

strands of DNA are contacted under hybridizing conditions with an array of up to many thousands of defined probe sequences immobilized to a solid support. Short duplexes formed in the procedure can be detected and analyzed by a computer for assembly into a contiguous sequence. Sequencing-by-synthesis procedures have taken various forms and also have been successful. Automation and improvements in the chemistry of labeled nucleotide addition, detection, and subsequent chemical processing have revolutionized procedures for acquisition of raw sequence information. In some cases, only relatively short stretches of nucleic acid sequence are determined, but massively parallel processing provides the information needed to deduce nucleic acid sequence on the scale of a genome.

Generally speaking, there is a need for extended sequencing read-length with improved base-calling accuracy across different sequencing platforms. The present invention addresses this need.

Brief Summary

In one aspect, the disclosure relates to a method of determining whether a test nucleotide is the next correct nucleotide including a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid. The method includes the steps of: (a) contacting the primed template nucleic acid with a first reaction mixture that includes a DNA polymerase; (b) contacting the primed template nucleic acid from step (a) with a second reaction mixture that includes a first test nucleotide under conditions for forming a complex comprising the primed template nucleic acid and the DNA polymerase; (c) contacting the complex from step (b) with a third reaction mixture that comprises the first test nucleotide and a second test nucleotide under conditions for forming a complex comprising the primed template nucleic acid and the DNA polymerase; (d) measuring binding of the primed template nucleic acid to the DNA polymerase of the first, second, and third reaction mixtures at one or more points during each of steps (a), (b) and (c), respectively, but without chemical incorporation of the test nucleotide into the primer; and (e) determining whether the test nucleotide is the next correct nucleotide using the measured binding results from step (d). According to one generally preferred embodiment, the test nucleotide can be a native nucleotide that does not include any added fluorescent label, and step (d) does not include measuring differences in fluorescence or absorbance signals from a conformationally sensitive

dye that changes optical properties as a result of the DNA polymerase binding to the test nucleotide. According to another generally preferred embodiment, the first reaction mixture does not include the test nucleotide. Preferably, the method further includes the step of (f) chemically incorporating the test nucleotide into the primer of the primed template nucleic acid by formation of a phosphodiester bond. More preferably, the method further involves repeating steps (a)-(f). For example, steps (a)-(f) can be repeated at least 50 times. Alternatively, the method can further include the step of (g) repeating steps (a)-(f) using a second test nucleotide in place of the first test nucleotide, where the first test nucleotide and the second test nucleotide are different from each other. In other embodiments, where the first reaction mixture does not include the test nucleotide, the method further includes the step of (f) replacing the second reaction mixture with a third reaction mixture that includes a reversible terminator nucleotide having a reversible terminator moiety, and a DNA polymerase different from the DNA polymerase of the second reaction mixture. More preferably, the method further includes the step of (g) incorporating the reversible terminator nucleotide into the primer by formation of a phosphodiester bond. Yet more preferably, the method further includes the step of (h) removing the reversible terminator moiety from the reversible terminator nucleotide that incorporated into the primer. Still yet more preferably, the method further involves repeating steps (a)-(h) at least 50 times. In other embodiments, where the first reaction mixture does not include the test nucleotide, the primed template nucleic acid can be immobilized to a solid support, and step (b) can involve moving the solid support from a vessel containing the first reaction mixture to a different vessel containing the second reaction mixture. In other embodiments, where the first reaction mixture does not include the first test nucleotide, the primed template nucleic acid can be immobilized to a solid support, and each of steps (a), (b), and (c) can include flowing reaction mixtures over the primed template nucleic acid. In other embodiments, where the first reaction mixture does not include the first test nucleotide, step (d) can include continuously measuring binding of the primed template nucleic acid to the DNA polymerase during each of steps (a), (b) and (c). In other embodiments, where the first reaction mixture does not include the first test nucleotide, step (d) can include measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the difference between the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the

