clarify, in particular, the plus strand and the minus strand of the template nucleic acid. Here, the amplification reaction is started and proceeds in a similar manner to that of FIG. 1 except that the S1 sequence, S2 sequence and S3 sequence in FIG. 1 are referred to as "B1 sequence", "B2 sequence" and "F1 sequence", and the first template nucleic acid is assigned as the plus strand (sign "(+)" is indicated for the sequence in each stage) and the second template nucleic acid is assigned as the minus strand (sign "(-)" is indicated for the sequence in each stage).

FIG. 4 shows an embodiment in which the first primer contains the S1 sequence and S2 sequence, and as well, the second primer contains, in addition to the S3 sequence, an S4 sequence on the 5' terminal side thereof. More specifically, the first primer is used as the forward primer, and contains the F1 sequence corresponding to the S1 sequence and the F2 sequence corresponding to the ~~F2~~ S2 sequence. On the other hand, the second primer is used as the reverse primer, and contains the B1 sequence corresponding to the S3 sequence and the B2 sequence corresponding to the S4 sequence.

Each of the above-described amplification steps is carried out in a similar manner to that of FIG. 1 except for the following. That is, with the amplification reaction shown in FIG. 1, two types of stem-and-loop structural bodies are obtained, whereas with the amplification reaction shown in FIG. 4, a total of four types of stem-and-loop structural bodies are obtained. Of these, two types each comprise the first replication strand corresponding to the elongated strand of the first primer and the second replication strand corresponding to the elongated strand of the second primer as in FIG. 1. The other two types each comprise the first replication strand corresponding to the elongated strand of the first primer and the second

replication strand corresponding to the elongated strand of the second primer, obtained in the amplification step shown in FIG. 3.

FIG. 5 shows the process of forming a replication strand corresponding to the elongated strand of the second primer shown in FIG. 1. This is shown as the subsequent amplification process following the reaction of FIG. 2, which further specifically illustrates FIG. 1. The replication strand used as the first template in FIG. 5 is a replication strand 210 corresponding to the elongated strand of the forward primer 206 in FIG. 2. In FIG. 5, the reverse primer 209 bound to the replication strand 210 is elongated to generate an elongated strand 502. The elongated strand 502 forms an intramolecular structure spontaneously while depending on the sequence thereof, and thus a stem-and-loop structural body 505 is formed. The stem-and-loop structural body 505 comprises an F2′ sequence located on the 3′ terminal (the sequence being complementary to the "F2 sequence"), an F1′ sequence adjacent thereto on the 5′ side (the sequence being complementary to the "F1 sequence"), a template sequence of a minus strand, adjacent thereto on the 5′ side, and a B1 sequence adjacent thereto on the 5′ side. The F2′ sequence contains therein a sequence complementary to a part of the template sequence of the minus strand, and therefore, an intramolecular binding occurs on the same strand. Thus, a double strand region containing a stem portion and a single strand region containing a loop portion are formed. Further, the 3′ terminal of the stem-and-loop structural body 505 is further elongated by polymerase in the reaction liquid. With this elongation, all the regions are double-stranded except for the loop portion ~~508~~507.

FIG. 6 shows the process of further forming a replication strand in which all the regions of the stem-and-loop structural body are double stranded

was detected more stably in the case where amplification products having a long stem portion are detected, as compared to the case of the amplification products having a short stem portion. In terms of the loop length, a sufficient signal increase was confirmed from 40bp to 60bp. Further, as to the products of the negative control, to which TE was added in place of the template, it was confirmed that there were no signal increases.

Example 2

Discussion on further modified examples which involve different target lengths and different high-rate PCR enzymes, will now be provided in connection the method of detecting a product amplified with use loop-forming primers by using a DNA chip. Further, the case where both of the F primers and R primers were used as the loop-forming primers was also discussed.

(1) Amplification

<Template>

A template similar to that used in Example 1 was used here. <Primers>

In order to obtain targets which give different target lengths each other and which show stem-and-loop structures having long stems, 5′-GCGTAGAGATGTGGAGGAAC-3′ (F primer: SEQ ID NO. 12) was prepared for targeting 161bp, and further 5′-GTAGGTGGCAAGCGTTGTCC-3′ (F primer: SEQ ID NO. 4) for targeting 322bp, 5′-AGGCAGCAGTAGGGAATCTTCC-3′ (F primer: SEQ ID NO. 13) for targeting 511bp and 5′-CCTGTAAGACTGGGATAACTCCG-3′ (F primer: SEQ ID NO. 14) for targeting 734bp were prepared. Further, in order to obtain products for the case where both of the F primers and R primers were used as the loop-forming primers, 5′-GCTCCTCAGCGTCAGTTACA-GCGTAGAGATGTGGAGGAAC-3′ (F primer: SEQ ID NO. 15) was prepared for targeting 161bp, and further 5′-GCTTTCACATCAGACTTAAGGAACC-GTAGGTGGCAAGCGTTGTCC-3′ (F primer: SEQ ID NO. 416) for

