

~~What is claimed is:~~ CLAIMS

1. A method for sequencing a nucleic acid molecule, which comprises the following steps:

(1) providing a nucleic acid molecule to be sequenced that is linked to a support, or linking a nucleic acid molecule to be sequenced to a support;

(2) adding a primer for initiating a nucleotide polymerization reaction, a polymerase for performing the nucleotide polymerization reaction, and four compounds to form a reaction system containing a solution phase and a solid phase; wherein, the four compounds are respectively derivatives of nucleotides A, (T/U), C and G, and have the ability of base complementary pairing; and, the hydroxyl (-OH) at the 3'-position of ribose or deoxyribose of the four compounds is protected by a protecting group; and,

the first compound is linked with a first molecular label,

the second compound is linked with a second molecular label,

the third compound is linked with a first molecular label and a second molecular label, or a part of the third compound is linked with a first molecular label and another part of the third compound is linked with a second molecular label,

the fourth compound is not linked with any molecular label;

(3) annealing the primer to the nucleic acid molecule to be sequenced, and forming a duplex linked to the support by using the primer as an initial growing nucleic acid chain together with the nucleic acid molecule to be sequenced;

(4) using the polymerase to carry out the nucleotide polymerization reaction under a condition that allows the polymerase to carry out the nucleotide polymerization reaction, thereby incorporating one of the four compounds into the 3'-end of the growing nucleic acid chain;

(5) allowing the duplex of the previous step to contact with two different luciferases and perform a ligation reaction, wherein the two luciferases can specifically be ligated with the first molecular label and the second molecular label, respectively; then, allowing the luciferase to undergo a ~~fluorescence~~-luminescence reaction in the presence of a substrate, and detecting an emitted ~~fluorescence~~-luminescence signal;

(6) removing the molecular label of each nucleotide;

(7) optionally, repeating steps (3) to (6) or (3) to (5) one or more times to obtain sequence information of the nucleic acid molecule.

2. The method according to claim 1, wherein in step (5), the duplex is contacted with the two different luciferases in a one-step reaction and undergoes the ligation reaction; or in step (5), the duplex is sequentially contacted with the two luciferases and undergoes the ligation reaction.

3. The method according to claim 1 or 2, which comprises the following steps:

(1) providing a nucleic acid molecule to be sequenced that is linked to a support, or linking a nucleic acid molecule to be sequenced to a support;

(2) adding a primer for initiating a nucleotide polymerization reaction, a polymerase for performing the nucleotide polymerization reaction, and four compounds to form a reaction system containing a solution phase and a solid phase; wherein, the four compounds are respectively derivatives of nucleotides A, (T/U), C and G, and have the ability of base complementary pairing; and, the hydroxyl (-OH) at the 3'-position of ribose or deoxyribose of the four compounds is protected by a protecting group; and,

the first compound is linked with a first molecular label,

the second compound is linked with a second molecular label,

the third compound is linked with a first molecular label and a second molecular label, or a part of the third compound is linked with a first molecular label and another part of the third compound is linked with a second molecular label,

the fourth compound is not linked with any molecular label;

(3) annealing the primer to the nucleic acid molecule to be sequenced, and forming a duplex linked to the support by using the primer as an initial growing nucleic acid chain together with the nucleic acid molecule to be sequenced;

(4) using the polymerase to carry out the nucleotide polymerization reaction under a condition that allows the polymerase to carry out the nucleotide polymerization reaction, thereby incorporating one of the four compounds into the 3'-end of the growing nucleic acid chain;

(5) removing the solution phase of the reaction system in the previous step, keeping the duplex linked to the support, and adding two different luciferases to carry out the ligation reaction, wherein the two luciferases can be specifically ligated with the first molecular label and the second molecular label, respectively;

(6) removing unbound luciferase by using an elution buffer;

(7) adding a substrate of the first luciferase and detecting ~~fluorescence~~luminescence signal at the same time;

(8) removing the solution of the previous step reaction;

(9) adding a substrate of the second luciferase and detecting ~~fluorescence~~luminescence signal at the same time;

(10) removing the solution of the previous step reaction;

(11) removing the molecular label and 3'-protecting group of each nucleotide;

(12) optionally removing the solution of the previous step reaction;

(13) optionally repeating steps (3) to (12) or (3) to (9) one or more times to obtain sequence information of the nucleic acid molecule.

