

reaction vessel without any sequence bias and any intervening cleaning steps are therefore highly desirable.

BRIEF DESCRIPTION

[0006] A method for generating a single-stranded DNA circle from a linear DNA is provided. The method comprises the steps of providing a linear DNA, end-repairing the linear DNA by incubating it with a polynucleotide kinase in the presence of a phosphate donor to generate a ligatable DNA sequence having a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end, and performing an intra-molecular ligation of the repaired, ligatable DNA sequence with a ligase in order to generate the single-stranded DNA circle. All steps of the method are performed in single reaction vessel without any intervening isolation or purification steps. The phosphate donor may be a guanosine triphosphate (GTP), a cytidine triphosphate (CTP), a uridine triphosphate (UTP), a deoxythymidine triphosphate (dTTP) or a combination thereof. The linear DNA may either be double-stranded or single-stranded DNA. DNA may be a fragmented DNA such as circulating DNA. The ligatable DNA, if in double-stranded form, needs to be denatured prior to intra-molecular ligation reaction. A pre-adenylated ligase that is capable of template-independent, intra-molecular ligation of single-stranded DNA sequences may be employed for the ligation reaction.

[0006a] In an aspect, there is provided a method for generating a single-stranded DNA circle, the method comprising:

- (a) providing a linear single-stranded DNA;
- (b) incubating the linear single-stranded DNA with a polynucleotide kinase in presence of a phosphate donor to generate a ligatable DNA sequence having a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end; and
- (c) incubating the ligatable DNA sequence with a ligase that is capable of template-independent, intra-molecular ligation of a single-stranded DNA sequence to generate the single-stranded DNA circle,

of the 5' adenylated DNA sequence to generate the single-stranded DNA circle. All steps of the method are performed in a single reaction vessel without any intervening isolation or purification steps. ATP may have to be removed from the reaction mixture (e.g., by treating the reaction mixture with a phosphatase) before the intra-molecular ligation reaction if the non-adenylated ligase is an ATP-dependent ligase. If the 5' adenylated DNA is in double-stranded form, it needs to be denatured prior to the intra-molecular ligation reaction.

[0007a] In another aspect, there is provided a method of generating a single-stranded DNA circle, the method comprising:

- (a) providing a linear single-stranded DNA;
- (b) optionally incubating the linear single-stranded DNA with a polynucleotide kinase in presence of adenosine triphosphate to generate a ligatable DNA sequence having a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end;
- (c) incubating the linear DNA sequence or the ligatable DNA sequence with an adenylating enzyme in presence of adenosine triphosphate to generate a 5' adenylated DNA sequence and treating the reaction mixture with a phosphatase to remove ATP from the reaction mixture; and
- (d) incubating the 5' adenylated DNA sequence with a non-adenylated ligase, which is capable of template-independent intra-molecular ligation of a 5' adenylated, single-stranded DNA sequence to generate the single-stranded DNA circle,

wherein all the steps of the method are performed in single reaction vessel without any intervening isolation or purification steps.

DRAWINGS

[0008] These and other features, aspects and advantages of the invention will become better understood when the following detailed description is read with reference to the accompanying figures.

[0009] FIG. 1 illustrates a schematic representation of an embodiment of a ligase-assisted whole-genome amplification of a fragmented dsDNA.

CLAIMS:

1. A method for generating a single-stranded DNA circle, the method comprising:
 - (a) providing a linear single-stranded DNA;
 - (b) incubating the linear single-stranded DNA with a polynucleotide kinase in presence of a phosphate donor to generate a ligatable DNA sequence having a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end; and
 - (c) incubating the ligatable DNA sequence with a ligase that is capable of template-independent, intra-molecular ligation of a single-stranded DNA sequence to generate the single-stranded DNA circle,wherein all the steps of the method are performed in a single reaction vessel without any intervening isolation or purification steps and wherein step (b) is performed using a phosphate donor other than adenosine triphosphate or deoxyadenosine triphosphate.
2. The method of claim 1, wherein the phosphate donor is selected from a group consisting of guanosine triphosphate, cytidine triphosphate, uridine triphosphate, deoxythymidine triphosphate and combinations thereof.
3. The method of claim 2, wherein the phosphate donor is guanosine triphosphate.
4. The method of claim 3, wherein the linear single-stranded DNA is incubated with the polynucleotide kinase in presence of less than 200 μM of guanosine triphosphate.
5. The method of claim 4, wherein the linear single-stranded DNA is incubated with the polynucleotide kinase in presence of up to 30 μM of guanosine triphosphate and up to

2.5 mM of manganese ion.