C L A I M S

1. A method of analyzing a nucleic acid in a sample,

the nucleic acid to be amplified comprising a first template nucleic acid and a second template nucleic acid,

the first template nucleic acid comprising a first sequence located on a 3′ terminal, a first template sequence adjacent to a 5′ side of the first sequence, and a second sequence adjacent to a 5′ side of the first template sequence and located on the 5′ terminal, and

the second template nucleic acid, which is a strand complementary to the first template nucleic acid, comprising a third sequence located on a 3′ terminal thereof, a second template sequence adjacent to a 5′ side of the third sequence, and a fourth sequence adjacent to a 5′ side of the second template sequence and located on the 5′ terminal,

the method comprising:

(a-1) preparing a first primer and a second primer configured to amplify the first template nucleic acid and the second template nucleic acid,

wherein the first primer comprises an S1 sequence complementary to the first sequence and an S2 sequence joined to a 5′ terminal side of the S1 sequence, the S2 sequence is a sequence, which is same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the first sequence on the first template nucleic acid and

the second primer comprises an S3 sequence complementary to the third sequence; and

a first replication strand corresponding to an elongated strand from the first primer forms a first stem-and-loop structural body, the first stem-and-loop structural body comprises a first double strand region and a first single strand region on one strand, the first double strand region comprises a first stem portion derived from the S2 sequence and a sequence derived from a complementary sequence of the first template sequence, the sequence derived from a complementary sequence of the first template sequence and the

first stem portion in the first double strand region are complementary each other, the first single strand region forms a first loop portion, and the first single strand region, which forms the first loop portion, comprises the S1 sequence; and

a second replication strand corresponding to an elongated strand from the second primer forms a second stem-and-loop structural body, the second stem-and-loop structural body comprises a second double strand region and a second single strand region on one strand, the second double strand region comprises a second stem portion derived from a complementary sequence of the S2 sequence and a sequence complementary to the second template sequence, the sequence complementary to the second template sequence and the second stem portion in the second double strand region are complementary each other, the second single strand region forms a second loop portion, and the second single strand region, which forms the second loop portion, comprises the S1′ sequence;

(a-2) preparing a first nucleic acid probe and/or a second nucleic acid probe configured to detect the first replication strand and/or the second replication strand,

wherein the first nucleic acid probe comprises a sequence of at least a part of the first single strand region forming the first loop portion of the first replication strand, and the second nucleic acid probe comprises a sequence of at least a part of the second single strand region forming the second loop portion of the second replication strand;

(b) amplifying the sample using the first primer and the second primer, wherein the amplification is by a PCR amplification reaction,

(c) reacting an amplification product obtained in (b) with the first nucleic acid probe and/or the second nucleic acid probe,

(d) detecting presence/absence of a hybridization and/or measuring a quantity of hybridization between the first nucleic acid probe and/or the second nucleic acid probe and the amplification product, and

(e) determining presence or absence of the target nucleic acid in the sample and/or determining a quantity of target nucleic acid presence, based on a result in (d).

~~2. The nucleic acid analysis method of claim 1, wherein the amplification is by a PCR amplification reaction.~~

~~3~~2. The nucleic acid analysis method of claim ~~2~~1, wherein the nucleic acid probe is immobilized on a substrate.

~~4~~3. The nucleic acid analysis method of claim ~~3~~2, wherein the detecting the hybridization and/or measuring a quantity of the hybridization between the nucleic acid probe immobilized on the substrate and the amplification product is carried out using an intercalating agent which indicates an electrochemical activity.

~~5~~4. The nucleic acid analysis method of claim ~~4~~3, wherein the sample comprises a gene and an object of the analysis is the gene.