4. The method according to claim 1 or 2, which comprises the following steps:

(1) providing a nucleic acid molecule to be sequenced that is linked to a support, or linking a nucleic acid molecule to be sequenced to a support;

(2) adding a primer for initiating a nucleotide polymerization reaction, a polymerase for performing the nucleotide polymerization reaction, and four compounds to form a reaction system containing a solution phase and a solid phase; wherein, the four compounds are respectively derivatives of nucleotides A, (T/U), C and G, and have the ability of base complementary pairing; and, the hydroxyl (-OH) at the 3'-position of ribose or deoxyribose of the four compounds is protected by a protecting group; and,

the first compound is linked with a first molecular label,

the second compound is linked with a second molecular label,

the third compound is linked with a first molecular label and a second molecular label, or a part of the third compound is linked with a first molecular label and another part of the third compound is linked with a second molecular label,

the fourth compound is not linked with any molecular label;

(3) annealing the primer to the nucleic acid molecule to be sequenced, and forming a duplex linked to the support by using the primer as an initial growing nucleic acid chain together with the nucleic acid molecule to be sequenced;

(4) using the polymerase to carry out the nucleotide polymerization reaction under a condition that allows the polymerase to carry out the nucleotide polymerization reaction, thereby incorporating one of the four compounds into the 3'-end of the growing nucleic acid chain;

(5) removing the solution phase of the reaction system in the previous step, keeping the duplex linked to the support, and adding a first luciferase to perform a ligation reaction, wherein the first luciferase can specifically bind to the first molecular label;

(6) removing unbound first luciferase by using an elution buffer;

(7) adding a substrate of the first luciferase and detecting luminescence ~~fluorescence~~ signal at the same time;

(8) removing the solution of the previous step reaction;

(9) adding a second luciferase to perform a ligation reaction, wherein the second luciferase can specifically bind to the second molecular label;

(10) removing unbound second luciferase by using an elution buffer;

(11) adding a substrate of the second luciferase and detecting luminescence ~~fluorescence~~ signal at the same time;

(12) removing the solution of the previous step reaction;

(13) optionally removing the molecular label of each nucleotide;

(14) optionally repeating steps (3) to (13) or (3) to (11) one or more times to obtain sequence information of the nucleic acid molecule.

5. The method according to claim 3 or 4, wherein the first luciferase and the second luciferase contain different luciferases.

6. The method according to claim 1 or 2, which comprises the following steps:

(1) providing a nucleic acid molecule to be sequenced that is linked to a support, or linking a nucleic acid molecule to be sequenced to a support;

(2) adding a primer for initiating a nucleotide polymerization reaction, a polymerase for performing the nucleotide polymerization reaction, and four compounds to form a reaction system containing a solution phase and a solid phase; wherein, the four compounds are respectively derivatives of nucleotides A, (T/U), C and G, and have the ability of base complementary pairing;

and, the hydroxyl (-OH) at the 3'-position of ribose or deoxyribose of the four compounds is protected by a protecting group; and,

the first compound is linked with a first molecular label,

the second compound is linked with a second molecular label,

the third compound is linked with a first molecular label and a second molecular label, or a part of the third compound is linked with a first molecular label and another part of the third compound is linked with a second molecular label,

the fourth compound is not linked with any molecular label;

(3) annealing the primer to the nucleic acid molecule to be sequenced, and forming a duplex linked to the support by using the primer as an initial growing nucleic acid chain together with the nucleic acid molecule to be sequenced;

(4) using the polymerase to carry out the nucleotide polymerization reaction under a condition that allows the polymerase to carry out the nucleotide polymerization reaction, thereby incorporating one of the four compounds into the 3'-end of the growing nucleic acid chain;