6. The method of claim 3, further comprising denaturing the ligatable DNA sequence prior to step (c) if the ligatable DNA sequence is in double-stranded form.
7. The method of claim 6, wherein the ligase is a pre-adenylated ligase.
8. The method of claim 7, wherein the pre-adenylated ligase is a pre-adenylated TS2126 RNA ligase.
9. The method of claim 8, wherein steps (a) to (c) are performed in a sequential manner in the single reaction vessel.
10. The method of claim 9, wherein all the steps of the method are performed in absence of adenosine triphosphate or deoxyadenosine triphosphate.
11. The method of claim 10, wherein all the steps of the method are performed in HEPES buffer.
12. The method of claim 1, wherein the linear single-stranded DNA is a circulating DNA, a DNA isolated from formalin fixed paraffin-embedded samples, a forensic DNA sample that have been exposed to environmental conditions, or an ancient DNA sample.
13. The method of claim 1, further comprising amplifying the single-stranded DNA circle under isothermal conditions.
14. The method of claim 13, wherein the single-stranded DNA circle is amplified via rolling circle amplification.

15. The method of claim 14, wherein the rolling circle amplification is performed using a nuclease-resistant primer.
16. The method of claim 15, wherein the nuclease-resistant primer comprises at least one modified nucleotide.
17. The method of claim 14, wherein the single-stranded DNA circle is amplified using a random primer mixture.
18. The method of claim 14, wherein the single-stranded DNA circle is amplified using a primer solution that consists essentially of a partially constrained primer mixture comprising a terminal mismatch primer-dimer structure.
19. A method of generating a single-stranded DNA circle, the method comprising:
- (a) providing a linear single-stranded DNA;
 - (b) optionally incubating the linear single-stranded DNA with a polynucleotide kinase in presence of adenosine triphosphate to generate a ligatable DNA sequence having a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end;
 - (c) incubating the linear single-stranded DNA sequence or the ligatable DNA sequence with an adenylating enzyme in presence of adenosine triphosphate to generate a 5' adenylated DNA sequence and treating the reaction mixture with a phosphatase to remove ATP from the reaction mixture; and
 - (d) incubating the 5' adenylated DNA sequence with a non-adenylated ligase, which is capable of template-independent intra-molecular ligation of a 5' adenylated, single-stranded DNA sequence to generate the single-stranded DNA circle,
- wherein all the steps of the method are performed in single reaction vessel without any intervening isolation or purification steps.

20. The method of claim 19, further comprising denaturing the 5' adenylated DNA sequence prior to step (d) if the 5' adenylated DNA sequence is in double-stranded form.
21. The method of claim 20, wherein the adenylating enzyme is an RNA ligase 1 derived from thermophilic archeabacteria, *Methanobacterium thermoautotrophicum*.
22. The method of claim 21, wherein the non-adenylated ligase is a mutant or engineered ligase, which is adenosine-triphosphate-independent and is defective in self-adenylation, de-adenylation and adenylate transfer.
23. The method of claim 22, wherein the mutant ligase is a mutant of RNA ligase 1 derived from thermophilic archeabacteria, *Methanobacterium thermoautotrophicum*.
24. The method claim 21, further comprising incubating the reaction mixture of step (c) comprising the 5' adenylated DNA sequence with a phosphatase to eliminate the adenosine triphosphate from the reaction mixture, wherein steps (a) to (d) are carried out in a sequential manner.
25. The method of claim 24, wherein the non-adenylated ligase is a TS2126 RNA ligase derived from thermus bacteriophage, TS2126.
26. The method of claim 19, wherein the linear single-stranded DNA is a circulating DNA, a DNA isolated from formalin fixed paraffin-embedded samples, a forensic DNA sample that have been exposed to environmental conditions, or an ancient DNA sample.