REAGENTS FOR MASSIVELY PARALLEL NUCLEIC ACID SEQUENCING

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/108,207, filed October 30, 2020, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Sequences of nucleic acid molecules may be determined using massively parallel sequencing. Massively parallel sequencing may be performed using sequencing by synthesis in which, in a primer extension reaction, a polymerizing enzyme adds nucleotides sequentially to a growing strand to yield a strand that is complementary to a template strand, and the nucleotides being incorporated are detected. Such sequences may be used in various applications, such as, for example, disease (e.g., cancer) diagnostics.

SUMMARY

[0002a] In one aspect, the present disclosure refers to a method of analyzing a nucleic acid sequence, the method comprising: (a) providing a fluorescently-labeled nucleotide conjugate comprising (i) a core, (ii) a plurality of nucleotide moieties coupled to the core, and (iii) one or more fluorophores coupled to the core; (b) bringing the nucleic acid sequence in contact with the fluorescently-labeled nucleotide conjugate and a polymerizing enzyme under conditions sufficient to form a binding complex comprising (i) the polymerizing enzyme and (ii) a nucleotide of the nucleic acid sequence bound to a nucleotide moiety of the plurality of nucleotide moieties of the fluorescently-labeled nucleotide conjugate, wherein the binding complex persists for a persistence time of 1 second or more before it is removed; (c) contacting the binding complex with an imaging reagent, wherein the imaging reagent comprises a photobleaching reducing agent; and (d) obtaining a fluorescent image of the binding complex in a presence of the imaging reagent.

[0003] Aspects disclosed herein provide methods of analyzing a nucleic acid, the method comprising: (a) bringing a primed nucleic acid sequence into contact with a fluorescently-labeled nucleotide conjugate under conditions sufficient to form a binding complex comprising a first nucleotide of the primed nucleic acid molecule bound to a second nucleotide of the fluorescently-labeled nucleotide conjugate; (b) contacting the binding complex with an imaging reagent; and (c) obtaining an image of the binding complex in a

presence of the imaging reagent, thereby reducing a risk of photo-bleaching of the fluorescently-labeled nucleotide conjugate as compared to a risk of photo-bleaching under like conditions in absence of the imaging reagent.

[0004] In some embodiments, the method further comprises identifying the first nucleotide by analyzing the image obtained in (c). In some embodiments, the imaging reagent comprises ascorbic acid. In some embodiments, the ascorbic acid comprises sodium ascorbate. In some embodiments, the concentration of the ascorbic acid is at least 20 mM. In some embodiments, the concentration of the ascorbic acid is between about 10mM and about 100 mM. In some embodiments, the concentration of the ascorbic acid is about 50 mM. In some embodiments, the fluorescently-labeled nucleotide conjugate comprises: (i) a core, (ii) a plurality of the second nucleotide coupled thereto, and (ii) one or more fluorophores directly coupled to the core. In some embodiments, the fluorescently-labeled nucleotide conjugate

CLAIMS

What is claimed:

1. A method of analyzing a nucleic acid sequence, the method comprising:
   (a) providing a fluorescently-labeled nucleotide conjugate comprising (i) a core, (ii) a plurality of nucleotide moieties coupled to the core, and (iii) one or more fluorophores coupled to the core;
   (b) bringing the nucleic acid sequence in contact with the fluorescently-labeled nucleotide conjugate and a polymerizing enzyme under conditions sufficient to form a binding complex comprising (i) the polymerizing enzyme and (ii) a nucleotide of the nucleic acid sequence bound to a nucleotide moiety of the plurality of nucleotide moieties of the fluorescently-labeled nucleotide conjugate, wherein the binding complex persists for a persistence time of 1 second or more before it is removed;
   (c) contacting the binding complex with an imaging reagent, wherein the imaging reagent comprises a photobleaching reducing agent; and
   (d) obtaining a fluorescent image of the binding complex in a presence of the imaging reagent.
2. The method of claim 1, wherein the photobleaching reducing agent comprises (i) ascorbate, (ii) Trolox, or (iii) a combination of the ascorbate and the Trolox.
3. The method of claim 2, wherein in (d), the fluorescent image exhibits no residual fluorescent signal from the fluorescently-labeled nucleotide conjugate of the binding complex when (i) the photobleaching reducing agent comprises 50 millimolar (mM) of the ascorbate and 2 mM of the Trolox, wherein the Trolox is non-aged Trolox and (ii) the obtaining comprises performing a 90 second exposure by a laser having a power of between 75-200 milliwatt (mW).
4. The method of claim 2, wherein the photobleaching reducing agent comprises ascorbate.
5. The method of claim 4, wherein a concentration of the ascorbate is at least 20 millimolar (mM).
6. The method of claim 4, wherein a concentration of the ascorbate is between about 10 millimolar (mM) and about 100 mM.

