

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

BRIEF DESCRIPTION

[0006] ~~In some embodiments, 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 from a linear DNA, the method comprising:

(a) providing the linear DNA;

(b) incubating the linear 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.

[0007] In some embodiments, a method for generating a single-stranded DNA circle from a linear DNA is provided, wherein the method employs a DNA pre-adenylation step prior to an intra-molecular ligation step. The linear DNA may optionally be incubated with a polynucleotide kinase in the presence of adenosine triphosphate (ATP) to generate a ligatable DNA sequence that comprises a phosphate group at a 5' terminal end and a hydroxyl group at a 3' terminal end. Generation of ligatable DNA sequence from the linear DNA may be preferred if the linear DNA is in a highly fragmented form. The linear DNA or the ligatable DNA sequence is then incubated with an adenylating enzyme in presence of ATP to generate a 5' adenylated DNA sequence. The 5' adenylated DNA sequence is then incubated with a non-adenylated ligase, which is capable of template-independent intra-molecular ligation

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 from a linear DNA, the method comprising:

(a) providing the linear DNA;

(b) optionally incubating the linear 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

(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.

[0010] FIG. 2 illustrates size profiles of circulating DNA isolated from blood plasma of healthy individuals.

[0011] FIG. 3 illustrates ligase-assisted whole-genome amplification of circulating DNA extracted from blood using different ligases.

[0012] FIG. 4 illustrates the effectiveness of ligase-assisted whole-genome amplification for sensitive and balanced DNA amplification of four different CODIS loci.

[0013] FIG. 5 illustrates the effectiveness of ligase-assisted whole-genome amplification for sensitive and balanced DNA amplification of twelve different CODIS loci.

[0014] FIG. 6 illustrates the efficiencies of ligase-assisted whole-genome amplification in different reaction and buffer conditions.