measured binding resulting from step (a). In other embodiments, where the first reaction mixture does not include the first test nucleotide, step (d) includes measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the ratio of the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the measured binding resulting from step (a). In other embodiments, where the first reaction mixture does not include the first test nucleotide, step (d) includes measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the time-dependent rate of change of the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the measured binding resulting from step (a). In other embodiments, where the first reaction mixture does not include the first test nucleotide, the test nucleotide can be a native nucleotide selected from the group consisting of dATP, dGTP, dCTP, dTTP, and dUTP. In other embodiments, where the first reaction mixture does not include the test nucleotide, the test nucleotide does not include an exogenous fluorescent label. In other embodiments, where the first reaction mixture does not include the first test nucleotide, the test nucleotide can be an unlabeled test nucleotide that does not include an exogenous label. In other embodiments, where the first reaction mixture does not include the first test nucleotide, the test nucleotide is a nucleotide analog. More preferably, the nucleotide analog includes a reversible terminator moiety. Still more preferably, the nucleotide analog that includes the reversible terminator moiety further includes a fluorescent label. In other embodiments, where the first reaction mixture does not include the first test nucleotide, the primed template nucleic acid is immobilized to a surface, and step (d) involves measuring changes in refractive index to measure binding of the primed template nucleic acid to the DNA polymerase. More preferably, step (d) involves measuring changes in refractive index by interferometry or surface plasmon resonance sensing. According to a different generally preferred embodiment, the DNA polymerase of the first reaction mixtures is free of added fluorescent label. According to a different generally preferred embodiment, the DNA polymerase of the first reaction mixtures is free of added fluorescent label that changes properties after the DNA polymerase binds to a nucleotide. In other embodiments, where the first reaction mixture does not include the first test nucleotide, and where the test nucleotide can be an unlabeled test nucleotide that does not include an exogenous label, the DNA polymerase of the first

reaction mixture can be free of added fluorescent label that changes properties after the DNA polymerase binds to a nucleotide. More preferably, the primed template nucleic acid is immobilized to a surface, and step (d) includes measuring changes in refractive index to measure binding of the primed template nucleic acid to the DNA polymerase. According to a different generally preferred embodiment, the first reaction mixture further includes the first test nucleotide at a concentration below that needed to achieve maximal ternary complex formation when the test nucleotide is the next correct nucleotide, and the second reaction mixture includes the test nucleotide at a concentration sufficient to achieve maximal ternary complex formation when the test nucleotide is the next correct nucleotide. According to a different generally preferred embodiment, step (e) includes determining that the test nucleotide is the next correct nucleotide if the measured binding resulting from step (b) exceeds the measured binding resulting from step (a).

In another aspect, the disclosure relates to a method of identifying the next correct nucleotide including a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid. The method includes the steps of: (a) contacting the primed template nucleic acid with a first reaction mixture that includes a DNA polymerase; (b) contacting the primed template nucleic acid from step (a) with a second reaction mixture that includes a first test nucleotide; (c) contacting the primed template nucleic acid from step (b) with a third reaction mixture that includes the first test nucleotide, and a second test nucleotide; (d) contacting the primed template nucleic acid from step (c) with a fourth reaction mixture that includes the first test nucleotide, the second test nucleotide, and a third test nucleotide; (e) contacting the primed template nucleic acid from step (d) with a fifth reaction mixture that includes the first test nucleotide, the second test nucleotide, the third test nucleotide, and a fourth test nucleotide; (f) measuring binding of the primed template nucleic acid to the DNA polymerase at one or more points during each of steps (a)-(e), without chemical incorporation of the first test nucleotide into the primer; and (g) identifying the next correct nucleotide as: (i) the first test nucleotide if the measured binding resulting from step (b) exceeds the measured binding resulting from step (a), or (ii) the second test nucleotide if the measured binding resulting from step (c) exceeds the measured binding resulting from step (b), or (iii) the third test nucleotide if the measured binding resulting from step (d) exceeds the measured binding resulting from step (c), or (iv) the fourth test

