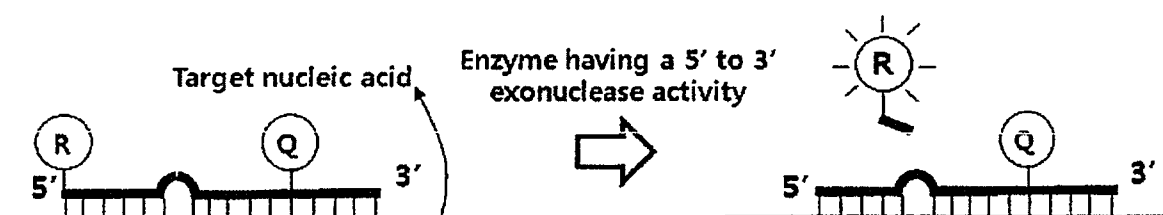
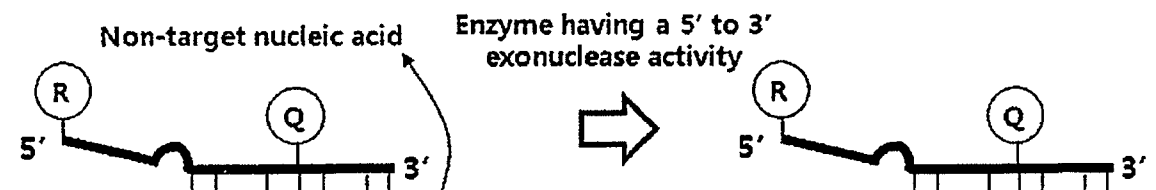


Fig. 1**A. Signal generation on target-specific hybridization**

When a dual-labeled target discriminative (TD) probe is hybridized with a target nucleic acid sequence, the 5'-second hybridization portion of the TD probe is digested by an enzyme having a 5' to 3' exonuclease activity.

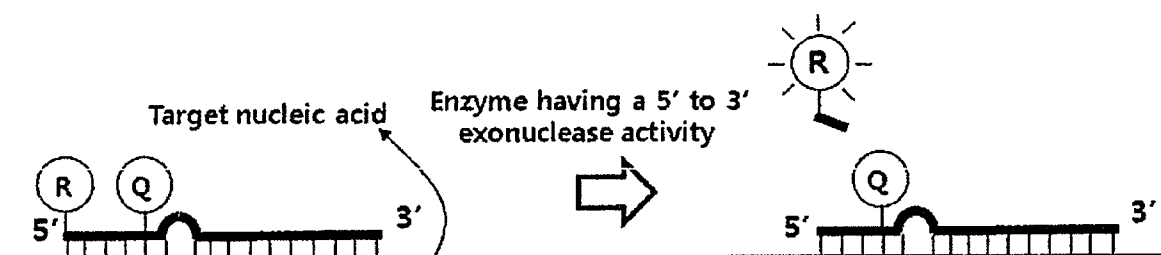
B. No signal on non-target hybridization

When a dual-labeled target discriminative (TD) probe is hybridized with a non-target nucleic acid sequence, the 5'-second hybridization portion of the TD probe forms a single strand such that the 5'-second hybridization portion is not digested by an enzyme having a 5' to 3' exonuclease activity.

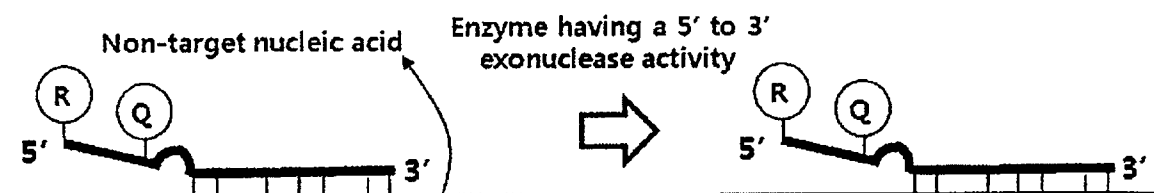
(R) : Reporter molecule

(Q) : Quencher molecule

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Fig. 2**A. Signal generation on target-specific hybridization**

When a dual-labeled target discriminative (TD) probe is hybridized with a target nucleic acid sequence, the 5'-second hybridization portion of the TD probe is digested by an enzyme having a 5' to 3' exonuclease activity.

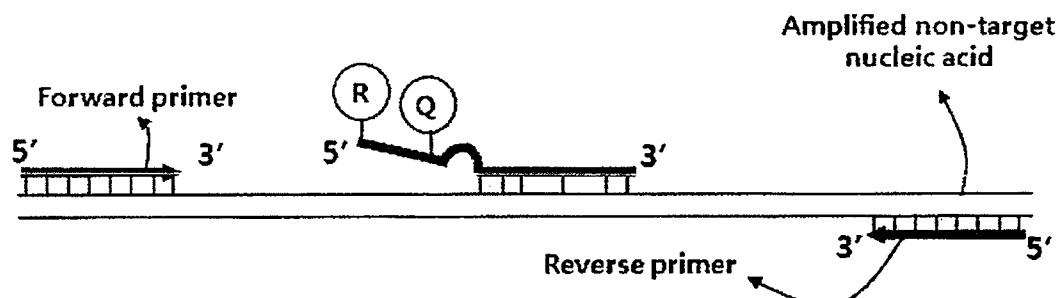
B. No signal on non-target hybridization

When a dual-labeled target discriminative (TD) probe is hybridized with a non-target nucleic acid sequence, the 5'-second hybridization portion of the TD probe forms a single strand such that the 5'-second hybridization portion is not digested by an enzyme having a 5' to 3' exonuclease activity.

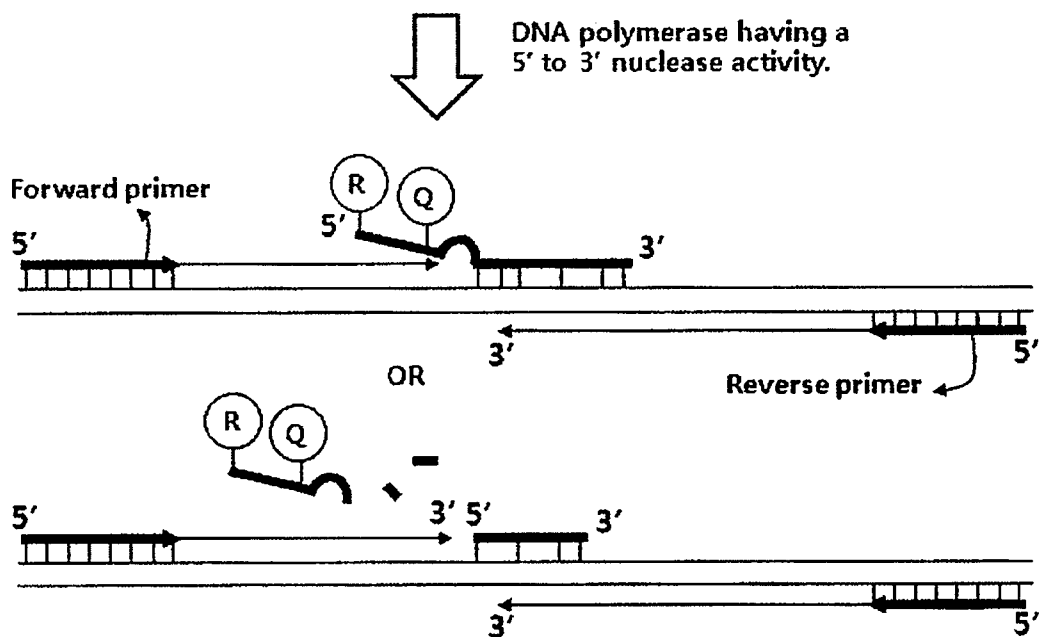
(R) : Reporter molecule

(Q) : Quencher molecule

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Fig. 3**Non-target hybridization in Real-time PCR**

When a dual-labeled target discriminative (TD) probe is hybridized with a non-target nucleic acid sequence, the 5'-second hybridization portion of the TD probe forms a single strand.

