

Claims

1. A method for amplifying circular DNA, comprising the following steps:
 - (1) forming a reaction mixture of circular DNA as a template with a reaction solution comprising:
 - a first enzyme group that catalyzes replication of circular DNA;
 - a second enzyme group that catalyzes an Okazaki fragment maturation and synthesizes two sister circular DNAs constituting a catenane;
 - a third enzyme group that catalyzes a separation of two sister circular DNAs;
 - a buffer;
 - ATP;
 - GTP, CTP and UTP;
 - dNTP;
 - a magnesium ion source; and
 - an alkali metal ion source, whereinwherein the circular DNA includes a replication origin sequence (origin of chromosome (*oriC*)) that can bind to an enzyme having DnaA activity; and
 - (2) incubating the reaction mixture formed in step (1) in a temperature cycle of repeating an incubation at 30°C or higher and an incubation at 27°C or lower.
2. The method according to claim 1, wherein the reaction solution further comprises a protein non-specific adsorption inhibitor and/or a nucleic acid non-specific adsorption inhibitor.
3. The method according to claim 1, wherein the reaction solution further comprises linear DNA-specific exonuclease and/or RecG-type helicase.
4. The method according to claim 1, wherein the reaction solution further comprises an ammonium salt.
5. The method according to claim 1, wherein

the first enzyme group comprises a combination of an enzyme having DnaA activity, one or more types of nucleoid protein, an enzyme or enzyme group having DNA gyrase activity, single-strand binding protein (SSB), an enzyme having DnaB-type helicase activity, an enzyme having DNA helicase loader activity, an enzyme having DNA primase activity, an enzyme having DNA clamp activity, and an enzyme or enzyme group having DNA polymerase III* activity;

the second enzyme group comprises a combination of an enzyme having DNA polymerase I activity and an enzyme having DNA ligase activity, and

the third enzyme group comprises an enzyme having topoisomerase III activity and/or an enzyme having topoisomerase IV activity.

6. The method according to claim 5, wherein the second enzyme group further comprises an enzyme having RNaseH activity.

7. The method according to claim 5, wherein the third enzyme group further comprises an enzyme having RecQ-type helicase activity.

8. The method according to claim 5, wherein

in the first enzyme group,

the one or more nucleoid proteins are IHF or HU,

the enzyme or the enzyme group having DNA gyrase activity is a complex of GyrA and GyrB,

the enzyme having DnaB-type helicase activity is DnaB helicase,

the enzyme having DNA helicase loader activity is DnaC helicase loader,

the enzyme having DNA primase activity is DnaG primase,

the enzyme having DNA clamp activity is DnaN clamp,

the enzyme or the enzyme group having DNA polymerase III* activity is an enzyme or an enzyme group comprising any of DnaX, HolA, HolB, HolC, HolD, DnaE, DnaQ, and HolE.

9. The method according to claim 1, wherein the reaction solution further comprises

RecG-type helicase and/or single-strand DNA-specific exonuclease.

10. The method according to claim 1, wherein the reaction solution further comprises linear DNA-specific exonuclease and/or single-strand DNA-specific exonuclease.

11. The method according to claim 1, wherein the reaction solution further comprises a DNA stabilizing factor.

12. The method according to claim 1, wherein

step (1) comprises:

(1-1) pre-incubating a reaction solution comprising:

a first enzyme group that catalyzes replication of circular DNA;

a second enzyme group that catalyzes an Okazaki fragment maturation and

synthesizes two sister circular DNAs constituting a catenane;

a third enzyme group that catalyzes a separation of two sister circular DNAs;

a buffer;

ATP;

GTP, CTP and UTP;

dNTP;

a magnesium ion source; and

an alkali metal ion source; and

(1-2) forming a reaction mixture of the reaction solution with circular DNA as a template.

13. The method according to claim 1, wherein step (2) is carried out in a water-in-oil emulsion.

14. The method according to claim 1, wherein, following step (2), the method further comprises:

(3) performing a post-reaction treatment, wherein

the post-reaction treatment is:

(i) a treatment of diluting the reaction mixture five or more times with the reaction

solution that does not contain the first to third enzyme groups;

(ii) a treatment with linear DNA-specific exonuclease and/or single-strand DNA-specific exonuclease; and/or

(iii) a treatment with a gap repair enzyme.

15. A composition for amplifying circular DNA to be used for the method according to any one of claims 1-14, wherein the composition comprises:

a first enzyme group that catalyzes replication of circular DNA;

a second enzyme group that catalyzes an Okazaki fragment maturation and synthesizes two sister circular DNAs constituting a catenane;

a third enzyme group that catalyzes a separation of two sister circular DNAs;

a buffer;

ATP;

GTP, CTP and UTP;

dNTP;

a magnesium ion source;

an alkali metal ion source; and

linear DNA-specific exonuclease and/or RecG-type helicase.

16. The composition according to claim 15, further comprising a protein non-specific adsorption inhibitor and/or a nucleic acid non-specific adsorption inhibitor.

17. The composition according to claim 15, further comprising single-strand DNA-specific exonuclease.

18. The composition according to claim 15, further comprising a DNA stabilizing factor.

19. A kit for amplifying circular DNA to be used for the method according to any one of claims 1-14, wherein the kit comprises a combination of:

a first enzyme group that catalyzes replication of circular DNA;

a second enzyme group that catalyzes an Okazaki fragment maturation and

synthesizes two sister circular DNAs constituting a catenane;

a third enzyme group that catalyzes a separation of two sister circular DNAs;

a buffer;

ATP;

GTP, CTP and UTP;

dNTP;

a magnesium ion source;

an alkali metal ion source and

linear DNA-specific exonuclease and/or RecG-type helicase.

20. The kit according to claim 19, further comprising a combination with a protein non-specific adsorption inhibitor and/or a nucleic acid non-specific adsorption inhibitor.

21. The kit according to claim 19, further comprising single-strand DNA-specific exonuclease.

22. The kit according to claim 19, further comprising a DNA stabilizing factor.

23. The kit according to claim 19, further comprising a gap repair enzyme.