

Patent Literature

[0006]

Patent Literature 1: JP Patent No. 5389638

Patent Literature 2: JP Patent Publication (Kokai) No. 2003-79375 A

Patent Literature 3: JP Patent No. 3972106

Summary

Technical Problem

[0007]

For a technique for genome information analysis, such as genetic linkage analysis conducted with the use of a DNA marker, production of a DNA library in a more convenient and highly reproducible manner is desired. As described above, a wide variety of techniques for producing a DNA library are known. To date, however, there have been no techniques known to be sufficient in terms of convenience and/or reproducibility. Under the above circumstances, it is an object of the present invention to provide a method for producing a DNA library with more convenience and higher reproducibility, and it is another object to provide a method for analyzing genomic DNA with the use of such DNA library.

Solution to Problem

[0008]

The present inventors have conducted concentrated studies in order to attain the above objects. As a result, they discovered that high reproducibility could be achieved by conducting PCR with the use of a random primer while designating the concentration of such random primer within a designated range in a reaction solution. This has led to the completion of the present invention.

In one aspect, the present disclosure refers to a method for analyzing genomic DNA, comprising steps of; preparing a DNA library, which is produced by the method comprising conducting a nucleic acid amplification reaction in a reaction solution containing genomic

DNA and a random primer comprising 9 to 30 nucleotides using genomic DNA as a template to obtain DNA fragments by the nucleic acid amplification reaction, wherein when the random primer contains 9 to 10 nucleotides, the concentration of the random primer is set to 40 to 60 μM ; when the random primer contains 10 to 14 nucleotides, the concentration of the random primer is set to 100 μM or less, and said concentration is defined by the formula $y > 3E + 08x^{-6.974}$, provided that the nucleotide length of the random primer is represented by "x" and the concentration of the random primer is represented by "y"; when the random primer contains 14 to 18 nucleotides, the concentration of the random primer is set to 4 to 100 μM ; when the random primer contains 18 to 28 nucleotides, the concentration of the random primer is set to 4 μM or more, and said concentration is defined by the formula $y < 8E + 08x^{-5.533}$; when the random primer contains 28 to 29 nucleotides, the concentration of the random primer is set to 6 to 10 μM ; and when the random primer contains 30 nucleotides, the concentration of the random primer is set to 6 μM ; determining the nucleotide sequence of DNA fragments contained in the DNA library produced; and confirming the presence or absence of specific DNA fragments based on the nucleotide sequence.

In another aspect, the present disclosure refers to a method for analyzing genomic DNA, comprising steps of: conducting a nucleic acid amplification reaction in a first reaction solution comprising genomic DNA and a random primer comprising 9 to 30 nucleotides at a high concentration to obtain first DNA fragments by the nucleic acid amplification reaction using the genomic DNA as a template, wherein when the random primer contains 9 to 10 nucleotides, the concentration of the random primer is set to 40 to 60 μM ; when the random primer contains 10 to 14 nucleotides, the concentration of the random primer is set to 100 μM or less, and said concentration is defined by the formula $y > 3E + 08x^{-6.974}$, provided that the nucleotide length of the random primer is represented by "x" and the concentration of the random primer is represented by "y"; when the random primer contains 14 to 18 nucleotides, the concentration of the random primer is set to 4 to 100 μM ; when the random primer contains 18 to 28 nucleotides, the concentration of the random primer is set to 4 μM or more, and said concentration is defined by the formula $y < 8E + 08x^{-5.533}$; when the random primer contains 28 to 29 nucleotides, the concentration of the random primer is set to 6 to 10 μM ;

and when the random primer contains 30 nucleotides, the concentration of the random primer is set to 6 μM ; conducting a nucleic acid amplification reaction in a second reaction solution comprising the obtained first DNA fragments and a primer, which has a 3'-end nucleotide sequence capable of hybridizing to a 5'-end nucleotide sequence of the first DNA fragments to obtain second DNA fragments; preparing a DNA library with the second DNA fragments or DNA fragments obtained by a nucleic acid amplification reaction using the second DNA fragments as templates or a nucleic acid amplification reaction to be conducted repeatedly; determining the nucleotide sequence of DNA fragments contained in the DNA library prepared; and confirming the presence or absence of specific DNA fragments based on the nucleotide sequence.

[0009]

The present invention includes the following.