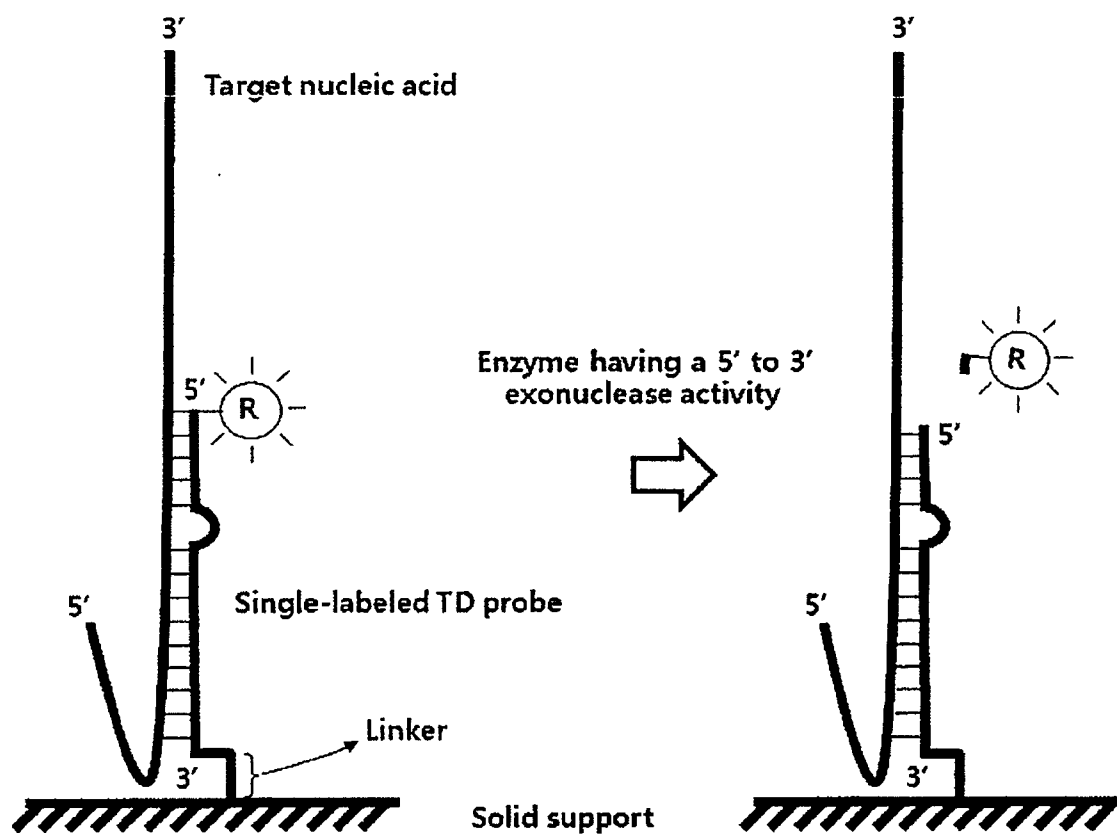


The 5'-second hybridization portion of the TD probe is not digested by a DNA polymerase having a 5' to 3' nuclease activity such that a false signal is not generated in real-time PCR reaction.

(R) : Reporter molecule

(Q) : Quencher molecule

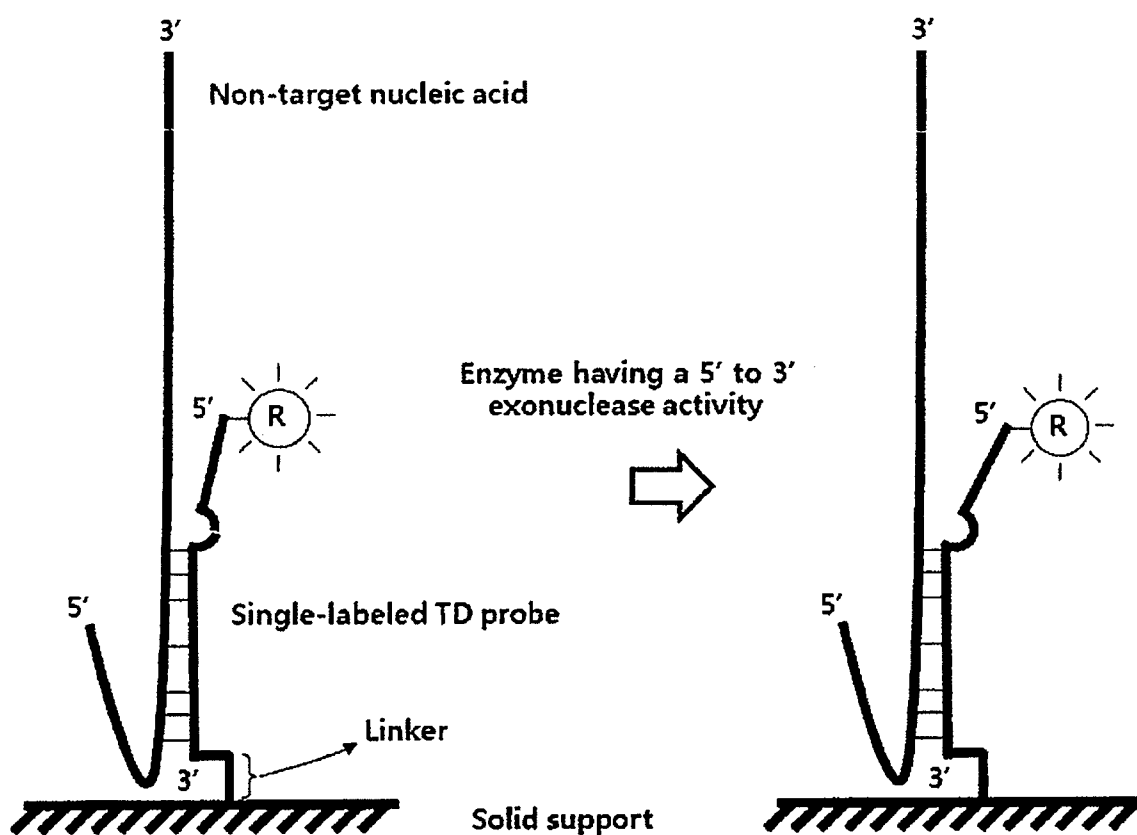
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Fig. 4A**Target-specific hybridization**

The 5'-second hybridization portion of a single-labeled target discriminative (TD) probe is digested by an enzyme having a 5' to 3' exonuclease activity.

