

CLAIMS

1. A probe for detecting a polynucleotide sequence having one or more polymorphisms,

wherein the probe is hybridized to the sequence; ~~and satisfies at least any one of the following requirements:~~

~~—— (1) the sequence contains one or more non-complementary bases at either end;~~

~~(2) the base portion corresponding to the polymorphisms that are not targeted for detection, among the plural polymorphisms contained in the sequence, contains universal bases; and~~

~~—— (3) the polymorphism that is targeted for detection is located at a position six or fewer bases away from any one terminus of the probe; and~~

~~the probe contains one or more of the sequences and non-complementary bases at either end.~~

2. The probe according to claim 1, wherein the probe portion corresponding to the polymorphisms that are not targeted for detection, among the plural polymorphisms contained in the sequence, contains universal bases.

32. A probe for detecting ~~that is hybridized to~~ a polynucleotide sequence having one or more polymorphisms, the polynucleotide sequence being a sequence in which the sum of the contents of guanine and cytosine in the sequence is 63% or more, wherein the probe is hybridized to the sequence; ~~and satisfies the following requirements (1) and/or (2):~~

the base portion corresponding to the polymorphism that is targeted for detection is located at a position six or fewer bases away from any one terminus of the probe; and

~~(1) the probe sequence contains one or more of the sequences and non-complementary bases at either end; and~~

4. The probe according to claim 3, wherein—(2) the probe portion corresponding to the polymorphisms that are not targeted for detection, among the plural polymorphisms contained in the sequence, contains universal bases.
53. A probe ~~for detecting~~that is hybridized to a polynucleotide sequence having one or more polymorphisms, the polynucleotide sequence being a sequence in which the sum of the contents of guanine and cytosine in the sequence is 45% or less, wherein the probe is hybridized to the sequence; and the base portion corresponding to the polymorphism that is intended for detection is located at a position six or fewer bases away from any one terminus of the probe.
64. The probe according to any one of claims 1 to 53, wherein the polynucleotide sequence having one or more polymorphisms is a human β -globin gene sequence.
75. The probe according to claim 32, wherein the sum of the contents of guanine and cytosine is 63% or more, and the polynucleotide sequence having one or more polymorphisms comprises a sequence set forth in SEQ ID NO:3, 4, 7, 8, 17 or 18.
6. ~~The probe according to claim 3, wherein the sum of the contents of guanine and cytosine is 45% or less, and the polynucleotide sequence having one or more polymorphisms comprises a sequence set forth in SEQ ID NO:11 or 12.~~
87. A microarray comprising at least one of the sequences set forth in SEQ ID NOs:3, 4, 7, 8, 11, 12, 17 and 18.
98. A probe group for detecting mutations in a β -globin gene, the probe group comprising genes having the sequences set forth in SEQ ID NOs:3, 4, 7, 8, 11, 12, 17, 18 and 25 to 66.

109. A microarray comprising at least one of the sequences set forth in SEQ ID NOs:3, 4, 7, 8, ~~11, 12,~~ 17, 18 and 25 to 66.

1140. A β -thalassemia detection kit, comprising at least the probe according to any one of claims 1 to 109.

1244. A β -thalassemia detection kit, comprising the microarray according to claim 87 or 109.

1342. A kit for β -globin gene mutation detection, the kit comprising:

(a) (i) an oligonucleotide primer having the sequence set forth in SEQ ID NO:21 and an oligonucleotide primer having the sequence set forth in SEQ ID NO:22, and/or (ii) an oligonucleotide primer having the sequence set forth in SEQ ID NO:23 and an oligonucleotide primer having the sequence set forth in SEQ ID NO:24; and

(b) the microarray according to claim 87 or 109.

~~13. A method for evaluating a microarray probe pair for polymorphism detection, the method comprising the following steps:~~

~~—— (1) plotting the fluorescence coordinates obtained by hybridizing a control nucleic acid for first polymorphism with a probe pair for polymorphism detection, in a fluorescence coordinate system which includes a Y-axis representing the signal intensity obtainable when the probe for first polymorphism detection is hybridized, and an X-axis representing the signal intensity obtainable when the probe for second polymorphism detection is hybridized;~~

~~—— (2) defining a value which is inversely proportional to the gradient of a straight line that passes through the intersection O between the Y-axis and the X-axis and the fluorescence coordinates plotted in step (1), as a correction value C; and~~

~~—— (3) a step of carrying out steps (1) and (2) on plural probe pairs for polymorphism detection, comparing the correction values C between the various probes;~~

and determining a probe pair having the minimum correction value C as probes appropriate for first polymorphism detection.

14. The method according to claim 13, wherein the fluorescence coordinate system has the Y axis and the X axis perpendicularly intersecting each other.

15. The method according to claim 13 or 14, wherein

— step (1) involves obtaining two or more points of fluorescence coordinates by performing hybridization between the control nucleic acid and the probe two or more times, and determining a representative value M for the various coordinates; and

— in step (2), the straight line is a median straight line that passes through the intersection O and the representative value M.

16. The method according to claim 14 or 15, wherein

— step (1) further involves a process of selecting a straight line having a difference in the gradient with the median straight line among plural straight lines that pass through the intersection O and the two or more points of fluorescence coordinates, and designating this as an error straight line; and

— step (2) includes:

— (a) a process of determining

— correction value $C = \pi/2 : \alpha$

— from the angle α (radian) between the median straight line and the X-axis; and

— (b) a process of determining

— correction error angle θ' (radian) = θ (radian) $\times$ correction value C

— from an error angle θ (radian) which is an angle formed by the median straight line and the error straight line.

17. The method according to claims 13 to 16, further comprising the following steps:

~~—— (4) plotting the fluorescence coordinates obtained by hybridizing a control nucleic acid for second polymorphism with a probe pair for second polymorphism detection;~~
~~—— (5) defining a value which is proportional to the gradient of a straight line that passes through the intersection O and the fluorescence coordinates plotted in step (4), as a correction value C_2 ; and~~
~~—— (6) performing steps (4) and (5) on plural probe pairs for second polymorphism detection, comparing the correction values C_2 between the various probes, and determining a probe pair having the minimum correction value C_2 as a probe appropriate for second polymorphism detection.~~

18. ~~The method according to claim 17, wherein~~
~~—— step (4) involves obtaining two or more points of fluorescence coordinates by performing hybridization between the control nucleic acid for second polymorphism and the probe for second polymorphism detection two or more times, and determining a representative value M_2 for the various coordinates; and~~
~~—— in step (5), the straight line is a second median straight line that passes through the intersection O and the representative value M_2 .~~

19. ~~The method according to claim 18, wherein~~
~~—— step (4) further involves a process of selecting a straight line having a difference in the gradient with the second median straight line among plural straight lines that pass through the intersection O and the two or more points of fluorescence coordinates, and designating this as an error straight line; and~~
~~—— step (5) includes:~~
~~—— (c) a process of determining~~
~~—— correction value $C_2 = \pi/2 \div \beta$~~
~~—— from the angle β (radian) between the second median straight line and the Y-axis; and~~
~~—— (d) a process of determining~~

~~second correction error angle θ_2' (radian) = θ_2 (radian) $\times$ correction value C_2~~

~~from a second error angle θ_2 (radian) which is an angle formed by the second median straight line and the error straight line.~~

- 5 20. ~~A genotype discrimination display program utilizing the method according to any one of claims 13 to 19.~~