nucleotide if the measured binding resulting from step (e) exceeds the measured binding resulting from step (d). According to one generally preferred embodiment, the method further includes, after step (f), the step of (h) chemically incorporating the next correct nucleotide into the primer by phosphodiester bond formation. More preferably, each of the test nucleotides is different from the others, and each of the test nucleotides is selected from the group consisting of dATP, dGTP, dCTP, dTTP, and dUTP. Alternatively, each of the test nucleotides is different from the others, and each test nucleotide is a native nucleotide selected from the group consisting of dATP, dGTP, dCTP, and dTTP. Alternatively, each of the test nucleotides is different from the others, and none of the test nucleotides includes an exogenous fluorescent label. Alternatively, each of the test nucleotides is different from the others, and where each test nucleotide is a nucleotide analog including a reversible terminator moiety. Alternatively, the next correct nucleotide incorporated into the primer includes a reversible terminator moiety. Preferably, the next correct nucleotide incorporated into the primer includes a reversible terminator moiety. According to a different generally preferred embodiment, when the method further includes, after step (f), the step of (h) chemically incorporating the next correct nucleotide into the primer by phosphodiester bond formation, none of the test nucleotides includes a reversible terminator moiety, and between step (f) and step (h) there is the step of (i) removing any test nucleotide that bound to the primed template nucleic acid, and then adding at least one nucleotide that includes a reversible terminator moiety. According to a different generally preferred embodiment, when the method further includes, after step (f), the step of (h) chemically incorporating the next correct nucleotide into the primer by phosphodiester bond formation, the polymerase used in steps (a)-(e) can be different from the polymerase used in step (h).

In another aspect, the disclosure relates to a method of determining whether a test nucleotide is the next correct nucleotide including a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid. The method includes the steps of: (a) contacting the primed template nucleic acid with a first reaction mixture that includes a first DNA polymerase; (b) contacting the primed template nucleic acid from step (a) with a second reaction mixture that includes a second DNA polymerase and the test nucleotide, where the second DNA polymerase includes a label that is absent from the first DNA polymerase; (c) measuring binding of the primed template nucleic acid to

the second DNA polymerase by detecting the label present in a complex including the primed template nucleic acid at one or more points during steps (a) and (b), respectively, but without chemical incorporation of the test nucleotide into the primer; and (d) determining whether the test nucleotide is the next correct nucleotide using the measured binding results from step (c). According to one generally preferred embodiment, the amino acid sequences of the first and second DNA polymerases can be the same. According to a different generally preferred embodiment, each of steps (a) and (b) occurs under buffer conditions that stabilize formation of ternary complexes, and that destabilize formation of binary complexes. Preferably, each of steps (a) and (b) occurs under high salt buffer conditions. Still more preferably, the first DNA polymerase does not include an exogenous detectable label. According to a different generally preferred embodiment, the method further includes repeating each of steps (a)-(c) three times, with a different test nucleotide each time. Preferably, the label of the second DNA polymerase is a fluorescent label. More preferably, step (c) does not include measuring a fluorescent signal from a conformationally sensitive dye that changes optical properties as a result of the second DNA polymerase binding to a cognate nucleotide. According to a different generally preferred embodiment, the method further includes removing the test nucleotide and any polymerase bound to the primed template nucleic acid. Preferably, there is the further step of contacting the primed template nucleic acid with a third reaction mixture that includes a third DNA polymerase and a reversible terminator nucleotide, and then incorporating the reversible terminator nucleotide into the primer using the third DNA polymerase to produce a reversibly terminated primer. More preferably, the method further includes repeating steps (a)-(d) using the primed template nucleic acid that includes the reversibly terminated primer in each of steps (a)-(c).

