fragment without the need of special equipment by amplifying the target nucleic acid in the form of a double-stranded nucleic acid having a single-stranded region at each end thereof, allowing the amplified fragment to bind to a solid phase with an oligonucleotide probe capable of hybridizing to one of the single-stranded regions, and detecting the binding. Thus, the present invention has been completed.

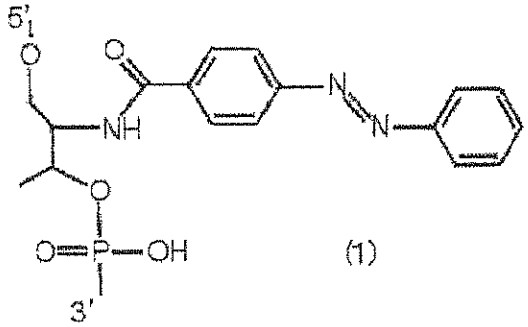
[0014]

In one aspect, there is provided a nucleic acid detection method, comprising the following steps (1) and (2):

(1) hybridizing an oligonucleotide probe immobilized on a solid phase with a single-stranded region of an amplified double-stranded DNA fragment, wherein the single-stranded region comprises natural nucleotides,

(2) detecting the amplified double-stranded DNA fragment,

wherein the amplified double-stranded DNA fragment is a product obtained by a nucleic acid amplification technique using a primer containing a tag region that is not made double-stranded by a nucleic acid amplification reaction and a nucleic acid represented by the following formula (1):



(1)

Specifically, the present ~~invention~~disclosure relates to a nucleic acid detection method, including the step of hybridizing a first oligonucleotide probe immobilized on a solid phase with one of single-stranded regions which contain natural nucleotides and are respectively located at opposite ends of an amplified double-stranded DNA fragment.

[0015]

Preferably, the method further includes the step of hybridizing a second oligonucleotide probe labeled with a labeling substance with the other single-stranded region of the amplified DNA fragment.

[0016]

Preferably, the labeling substance includes a colored carrier that allows the amplified DNA fragment to be visually detected.

[0017]

Preferably, the method includes the step of detecting the presence of the amplified DNA fragment on a nucleic acid detection device.

[0018]

Preferably, the presence of the amplified DNA fragment is detected by chromatography.

[0019]

Preferably, the method includes the following steps (a) to (c):

(a) placing the amplified DNA fragment in a zone on the nucleic acid detection device which is different from a zone

CLAIMS

Claim 1. A nucleic acid detection method, comprising the step of hybridizing a first oligonucleotide probe immobilized on a solid phase with one of single-stranded regions which comprise natural nucleotides and are respectively located at opposite ends of an amplified double-stranded DNA fragment.

Claim 2. The nucleic acid detection method according to claim 1, further comprising the step of hybridizing a second oligonucleotide probe labeled with a labeling substance with the other single-stranded region of the amplified DNA fragment.

Claim 3. The nucleic acid detection method according to claim 2,

wherein the labeling substance comprises a colored carrier that allows the amplified DNA fragment to be visually detected.

Claim 4. The nucleic acid detection method according to any one of claims 1 to 3, comprising the step of detecting the presence of the amplified DNA fragment on a nucleic acid detection device.

Claim 5. The nucleic acid detection method according to any one of claims 1 to 4,

wherein the presence of the amplified DNA fragment is detected by chromatography.

Claim 6. The nucleic acid detection method according to claim 4 or 5, comprising the following steps (a) to (c):

(a) placing the amplified DNA fragment in a zone on the nucleic acid detection device which is different from a zone where the first oligonucleotide probe is immobilized;

(b) diffusing the amplified DNA fragment with a solvent

on the device toward the zone where the first oligonucleotide probe is immobilized; and

(c) hybridizing the first oligonucleotide probe with the amplified DNA fragment in the zone where the first oligonucleotide probe is immobilized.

Claim 7. The nucleic acid detection method according to claim 6, further comprising the step of hybridizing the second oligonucleotide probe labeled with the labeling substance with the amplified DNA fragment before the step (c).

Claim 8. The nucleic acid detection method according to claim 4 or 5, comprising the following steps (d) to (h):

(d) placing the amplified DNA fragment and the second oligonucleotide probe labeled with the labeling substance respectively in discrete zones on the nucleic acid detection device which are different from a zone where the first oligonucleotide probe is immobilized;

(e) diffusing the amplified DNA fragment with a solvent toward the zone where the second oligonucleotide probe labeled with the labeling substance is placed;

(f) hybridizing the second oligonucleotide probe labeled with the labeling substance with the amplified DNA fragment in the zone where the second oligonucleotide probe labeled with the labeling substance is placed;

(g) diffusing a hybridization complex obtained in the step (f) on a development medium toward the zone where the first oligonucleotide probe is placed; and

(h) hybridizing the first oligonucleotide probe with the complex in the zone where the first oligonucleotide probe is immobilized.

Claim 9. The nucleic acid detection method according to any one of claims 1 to 8,

wherein each of the single-stranded regions comprising

natural nucleotides has a sequence in the same orientation as the double-stranded DNA region.