(R) : Reporter molecule

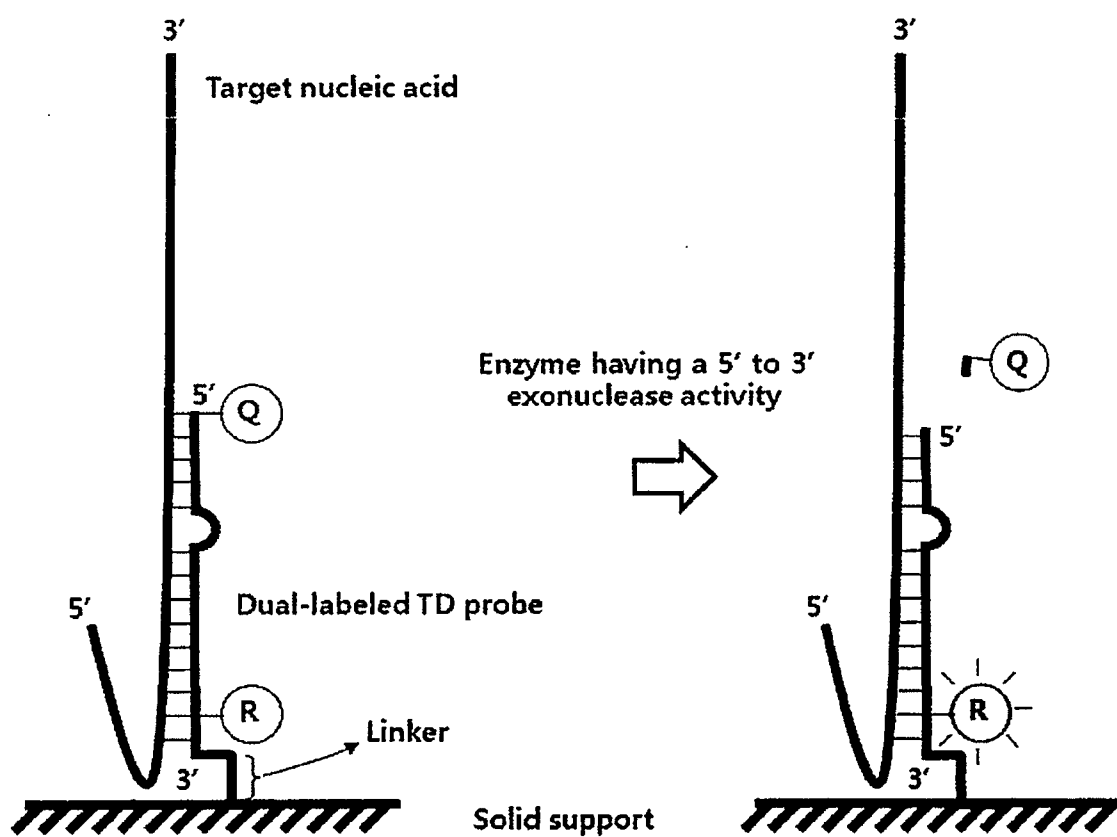
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Fig. 4B**Non-target hybridization**

The 5'-second hybridization portion of a single-labeled target discriminative (TD) probe is not digested by an enzyme having a 5' to 3' exonuclease activity.

(R) : Reporter molecule

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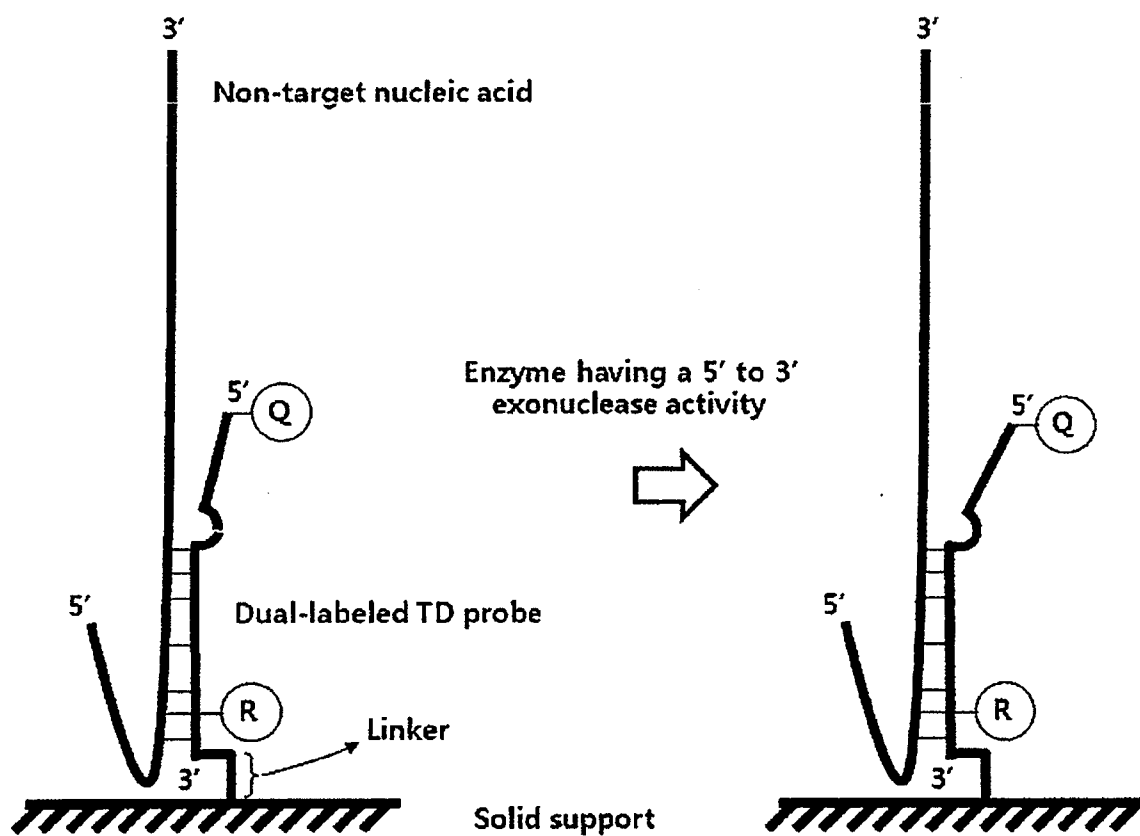
Fig. 5A**Signal generation on target-specific hybridization**

The 5'-second hybridization portion of a dual-labeled target discriminative (TD) probe is digested by an enzyme having a 5' to 3' exonuclease activity.

(R) : Reporter molecule

(Q) : Quencher molecule

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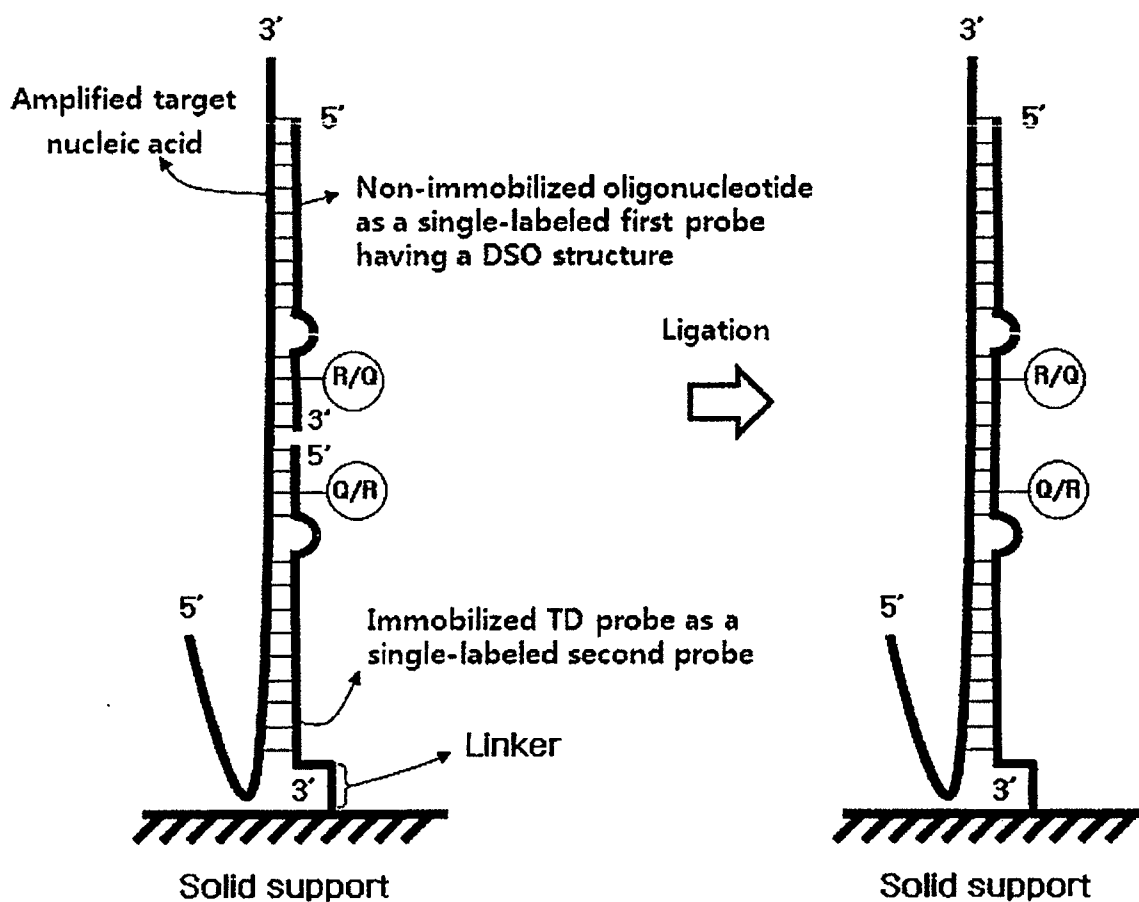
Fig. 5B**No signal on non-target hybridization**

The 5'-second hybridization portion of a dual-labeled target discriminative (TD) probe is not digested by an enzyme having a 5' to 3' exonuclease activity.