(5) removing the solution phase of the reaction system in the previous step, keeping the duplex linked to the support, and adding a first luciferase to perform a ligation reaction, wherein the first luciferase can specifically bind to the first molecular label;

(6) removing unbound first luciferase by using an elution buffer;

(7) adding a substrate of the luciferase and detecting luminescence ~~fluorescence~~-signal at the same time;

(8) removing the solution of the previous step reaction;

(9) adding a reagent for denaturing the luciferase;

(10) removing the solution of the previous step reaction;

(11) adding a second luciferase to perform a ligation reaction, wherein the second luciferase can specifically bind to the second molecular label;

(12) removing unbound second luciferase by using an elution buffer;

(13) adding a substrate of the luciferase and detecting luminescence ~~fluorescence~~-signal at the same time;

(14) optionally removing the solution of the previous step reaction;

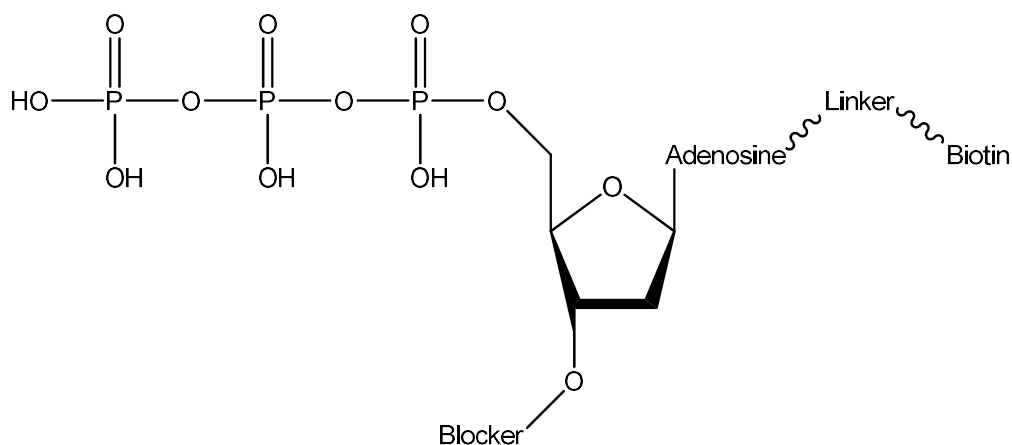
(15) optionally removing the molecular label and 3'-protecting group of each nucleotide;

(16) optionally repeating steps (3) to (15) or (3) to (13) one or more times to obtain sequence information of the nucleic acid molecule.

~~7. The method according to claim 6, wherein the first luciferase and the second luciferase may contain the same luciferase.~~

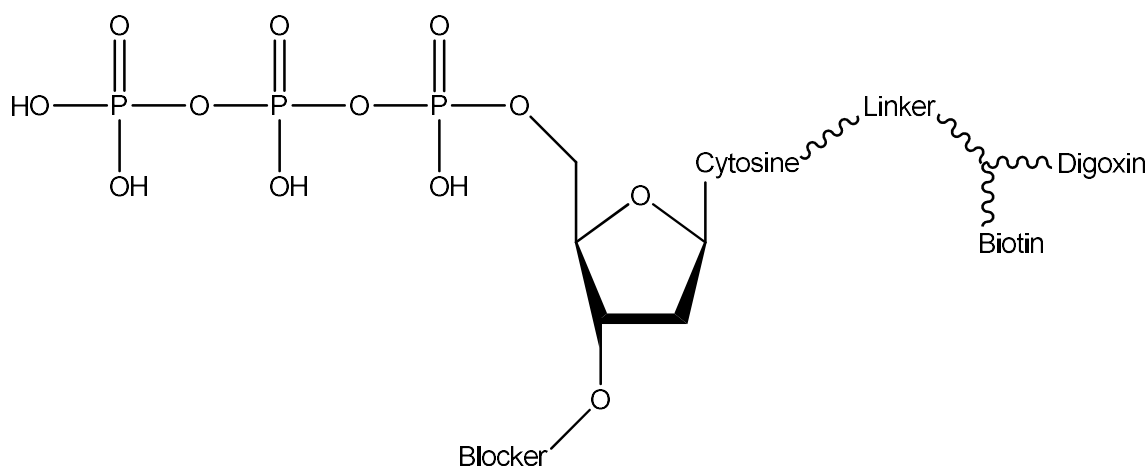
~~87.~~ The method according to any one of claims 1-~~76~~, wherein the first molecular label and the second molecular label are selected from the group consisting of biotin, digoxin, N3G or FITC, and the first luciferase and the second luciferase are respectively labeled with streptavidin, digoxin antibody, N3G antibody or FITC antibody.

