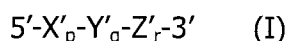


What is claimed is:

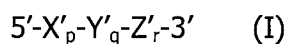
1. A target discriminative probe (TD probe) having a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I to allow for discrimination of a target nucleic acid sequence from a non-target nucleic acid sequence:



wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; p, q and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions of X'_p , Y'_q and Z'_r ; the length of the 5'-second hybridization portion is 3-15 nucleotides, the length of the 3'-first hybridization portion is 15-60 nucleotides and the 3'-first hybridization portion is longer than the 5'-second hybridization portion; the TD probe has a label or an interactive label system containing a plurality of labels to generate a detectable signal; the label is a chemical label, an enzymatic label, a fluorescent label, a luminescent label, a chemiluminescent label or a metal label; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe are determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the overall hybridization specificity of the TD probe is enhanced; wherein when the TD probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, both of the 5'-second hybridization portion and the separation portion form a single strand, whereby the TD probe allows discriminating the target nucleic acid sequence from the non-target nucleic

acid sequence.

2. The TD probe according to claim 1, wherein the T_m of the 3'-first hybridization portion ranges from 40°C to 80°C.
3. The TD probe according to claim 1, wherein the T_m of the 5'-second hybridization portion ranges from 6°C to 40°C.
4. The TD probe according to claim 1, wherein the T_m of the separation portion ranges from 2°C to 15°C.
5. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids using a target discriminative probe (TD probe), which comprises the steps of:
 - (a) hybridizing the target nucleic acid sequence with the TD probe having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; wherein the TD probe has a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I:



wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the TD probe is dually labeled with a fluorescent reporter molecule and a quencher molecule capable of quenching the fluorescence of the reporter molecule; at least one of the fluorescent reporter molecule and the quencher molecule is positioned on the 5'-second hybridization portion; p, q and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions

of X'_p , Y'_q and Z'_r ; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe is determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the overall hybridization specificity of the TD probe is enhanced;

wherein when the TD probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence and the 5'-second hybridization portion is digested by an enzyme having a 5' to 3' exonuclease activity; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, both the 5'-second hybridization portion and the separation portion form a single strand such that the 5'-second hybridization portion is not digested by the enzyme having the 5' to 3' exonuclease activity, whereby the TD probe allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence;

(b) contacting the resultant of step (a) to the enzyme having the 5' to 3' exonuclease activity; wherein when the TD probe is hybridized with the target nucleic acid sequence, the 5'-second hybridization portion is digested by the enzyme having the 5' to 3' exonuclease activity to separate the fluorescent reporter molecule from the quencher molecule on the TD probe, resulting in generation of a fluorescence signal; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, the 5'-second hybridization portion is not digested by the enzyme having the 5' to 3' exonuclease activity, resulting in no fluorescence signal; and

(c) detecting the fluorescence signal, such that the fluorescence signal generated by the digestion on the 5'-second hybridization portion is indicative of the presence of the target nucleic acid sequence.

6. The method according to claim 5, wherein the enzyme having the 5' to 3' exonuclease activity is a template-dependent nucleic acid polymerase having the 5' to 3' exonuclease activity.

7. The method according to claim 5, wherein the step (a) is carried out using the TD probe together with an upstream primer to be hybridized with a site downstream of a hybridized site of the TD probe and the enzyme having the 5' to 3' exonuclease activity is a template-dependent nucleic acid polymerase having the 5' to 3' exonuclease activity such that the upstream primer is extended by the template-dependent nucleic acid polymerase in the step (b).

8. The method according to claim 5, wherein the step (a) is carried out using the TD probe together with a reverse primer and the enzyme having the 5' to 3' exonuclease activity is a template-dependent nucleic acid polymerase having the 5' to 3' exonuclease activity such that the step (b) produces the target nucleic acid sequence hybridizable with the TD probe by an extension reaction of the reverse primer by the template-dependent nucleic acid polymerase.

9. The method according to claim 5, wherein the fluorescent reporter molecule and the quencher molecule all are positioned on the 5'-second hybridization portion or the fluorescent reporter molecule and the quencher molecule each is positioned on each different portion of the 5'-second hybridization portion and the separation portion.

10. The method according to claim 6, wherein the fluorescent reporter molecule and the quencher molecule each is positioned on each different portion of the 5'-second hybridization portion and the 3'-first hybridization portion.

11. The method according to claim 5, wherein the method further comprises repeating the steps (a)-(b) or (a)-(c) with denaturation between repeating cycles.

12. The method according to claim 6, wherein the target nucleic acid sequence comprises at least two types of nucleic acid sequences and the TD probe comprises at least two types of probes.