(R) : Reporter molecule

(Q) : Quencher molecule

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Fig. 6A**Target-specific hybridization**

When a non-immobilized first probe and an immobilized second probe are hybridized with a target nucleic acid sequence, the first and the second probes are ligated by a ligase.

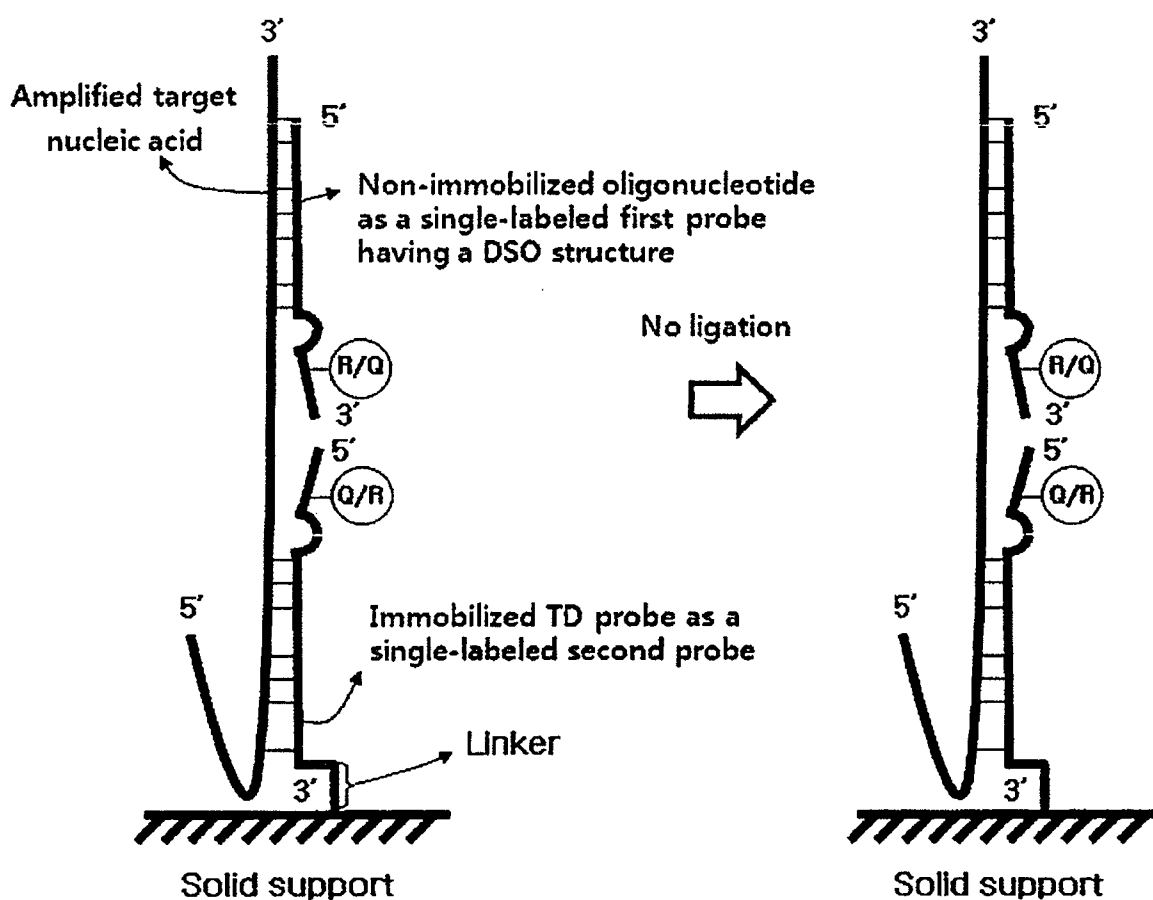
(R/Q) : Reporter or Quencher

DSO : Dual Specificity Oligonucleotide

(Q/R) : Quencher or Reporter

TD probe : Target Discriminative probe

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Fig. 6B**Non-target hybridization**

When a non-immobilized first probe and an immobilized second probe are hybridized with a non-target nucleic acid sequence, the first and the second probes are not ligated.

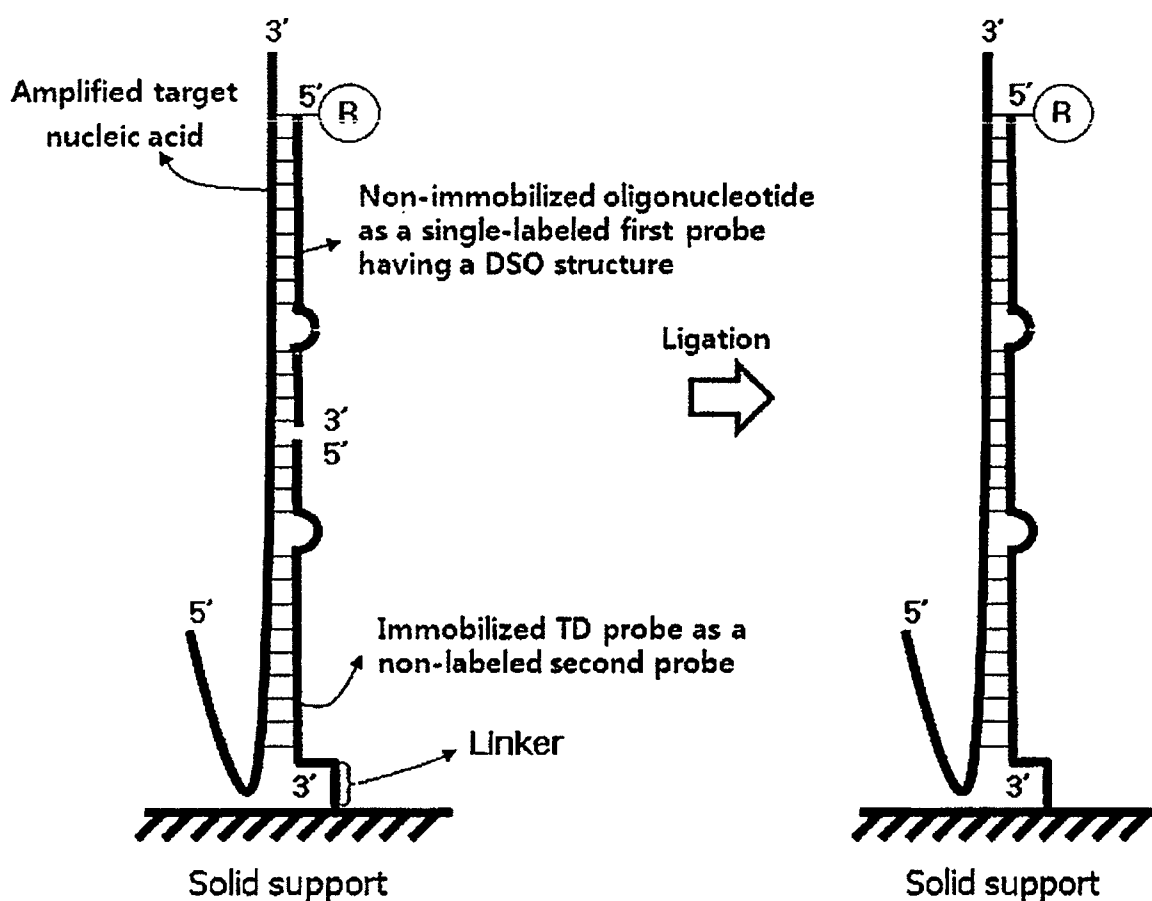
(R/Q) : Reporter or Quencher

DSO : Dual Specificity Oligonucleotide

(Q/R) : Quencher or Reporter

TD probe : Target Discriminative probe

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Fig. 7**Target-specific hybridization**