- (1) A method for producing a DNA library, comprising conducting a nucleic acid amplification reaction in a reaction solution containing genomic DNA and a random primer at a high concentration using genomic DNA as a template to obtain DNA fragments.
- (2) The method for producing a DNA library according to (1), wherein the reaction solution comprises the random primer at a concentration of 4 to 200 μM .
- (3) The method for producing a DNA library according to (1), wherein the reaction solution comprises the random primer at a concentration of 4 to 100 μM .
- (4) The method for producing a DNA library according to (1), wherein the random primer comprises 9 to 30 nucleotides.
- (5) The method for producing a DNA library according to (1), wherein the DNA fragments each comprise 100 to 500 nucleotides.
- (6) A method for analyzing genomic DNA, comprising using a DNA library produced by the method for producing a DNA library according to any one of (1) to (5) as a DNA marker.
- (7) The method for analyzing genomic DNA according to (6), which comprises determining the nucleotide sequence of the DNA library produced by the method for

producing a DNA library according to any one of (1) to (5) and confirming the presence or absence of the DNA marker based on the nucleotide sequence.

(8) The method for analyzing genomic DNA according to (7), wherein the presence or absence of the DNA marker is confirmed based on the number of reads of the nucleotide sequence of the DNA library in the step of confirming the presence or absence of the DNA marker.

(9) The method for analyzing genomic DNA according to (7), wherein the nucleotide sequence of the DNA library is compared with known sequence information or with the nucleotide sequence of a DNA library produced using genomic DNA from a different organism or tissue, and the presence or absence of the DNA marker is confirmed based on differences in the nucleotide sequences.

(10) The method for analyzing genomic DNA according to (6), which comprises:
a step of preparing a pair of primers for specifically amplifying the DNA marker based on the nucleotide sequence of the DNA marker;
a step of conducting a nucleic acid amplification reaction using genomic DNA extracted from a target organism as a template and the pair of primers; and
a step of confirming the presence or absence of the DNA marker in the genomic DNA based on the results of the nucleic acid amplification reaction.

(11) A method for producing a DNA library, comprising:
a step of conducting a nucleic acid amplification reaction in a first reaction solution comprising genomic DNA and a random primer at a high concentration to obtain first DNA fragments by the nucleic acid amplification reaction using the genomic DNA as a template; and
a step of conducting a nucleic acid amplification reaction in a second reaction solution comprising the obtained first DNA fragments and a nucleotide, as a primer, which has a 3'-end nucleotide sequence having 70% identity to at least a 5'-end nucleotide sequence of the random primer to ligate the nucleotides to the first DNA fragments, thereby obtaining second DNA fragments.

- (12) The method for producing a DNA library according to (11), wherein the first reaction solution comprises the random primer at a concentration of 4 to 100 μ M.
- (13) The method for producing a DNA library according to (11), wherein the first reaction solution comprises the random primer at a concentration of 4 to 100 μ M.
- (14) The method for producing a DNA library according to (11), wherein the random primer comprises 9 to 30 nucleotides.
- (15) The method for producing a DNA library according to (11), wherein the first DNA fragments each comprise 100 to 500 nucleotides.
- (16) The method for producing a DNA library according to (11), wherein the primer for amplifying the second DNA fragments comprises a region used for a nucleotide sequencing reaction, or the primer used for a nucleic acid amplification reaction using the second DNA fragments as templates or a nucleic acid amplification reaction to be conducted repeatedly comprises a region used for a nucleotide sequencing reaction.

CLAIMS

1. A method for analyzing genomic DNA, comprising steps of;
preparing a DNA library, which is produced by the method comprising conducting a nucleic acid amplification reaction in a reaction solution containing genomic DNA and a random primer comprising 9 to 30 nucleotides using genomic DNA as a template to obtain DNA fragments by the nucleic acid amplification reaction, wherein
 - when the random primer contains 9 to 10 nucleotides, the concentration of the random primer is set to 40 to 60 μM ;
 - when the random primer contains 10 to 14 nucleotides, the concentration of the random primer is set to 100 μM or less, and said concentration is defined by the formula $y > 3E + 08x^{-6.974}$, provided that the nucleotide length of the random primer is represented by "x" and the concentration of the random primer is represented by "y";
 - when the random primer contains 14 to 18 nucleotides, the concentration of the random primer is set to 4 to 100 μM ;
 - when the random primer contains 18 to 28 nucleotides, the concentration of the random primer is set to 4 μM or more, and said concentration is defined by the formula $y < 8E + 08x^{-5.533}$;
 - when the random primer contains 28 to 29 nucleotides, the concentration of the random primer is set to 6 to 10 μM ; and
 - when the random primer contains 30 nucleotides, the concentration of the random primer is set to 6 μM ;determining the nucleotide sequence of DNA fragments contained in the DNA library produced; and
confirming the presence or absence of specific DNA fragments based on the nucleotide sequence.