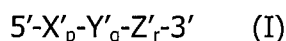
13. The method according to claim 7, wherein the target nucleic acid sequence comprises at least two types of nucleic acid sequences, the TD probe comprises at least two types of probes and the upstream primer comprises at least two types of primers or the reverse primer comprises at least two types of primers.

14. The method according to claim 5, wherein the target nucleic acid sequence comprises a nucleotide variation and the nucleotide variation on the target nucleic acid sequence is present at a site opposite to the 5'-second hybridization portion of the TD probe.

15. The method according to claim 5, wherein the TD probe has a blocker site containing as a blocker at least one nucleotide resistant to cleavage by the enzyme having 5' to 3' exonuclease activity and the blocker site is positioned at the 3'-first hybridization portion of the TD probe.

16. A method for detecting a target nucleic acid sequence on a solid phase from a DNA or a mixture of nucleic acids using a target discriminative probe (TD probe), which comprises the steps of:

(a) hybridizing the target nucleic acid sequence with the TD probe having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; wherein the TD probe is immobilized through its 3'-end on the surface of the solid substrate; wherein the TD probe has a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I:



wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the TD probe has a label generating a detectable signal and the label is positioned on the 5'-second hybridization portion of the TD probe; p, q

and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions of X'_p , Y'_q and Z'_r ; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe are determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the overall hybridization specificity of the TD probe is enhanced;

wherein when the TD probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence and the 5'-second hybridization portion is digested by the enzyme having the 5' to 3' exonuclease activity; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, both the 5'-second hybridization portion and the separation portion form a single strand such that the 5'-second hybridization portion is not digested by the enzyme having the 5' to 3' exonuclease activity, whereby the TD probe allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence;

(b) contacting the resultant of step (a) to the enzyme having the 5' to 3' exonuclease activity; wherein when the TD probe is hybridized with the target nucleic acid sequence, its 5'-second hybridization portion is digested by the enzyme having the 5' to 3' exonuclease activity to release the label from the TD probe, resulting in a signal change on the TD probe immobilized on the solid substrate; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, the 5'-second hybridization portion is not digested by the enzyme having the 5' to 3' exonuclease activity, resulting in no signal change on the TD probe immobilized on the solid substrate; whereby the signal change on the solid substrate is detected to determine the presence of the target nucleic acid sequence; and

(c) detecting the signal change on the solid substrate, such that the signal change by the digestion on the 5'-second hybridization portion is indicative of the presence of the

target nucleic acid sequence.

17. The method according to claim 16, wherein the label is a fluorescent reporter molecule and the signal change is the decrease or elimination of fluorescent signals on the solid substrate.

18. The method according to claim 16, wherein the label is the interactive label system comprising a pair of a fluorescent reporter molecule and a quencher molecule and the TD probe has one of the reporter molecule and the quencher molecule at a site on the 5'-second hybridization portion to be digested by the enzyme having the 5' to 3' exonuclease activity and the other on a site not to be digested by the enzyme having the 5' to 3' exonuclease activity.

19. The method according to claim 18, wherein the quencher molecule is positioned at a site on the 5'-second hybridization portion of the TD probe to be digested by the enzyme having the 5' to 3' exonuclease activity and the fluorescent reporter molecule is positioned on a site not to be digested by the enzyme having the 5' to 3' exonuclease activity; wherein when the TD probe is hybridized with the target nucleic acid sequence, its 5'-second hybridization portion is digested by the enzyme having the 5' to 3' exonuclease activity to separate the fluorescent reporter molecule from the quencher molecule on the TD probe, resulting in generation of a fluorescence signal; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, the 5'-second hybridization portion is not digested by the enzyme having the 5' to 3' exonuclease activity, resulting in no fluorescence signal; whereby the fluorescent signal on the solid substrate is detected to determine the presence of the target nucleic acid sequence.

20. The method according to claim 24, wherein the method further comprises repeating the steps (a)-(b) or (a)-(c) with denaturation between repeating cycles.

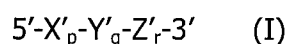
21. The method according to claim 16, wherein the target nucleic acid sequence comprises at least two types of nucleic acid sequences and the TD probe comprises at least two types of probes.

22. The method according to claim 16, wherein the target nucleic acid sequence comprises a nucleotide variation and the nucleotide variation on the target nucleic acid sequence is present at a site opposite to the 5'-second hybridization portion of the TD probe.

23. The method according to claim 16, wherein the TD probe has a blocker site containing as a blocker at least one nucleotide resistant to cleavage by the enzyme having 5' to 3' exonuclease activity and the blocker site is positioned at the 3'-first hybridization portion of the TD probe.

24. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids using a target discriminative probe (TD probe) and a polymerase chain reaction (PCR), which comprises the steps of:

(a) preparing a PCR mixture containing (i) the target nucleic acid sequence, (ii) the TD probe having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence, (iii) a primer pair composed of two primers as a forward primer and a reverse primer each having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence, and (iv) a template-dependent nucleic acid polymerase having a 5' to 3' exonuclease activity; wherein the TD probe is hybridized with a site between the two primers; wherein the TD probe has a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I:



wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the

target nucleic acid sequence; the TD probe is dually labeled with a fluorescent reporter molecule and a quencher molecule capable of quenching the fluorescence of the reporter molecule; the fluorescent reporter molecule and the quencher molecule all are positioned on the 5'-second hybridization portion, or the reporter molecule and the quencher molecule each is positioned on each different portion of the 5'-second hybridization portion and the separation portion; p , q and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions of X'_p , Y'_q and Z'_r ; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe are determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the overall hybridization specificity of the TD probe is enhanced;

wherein when the TD probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence and the 5'-second hybridization portion is digested by the 5' to 3' exonuclease activity of the template-dependent nucleic acid polymerase; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, both the 5'-second hybridization portion and the separation portion form a single strand such that the 5'-second hybridization portion is not digested by the 5' to 3' exonuclease activity of the template-dependent nucleic acid polymerase, whereby the TD probe allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence;

(b) amplifying the target nucleic acid sequence using the PCR mixture by performing at least two cycles of primer annealing, primer extending and denaturing, wherein the two primers are extended by a polymerase activity of the template-dependent nucleic acid polymerase to amplify the target nucleic acid sequence; wherein when the TD probe is hybridized with the target nucleic acid sequence, the 5'-second hybridization

portion is digested by the 5' to 3' exonuclease activity of the template-dependent nucleic acid polymerase to separate the fluorescent reporter molecule from the quencher molecule on the TD probe, resulting in generation of a fluorescence signal; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, the 5'-second hybridization portion is not digested by the 5' to 3' exonuclease activity of the template-dependent nucleic acid polymerase such that the fluorescent reporter molecule is not separated from the quencher molecule on the TD probe, resulting in no fluorescence signal; and

(c) detecting the fluorescence signal, such that the fluorescence signal generated is indicative of the presence of the target nucleic acid sequence.

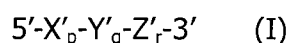
25. The method according to claim 24, wherein the target nucleic acid sequence comprises at least two types of nucleic acid sequences, the TD probe comprises at least two types of probes, the forward primer comprises at least two types of primers and the reverse primer comprises at least two types of primers.

26. The method according to claim 24, wherein the target nucleic acid sequence comprises a nucleotide variation and the nucleotide variation on the target nucleic acid sequence is present at a site opposite to the 5'-second hybridization portion of the TD probe.

27. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids using a target discriminative probe (TD probe) by a ligation reaction, which comprises the steps of:

(a) hybridizing the target nucleic acid sequence with a first probe having a hybridizing nucleotide sequence complementary to a first site of the target nucleic acid sequence and a second probe having a hybridizing nucleotide sequence complementary to a second site of the target nucleic acid sequence which is positioned upstream of the first site; wherein at least one of the first probe and the second probe has a label to generate a detectable signal; wherein the second probe is a TD probe; wherein the TD probe has

a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I:



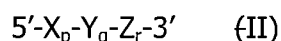
wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; p , q and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions of X'_p , Y'_q and Z'_r ; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe are determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the overall hybridization specificity of the TD probe is enhanced;

wherein when the second probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion of the second probe are hybridized with the target nucleic acid sequence to allow ligation of the first probe and the second probe; wherein when the second probe is hybridized with the non-target nucleic acid sequence, both of the 5'-second hybridization portion and the separation portion of the second probe form a single strand such that the first probe and the second probe are not ligated, whereby the second probe allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence;

- (b) ligating the first probe and the second probe hybridized with the target nucleic acid sequence such that a ligated probe is produced;
- (c) denaturing the resultant of step (b);
- (d) detecting the signal from the label on the ligated probe, such that the signal is indicative of the presence of the target nucleic acid sequence.

28. The method according to claim 27, wherein the label is the interactive label system comprising a pair of a reporter molecule and a quencher molecule.

29. The method according to claim 28, wherein the first probe has a dual specificity oligonucleotide (DSO) structure represented by the following general formula II:



wherein, X_p represents a 5'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid; Y_q represents a separation portion comprising at least three universal bases, Z_r represents a 3'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid; p , q and r represent the number of nucleotides, and X , Y , and Z are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-first hybridization portion is higher than that of the 3'-second hybridization portion and the separation portion has the lowest T_m in the three portions; the separation portion separates the 5'-first hybridization portion from the 3'-second hybridization portion in terms of hybridization events to the target nucleic acid, whereby the hybridization specificity of the oligonucleotide are determined dually by the 5'-first hybridization portion and the 3'-second hybridization portion such that the overall hybridization specificity of the oligonucleotide is enhanced.