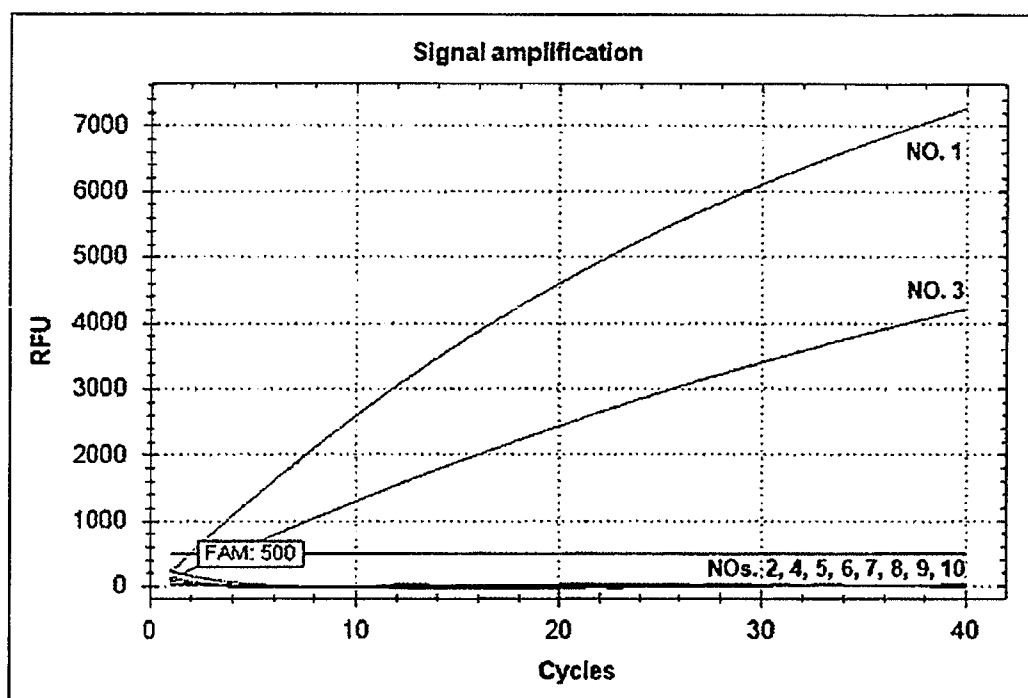
When a non-immobilized first probe and an immobilized second probe are hybridized with a target nucleic acid sequence, the first and the second probes are ligated by a ligase.

(R) : Reporter molecule

DSO : Dual Specificity Oligonucleotide

TD probe : Target Discriminative probe

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Fig. 8

No.	Template ¹⁾	Probe ²⁾	Ct value
1	+	SA_P0 ³⁾	1.93
2	-	SA_P0	-
3	+	SA_P1 ⁴⁾	3.81
4	-	SA_P1	-
5	+	SA_P3 ⁵⁾	-
6	-	SA_P3	-
7	+	SA_P6 ⁶⁾	-
8	-	SA_P6	-
9	+	SA_P9 ⁷⁾	-
10	-	SA_P9	-

¹⁾ Template is a synthetic oligonucleotide for *Staphylococcus aureus* gene.

²⁾ Probe has a reporter molecule at its 5'-end and a quencher molecule at its 3'-end portion.

³⁾ SA_P0 has a matched sequence at its 5'-end portion.

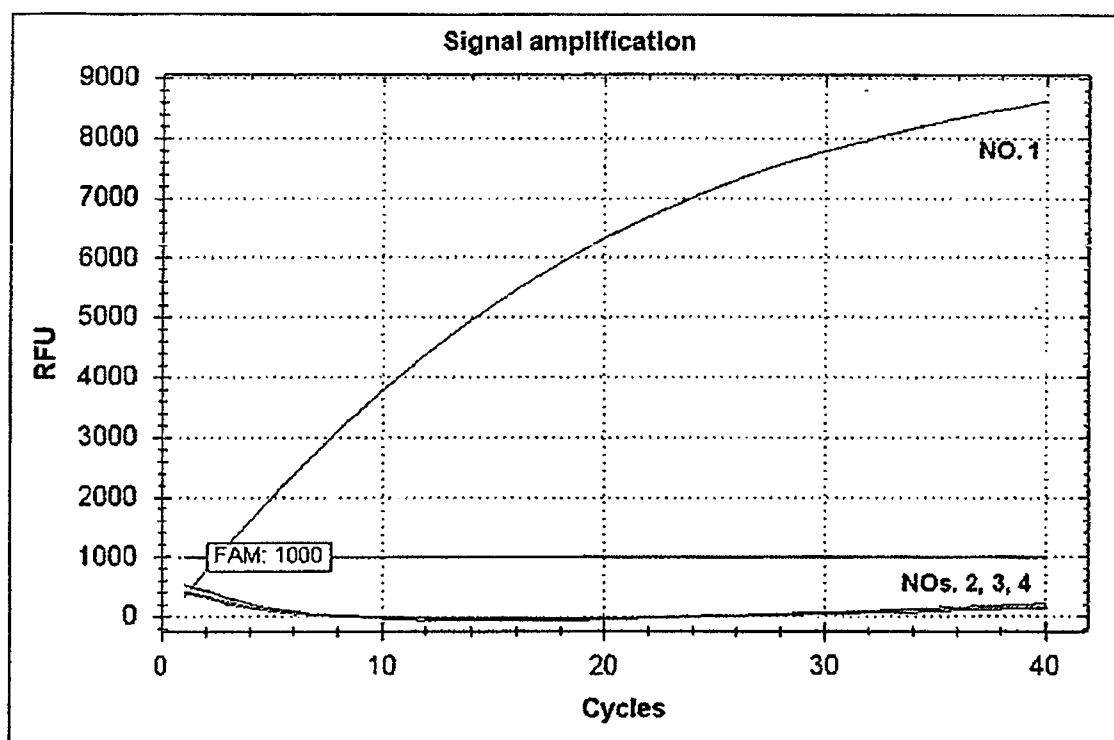
⁴⁾ SA_P1 has a single mismatched nucleotide at its 5'-end.

⁵⁾ SA_P3 has three mismatched nucleotides at its 5'-end portion.

⁶⁾ SA_P6 has six mismatched nucleotides at its 5'-end portion.

⁷⁾ SA_P9 has nine mismatched nucleotides at its 5'-end portion.