Claim 10. The nucleic acid detection method according to any one of claims 1 to 9,

wherein the amplified DNA fragment is a product obtained by a nucleic acid amplification technique using two primers each containing a tag region that is not made double-stranded by a nucleic acid amplification reaction.

Claim 11. The nucleic acid detection method according to any one of claims 1 to 9,

wherein the amplified DNA fragment is a product obtained by a nucleic acid amplification technique using a first primer set including primers each comprising a sequence capable of hybridizing to a template of the target nucleic acid and a common sequence incapable of hybridizing to the template, and a second primer set including primers each comprising a sequence capable of hybridizing to a complementary sequence to the corresponding common sequence and a tag region that is not made double-stranded by a nucleic acid amplification reaction.

Claim 12. The nucleic acid detection method according to claim 11,

wherein the tag region that is not made double-stranded by a nucleic acid amplification reaction comprises natural nucleotides, and has a sequence in the same orientation as the sequence capable of hybridizing to a complementary sequence to the corresponding common sequence.

Claim 13. A detection device for use in the nucleic acid detection method according to any one of claims 1 to 12, the device comprising:

a zone where the amplified DNA fragment is placed;

a chromatographic carrier having the first

oligonucleotide probe capable of binding to the amplified DNA fragment; and

the second oligonucleotide probe labeled with the labeling substance.

Claim 14. An amplified double-stranded DNA fragment which is produced by a nucleic acid amplification technique using two primers each containing a tag region that is not made double-stranded by a nucleic acid amplification reaction, and which has at each end thereof a single-stranded region comprising natural nucleotides.

Claim 15. The amplified double-stranded DNA fragment according to claim 14,

wherein the tag region of each primer that is not made double-stranded by a nucleic acid amplification reaction comprises natural nucleotides, and

the entire sequence of each primer is in the same orientation.

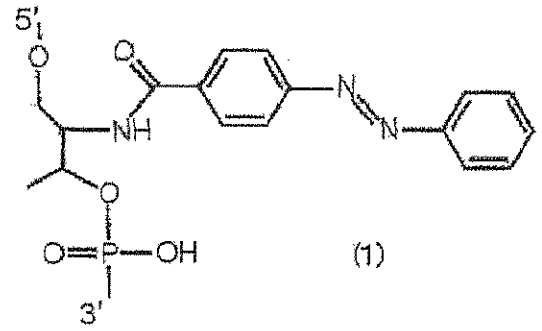
Claims

1. A nucleic acid detection method, comprising the following steps (1) and (2):

(1) hybridizing an oligonucleotide probe immobilized on a solid phase with a single-stranded region of an amplified double-stranded DNA fragment, wherein the single-stranded region comprises natural nucleotides,

(2) detecting the amplified double-stranded DNA fragment,

wherein the amplified double-stranded DNA fragment is a product obtained by a nucleic acid amplification technique using a primer containing a tag region that is not made double-stranded by a nucleic acid amplification reaction and a nucleic acid represented by the following formula (1):



(1)

2. The nucleic acid detection method according to Claim 1,

wherein the length of the single-stranded region comprising natural nucleotides is not less than 20 bases.

3. The nucleic acid detection method according to Claim 1 or 2,

wherein the amplified double-stranded DNA fragment is detected by allowing the double-stranded DNA with a labeling substance, and visually detecting the labeling substance.

4. The nucleic acid detection method according to any one of Claims 1 to 3,

comprising the step of detecting the presence of the amplified DNA fragment on a nucleic acid detection device.

5. The nucleic acid detection method according to

Claim 4,

comprising the following steps (a) to (c);

(a) placing the amplified DNA fragment in a zone on the nucleic acid detection device which is different from a zone where the oligonucleotide probe is immobilized;

(b) diffusing the amplified DNA fragment with a solvent on the device toward the zone where the oligonucleotide probe is immobilized; and

(c) hybridizing the oligonucleotide probe with the amplified DNA fragment in the zone where the oligonucleotide probe is immobilized.

6. The nucleic acid detection method according to Claim 5,

wherein the nucleic acid detection device is a chromatography device.

7. The nucleic acid detection method according to any one of Claims 1 to 6,

wherein the single-stranded region comprising natural nucleotides has a sequence in the same orientation as the double-stranded DNA region.

8. The nucleic acid detection method according to any one of Claims 1 to 7,

wherein the amplified DNA fragment is a product obtained by a nucleic acid amplification technique using

a first primer set including primers each comprising a sequence capable of hybridizing to a template of the target nucleic acid and a common sequence incapable of hybridizing to the template, and

a second primer set including primers each comprising a sequence capable of hybridizing to a complementary sequence to the corresponding common sequence and a tag region that is not made double-stranded by a nucleic acid amplification reaction.

9. The nucleic acid detection method according to Claim 8,

wherein the tag region that is not made double-stranded by a nucleic acid amplification reaction comprises natural

nucleotides, and has a sequence in the same orientation as the sequence capable of hybridizing to a complementary sequence to the corresponding common sequence.