In yet another aspect, the disclosure relates to a method of incorporating a nucleotide with a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid molecule. The method includes the steps of: (a) contacting the primed template nucleic acid molecule with a first reaction mixture that includes a DNA polymerase; (b) contacting the primed template nucleic acid molecule from step (a) with a second reaction mixture that includes a first test nucleotide, without incorporating the first test nucleotide into the primer; (c) measuring binding of the primed template nucleic acid molecule to the DNA polymerase at one or more points during each of steps (a) and

(b), without chemical incorporation of the first test nucleotide into the primer, to establish whether a ternary complex formed in step (b); and (d) selecting one of the following two options, (i) if it is established in step (c) that the ternary complex did not form in step (b), then contacting the primed template

WHAT IS CLAIMED IS:

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, said method comprising the steps of:

(a) contacting the primed template nucleic acid with a first reaction mixture that comprises a DNA polymerase, thereby forming a binary complex comprising the primed template nucleic acid and the polymerase;

(b) contacting the binary complex from step (a) with a second reaction mixture that comprises a first test nucleotide under conditions for forming a complex comprising the primed template nucleic acid and the DNA polymerase;

(c) contacting the complex from step (b) with a third reaction mixture that comprises the first test nucleotide and a second test nucleotide under conditions for forming a complex comprising the primed template nucleic acid and the DNA polymerase;

(d) measuring binding of the primed template nucleic acid to the DNA polymerase of the first, second, and third reaction mixtures at one or more points during each of steps (a), (b) and (c), respectively, but without chemical incorporation of the test nucleotide into the primer; and

(e) determining whether the test nucleotide is the next correct nucleotide using the measured binding results from step (d),

wherein either

(i) the first reaction mixture is free of the first test nucleotide, or

(ii) the first and second reaction mixtures each comprise the first test nucleotide, the first test nucleotide of the first reaction mixture being present at a concentration lower than the concentration of the first test nucleotide of the second reaction mixture.

2. The method of claim 1, wherein the test nucleotide is a native nucleotide that does not comprise any added fluorescent label, and wherein step (d) does not comprise measuring differences in fluorescence or absorbance signals from a conformationally sensitive dye that changes optical properties as a result of the DNA polymerase binding to the test nucleotide.

3. The method of claim 1, wherein the first reaction mixture is free of the first test nucleotide.
4. The method of claim 3, further comprising the step of (f) chemically incorporating the test nucleotide into the primer of the primed template nucleic acid by formation of a phosphodiester bond.
5. The method of claim 4, further comprising repeating steps (a)-(f).
6. The method of claim 5, wherein steps (a)-(f) are repeated at least 50 times.
7. The method of claim 4, further comprising the step of (g) repeating steps (a)-(f) using a second test nucleotide in place of the first test nucleotide, wherein the first test nucleotide and the second test nucleotide are different from each other.
8. The method of claim 3, further comprising the step of (f) replacing the second reaction mixture with a third reaction mixture that comprises a reversible terminator nucleotide comprising a reversible terminator moiety, and a DNA polymerase different from the DNA polymerase of the second reaction mixture.
9. The method of claim 8, further comprising the step of (g) incorporating the reversible terminator nucleotide into the primer by formation of a phosphodiester bond.
10. The method of claim 9, further comprising the step of (h) removing the reversible terminator moiety from the reversible terminator nucleotide that incorporated into the primer.
11. The method of claim 10, further comprising repeating steps (a)-(h) at least 50 times.
12. The method of claim 3, wherein the primed template nucleic acid is immobilized to a solid support, and wherein step (b) comprises moving the solid support from a vessel containing the first reaction mixture to a different vessel containing the second reaction mixture.

13. The method of claim 3, wherein the primed template nucleic acid is immobilized to a solid support, and wherein each of steps (a), (b), and (c) comprise flowing reaction mixtures over the primed template nucleic acid.

14. The method of claim 3, wherein step (d) comprises continuously measuring binding of the primed template nucleic acid to the DNA polymerase during each of steps (a), (b), and (c).