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Fig. 9A

No.	Template ¹⁾	TD probe ²⁾	Ct value
1	+	SA_TD_M ³⁾	2.53
2	-	SA_TD_M	-
3	+	SA_TD_m ⁴⁾	-
4	-	SA_TD_m	-

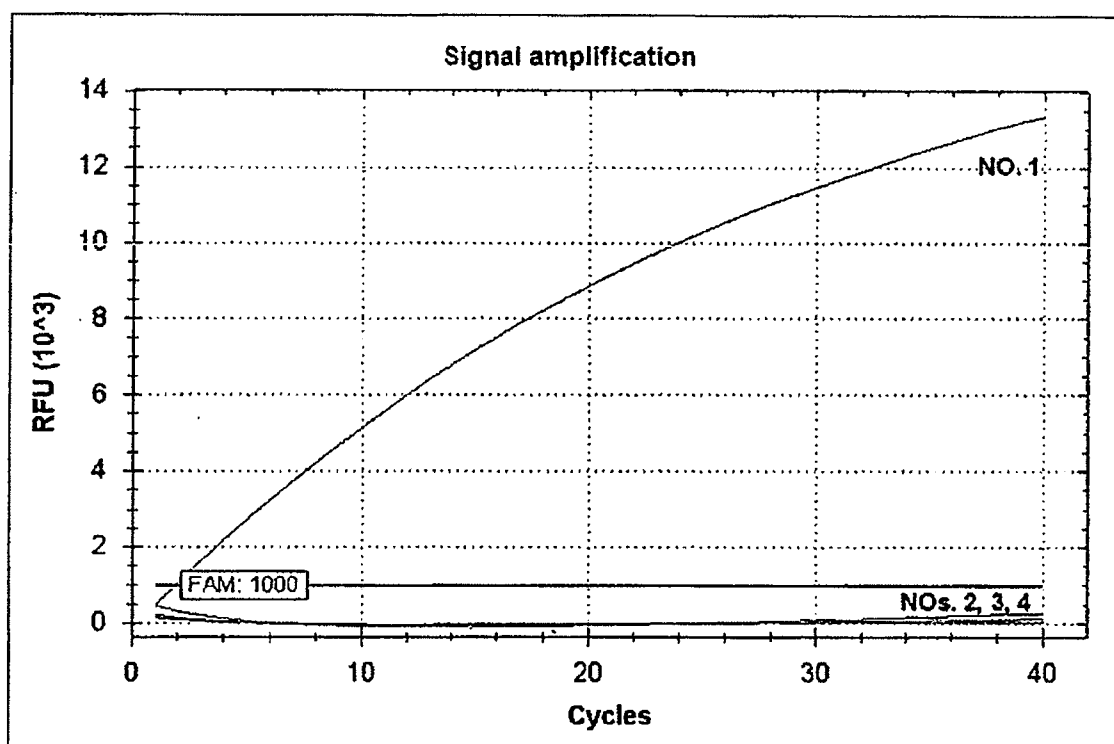
¹⁾ Template is a synthetic oligonucleotide for *Staphylococcus aureus* gene.

²⁾ TD probe has a reporter molecule at its 5'-end and a quencher molecule at its 3'-first hybridization portion.

³⁾ SA_TD_M has a matched sequence at its 5'-second hybridization portion.

⁴⁾ SA_TD_m has a mismatched sequence at its 5'-second hybridization portion.

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Fig. 9B

No.	Template ¹⁾	TD probe ²⁾	Ct value
1	+	NG_TD_M ³⁾	1.88
2	-	NG_TD_M	-
3	+	NG_TD_m ⁴⁾	-
4	-	NG_TD_m	-

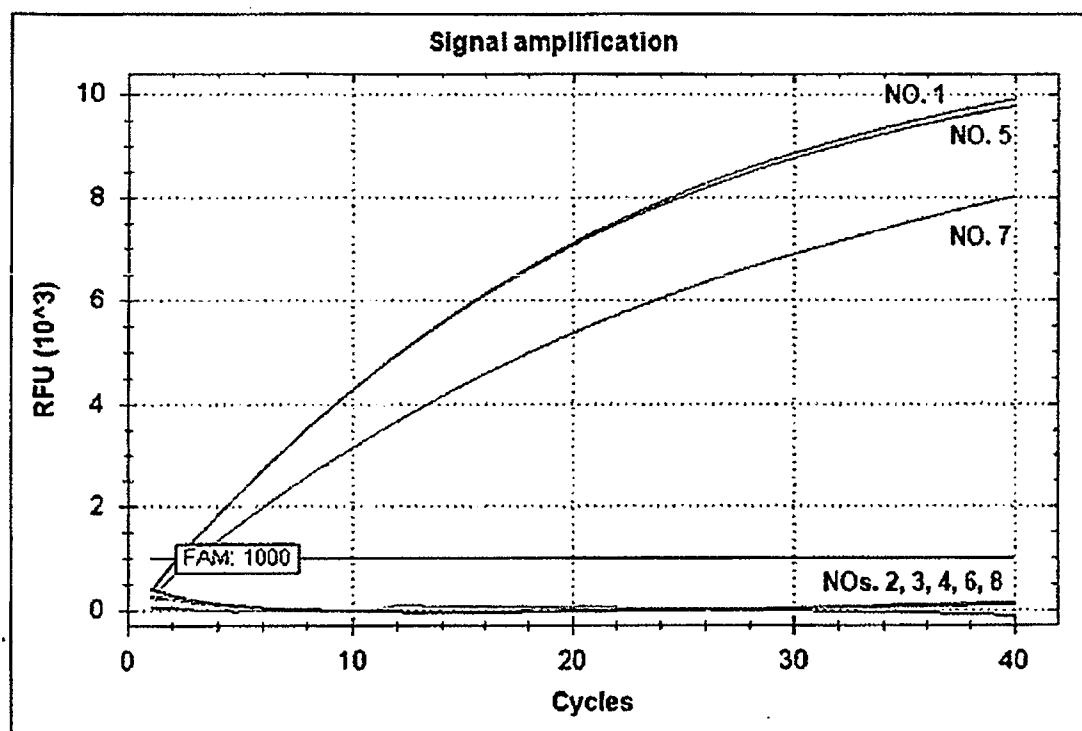
¹⁾ Template is a synthetic oligonucleotide for *Neisseria gonorrhoeae* gene.

²⁾ TD probe has a reporter molecule at its 5'-end and a quencher molecule at its 3'-first hybridization portion.

³⁾ NG_TD_M has a matched sequence at its 5'-second hybridization portion.

⁴⁾ NG_TD_m has a mismatched sequence at its 5'-second hybridization portion.

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Fig. 10

No.	Template ¹⁾	Probe ²⁾	Ct value
1	+	SA_TD_M ³⁾	2.19
2	-	SA_TD_M	-
3	+	SA_TD_m1 ⁴⁾	-
4	-	SA_TD_m1	-
5	+	SA_Con_M ⁵⁾	2.24
6	-	SA_Con_M	-
7	+	SA_Con_m1 ⁶⁾	2.97
8	-	SA_Con_m1	-

¹⁾ Template is a synthetic oligonucleotide for *Staphylococcus aureus* gene.

²⁾ Probe has a reporter molecule at its 5'-end and a quencher molecule at its 3'-end portion.

