

~~What is claimed is:~~ 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 a nucleotide under a condition that allows the polymerase to carry out a nucleotide polymerization reaction, so that the nucleotide is incorporated into the primer,

wherein the nucleotide ~~is selected from one or more of~~ comprises the followings: a first nucleotide component, a second nucleotide component, a third nucleotide component and a fourth nucleotide component, wherein the first nucleotide component comprises a first nucleotide labeled with a first label and optionally an unlabeled first nucleotide, the second nucleotide component comprises a second nucleotide labeled with a second label and optionally an unlabeled second nucleotide, the third nucleotide component comprises ~~is selected from: (1) a third nucleotide labeled with the first label and a third nucleotide labeled with the second label, or (2) a third nucleotide simultaneously labeled with the first label and the second label,~~ and the fourth nucleotide component comprises an unlabeled fourth nucleotide,

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.

2. The method according to claim 1, wherein the first label is a luminescent label.

3. The method according to claim 1, wherein the step (d) comprises contacting the partial duplex of the step (c) with a 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,

optionally, the ligand is removed when removing the protecting group and the label on the nucleotide incorporated in the partial duplex of the step (c).

4. The method according to any one of claims 1 to 3, wherein the step (e) comprises contacting the partial duplex of the step (c) with a 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.

5. The method according to claim 4, wherein ~~for example~~, the step (e) is performed after the step (d).

6. The method according to ~~any one of claims 2 to~~ claim 4, wherein the luminescent labels ~~are for labeling the 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 ligand that specifically binds to the first label~~.

7. The method according to any one of claims 2 to 6, wherein the luminescent label is a fluorescent label.

8. The method according to claim 7, wherein the luminescent label ~~is~~ ~~such as~~ a fluorophore.

9. The method according to claim 7, wherein the luminescent label ~~is~~ ~~for example~~ selected from coumarin, AlexaFluor, Bodipy, fluorescein, tetramethylrhodamine, Phenoxazine, acridine, Cy5, Cy3, AF532, Texas red and derivatives thereof.

10. The method according to any one of claims 1 to 9, wherein as for the first nucleotide ~~component~~, the first nucleotide labeled with the first label and the unlabeled first nucleotide have a ratio of 4:1 to 3:2.

~~8~~11. The method ~~according to~~f any one of claims 1-~~7~~10, wherein as for the second nucleotide component, the second nucleotide labeled with the second label and the unlabeled second nucleotide have a ratio of 4:1 to 3:2.

~~9~~12. A kit for sequencing a polynucleotide, comprising: (a) ~~one or more nucleotides selected from the followings:~~ a first nucleotide component, a second nucleotide component, a third nucleotide component and a fourth nucleotide component, wherein the first nucleotide component comprises a first nucleotide labeled with a first label and optionally an unlabeled first nucleotide, the second nucleotide component comprises a second nucleotide labeled with a second label and optionally an unlabeled second nucleotide, the third nucleotide ~~component comprises~~is selected from: (1) a third nucleotide labeled with the first label and a third nucleotide labeled with the second label, ~~or (2) a third nucleotide simultaneously labeled with the first label and the second label,~~ and the fourth nucleotide component comprises an unlabeled fourth nucleotide; and (b) a packaging material for them,

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~~13. The kit according to claim ~~9~~12, wherein the first label is a luminescent label.

~~11~~14. The kit according to claim ~~9~~12, which further comprises a ligand that is labeled with a luminescent label and specifically binds to the first label.

~~12~~15. The kit according to any one of claims ~~9-12~~9-12 to ~~11~~14, which further comprises a ligand that is labeled with a luminescent label and specifically binds to the second label.

~~13~~16. The kit according to ~~any one of claims 9 to 12~~claim 15, wherein the luminescent labels for labeling the ligand that specifically binds to the second label is ~~are~~ the same as the luminescent

label used as the first label or the luminescent for labeling the ligand that specifically binds to the first label.

1417. The kit according to any one of claims 913 to 1316, wherein the luminescent label is a fluorescent label.5

18. The kit according to claim 17, wherein the luminescent label is such as a fluorophore.5

19. The kit according to claim 17, wherein the luminescent label is for example selected from coumarin, AlexaFluor, Bodipy, fluorescein, tetramethylrhodamine, Phenoxazine, acridine, Cy5, Cy3, AF532, Texas red and derivatives thereof.

1520. The kit according to any one of claims 129 to 1419, which further comprises an enzyme and a buffer suitable for the enzyme to function.