[Claims]

[Claim 1]

A preparation method of a DNA fragment, the DNA fragment having at least one insert DNA unit comprising: a DNA comprising a replication origin effective in a host; and an insert DNA in which unit DNAs are linked, characterized in that the method comprises:

(A) processing the plurality of types of plasmids comprising an insert DNA unit in which a plurality of types of unit DNAs capable of being linked in a specific linking order are linked with a restriction enzyme suitable for each plasmid to prepare a plurality of types of unit DNA mixture solutions, each comprising the plurality of types of unit DNAs linked on each of the plasmids; and

(B) re-assembling the plurality of types of unit DNAs by OGAB method using the plurality of types of unit DNA mixture solutions obtained in step (A) to prepare a long-chain DNA fragment.

[Claim 2]

The preparation method of a DNA fragment of claim 1, wherein the DNA fragment is a DNA fragment for cell transformation.

[Claim 3]

The preparation method of a DNA fragment of claim 2, wherein the replication origin is effective in a host microorganism, and the DNA fragment for cell transformation is for microbial cell transformation.

[Claim 4]

The preparation method of a DNA fragment of any one of claims 1 to 3 further comprising preparing the plurality of types of plasmids prior to step (A).

[Claim 5]

The preparation method of a DNA fragment of any one of claims 1 to 4, wherein the plurality of types of plasmids is prepared by OGAB method.

[Claim 6]

The preparation method of a DNA fragment of any one of claims 1 to 5, characterized in that all the ratios between molar concentrations for DNA fragments in the plurality of types of unit DNA mixture solutions obtained in step (A) are 0.8 to 1.2.

[Claim 7]

The preparation method of a DNA fragment of any one of claims 1 to 6, characterized in that in the plurality of types of plasmids, the number of types of unit DNAs comprised in one type of insert DNA unit is 3 to 60.

[Claim 8]

The preparation method of a DNA fragment of any one of claims 1 to 7, wherein the number of types of the restriction enzymes used in step (A) is three or less.

[Claim 9]

The preparation method of a DNA fragment of any one of claims 1 to 8, wherein the restriction enzyme is a restriction enzyme that produces an overhang end.

[Claim 10]

A preparation method of a DNA fragment, the DNA fragment having at least one
insert DNA unit comprising: a DNA comprising a replication origin effective in a host; and an insert DNA in which unit DNAs are linked, characterized in that the method comprises:

(A') preparing a plurality of types of plasmids by the preparation method of a DNA fragment of any one of claims 1 to 9,

(B') processing the plurality of types of plasmids prepared in step (A') with a restriction enzyme suitable for each plasmid to cleave the plasmids into a plurality of types of unit DNAs and preparing a plurality of types of unit DNA mixture solutions; and

(C) re-assembling the plurality of types of unit DNAs by OGAB method using the unit DNA mixture solutions obtained in step (B') to prepare a long-chain DNA fragment.

[Claim 11]

The preparation method of a DNA fragment of claim 10, characterized by selecting a plurality of types of plasmids comprising the obtained long-chain DNA fragment and reusing the plasmids as the plasmids in step (B').

[Claim 12]

A preparation method of a plasmid comprising

preparing a DNA fragment by the preparation method of a DNA fragment of any one of claims 1 to 11, and

allowing a plasmid to comprise the DNA fragment.

[Claim 13]

A method for constructing a chimeric plasmid library, using the preparation method of a DNA fragment of any one of claims 1 to 11 or the preparation method of a plasmid of claim 12.

[Claim 14]

A method of cell transformation comprising

preparing a DNA fragment by the preparation method of a DNA fragment of any one of claims 1 to 11 or preparing a plasmid by the preparation method of a plasmid of claim 12, and

transforming a cell with the DNA fragment or the plasmid.

[Claim 15]

A method of transforming a cell with a DNA fragment of interest, wherein the DNA fragment has at least one insert DNA unit comprising: a DNA comprising a replication origin effective in a host of the cell; and an insert DNA, wherein the insert DNA is formed by linking a plurality of types of unit DNAs capable of being linked in a specific linking order,

wherein the method comprises

(A) processing a plurality of types of plasmids comprising the insert DNA unit with a restriction enzyme suitable for each plasmid to prepare a plurality of types of unit DNA mixture solutions, each comprising the plurality of types of unit DNAs linked on each of the plasmids;

(B) re-assembling the plurality of types of unit DNAs by

OGAB method using the plurality of types of unit DNA mixture solutions obtained in step (A) to prepare a long-chain DNA fragment; and

(C) transforming the cell with the long-chain DNA fragment.

[Claim 16]

A method of producing a cell comprising a DNA fragment of interest, wherein the DNA fragment has at least one insert DNA unit comprising: a DNA comprising a replication origin effective in a host of the cell; and an insert DNA, wherein the insert DNA is formed by linking a plurality of types of unit DNAs capable of being linked in a specific linking order,

wherein the method comprises

(A) processing a plurality of types of plasmids comprising the insert DNA unit with a restriction enzyme suitable for each plasmid to prepare a plurality of types of unit DNA mixture solutions, each comprising the plurality of types of unit DNAs linked on each of the plasmids;

(B) re-assembling the plurality of types of unit DNAs by OGAB method using the plurality of types of unit DNA mixture solutions obtained in step (A) to prepare a long-chain DNA fragment; and

(C) transforming the cell with the long-chain DNA fragment.