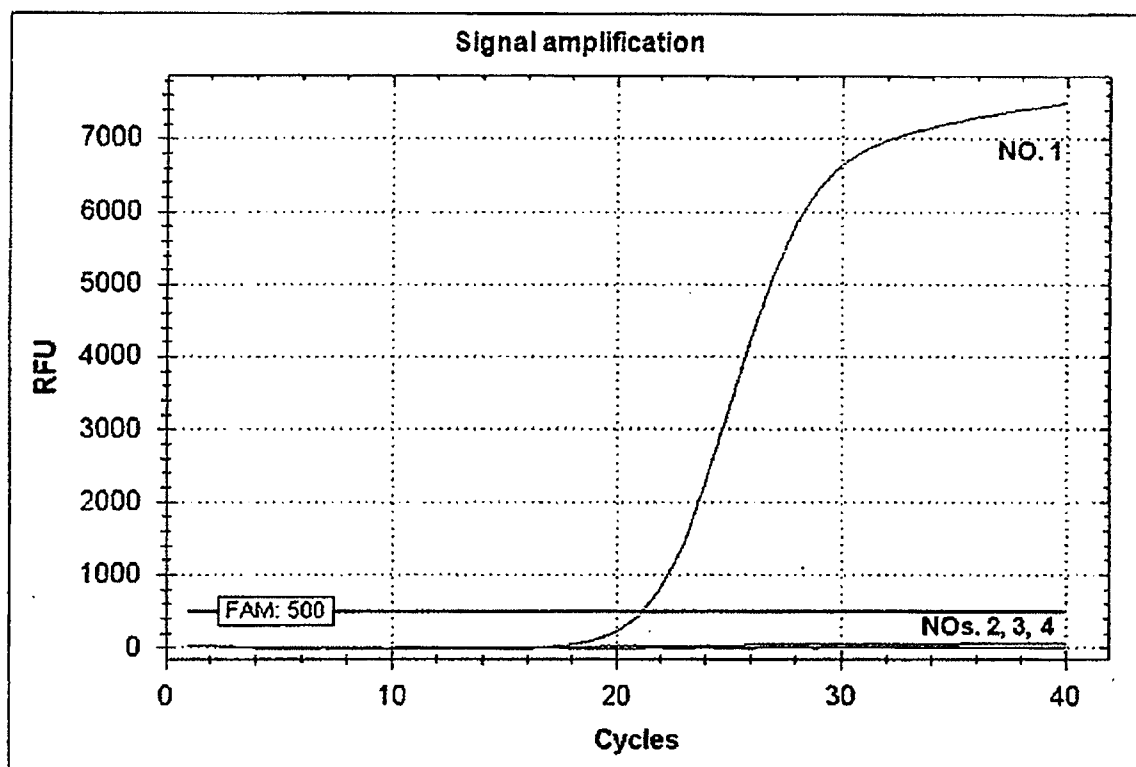
³⁾ SA_TD_M is a TD probe and has a matched sequence at its 5'-second hybridization portion.

⁴⁾ SA_TD_m1 is a TD probe and has three mismatched nucleotides at its 5'-second hybridization portion.

⁵⁾ SA_Con_M is a conventional probe and has a matched sequence at its 5'-end portion.

⁶⁾ SA_Con_m1 is a conventional probe and has three mismatched nucleotides at its 5'-end portion.

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Fig. 11A

No.	Template ²⁾	TD probe ²⁾	Ct value
1	+	SA_TD2_M ³⁾	21.17
2	-	SA_TD2_M	-
3	+	SA_TD2_m ⁴⁾	-
4	-	SA_TD2_m	-

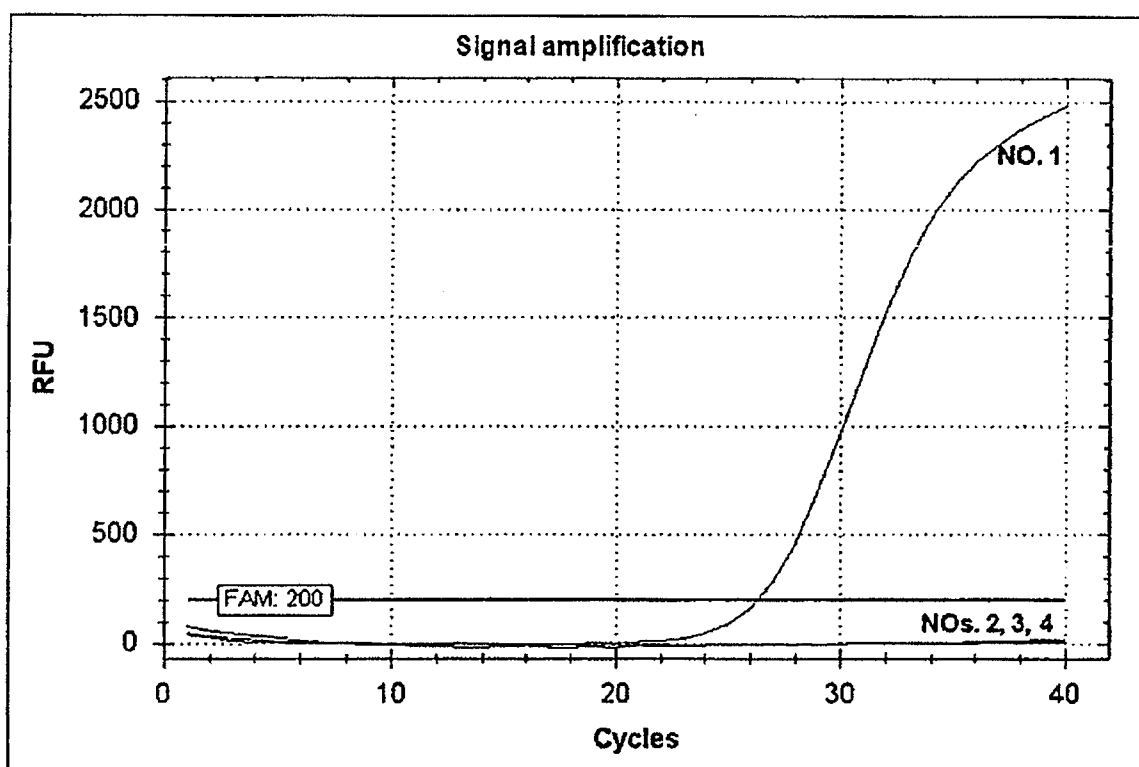
²⁾ Template is a genomic DNA of *Staphylococcus aureus*.

²⁾ TD probe has both a reporter molecule and a quencher molecule at its 5'-second hybridization portion.

³⁾ SA_TD2_M has a matched sequence at its 5'-second hybridization portion.

⁴⁾ SA_TD2_m has a mismatched sequence at its 5'-second hybridization portion.

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Fig. 11B

No.	Template ¹⁾	TD probe ²⁾	Ct value
1	+	NG_TD2_M ³⁾	26.31
2	-	NG_TD2_M	-
3	+	NG_TD2_m ⁴⁾	-
4	-	NG_TD2_m	-

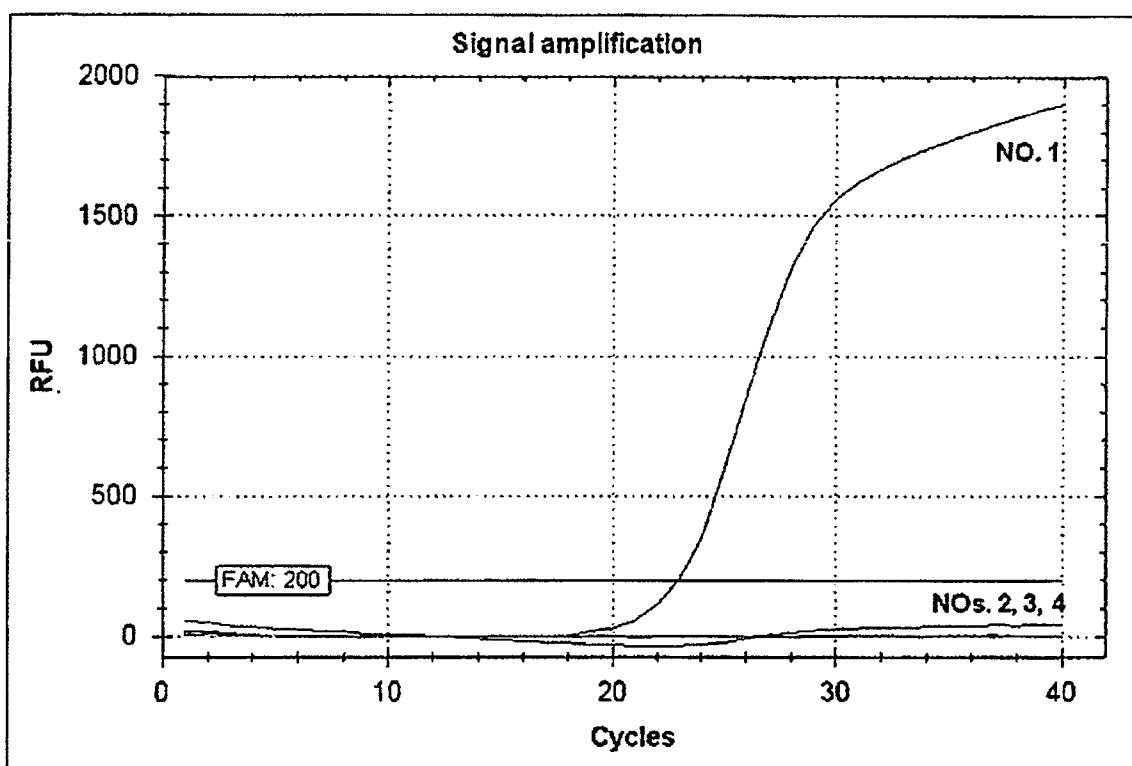
¹⁾ Template is a genomic DNA of *Neisseria gonorrhoeae*.

