Claims

1. A method for the differential quantitative detection of two or more, closely linked, homologous genes in a sample comprising the steps of :

(i) subjecting the sample to separate amplification reactions using (a) a pair of forward and reverse primers specific for the head region of each of said two or more, closely linked, homologous genes and (b) a pair of forward and reverse primers specific for the tail region of each of said two or more, closely linked, homologous genes; and

(ii) detecting and quantifying amplification products relative to a control product.

2. The method according to claim 1, wherein the sample is genomic DNA.

3. The method according to claims 1 or 2, wherein the amplification is performed by RT-PCR.

4. The method of any preceding claim, wherein the pair of forward and reverse primers specific for the head region of each of said two or more, closely linked, homologous genes are each a nucleic acid sequence between 10 and 100 nucleotides in length capable of hybridizing under very high stringency conditions to the head region of said two or more, closely linked, homologous genes, and the pair of forward and reverse primers specific for the tail region of each of said two or more, closely linked, homologous genes are each a nucleic acid sequence between 10 and 100 nucleotides in length capable of hybridizing under very high stringency conditions to

the head region of said two or more, closely linked, homologous genes.

5. A method according to any preceding claim, wherein the two or more, closely linked, homologous genes represent thalassemia genes α1 and α2.

6. A method according to claim 5 comprising the steps of:

(i) amplifying a first portion of the sample with a pair of forward and reverse primers specific for the head region of α1;

(ii) amplifying a second portion of the sample with a pair of forward and reverse primers specific for the head region of α2;

(iii) amplifying a third portion of the sample with a pair of forward and reverse primers specific for the tail region of α1;

(iv) amplifying a fourth portion of the sample with a pair of forward and reverse primers specific for the tail region of α2; and

(v) detecting and quantifying amplification products relative to a control product.

7. The method of claims 5 or 6, wherein the head region of α1 comprises about nucleotides 163708 to 167099 and/or the head region of α2 comprises about about nucleotides 158640 to 162791 and/or the tail region of α1 comprises about about nucleotides 167099 to 170335 and/or the tail region of α2 comprises about nucleotides 162791 to 166679 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462.

8. The method according to any preceding claim, wherein the control product is obtained by amplifying an endogenous control sequence (i) in each of the separate amplification reactions or (ii) in a separate amplification reaction, using a pair of forward and reverse primers specific for said endogeneous control sequence.

9. A method of screening for an α-thalassemia carrier comprising the steps of :

(i) obtaining a genomic sample,

(ii) subjecting said sample to four separate amplification reactions by RT-PCR using

(a) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 163708 to nucleotides 167099 of the α-globin gene cluster as identified in GenBank Accession No. AE006462, preferably a primer pair such as SEQ ID NO: 1 and 2,

(b) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 158640 to nucleotides 162791 of the α-globin gene cluster as identified in GenBank Accession No. AE006462, preferably a primer pair such as SEQ ID NO: 5 and 6,

(c) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about

nucleotides 167099 to nucleotides 170335 of the α-globin gene cluster as identified in GenBank Accession No. AE006462, preferably a primer pair such as SEQ ID NO: 3 and 4,

(d) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 162791 to nucleotides 166679 of the α-globin gene cluster as identified in GenBank Accession No. AE006462, preferably a primer pair such as SEQ ID NO: 7 and 8, and

(iii) detecting and quantifying the amplification products relative to a control product.

10. The method of any preceding claim, wherein the pair of forward and reverse primers specific for the head region of each of said genes and the pair of forward and reverse primers specific for the tail region of each of said genes are immobilized in an array.

11. A pair of forward and reverse primers for the amplification of

(i) the head region of α1 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about nucleotides 163708 to 167099 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462, preferably SEQ ID NO: 1 and 2,

(ii) the tail region of α1 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about 167099 to 170335 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462, preferably SEQ ID NO: 3 and 4,

(iii) the head of α2 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about nucleotides 158640 to 162791 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462, preferably SEQ ID NO: 5 and 6,

(iv) the tail region of α2 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about 162791 to 166679 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462, preferably SEQ ID NO: 7 and 8.

12. A kit for the differential quantitative detection of two or more closely linked, homologous genes in a sample, comprising in separate compartments (a) a pair of forward and reverse primers specific for the head region of each of said two or more, closely linked, homologous genes and (b) a pair of forward and reverse primers specific for the tail region of each of said two or more, closely linked, homologous genes.