15. The method of claim 3, wherein step (d) comprises measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the difference between the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the measured binding resulting from step (a).

16. The method of claim 3, wherein step (d) comprises measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the ratio of the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the measured binding resulting from step (a).

17. The method of claim 3, wherein step (d) comprises measuring optical signals indicating binding of the primed template nucleic acid to the DNA polymerase and then calculating the time-dependent rate of change of the measured optical signals to determine whether the measured binding resulting from step (b) exceeds the measured binding resulting from step (a).

18. The method of claim 3, wherein the test nucleotide is a native nucleotide selected from the group consisting of dATP, dGTP, dCTP, dTTP, and dUTP.

19. The method of claim 3, wherein the test nucleotide does not comprise an exogenous fluorescent label.

20. The method of claim 3, wherein the test nucleotide is an unlabeled test nucleotide that does not comprise an exogenous label.

21. The method of claim 3, wherein the test nucleotide is a nucleotide analog.

22. The method of claim 21, wherein the nucleotide analog comprises a reversible terminator moiety.

23. The method of claim 22, wherein the nucleotide analog that comprises the reversible terminator moiety further comprises a fluorescent label.

24. The method of claim 3, wherein the primed template nucleic acid is immobilized to a surface, and wherein step (d) comprises measuring changes in refractive index to measure binding of the primed template nucleic acid to the DNA polymerase.

25. The method of claim 24, wherein step (d) comprises measuring changes in refractive index by interferometry or surface plasmon resonance sensing.

26. The method of claim 1, wherein the DNA polymerase of the first reaction mixtures is free of added fluorescent label.

27. The method of claim 1, wherein the DNA polymerase of the first reaction mixtures is free of added fluorescent label that changes properties after the DNA polymerase binds to a nucleotide.

28. The method of claim 20, wherein the DNA polymerase of the first reaction mixture is free of added fluorescent label that changes properties after the DNA polymerase binds to a nucleotide.

29. The method of claim 28, wherein the primed template nucleic acid is immobilized to a surface, and wherein step (d) comprises measuring changes in refractive index to measure binding of the primed template nucleic acid to the DNA polymerase.

30. The method of claim 1, wherein the first and second reaction mixtures each comprise the test nucleotide, wherein the first reaction mixture comprises the test nucleotide at a concentration below that needed to achieve maximal ternary complex formation when the test nucleotide is the next correct nucleotide, and wherein the second reaction mixture comprises the test nucleotide at a concentration sufficient to achieve maximal ternary complex formation when the test nucleotide is the next correct nucleotide.

31. The method of claim 1, wherein step (e) comprises determining that the test nucleotide is the next correct nucleotide if the measured binding resulting from step (b) exceeds the measured binding resulting from step (a).

32. A method of identifying 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, the method comprising the steps of:

(a) contacting the primed template nucleic acid with a first reaction mixture that comprises a DNA polymerase, thereby forming a first complex comprising the primed template nucleic acid and the DNA polymerase;

(b) contacting the first complex from step (a) with a second reaction mixture that comprises a first test nucleotide, thereby forming a second complex comprising the primed template nucleic acid and the DNA polymerase;

(c) contacting the second complex from step (b) with a third reaction mixture that comprises the first test nucleotide, and a second test nucleotide, thereby forming a third complex comprising the primed template nucleic acid and the polymerase;

(d) contacting the third complex from step (c) with a fourth reaction mixture that comprises the first test nucleotide, the second test nucleotide, and a third test nucleotide, thereby forming a fourth complex comprising the primed template nucleic acid and the DNA polymerase;

(e) contacting the fourth complex from step (d) with a fifth reaction mixture that comprises the first test nucleotide, the second test nucleotide, the third test nucleotide, and a fourth test nucleotide, thereby forming a fifth complex comprising the primed template nucleic acid and the DNA polymerase;

(f) measuring binding of the primed template nucleic acid to the DNA polymerase at one or more points during each of steps (a)-(e), without chemical incorporation of the first test nucleotide into the primer;

