second chain extending enzyme configured to incorporate an appropriate one of the blocked, unlabeled nucleotides into the copy polynucleotide strands, based on a sequence of the template strands

to which the copy polynucleotide strands correspond, provided that the previously incorporated nucleotide in the copy strand, if any, is not blocked.

12. The method of claim 11, wherein the first chain extending enzyme and the second chain extending enzyme are the same enzymes.

13. The method of claim 1, wherein the buffer comprises Tris buffer, wherein the detergent comprises CHAPS, and wherein the antioxidant includes ascorbate, acetovanillone, or ascorbate and acetovanillone.

14. The method of claim 13, wherein the composition comprises ascorbate and acetovanillone.

15. The method of claim 14, wherein the composition comprises 3 to 20 mM ascorbate and 10 to 15 mM acetovanillone.

16. The method of claim 1, wherein the first chain extending enzyme comprises Pol812.

17. The method of claim 1, wherein the error rate of detected nucleotide incorporation is 0.35% or less over 80 cycles when step (a) is performed for 7.5 seconds in each cycle.