30. The method according to claim 27, wherein the method further comprises repeating the steps (a)-(c) or (a)-(d).

31. The method according to claim 27, wherein the method is performed on a solid phase; wherein the first probe is immobilized through its 5'-end on the surface of a solid substrate and the second probe is not immobilized.

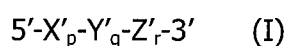
32. The method according to claim 27, wherein the method is performed on a solid phase; wherein the second probe is immobilized through its 3'-end on the surface of the solid substrate and the first probe is not immobilized.

33. The method according to claim 27, wherein the target nucleic acid sequence comprises at least two types of nucleic acid sequences and the first probe and the second probe each comprises at least two types of probes.

34. The method according to claim 27, wherein the target nucleic acid sequence comprises a nucleotide variation and the nucleotide variation on the target nucleic acid sequence is present at a site opposite to the 5'-second hybridization portion of the second probe.

35. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids using a target discriminative probe (TD probe), which comprises the steps of:

(a) hybridizing the target nucleic acid sequence with the TD probe having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; wherein the TD probe has a modified dual specificity oligonucleotide (mDSO) structure represented by the following general formula I:



wherein, X'_p represents a 5'-second hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; Y'_q represents a separation portion comprising at least three universal bases, Z'_r represents a 3'-first hybridization portion having a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the TD probe is labeled with a fluorescent reporter molecule on the 5'-second hybridization portion; p, q and r represent the number of nucleotides; and X' , Y' and Z' are deoxyribonucleotides or ribonucleotides; the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions of X'_p , Y'_q and Z'_r ; the separation portion separates the 5'-second hybridization portion from the 3'-first hybridization portion in terms of hybridization events to the target nucleic acid sequence, whereby the hybridization specificity of the TD probe is determined dually by the 5'-second hybridization portion and the 3'-first hybridization portion such that the

overall hybridization specificity of the TD probe is enhanced;

wherein when the TD probe is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence to induce a change in fluorescence from the fluorescent reporter molecule; wherein when the TD probe is hybridized with the non-target nucleic acid sequence, both the 5'-second hybridization portion and the separation portion form a single strand to induce no change in fluorescence from the fluorescent reporter molecule, whereby the TD probe allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence; and

(b) detecting the fluorescence change, such that the fluorescence change is indicative of the presence of the target nucleic acid sequence.

36. The method according to claim 35, wherein the step (a) is carried out using the TD probe together with a reverse primer and a template-dependent nucleic acid polymerase such that the target nucleic acid sequence hybridizable with the TD probe is additionally generated to enhance the fluorescence change indicative of the presence of the target nucleic acid sequence.

37. The method according to claim 35, wherein the step (a) is carried out using the TD probe together with a primer pair composed of two primers as a forward primer and a reverse primer and a template-dependent nucleic acid polymerase such that the target nucleic acid sequence hybridizable with the TD probe is amplified by PCR to enhance the fluorescence change indicative of the presence of the target nucleic acid sequence.

38. The method according to claim 35, wherein the TD probe is additionally labeled with a quencher molecule capable of quenching the fluorescence of the reporter molecule.

39. A method for enabling a probe molecule to discriminate a target nucleic acid sequence from a non-target nucleic acid sequence, which comprises the steps of:

- (a) selecting a target nucleic acid sequence;
- (b) designing a sequence of a probe molecule having (i) a hybridizing sequence complementary to the target nucleic acid and (ii) a separation portion comprising at least three universal bases, such that the separation portion intervenes in the hybridizing sequence to form three portions in the probe molecule; and
- (c) determining the position of the separation portion in the probe molecule to allow a portion at the 5'-direction of the separation portion to have a lower T_m than a portion at the 3'-direction of the separation portion and to allow the separation portion to have the lowest T_m in the three portions, thereby providing the probe molecule having three distinct portions with different T_m values from one another in which (i) a 5'-second hybridization portion of the probe molecule has a hybridizing nucleotide sequence complementary to the target nucleic acid, (ii) a 3'-first hybridization portion of the probe molecule has a hybridizing nucleotide sequence complementary to the target nucleic acid; and (iii) the separation portion of the probe molecule between the 5'-second hybridization portion and the 3'-first hybridization portion comprises at least three universal bases; and the T_m of the 5'-second hybridization portion is lower than that of the 3'-first hybridization portion and the separation portion has the lowest T_m in the three portions,

wherein when the probe molecule is hybridized with the target nucleic acid sequence, both of the 5'-second hybridization portion and the 3'-first hybridization portion are hybridized with the target nucleic acid sequence; wherein when the probe molecule is hybridized with the non-target nucleic acid sequence, both of the 5'-second hybridization portion and the separation portion form a single strand, whereby the probe molecule allows discriminating the target nucleic acid sequence from the non-target nucleic acid sequence.