2. The method for analyzing genomic DNA according to claim 1, wherein the presence or absence of the specific DNA fragments is confirmed based on the number of reads of the nucleotide sequence of the specific DNA fragments.

3. The method for analyzing genomic DNA according to claim 1, wherein the nucleotide sequence of DNA fragments contained in the DNA library is compared with known sequence information or with the nucleotide sequence of DNA fragments contained in a DNA library produced using genomic DNA from a different organism or tissue, and the presence or absence of the specific DNA fragments is confirmed based on differences in the nucleotide sequences.

4. The method for analyzing genomic DNA according to claim 1, further comprising steps of:

preparing a pair of primers for specifically amplifying the specific DNA fragments;
conducting a nucleic acid amplification reaction using genomic DNA extracted from a target organism as a template and the pair of primers; and

confirming the presence or absence of the specific DNA fragments in the genomic DNA based on the results of the nucleic acid amplification reaction.

5. The method for analyzing genomic DNA according to claim 1, wherein the DNA fragments each comprise 100 to 500 nucleotides.

6. A method for analyzing genomic DNA, comprising steps of:

conducting a nucleic acid amplification reaction in a first reaction solution comprising genomic DNA and a random primer comprising 9 to 30 nucleotides at a high concentration to obtain first DNA fragments by the nucleic acid amplification reaction using the genomic DNA as a template, wherein

when the random primer contains 9 to 10 nucleotides, the concentration of the random primer is set to 40 to 60 μM ;

when the random primer contains 10 to 14 nucleotides, the concentration of the random primer is set to 100 μ M or less, and said concentration is defined by the formula $y > 3E + 08x^{-6.974}$, provided that the nucleotide length of the random primer is represented by "x" and the concentration of the random primer is represented by "y";

when the random primer contains 14 to 18 nucleotides, the concentration of the random primer is set to 4 to 100 μ M;

when the random primer contains 18 to 28 nucleotides, the concentration of the random primer is set to 4 μ M or more, and said concentration is defined by the formula $y < 8E + 08x^{-5.533}$;

when the random primer contains 28 to 29 nucleotides, the concentration of the random primer is set to 6 to 10 μ M; and

when the random primer contains 30 nucleotides, the concentration of the random primer is set to 6 μ M;

conducting a nucleic acid amplification reaction in a second reaction solution comprising the obtained first DNA fragments and a primer, which has a 3'-end nucleotide sequence capable of hybridizing to a 5'-end nucleotide sequence of the first DNA fragments to obtain second DNA fragments;

preparing a DNA library with the second DNA fragments or DNA fragments obtained by a nucleic acid amplification reaction using the second DNA fragments as templates or a nucleic acid amplification reaction to be conducted repeatedly;

determining the nucleotide sequence of DNA fragments contained in the DNA library prepared; and

confirming the presence or absence of specific DNA fragments based on the nucleotide sequence.

7. The method for analyzing genomic DNA according to claim 6, wherein the primer for amplifying the second DNA fragments comprises a region used for a nucleotide sequencing reaction, or the primer used for a nucleic acid amplification reaction using the

second DNA fragments as templates or a nucleic acid amplification reaction to be conducted repeatedly comprises a region used for a nucleotide sequencing reaction.

8. The method for analyzing genomic DNA according to claim 6, wherein the presence or absence of specific DNA fragments is confirmed based on the number of reads of the nucleotide sequence of the specific DNA fragments.

9. The method for analyzing genomic DNA according to claim 6, wherein the nucleotide sequence of DNA fragments contained in the DNA library is compared with known sequence information or with the nucleotide sequence of DNA fragments contained in a DNA library produced using genomic DNA from a different organism or tissue, and the presence or absence of the specific DNA fragments is confirmed based on differences in the nucleotide sequences.

10. The method for analyzing genomic DNA according to claim 6, which further comprises steps of:

- preparing a pair of primers for specifically amplifying the specific DNA fragments;
- conducting a nucleic acid amplification reaction using genomic DNA extracted from a target organism as a template and the pair of primers; and
- confirming the presence or absence of the specific DNA fragments in the genomic DNA based on the results of the nucleic acid amplification reaction.

11. The method for analyzing genomic DNA according to claim 6, wherein the first DNA fragments each comprise 100 to 500 nucleotides.