~~6~~5. An assay kit used for the nucleic acid analysis method of claim 1, the assay kit comprising:

a primer set and a nucleic acid probe,

wherein a first template nucleic acid to be amplified with the primer set comprises a first sequence located on a 3′ terminal, a first template sequence adjacent to a 5′ side of the first sequence, and a second sequence adjacent to a 5′ side of the first template sequence and located on the 5′ terminal, and

a second template nucleic acid to be amplified with the primer set is a strand complementary to the first template nucleic acid, and comprises a third sequence located on a 3′ terminal thereof, a second template sequence adjacent to a 5′ side of the third sequence, and a fourth sequence adjacent to a 5′ side of the second template sequence and located on the 5′ terminal thereof,

the primer set satisfies the following (1) and/or (2):

(1) the first primer comprises an S1 sequence and an S2 sequence, the S1 sequence being complementary to the first sequence, the S2 sequence joined to a 5′ terminal side of the S1 sequence, and the S2 sequence being a sequence, which is

same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the first sequence on the first template nucleic acid, and

the second primer comprises an S3 sequence complementary to the third sequence;

(2) the first primer comprises an S1 sequence and an S2 sequence, the S1 sequence being complementary to the first sequence, the S2 sequence joined to a 5′ side of the S1 sequence, and the S2 sequence being a sequence, which is same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the first sequence on the first template nucleic acid, and

the second primer comprises an S3 sequence and an S4 sequence, the S3 sequence being complementary to the third sequence, the S4 sequence joined to a 5′ side of the S3 sequence, and the S4 sequence being a sequence, which is same as a sequence of at least a part of the second template sequence, located adjacent to or in a vicinity of a 5′ side of the third sequence on the second template nucleic acid;

the nucleic acid probe is at least one type selected from the group consisting of:

a nucleic acid probe comprising a sequence of at least a part of the S1 sequence;

a nucleic acid probe comprising a sequence of at least a part of the complementary sequence of the S1 sequence;

a nucleic acid probe comprising a sequence of at least a part of the S3 sequence; and

a nucleic acid probe comprising a sequence of at least a part of the complementary sequence of the S3 sequence.

~~7~~6. A method of analyzing nucleic acids in a sample,

the nucleic acids to be amplified comprising a first template nucleic acid and a second template nucleic acid,

the first template nucleic acid comprising a first sequence located on a 3′ terminal, a first template sequence adjacent to a 5′ side of the first sequence, and a second sequence adjacent to a 5′ side of the first template sequence and located on the 5′ terminal, and

the second template nucleic acid, which is a strand complementary to the first template nucleic acid, comprising a third sequence located on a 3′ terminal thereof, a second template sequence adjacent to a 5′ side of the third sequence, and a fourth sequence adjacent to a 5′ side of the second template sequence and located on the 5′ terminal,

the method comprising:

(a-1) preparing a first primer and a second primer configured to amplify the first template nucleic acid and the second template nucleic acid,

wherein the first primer comprises an S1 sequence complementary to the first sequence and an S2 sequence joined to a 5′ terminal side of the S1 sequence, the S2 sequence is a sequence, which is same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the first sequence on the first template nucleic acid and

the second primer comprises an S3 sequence complementary to the third sequence and an S4 sequence joined to a 5′ side of the S3 sequence, the S4 sequence is a sequence, which is same as a sequence of at least a part of the second template sequence, located adjacent to or in a vicinity of a 5′ side of the third sequence on the second template nucleic acid;

further, a first replication strand corresponding to an elongated strand from the first primer forms a first stem-and-loop structural body, the first stem-and-loop structural body comprises a first double strand region and a first single strand region on one strand, the first double strand region comprises a first stem portion derived from the S2 sequence and a sequence derived from a complementary sequence of the first template sequence, the sequence derived from a complementary sequence of the first template sequence is complementary to the first stem portion, the first single strand region forms a first loop portion, and the first single strand region, which forms the first loop portion, comprises the S1 sequence;

a second replication strand corresponding to an elongated

strand from the first primer forms a second stem-and-loop structural body, the second stem-and-loop structural body comprises a second double strand region and a second single strand region on one strand, the second double strand region comprises a second stem portion derived from a complementary sequence of the S4 sequence and a sequence derived from a complementary sequence of the first template sequence, the sequence derived from a complementary sequence of the first template sequence is complementary to the second stem portion, the second single strand region forms a second loop portion, and the second single strand region, which forms the second loop portion, comprises a complementary sequence to the S3 sequence;

a third replication strand corresponding to an elongated strand from the second primer forms a third stem-and-loop structural body, the third stem-and-loop structural body comprises a third double strand region and a third single strand region on one strand, the third double strand region comprises a third stem portion derived from the S4 sequence and a sequence derived from a complementary sequence of the second template sequence, the sequence derived from a complementary sequence of the second template sequence is complementary to the third stem portion, the third single strand region forms a third loop portion, and the third single strand region, which forms the third loop portion, comprises the S3 sequence; and

a fourth replication strand corresponding to an elongated strand from the second primer forms a fourth stem-and-loop structural body, the fourth stem-and-loop structural body comprises a fourth double strand region and a fourth single strand region on one strand, the fourth double strand region comprises a fourth stem portion derived from a complementary sequence of the S2 sequence and a sequence derived from a complementary sequence of the second template sequence, the sequence derived from a complementary sequence of the second template sequence is complementary to the fourth stem portion, the fourth single strand region forms a fourth loop portion,

and the fourth single strand region, which forms the fourth loop portion, comprises a complementary sequence to the S1 sequence;

