

Claims

1. A method for determining a sequence of a target polynucleotide, which comprises:

(a) providing a target polynucleotide,

(b) contacting the target polynucleotide with a primer so that the primer hybridizes to the target polynucleotide, thereby forming a partial duplex of the target polynucleotide and the primer,

(c) contacting the partial duplex with a polymerase and four groups of nucleotides under a condition that allows the polymerase to carry out a nucleotide polymerization reaction, so that a nucleotide is incorporated into the primer,

wherein the four groups of nucleotides comprises the followings: a first nucleotide group, a second nucleotide group, a third nucleotide group and a fourth nucleotide group, wherein the first nucleotide group comprises a first nucleotide labeled with a first label and optionally an unlabeled first nucleotide, the second nucleotide group comprises a second nucleotide labeled with a second label and optionally an unlabeled second nucleotide, the third nucleotide group comprises a third nucleotide labeled with the first label and a third nucleotide labeled with the second label, and the fourth nucleotide group comprises an unlabeled fourth nucleotide,

wherein the first label is a luminescent label; or, the first label is a label that can specifically binds to a first ligand that is labeled with a luminescent label; the second label is a label that can specifically binds to a second ligand that is labeled with a luminescent label;

wherein each nucleotide has a ribose or deoxyribose moiety that contains a protecting group attached thereto via a 2' or 3' oxygen atom,

(d) detecting the presence of the first label on the partial duplex of the step (c),

(e) detecting the presence of the second label on the partial duplex of the step (c),

(f) optionally removing the protecting group and the label on the nucleotide incorporated in the partial duplex of the step (c),

(g) optionally repeating the steps (c) to (f) one or more times to obtain sequence information of the target polynucleotide,

wherein the presence of the first label and the second label is detected by the same luminescence signal wherein (i) the first label is a luminescent label, and the step (d) comprises detecting the presence of the luminescent label on the partial duplex of the step (c); or, (ii) the first label is not a luminescent label, and the step (d) comprises contacting the partial duplex of the step (c) with a first ligand that is labeled with a luminescent label and specifically binds to the first

label, and then detecting the presence of the luminescent label on the partial duplex; wherein the step (e) comprises contacting the partial duplex of the step (c) with a second ligand that is labeled with a luminescent label and specifically binds to the second label, and then detecting the presence of the luminescent label on the partial duplex.

2. The method according to claim 1, wherein the step (e) is performed after the step (d).

3. The method according to claim 1, wherein the luminescent label for labeling the second ligand that specifically binds to the second label is the same as the luminescent label used as the first label or the luminescent label for labeling the first ligand that specifically binds to the first label.

4. The method according to any one of claims 1 to 3, wherein the luminescent label is a fluorescent label.

5. The method according to claim 4, wherein the luminescent label is a fluorophore.

6. The method according to claim 4, wherein the luminescent label is selected from coumarin, AlexaFluor, Bodipy, fluorescein, tetramethylrhodamine, Phenoxazine, acridine, Cy5, Cy3, AF532, Texas red and derivatives thereof.

7. The method according to any one of claims 1 to 6, wherein as for the first nucleotide group, the first nucleotide labeled with the first label and the unlabeled first nucleotide have a ratio of 4:1 to 3:2.

8. The method according to any one of claims 1-7, wherein as for the second nucleotide group, the second nucleotide labeled with the second label and the unlabeled second nucleotide have a ratio of 4:1 to 3:2.

9. A kit for sequencing a polynucleotide, comprising: (a) a first nucleotide group, a second nucleotide group, a third nucleotide group and a fourth nucleotide group, wherein the first nucleotide group comprises a first nucleotide labeled with a first label and optionally an unlabeled first nucleotide, the second nucleotide group comprises a second nucleotide labeled with a second label and optionally an unlabeled second nucleotide, the third nucleotide group comprises a third nucleotide labeled with the first label and a third nucleotide labeled with the second label, and the fourth nucleotide group comprises an unlabeled fourth nucleotide; and (b) a packaging material for them,

wherein the first label is a luminescent label; or, the first label is a label that can specifically binds to a first ligand that is labeled with a luminescent label; the second label is a label that can specifically binds to a second ligand that is labeled with a luminescent label;

wherein the nucleotide each comprises a ribose or deoxyribose moiety that contains a protecting group attached via a 2' or 3' oxygen atom;

wherein the presence of the first label and the second label is detected by the same luminescence signal.

10. The kit according to claim 9, wherein (i) the first label is a luminescent label; or (ii) the first label is not a luminescent label, and the kit further comprises a first ligand that is labeled with a luminescent label and specifically binds to the first label.

11. The kit according to claim 9 or 10, which further comprises a second ligand that is labeled with a luminescent label and specifically binds to the second label.

12. The kit according to claim 11, wherein the luminescent label for labeling the second ligand that specifically binds to the second label is the same as the luminescent label used as the first label or the luminescent label for labeling the first ligand that specifically binds to the first label.

13. The kit according to any one of claims 10 to 12, wherein the luminescent label is a fluorescent label.

14. The kit according to claim 13, wherein the luminescent label is a fluorophore.

15. The kit according to claim 13, wherein the luminescent label is selected from coumarin, AlexaFluor, Bodipy, fluorescein, tetramethylrhodamine, Phenoxazine, acridine, Cy5, Cy3, AF532, Texas red and derivatives thereof.

16. The kit according to any one of claims 9 to 15, which further comprises an enzyme and a buffer suitable for the enzyme to function.