

WHAT IS CLAIMED:

1. A method for detecting at least two genomic alleles associated with corneal dystrophy in a sample from a subject, the method comprising:
 - (A) providing epithelial cells of a subject adhered to a tip of a substrate;
 - (B) agitating the tip of the substrate in a lysis solution that lyses cells adhered to the substrate;
 - (C) removing the substrate from the lysis solution upon completion of the agitating (B);
 - (D) incubating the lysis solution after the removing (C);
 - (E) isolating genomic DNA from the lysis solution to form a gDNA solution; and
 - (F) determining an identity of at least two nucleotides present in the TGF β I gene using at least two oligonucleotide primer pairs and the gDNA solution, wherein the at least two nucleotides are located at respective independent positions of the TGF β I gene corresponding to respective independent single nucleotide polymorphisms (SNPs) associated with corneal dystrophy.
2. The method according to claim 1, wherein the at least two nucleotides present in the TGF β I gene are separated in the human genome by at least one nucleotide.
3. The method according to any one of the preceding claims, wherein at least one pair of the at least two oligonucleotide primer pairs comprises a forward PCR primer having a nucleotide sequence comprising SEQ ID NO:1 and a reverse PCR primer having a nucleotide sequence comprising SEQ ID NO:2.
4. The method according to any one of the preceding claims, wherein at least one pair of the at least two oligonucleotide primer pairs comprises a forward PCR primer having a nucleotide sequence comprising SEQ ID NO:43 and a reverse PCR primer having a nucleotide sequence comprising SEQ ID NO:44.
5. The method according to any one of the preceding claims, wherein the at least two oligonucleotide primer pairs comprise a first amplification primer pair and a second amplification primer pair, wherein:

the first amplification primer pair comprises a forward PCR primer having a nucleotide sequence comprising SEQ ID NO:1 and a reverse PCR primer having a nucleotide sequence comprising SEQ ID NO:2; and

the second amplification primer pair comprises a forward PCR primer having a nucleotide sequence comprising SEQ ID NO:43 and a reverse PCR primer having a nucleotide sequence comprising SEQ ID NO:44.

6. The method according to any one of the preceding claims, wherein the determining (F) further comprises using:

(i) a first wild type detection probe having a nucleotide sequence comprising SEQ ID NO:25 and a first mutant detection probe having a nucleotide sequence comprising SEQ ID NO:26, SEQ ID NO:48, or SEQ ID NO:49; and

(ii) a second wild type detection probe having a nucleotide sequence comprising SEQ ID NO:45 or SEQ ID NO:47 and a second mutant detection probe having a nucleotide sequence comprising SEQ ID NO:46 or SEQ ID NO:50.

7. A method for detecting corneal dystrophy comprising:

(A) amplifying at least two TGF β I gene sequences, including a first TGF β I gene sequence comprising nucleotides encoding amino acid residue 124 and a second TGF β I gene sequence comprising nucleotides encoding amino acid residue 555, from a biological sample from a human subject using a reaction mixture comprising at least a first amplification primer pair and at least a second amplification primer pair;

(B) hybridizing a first detection probe of a first detection oligonucleotide probe pair to the first TGF β I gene sequence;

(C) hybridizing a second detection probe of a second detection oligonucleotide probe pair to the second TGF β I gene sequence; and

(D) detecting one or more mutations in the first TGF β I gene sequence and/or the second TGF β I gene sequence based on a use of at least two detection probe pairs, including the first detection oligonucleotide probe pair and the second detection oligonucleotide probe pair.

8. The method according to claim 7, wherein detecting the one or more mutations in the first TGF β I gene sequence and/or the second TGF β I gene sequence includes detecting two or more mutations in the first TGF β I gene sequence and/or the second TGF β I gene sequence and the two or more mutations are separated in the human genome by at least one nucleotide.