7. The method of claim 4, wherein a concentration of the ascorbate is about 50 millimolar (mM).
8. The method of claim 1, further comprising bringing the nucleic acid sequence into contact with a second fluorescently-labeled nucleotide conjugate under conditions sufficient to form a second binding complex comprising a second nucleotide of the nucleic acid sequence bound to a second nucleotide moiety of the fluorescently-labeled nucleotide conjugate, wherein the nucleotide is adjacent to the second nucleotide in the nucleic acid sequence.
9. The method of claim 1, wherein the nucleic acid sequence is comprised in a concatemer nucleic acid molecule comprising tandem repeats of the nucleic acid sequence.
10. The method of claim 1, wherein the nucleic acid sequence further comprises at least one universal adapter sequence.
11. The method of claim 1, wherein the fluorescently-labeled nucleotide conjugate further comprises a core attachment moiety linking the plurality of the nucleotide moieties to the core.
12. The method of claim 1, wherein the nucleotide moiety is selected from the group consisting of dATP, dGTP, dCTP, dTTP and dUTP.
13. The method of claim 1, further comprising bringing the nucleic acid sequence into contact with a plurality of fluorescently-labeled nucleotide conjugates comprising the fluorescently-labeled nucleotide conjugate and an additional fluorescently-labeled nucleotide conjugate, wherein the nucleotide moiety of the fluorescently-labeled nucleotide conjugate is different than an additional nucleotide moiety of the additional fluorescently-labeled nucleotide conjugate.
14. The method of claim 13, wherein the additional nucleotide moiety is selected from the group consisting of dATP, dGTP, dCTP, dTTP and dUTP.
15. The method of claim 1, wherein the nucleotide moiety comprises between about 3 and about 10 phosphate groups.
16. The method of claim 1, wherein the nucleotide of the nucleic acid sequence comprises a removable chain terminating moiety.
17. The method of claim 16, wherein the removable chain terminating moiety has a blocking moiety at the 3' sugar position of the nucleotide configured to prevent incorporation of the nucleotide moiety into the nucleic acid sequence.

18. The method of claim 1, wherein the polymerizing enzyme is a DNA polymerase.
19. The method of claim 1, wherein the polymerizing enzyme lacks a detectable label.
20. The method of claim 1, wherein the binding complex is immobilized to a surface of a support, and wherein the surface comprises a hydrophilic coating layer coupled thereto.
21. The method of claim 20, wherein the image of the surface exhibits a contrast to noise ratio (CNR) that is greater than or equal to about 20 when the fluorescently-labeled nucleotide conjugate of the binding complex comprises a detectable label comprising Cyanine dye-3 (Cy3) and when the image of the surface is acquired using an inverted fluorescence microscope and a camera under non-signal saturating conditions while the surface is immersed in a buffer.
22. The method of claim 20, wherein the hydrophilic coating layer comprises a water contact angle of less than 50 degrees.
23. The method of claim 20, wherein a plurality of the nucleic acid sequence is immobilized to the surface, and wherein a surface density of the plurality of the nucleic acid sequence comprises about 103 molecules/ millimeter $(mm)^2$ to about 5×10^5 molecules/mm^2.
24. The method of claim 1, wherein the imaging reagent comprises a non-catalytic divalent cation that inhibits incorporation of the nucleotide moiety of the fluorescently-labeled nucleotide conjugate.
25. The method of claim 24, wherein the non-catalytic divalent cation comprises strontium, barium, scandium, titanium, calcium, vanadium, chromium, iron, cobalt, nickel, copper, zinc, gallium, germanium, arsenic, selenium, rhodium, europium, tin or terbium ions.
26. The method of claim 1, wherein the nucleic acid sequence comprises a template nucleic acid sequence for a sequencing reaction and at least one universal adaptor sequence.
27. The method of claim 1, wherein the imaging reagent further comprises 1-methyl-4-(1-methylpyridin-1-ium-4-yl)pyridin-1-ium;dichloride, Tris-HCl EDTA, NaCl, a non-ionic detergent, sucrose, strontium acetate or glycerol, or any combination thereof.
28. The method of claim 1, wherein said persistence time is more than 1 second.
29. The method of claim 1, further comprising identifying the nucleotide by analyzing the image obtained in (d).