CLAIMS

1. A method for replicating circular DNA in a cell-free system, 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, and

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

(2) reacting the reaction mixture formed in step (1), wherein

the circular DNA includes a replication origin sequence (origin of chromosome (*oriC*)) that can bind to an enzyme having DnaA activity, and further includes a pair of ter sequences that are each inserted outward with respect to *oriC*, and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, wherein

the ter sequences are present in a region close to or adjacent to ori C, wherein

when the circular DNA has the ter sequences, the reaction solution in step (1) further comprises a protein having an activity of inhibiting replication by binding to the ter sequences, and when the circular DNA has the nucleotide sequence recognized by a DNA multimer separation enzyme, the reaction solution in step (1) further comprises the DNA multimer separation enzyme, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the protein having an activity of inhibiting replication by binding to the ter sequences is a Tus protein or an RTP protein.

2. The circular DNA is present in a concentration of 10 ng/μl or less in the reaction solution at the initiation of the reaction.

3. The method according to any one of claim 1 or 2, wherein the pair of ter sequences that are each inserted outward with respect to *oriC* comprises: a sequence comprising any one

of sequences shown in SEQ ID NOS: 1 to 14 which is inserted as one ter sequence into the 5'-terminal side of *oriC*; and a sequence comprising a complementary sequence to any one of sequences shown in SEQ ID NOS: 1 to 14 which is inserted as the other ter sequence into the 3'-terminal side of *oriC*.

4. The method according to claim 1, wherein the nucleotide sequence recognized by XerCD is a sequence comprising any one of sequences shown in SEQ ID NOS: 15 to 24, or a complementary sequence thereto.
5. The method according to claim 1, wherein nucleotide sequence recognized by Cre is a sequence comprising any one of sequences shown in SEQ ID NOS: 30 to 35, or a complementary sequence thereto.
6. A nucleic acid, which is linear DNA having a length of 273 bp to 2.0 kb, and comprises *oriC*, and a pair of ter sequences that are each inserted outward with respect to the *oriC* and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, wherein the ter sequences are present in a region close to or adjacent to ori C, wherein the DNA multimer separation enzyme is Cre or XerCD, and wherein the replication of the ter sequences is inhibited when the sequences are bounded by Tus protein or an RTP protein.
7. A method for replicating circular DNA in a cell-free system, comprising the following steps:

(1) preparing circular DNA comprising *oriC* by:

adding an *oriC* transposon and transposase into a buffer to form an *oriC* transposome, wherein the *oriC* transposon is linear DNA comprising a replication origin sequence (origin of chromosome (*oriC*)) that can bind to an enzyme having DnaA activity, and comprising outside end (OE) sequences at both termini thereof; and

reacting the *oriC* transposome with circular DNA comprising no *oriC* in a buffer to carry out a transfer reaction,

(2) forming a reaction mixture of the circular DNA comprising *oriC* obtained in step (1) 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, and

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

(3) reacting the reaction mixture formed in step (2), wherein

the circular DNA comprising *oriC* further comprises a pair of ter sequences that are each inserted outward with respect to *oriC*, and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, wherein

the ter sequences are present in a region close to or adjacent to ori C, wherein

when the circular DNA has the ter sequences, the reaction solution in step (2) further comprises a protein having an activity of inhibiting replication by binding to the ter sequences, and when the circular DNA has the nucleotide sequence recognized by a DNA multimer separation enzyme, the reaction solution in step (2) further comprises the DNA multimer separation enzyme, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the protein having an activity of inhibiting replication by binding to the ter sequences is a Tus protein or an RTP protein.

8. A method for replicating circular DNA in a cell-free system, comprising the following steps:

(1) preparing circular DNA comprising *oriC* by:

adding an *oriC* transposon and transposase into a buffer to form an *oriC* transposome, wherein the *oriC* transposon is linear DNA comprising a replication origin sequence (origin of chromosome (*oriC*)) that can bind to an enzyme having DnaA activity, and comprising outside end (OE) sequences at both termini thereof; and

reacting the *oriC* transposome with circular DNA comprising no *oriC* in a buffer to carry out a transfer reaction,

(2) forming a reaction mixture of the circular DNA comprising *oriC* obtained in step (1) 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, and

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

(3) reacting the reaction mixture formed in step (2), wherein

the *oriC* transposon in step (1) further comprises a pair of ter sequences that are each inserted outward with respect to *oriC*, and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, wherein

the ter sequences are present in a region close to or adjacent to ori C, wherein

when the linear DNA has the ter sequences, the reaction solution in step (2) further comprises a protein having an activity of inhibiting replication by binding to the ter sequences, and when the circular DNA has the nucleotide sequence recognized by a DNA multimer separation enzyme, the reaction solution in step (2) further comprises the DNA multimer separation enzyme, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the protein having an activity of inhibiting replication by binding to the ter sequences is a Tus protein or an RTP protein.

9. The method according to claim 7 or 8, wherein the OE sequence comprises the sequence shown in SEQ ID NO: 25 (5’-CTGTCTCTTATACACATCT-3’) and a complementary sequence thereto, and the OE sequence comprising the sequence shown in SEQ ID NO: 25 is inserted into the 5’-terminus of the linear DNA in step (1), and the OE sequence comprising a complementary sequence to the sequence shown in SEQ ID NO: 25 is inserted into the 3’-terminus of the linear DNA.

10. The method according to any one of claims 7 to 9, further comprising:

(4) removing the *oriC* transposon from the circular DNA replicated or amplified in the reaction product in step (3).

11. A nucleic acid, which is linear DNA having a length of 311 bp to 2.0 kb, and comprises *oriC*, and a pair of ter sequences that are each inserted outward with respect to the

oriC and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, and also comprises outside end (OE) sequences at both termini thereof, wherein

the ter sequences are present in a region close to or adjacent to ori C, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the replication of the ter sequences is inhibited when the sequences are bounded by Tus protein or an RTP protein.

12. A kit for replicating circular DNA to be used for the method according to any one of Claims 1 to 10, comprising 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;

an *oriC* transposon, which is linear DNA comprising a replication origin sequence (origin of chromosome (*oriC*)) that can bind to an enzyme having DnaA activity, and comprising outside end (OE) sequences at both termini thereof; and

transposase, wherein

the *oriC* transposon further comprises a pair of ter sequences that are each inserted outward with respect to *oriC*, and/or a nucleotide sequence recognized by a DNA multimer separation enzyme, wherein

the ter sequences are present in a region close to or adjacent to ori C, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the replication of the ter sequences is inhibited when the sequences are bounded by Tus protein or an RTP protein.

13. The kit according to claim 12, further comprising:

a protein having an activity of inhibiting replication by binding to the ter sequences; and/or

a DNA multimer separation enzyme, wherein

the DNA multimer separation enzyme is Cre or XerCD, and wherein

the protein having an activity of inhibiting replication by binding to the ter sequences is a Tus protein or an RTP protein.