13. A kit according to claim 12 for the differential quantitative detection of the thalassemia genes α1 and α2 in a sample, comprising in separate compartment pairs of forward and reverse primers according to claim 11.

14. The kit according to claims 12 or 13, further comprising a pair of forward and reverse primers specific for an endogeneous control sequence.

Claims

1. A method of screening for an α-thalassemia carrier comprising the steps of:

(i) obtaining a genomic sample,

(ii) subjecting said sample to four separate amplification reactions by RT-PCR using

(a) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 163708 to nucleotides 167099 of the α-globin gene cluster as identified in GenBank Accession No. AE006462,

(b) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 158640 to nucleotides 162791 of the α-globin gene cluster as identified in GenBank Accession No. AE006462,

(c) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 167099 to nucleotides 170335 of the α-globin gene cluster as identified in GenBank Accession No. AE006462,

(d) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 162791 to nucleotides 166679 of the α-globin gene cluster as identified in GenBank Accession No. AE006462, and

(iii) detecting and quantifying the amplification products relative to a control product, wherein the pair of primers comprises:

in step a), a primer pair comprising (the sequences comprising/consisting of) SEQ ID NO: 1 and 2;

in step b), a primer pair comprising SEQ ID NO: 3 and 4;

in step c), a primer pair comprising SEQ ID NO: 5 and 6; and

in step d), a primer pair comprising SEQ ID NO: 7 and 8.

2. The method of claim 1, wherein the pairs of primers are immobilized in an array.

3. A set of pairs of forward and reverse primers and pairs of hybridization probes, for screening for an α-thalassemia carrier by RT-PCR through the amplification of

(i) the head region of α1 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about nucleotides 163708 to 167099 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462,

(ii) the tail region of α1 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about 167099 to 170335 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462,

(iii) the head of α2 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about nucleotides 158640 to 162791 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462, and

(iv) the tail region of α2 comprising a nucleic acid 10 to 100 nucleotides in length capable of hybridizing under high stringency conditions to about 162791 to 166679 of the α-globin gene cluster as set forth in GenBank Accession No. AE006462,

wherein the pair of primers an the pairs of hybridization probes comprise:

in step i), a primer pair comprising SEQ ID NO: 1 and 2, and a hybridisation probe pair comprising SEQ ID 9 and 10;

in step ii), a primer pair comprising SEQ ID NO: 3 and 4, and a hybridisation probe pair comprising SEQ ID 11 and 12;

in step iii), a primer pair comprising SEQ ID NO: 5 and 6, and a hybridisation probe pair comprising SEQ ID 13 and 14; and

in step iv), a primer pair comprising SEQ ID NO: 7 and 8, and a hybridisation probe pair comprising SEQ ID 15 and 16.

4. A kit for screening for an α-thalassemia carrier by RT-PCR, comprising in separate compartments

(a) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 163708 to nucleotides 167099 of the α- globin gene cluster as identified in GenBank Accession No. AE006462 and a hybridisation probe pair,

(b) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 158640 to nucleotides 162791 of the α-globin gene cluster as identified in GenBank Accession No. AE006462 and a hybridisation probe pair,

(c) a primer pair that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 167099 to nucleotides 170335 of the α-globin gene cluster as identified in GenBank Accession No. AE006462 and a hybridisation probe pair,

(d) a primer pair having a sequence that specifically hybridizes under conditions suitable for a PCR with a sequence between about nucleotides 162791 to nucleotides 166679 of the *α*-globin gene cluster as identified in GenBank Accession No. AE006462 and a hybridisation probe pair

wherein the primer pair and the hybridisation probe pair comprises:

in compartment (a), a primer pair comprising SEQ ID NO: 1 and 2, and a hybridisation probe pair comprising SEQ ID 9 and 10;

in compartment (b), a primer pair comprising SEQ ID NO: 3 and 4, and a hybridisation probe pair comprising SEQ ID 11 and 12;

in compartment (c), a primer pair comprising SEQ ID NO: 5 and 6, and a hybridisation probe pair comprising SEQ ID 13 and 14; and

in compartment (d), a primer pair comprising SEQ ID NO: 7 and 8, and a hybridisation probe pair comprising SEQ ID 15 and 16.

5. The method according to claim 1, wherein the sample is genomic DNA.

6. The method according to claim 1, wherein the control product is obtained by amplifying an endogenous control sequence (i) in each of the separate amplification reactions or (ii) in a separate amplification reaction, using a pair of forward and reverse primers specific for said endogeneous control sequence.