8. The method according to any one of claims 1-7, wherein the four compounds are shown in the following Formula I, Formula II, Formula III, and Formula IV:



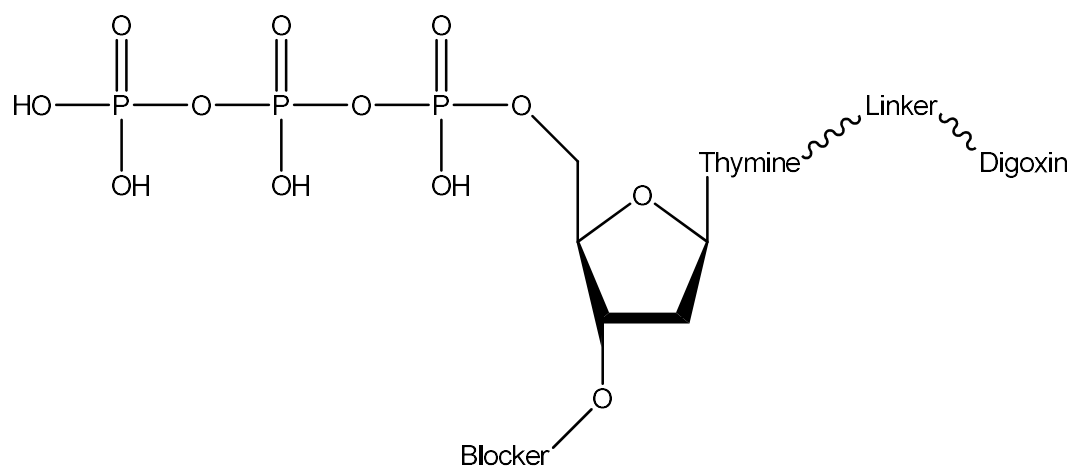
Formula I

2



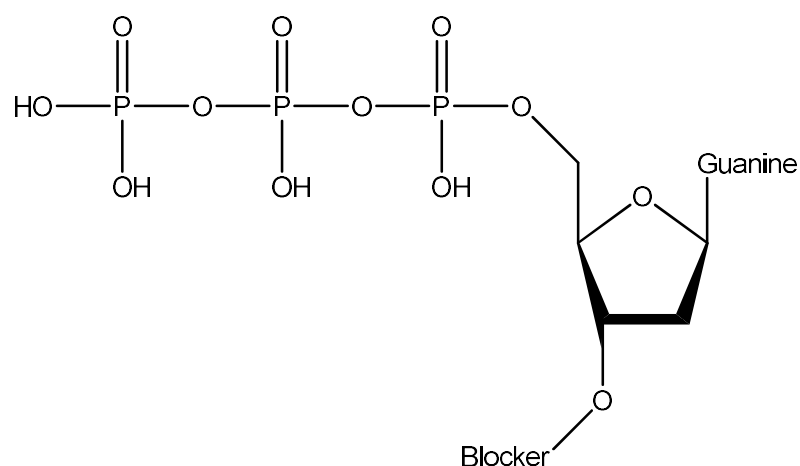
Formula II

2



Formula III

2



Formula IV

9. A kit for sequencing a polynucleotide, which comprises:

- (a) four compounds, the four compounds are respectively derivatives of nucleotides A, (T/U), C and G, and have the ability of base complementary pairing; and, the hydroxyl (-OH) at the 3'-position of ribose or deoxyribose of the four compounds is protected by a protecting group; and, the first compound is linked with a first molecular label, the second compound is linked with a second molecular label,