9. The method according to claim 7 or 8, wherein the at least first amplification primer pair comprises a first amplification primer and a second amplification primer, wherein the first amplification primer comprises nucleotide sequence SEQ ID NO:1 and wherein the second amplification primer comprises nucleotide sequence SEQ ID NO:2.
10. The method according to any one of claims 7-9, wherein the at least second amplification primer pair comprises a third amplification primer and a fourth amplification primer, wherein the third amplification primer is represented by nucleotide sequence SEQ ID NO:43, and wherein the fourth amplification primer is represented by nucleotide sequence SEQ ID NO:44.
11. The method according to claim 7 or 8, wherein:
 - the first amplification primer pair comprises a first amplification primer and a second amplification primer,
 - the first amplification primer comprises nucleotide sequence SEQ ID NO:1,
 - the second amplification primer comprises nucleotide sequence SEQ ID NO:2,
 - the second amplification primer pair comprises a third amplification primer and a fourth amplification primer,
 - the third amplification primer comprises nucleotide sequence SEQ ID NO:43, and
 - the fourth amplification primer comprises nucleotide sequence SEQ ID NO:44.
12. The method according to any one of claims 7-11, wherein one of the one or more mutations corresponds to, at amino acid 124, arginine mutated to a cysteine (R124C), arginine mutated to a histidine (R124H), and/or arginine mutated to a leucine (R124L) in the encoded TGFBI protein.
13. The method according to any one of claims 7-12, wherein one of the one or more mutations corresponds to, at amino acid 555, arginine mutated to a tryptophan (R555W) and/or arginine mutated to a glutamine (R555Q) in the encoded TGFBI protein.
14. The method according to any one of claims 7-13, wherein each of the at least two detection oligonucleotide probe pairs individually includes a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe comprise

a nucleotide sequence pair individually selected from the group consisting of SEQ ID NOs:25-26, SEQ ID NOs:25 and 48, SEQ ID NOs:25 and 49, SEQ ID NOs:27-28, SEQ ID NOs:29-30, SEQ ID NOs:31-32, SEQ ID NOs:33-34, SEQ ID NOs:35-36, SEQ ID NOs:37-38, SEQ ID NOs:39-40, SEQ ID NOs:41-42, SEQ ID NOs:45-46 and SEQ ID NOs:46-47, SEQ ID NOs: 45 and 50, and SEQ ID NOs: 47 and 50.

15. The method according to any one of claims 7-14, wherein the first detection oligonucleotide probe pair comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:26.
16. The method according to any one of claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:45 and SEQ ID NO:46.
17. The method according to any one of the claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:47 and SEQ ID NO:46.
18. The method according to any one of the claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:48.
19. The method according to any one of the claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the

fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:49.

20. The method according to any one of the claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:45 and SEQ ID NO:50.
21. The method according to any one of the claims 7-15, wherein the second detection oligonucleotide probe pair comprises a third detection oligonucleotide probe and a fourth detection oligonucleotide probe, wherein the third detection oligonucleotide probe and the fourth detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:47 and SEQ ID NO:50.
22. The method according to any one of claims 7-21, wherein the first detection probe is coupled with a first label and the second detection probe is coupled to a second label.
23. The method according to claim 22, wherein the first label is VIC and the second label is FAM.
24. The method of claim 7, wherein the hybridizing (B) and the hybridizing (C) are performed concurrently in a same solution.
25. The method of claim 7, wherein the hybridizing (B) and the hybridizing (C) are performed concurrently or at different time in a same solution or different solutions.
26. A reaction mixture for detecting corneal dystrophy in a human subject, the reaction mixture comprising:
 - (A) at least a first amplification primer pair and a second amplification primer pair for amplifying and determining (1) a first TGF β I gene sequence of at least two TGF β I gene sequences that comprises nucleotides encoding amino acid residue 124 from a biological sample from the subject and (2) a second TGF β I gene sequence of the at least two TGF β I

gene sequences that comprises nucleotides encoding amino acid residue 555 from a biological sample from the subject; and

(B) at least two detection probe pairs, wherein a detection probe in each of the at least two detection probe pairs hybridizes to at least one of the at least two TGF β I gene sequences.

27. The reaction mixture according to claim 26, wherein the first amplification primer pair comprises a first amplification primer and a second amplification primer, the first amplification primer comprises a nucleotide sequence SEQ ID NO:1, and the second amplification primer comprises a nucleotide sequence SEQ ID NO:2.
28. The reaction mixture according to claim 26 or 27, wherein the second amplification primer pair comprises a third amplification primer and a fourth amplification primer, the third amplification primer comprises a nucleotide sequence SEQ ID NO:43, and the fourth amplification primer comprises a nucleotide sequence SEQ ID NO:44.
29. The reaction mixture according to any one of claims 26-28, wherein at least one of the at least two detection probe pairs is used to detect a mutation that corresponds to, at amino acid 124, arginine mutated to a cysteine (R124C), arginine mutated to a histidine (R124H), and/or arginine mutated to a leucine (R124L) in the encoded TGFBI protein.
30. The reaction mixture according to any one of claims 26-29, wherein at least one of the at least two detection probe pairs is used to detect a mutation that corresponds to, at amino acid 555, arginine mutated to a tryptophan (R555W) and/or arginine mutated to a glutamine (R555Q) in the encoded TGFBI protein.
31. The reaction mixture according to any one of claims 26-30, wherein respective detection probe pairs of the at least two detection probe pairs individually include a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe comprise a nucleotide sequence pair individually selected from the group consisting of SEQ ID NOs:25-26, SEQ ID NOs:25 and 48, SEQ ID NOs:25 and 49, SEQ ID NOs:27-28, SEQ ID NOs:29-30, SEQ ID NOs:31-32, SEQ ID NOs:33-34, SEQ ID NOs:35-36, SEQ ID NOs:37-38, SEQ ID NOs:39-40, SEQ ID NOs:41-42, SEQ ID NOs: 45-46 and SEQ ID NOs: 46-47, SEQ ID NOs: 45 and 50, and SEQ ID NOs: 47 and 50.