(g) identifying the next correct nucleotide as:

(i) the first test nucleotide if the measured binding resulting from step (b) exceeds the measured binding resulting from step (a), or

(ii) the second test nucleotide if the measured binding resulting from step (c) exceeds the measured binding resulting from step (b), or

(iii) the third test nucleotide if the measured binding resulting from step (d) exceeds the measured binding resulting from step (c), or

(iv) the fourth test nucleotide if the measured binding resulting from step (e) exceeds the measured binding resulting from step (d),

wherein the first reaction mixture is free of the first test nucleotide, the second test nucleotide, the third test nucleotide, and the fourth test nucleotide.

33. The method of claim 32, further comprising, after step (f), step (h) chemically incorporating the next correct nucleotide into the primer by phosphodiester bond formation.

34. The method of claim 33, wherein each of the test nucleotides is different from the others, and wherein each of the test nucleotides is selected from the group consisting of dATP, dGTP, dCTP, dTTP, and dUTP.

35. The method of claim 33, wherein each of the test nucleotides is different from the others, and wherein each test nucleotide is a native nucleotide selected from the group consisting of dATP, dGTP, dCTP, and dTTP.

36. The method of claim 33, wherein each of the test nucleotides is different from the others, and wherein none of the test nucleotides comprises an exogenous fluorescent label.

37. The method of claim 33, wherein each of the test nucleotides is different from the others, and wherein each test nucleotide is a nucleotide analog comprising a reversible terminator moiety.

38. The method of claim 33, wherein the next correct nucleotide incorporated into the primer comprises a reversible terminator moiety.

39. The method of claim 34, wherein the next correct nucleotide incorporated into the primer comprises a reversible terminator moiety.

40. The method of claim 33, wherein none of the test nucleotides comprises a reversible terminator moiety, and wherein between step (f) and step (h) there is step (i) removing any test nucleotide that bound to the primed template nucleic acid, and then adding at least one nucleotide that comprises a reversible terminator moiety.

41. The method of claim 33, wherein the polymerase used in steps (a)-(e) is different from the polymerase used in step (h).

42. 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, said method comprising the steps of:

(a) contacting the primed template nucleic acid with a first reaction mixture that comprises a first DNA polymerase, thereby forming a binary complex comprising the primed template nucleic acid and the first DNA polymerase;

(b) contacting the binary complex from step (a) with a second reaction mixture that comprises a second DNA polymerase, but not the first DNA polymerase, and the test nucleotide, wherein the second DNA polymerase comprises a label that is absent from the first DNA polymerase;

(c) detecting binding of the primed template nucleic acid to the second DNA polymerase and the test nucleotide by detecting the label of the second polymerase in a ternary complex comprising the primed template nucleic acid without chemical incorporation of the test nucleotide into the primer; and

(d) determining whether the test nucleotide is the next correct nucleotide using the measured binding results from step (c),

wherein either

- (i) the first reaction mixture is free of the test nucleotide, or
- (ii) the first and second reaction mixtures each comprise the test nucleotide, the test nucleotide of the first reaction mixture being present at a concentration lower than the concentration of the test nucleotide of the second reaction mixture.

43. The method of claim 42, wherein the amino acid sequences of the first and second DNA polymerases are the same.

44. The method of claim 42, wherein each of steps (a) and (b) occur under buffer conditions that stabilize formation of ternary complexes, and that destabilize formation of binary complexes.

45. The method of claim 44, wherein each of steps (a) and (b) occur under high salt buffer conditions.

46. The method of claim 45, wherein the first DNA polymerase does not comprise an exogenous detectable label.

47. The method of claim 42, further comprising repeating each of steps (a)-(c) three times, with a different test nucleotide each time.

48. The method of claim 47, wherein the label of the second DNA polymerase is a fluorescent label.

49. The method of claim 48, wherein the step (c) does not comprise measuring a fluorescent signal from a conformationally sensitive dye that changes optical properties as a result of the second DNA polymerase binding to a cognate nucleotide.