the third compound is linked with a first molecular label and a second molecular label, or a part of the third compound is linked with a first molecular label and another part of the third compound is linked with a second molecular label,

the fourth compound is not linked with any molecular label; and

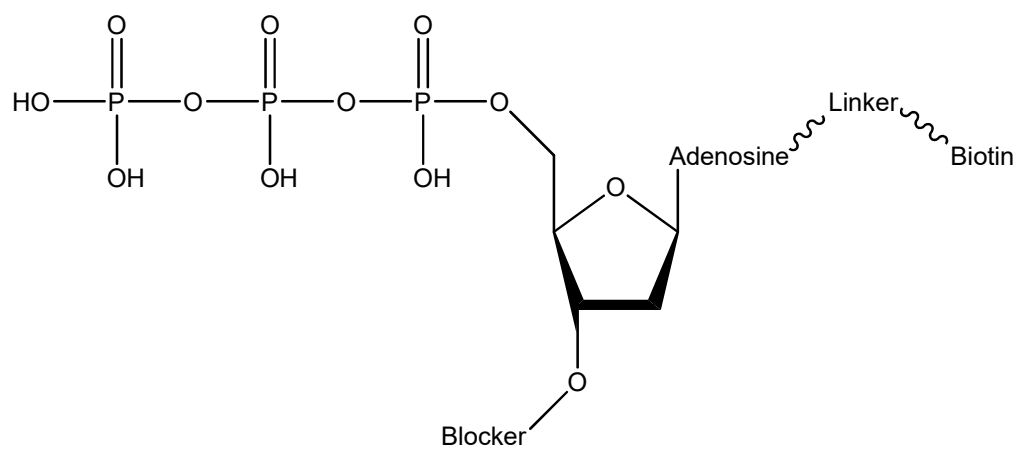
(b) two different luciferases, the two luciferases can specifically bind to the first molecular label and the second molecular label, respectively.

~~10. The kit according to claim 9, wherein the two different luciferases can contain the same luciferase or different luciferases.~~

~~11~~10. The kit according to claim 9 ~~or 10~~, wherein the first molecular label and the second molecular label are selected from the group consisting of biotin, digoxin, N3G or FITC, and the first luciferase and the second luciferase are respectively labeled with streptavidin, digoxin antibody, N3G antibody or FITC antibody.

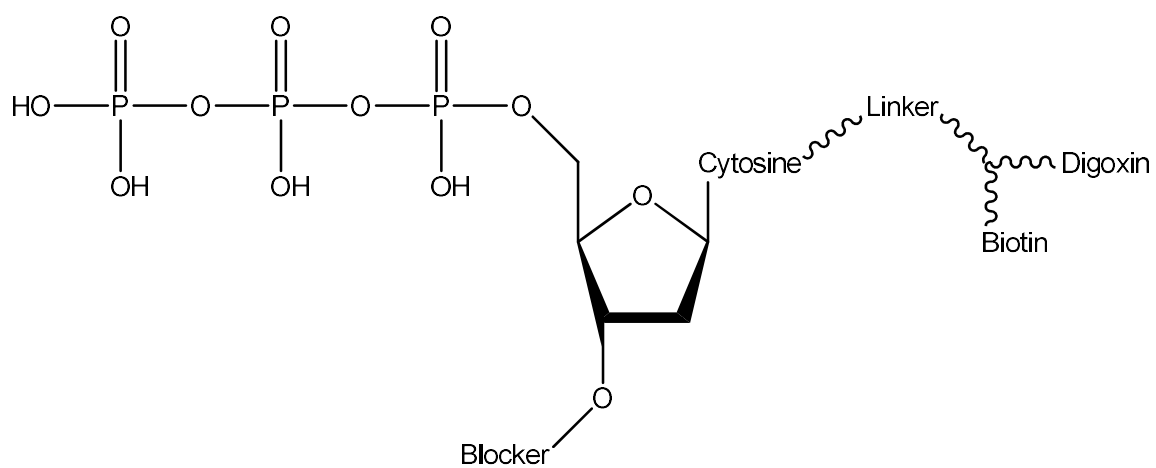
~~12~~11. The kit according to any one of claims 9 to ~~11~~10, further comprising: a reagent and/or device for extracting a nucleic acid molecule from a sample; a reagent for pretreating the nucleic acid molecule; a support for linking the nucleic acid molecule to be sequenced; a reagent for linking ~~(for example, covalently or non-covalently linking)~~ the nucleic acid molecule to be sequenced to the support; a primer for initiating a nucleotide polymerization reaction; a polymerase for performing the nucleotide polymerization reaction; one or more buffer solutions; one or more washing solutions; or any combination thereof.