²⁾ TD probe has both a reporter molecule and a quencher molecule at its 5'-second hybridization portion.

³⁾ NG_TD2_M has a matched sequence at its 5'-second hybridization portion.

⁴⁾ NG_TD2_m has a mismatched sequence at its 5'-second hybridization portion.

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Fig. 12

No.	Template ¹⁾	TD probe ²⁾	Ct value
1	+	SA_TD_S_M ³⁾	22.95
2	-	SA_TD_S_M	-
3	+	SA_TD_S_m ⁴⁾	-
4	-	SA_TD_S_m	-

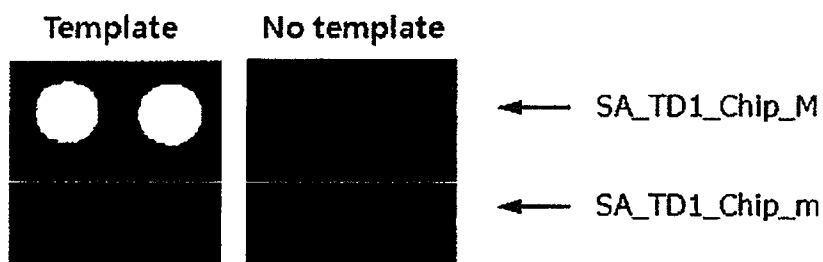
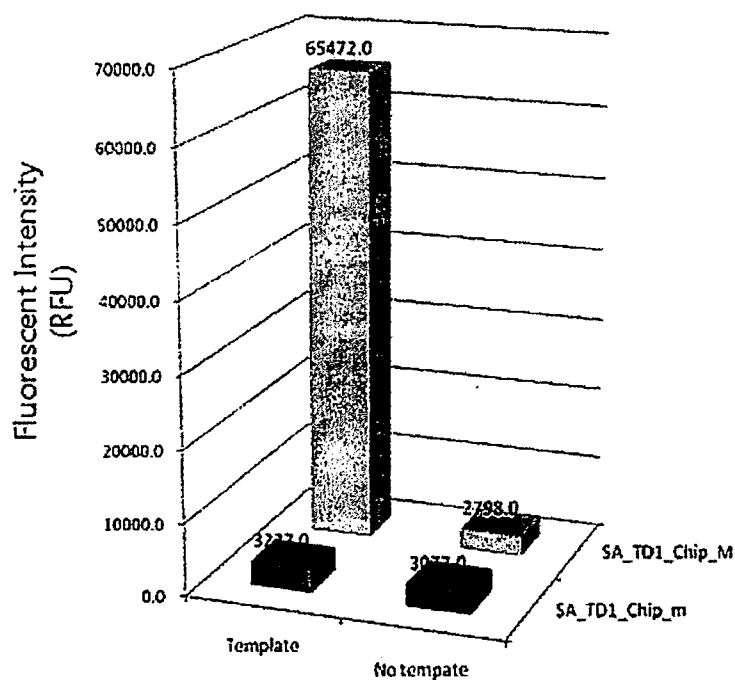
¹⁾ Template is a genomic DNA of *Staphylococcus aureus*.

²⁾ TD probe has both a reporter molecule and a quencher molecule at its 5'-second hybridization portion.

³⁾ SA_TD_S_M has a matched sequence at its 5'-second hybridization portion.

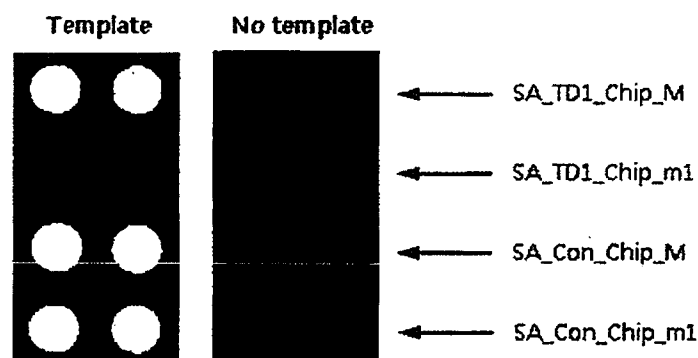
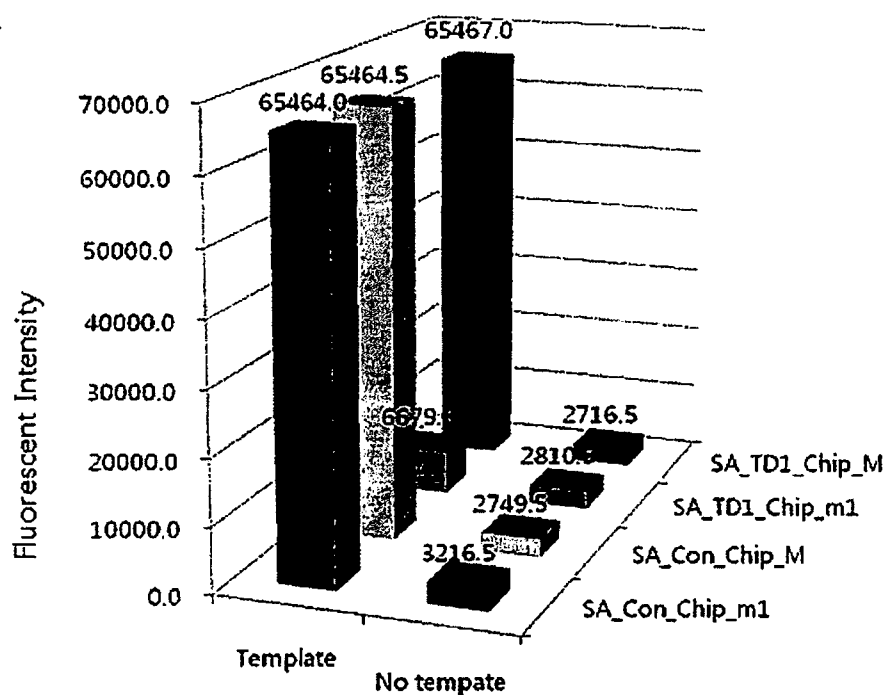
⁴⁾ SA_TD_S_m has a single mismatched nucleotide at its 5'-second hybridization portion.

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Fig. 13**A.****B.**

- SA_TD1_Chip_M is a TD probe having a matched sequence at its 5'-second hybridization portion.
- SA_TD1_Chip_m is a TD probe having a mismatched sequence at its 5'-second hybridization portion.
- Each spot was duplicated for the test of reproducibility.
- The fluorescence intensity indicates the average value of the duplicated spots.

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Fig. 14**A.****B.**

- SA_TD1_Chip_M is a TD probe having a matched sequence at its 5'-second hybridization portion.
- SA_TD1_Chip_m1 is a TD probe having three mismatched nucleotides at its 5'-second hybridization portion.
- SA_Con_Chip_M is a conventional probe having a matched sequence at its 5'-end portion.
- SA_Con_Chip_m1 is a conventional probe having three mismatched nucleotides at its 5'-end portion.