50. The method of claim 42, further comprising removing the test nucleotide and any polymerase bound to the primed template nucleic acid.

51. The method of claim 50, further comprising contacting the primed template nucleic acid with a third reaction mixture that comprises a third DNA polymerase and a reversible terminator nucleotide, and then incorporating the reversible terminator nucleotide into the primer using the third DNA polymerase to produce a reversibly terminated primer.

52. The method of claim 51, further comprising repeating steps (a)-(d) using the primed template nucleic acid comprising the reversibly terminated primer in each of steps (a)-(c).

53. A method of incorporating a nucleotide with a base complementary to the next base in a template strand immediately downstream of a primer in a primed template nucleic acid molecule, the method comprising the steps of:

(a) contacting the primed template nucleic acid molecule with a first reaction mixture that comprises a DNA polymerase, thereby forming a binary complex comprising the primed template nucleic acid molecule and the DNA polymerase;

(b) contacting the binary complex from step (a) with a second reaction mixture that comprises a first test nucleotide, without incorporating the first test nucleotide into the primer, thereby forming a second complex comprising the primed template nucleic acid molecule and the DNA polymerase;

(c) measuring binding of the primed template nucleic acid molecule to the DNA polymerase at one or more points during each of steps (a) and (b), without chemical incorporation of the first test nucleotide into the primer, to establish whether the second complex that formed in step (b) is a ternary complex comprising the first test nucleotide;

(d) selecting one of the following two options,

(i) if it is established in step (c) that the second complex is not the ternary complex, then contacting the second complex from step (b) with a third reaction mixture that comprises the DNA polymerase in combination with the first test nucleotide and a second test nucleotide, the second test nucleotide being different from the first test nucleotide; and

(ii) if it is established in step (c) that the second complex is the ternary complex, then performing an incorporation reaction to incorporate the first test nucleotide into the primer without first contacting the primed template nucleic acid molecule from step (b) with the third reaction mixture,

wherein either

the first reaction mixture is free of the first test nucleotide, or
the first and second reaction mixtures each comprise the first test nucleotide, the first test nucleotide of the first reaction mixture being present at a concentration lower than the concentration of the first test nucleotide of the second reaction mixture.

54. The method of claim 53, wherein the primed template nucleic acid molecule is immobilized to a solid support.

55. The method of claim 54, wherein the fourth reaction mixture comprises a polymerase different from the polymerase of the first and second reaction mixtures.

56. The method of claim 54, wherein the fourth reaction mixture comprises a polymerase different from the polymerase of the first and second reaction mixtures, and wherein the nucleotide incorporated into the primer in step (d)(ii) comprises a reversible terminator moiety.

57. The method of claim 53, wherein the primed template nucleic acid molecule is immobilized to a solid support, and wherein contacting steps (a) and (b) each comprise flowing the reaction mixtures over the primed template nucleic acid molecule immobilized to the solid support.

58. The method of claim 53, wherein the primed template nucleic acid molecule is immobilized to a solid support, and wherein contacting steps (a) and (b) each comprise moving the solid support from a vessel containing the first reaction mixture to a vessel containing the second reaction mixture.

59. The method of claim 53, wherein the first and second reaction mixtures each comprise the same concentration of the polymerase.

60. The method of claim 53, wherein the fourth reaction mixture comprises a polymerase different from the polymerase of the first and second reaction mixtures.
61. The method of claim 53, wherein step (c) comprises measuring by either of interferometry or surface plasmon resonance sensing.
62. The method of claim 53, wherein the first test nucleotide is a native nucleotide.
63. The method of claim 53, wherein the first test nucleotide comprises a reversible terminator moiety.
64. The method of claim 53, wherein the nucleotide incorporated in step (d)(ii) is selected from the group consisting of a native nucleotide and a nucleotide comprising a reversible terminator.