~~13~~12. ~~A~~The kit according to any one of claims 9 to 11, wherein the four compounds are compound as shown in Formula I, Formula II, Formula III, or Formula IV₅.



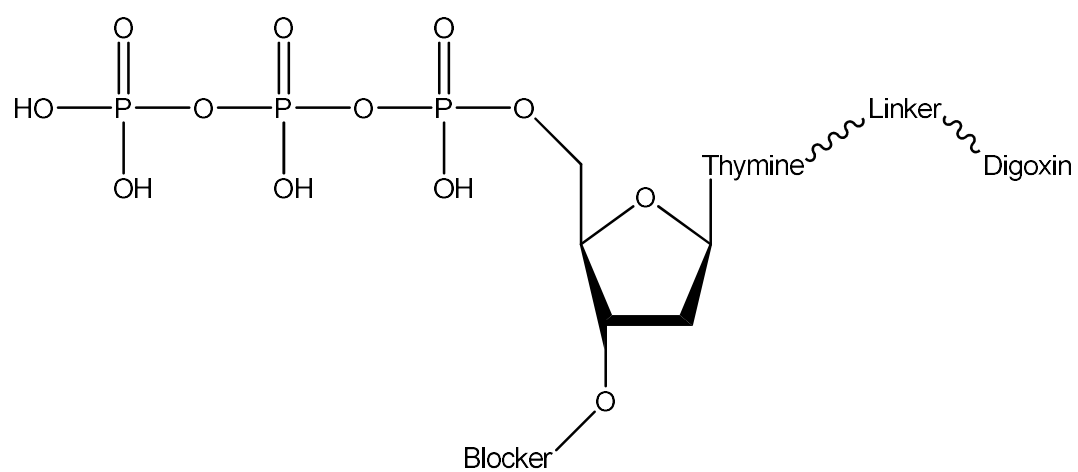
Formula I

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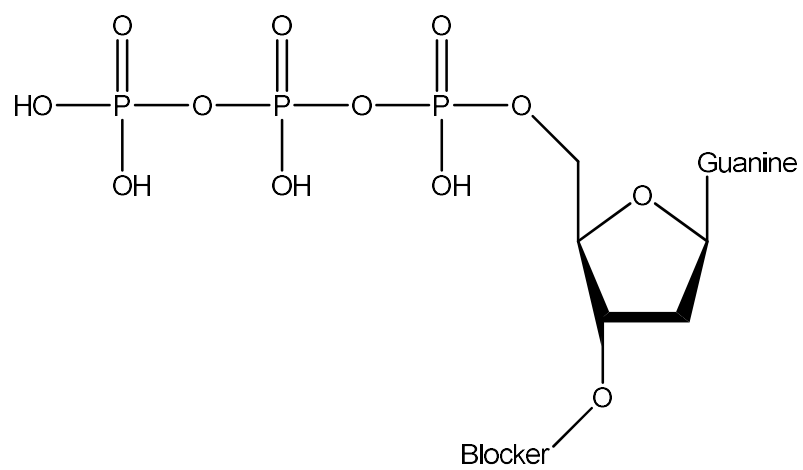
Formula II

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Formula III

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Formula IV