plasmids are constructed for other choice gene fragments to prepare seed plasmids, whose number of types is the same as the maximum number of choices. Cleaving each seed plasmid by a restriction enzyme gives a solution in which the gene fragments are mixed in an equimolar state. Equimolarity of this solution is maintained even when it is mixed with another seed plasmid. Various gene fragments contained in such solution are then linearly linked to give a polymeric DNA in a pseudo-tandem repeat state in which a plasmid vector portion periodically appears, and this DNA is used to transform Bacillus subtilis. A combinatorial library is efficiently constructed by circularization in the body of Bacillus subtilis utilizing homology to the plasmid vector portion.

[0012]

This method has a feature in that gene fragments at an equal molar concentration necessary for construction of a combinatorial library can be very easily and certainly prepared and the scale of the library construction can be enlarged more than ever before. In addition, with this method, a long-chain DNA combinatorial library of the next cycle can be constructed even without confirming the genotype of the obtained plasmid. Specifically, the present invention is summarized as follows.

[0013]

[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 microorganism; and an insert DNA in which unit DNAs are linked, characterized in that the method comprises:

(A) processing a 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 wherein the DNA unit comprises a DNA comprising a replication origin effective in a host microorganism; 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, wherein all the ratios between molar concentrations for unit DNAs in the plurality of types of unit DNA mixture solutions obtained in step (A) are 0.8 to 1.2, and the restriction enzyme is a restriction enzyme that produces an overhang end.

[2] The preparation method of a DNA fragment for microbial cell transformation of [1], characterized in that in step (A), the number of types of unit DNAs comprised in one type of insert DNA unit is 3 to 60.

[3] A plasmid comprising a DNA fragment for microbial cell transformation obtained by the preparation method of any of [1] or [2].

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

(B') processing the plurality of types of plasmids of [3] with a restriction enzyme suitable for each plasmid to cleave the plasmids into unit DNAs and preparing a plurality of types of unit DNA mixture solutions; and

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

[5] The preparation method of a DNA fragment for microbial cell transformation of [4], 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').

[6] A method for constructing a chimeric plasmid library, using the preparation method of a DNA fragment for microbial cell transformation of any of [1], [2], [4], and [5].

[Advantageous Effects of Invention]

[0014] According to the present invention, it is possible to quickly and efficiently construct a long-chain DNA combinatorial library. It is also possible to reuse a plurality of plasmids which are selected from the same library and whose base sequences have not been confirmed for construction of a new chimeric library.

[Brief Description of Drawings]

[0015] [Figure 1] Figure 1 shows the structure of pGETS302—SfiIpBR, which is a plasmid vector for assembling.

[Figure 2] Figure 2 shows the detailed structure of a unit DNA constituting an insert unit.

[Figure 3] Figure 3 shows an artificial metabolic pathway of budding yeast which is designed to highly produce isobutanol.

[Figure 4] Figure 4 schematically shows the method for constructing a chimeric plasmid library of the present invention.

Claims

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 microorganism; and an insert DNA in which unit DNAs are linked, characterized in that the method comprises:

(A) processing a 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 wherein the DNA unit comprises a DNA comprising a replication origin effective in a host microorganism; 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,

wherein all the ratios between molar concentrations for unit DNAs in the plurality of types of unit DNA mixture solutions obtained in step (A) are 0.8 to 1.2, and

the restriction enzyme is a restriction enzyme that produces an overhang end.

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

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.

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).

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.

6. The preparation method of a DNA fragment of any one of claims 1 to 5, 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.

7. The preparation method of a DNA fragment of any one of claims 1 to 6, wherein the number of types of the restriction enzymes used in step (A) is three or less.

8. 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 microorganism; 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 7,

(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.

9. The preparation method of a DNA fragment of claim 8, 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').

10. 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 9, and
allowing a plasmid to comprise the DNA fragment.

11. A method for constructing a chimeric plasmid library, using the preparation method of a DNA fragment of any one of claims 1 to 9 or the preparation method of a plasmid of claim 10.

12. A method of microbial cell transformation comprising
preparing a DNA fragment by the preparation method of a DNA fragment of any one of claims 1 to 9 or preparing a plasmid by the preparation method of a plasmid of claim 10, and
transforming a microbial cell with the DNA fragment or the plasmid.

13. A method of transforming a microbial 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 microorganism of the microbial 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 microbial cell with the long-chain DNA fragment.

14. A method of producing a microbial 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 microorganism of the microbial 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 microbial cell with the long-chain DNA fragment.