(a-2) preparing at least one from the group consisting of a first nucleic acid probe, a second nucleic acid probe, a third nucleic acid probe and a fourth nucleic acid probe, configured to detect the first replication strand and/or the second replication strand,

wherein the first nucleic acid probe comprises a sequence of at least a part of the first single strand region forming the first loop portion in the first replication strand,

the second nucleic acid probe comprises a sequence of at least a part of the second single strand region forming the second loop portion in the second replication strand,

the third nucleic acid probe comprises a sequence of at least a part of the third single strand region forming the third loop portion in the third replication strand, the sequence comprising a sequence of at least a part of the complementary sequence to the S3 sequence, and

the fourth nucleic acid probe comprises a sequence of at least a part of the fourth single strand region forming the fourth loop portion in the fourth replication strand, the sequence comprising a sequence of at least a part of the S1 sequence;

(b) amplifying the sample using the first primer and the second primer, wherein the amplification is by a PCR amplification reaction,

(c) reacting an amplification product obtained in (b) with the at least one from the group consisting of the first nucleic acid probe, the second nucleic acid probe, the third nucleic acid probe and the fourth nucleic acid probe,

(d) detecting presence/absence of a hybridization and/or measuring a quantity of the hybridization occurred due to the reaction in the (C), and

(e) determining presence/absence of the target nucleic acid in the sample and/or determining a quantity of target nucleic acid presence, based on a result in the (d).

~~8. The nucleic acid analysis method of claim 7, wherein the amplification is by a PCR amplification reaction.~~

~~9~~7. The nucleic acid analysis method of claim ~~8~~6, wherein the nucleic acid probe is immobilized on a substrate.

~~10~~8. The nucleic acid analysis method of claim ~~9~~7, wherein the detecting the hybridization and/or measuring the quantity of the hybridization between the nucleic acid probe immobilized on the substrate and the amplification product is carried out using an intercalating agent which indicates an electrochemical activity.

~~11~~9. The nucleic acid analysis method of claim ~~10~~8, wherein the sample comprises a gene and an object of the analysis is the gene.

~~12~~10. An assay kit used for the nucleic acid analysis method of claim ~~7~~5, the assay kit comprising:

a primer set and a nucleic acid probe,

wherein a first template nucleic acid to be amplified with the primer set comprises a first sequence located on a 3′ terminal, a first template sequence adjacent to a 5′ side of the first sequence, a second sequence adjacent to a 5′ side of the first template sequence and located on the 5′ terminal, and a second template nucleic acid to be amplified with the primer set is a strand complementary to the first template nucleic acid, and comprises a third sequence, located on a 3′ terminal thereof, a second template sequence adjacent to a 5′ side of the third sequence, and a fourth sequence adjacent to a 5′ side of the second template sequence and located on the 5′ terminal,

the primer set satisfies the following (1) and/or (2):

(1) the first primer comprises an S1 sequence and an S2 sequence, the S1 sequence being complementary to the first sequence, the S2 sequence joined to a 5′ terminal side of the S1 sequence, the S2 sequence being a sequence, which is same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the first sequence on the first template nucleic acid, and

the second primer comprises an S3 sequence complementary

to the third sequence;

(2) the first primer comprises an S1 sequence and an S2 sequence, the S1 sequence being complementary to the first sequence, and the S2 sequence joined to a 5′ side of the S1 sequence, the S2 sequence being a sequence, which is same as a sequence of at least a part of the first template sequence, located adjacent to or in a vicinity of a 5′ side of the ~~S1~~ first sequence on the first template nucleic acid, and

the second primer comprises an S3 sequence and an S4 sequence, the S3 sequence being complementary to the third sequence, the S4 sequence joined to a 5′ side of the S3 sequence, the S4 sequence being a sequence, which is same as a sequence of at least a part of the second template sequence, located adjacent to or in a vicinity of a 5′ side of the third sequence on the second template nucleic acid;

the nucleic acid probe is at least one type selected from the group consisting of:

a nucleic acid probe comprising a sequence of at least a part of the S1 sequence;

a nucleic acid probe comprising a sequence of at least a part of the complementary sequence of the S1 sequence;

a nucleic acid probe comprising a sequence of at least a part of the S3 sequence; and

a nucleic acid probe comprising a sequence of at least a part of the complementary sequence of the S3 sequence.