32. The reaction mixture according any one of the claims 26-31, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:26.
33. The reaction mixture according to any one of claims 26-32, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:45 and SEQ ID NO:46.
34. The reaction mixture according to any one of claims 26-32, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:47 and SEQ ID NO:46.
35. The reaction mixture according to any one of claims 26-34, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:48.
36. The reaction mixture according to any one of claims 26-35, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:25 and SEQ ID NO:49.
37. The reaction mixture according to any one of claims 24-36, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the

second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:45 and SEQ ID NO:50.

38. The reaction mixture according to any one of claims 24-36, wherein one of the at least two detection probe pairs comprises a first detection oligonucleotide probe and a second detection oligonucleotide probe, wherein the first detection oligonucleotide probe and the second detection oligonucleotide probe respectively comprise nucleotide sequence pair SEQ ID NO:47 and SEQ ID NO:50.
39. The reaction mixture according to any one of claims 26-38, wherein a first detection probe of the at least two detection probes is coupled with a first label and a second detection probe of the at least two detection probes is coupled to a second label.
40. The reaction mixture according to claim 39, wherein the first label is VIC and the second label is FAM.
41. A reaction mixture for detecting corneal dystrophy in a human subject, the reaction mixture comprising:
- (A) at least a first amplification primer pair for amplifying and determining a TGF β I gene sequence from a biological sample from the subject; and
 - (B) a set of at least three detection probes, wherein one detection probe of the set of at least three detection probes hybridizes to the TGF β I gene sequence when exposed to the TGF β I gene sequence.
42. The reaction mixture according to claim 41, wherein the TGF β I gene sequence comprises nucleotides encoding amino acid residue 124 from the biological sample from the subject.
43. The reaction mixture according to claim 41 or 42, wherein the set of at least three detection probes includes at least one detection probe selected from the group consisting of SEQ ID NOs:25-42 and 48-49.
44. The reaction mixture according to any one of claims 41-43, wherein the set of at least three detection probes includes at least two or more detection probes selected from the group consisting of SEQ ID NOs:25-42 and 48-49.

45. The reaction mixture according to any one of claims 41-44, wherein the set of at least three detection probes includes at least three or more detection probes selected from the group consisting of SEQ ID NOs:25-42 and 48-49.
46. The reaction mixture according to any one of claims 41-45, wherein the set of at least three detection probes include a first detection probe SEQ ID NO:25 and a second detection probe SEQ ID NO:26.
47. The reaction mixture according to claim 46, wherein the set of at least three detection probes includes a third detection probe selected from the group consisting of SEQ ID NO:48 and SEQ ID NO:49.
48. The reaction mixture according to claim 47, wherein the set of at least three detection probes include a fourth detection probe that is distinct from the third detection probe and that is selected from the group consisting of SEQ ID NO:48 and SEQ ID NO:49.
49. The reaction mixture according to any one of claims 41-45, wherein the set of at least three detection probes include two or more detection probes selected from the group consisting of SEQ ID NOs:26 and 48-49.
50. The reaction mixture according to any one of claims 41-49, wherein the first amplification primer pair comprises a first amplification primer and a second amplification primer, the first amplification primer comprises nucleotide sequence SEQ ID NO:1, and the second amplification primer comprises nucleotide sequence SEQ ID NO:2.
51. The reaction mixture according to any one of claims 41-50, further comprising:
- (C) at least a second amplification primer pair for amplifying and determining a second TGF β I gene sequence from the biological sample; and
 - (D) a second set of at least three detection probes, wherein one detection probe of the second set of at least three detection probes hybridizes the second TGF β I gene sequence when exposed to the second TGF β I gene sequence.

52. The reaction mixture according to claim 51, wherein the second TGF β I gene sequence comprises nucleotides encoding amino acid residue 555 from the biological sample from the subject.
53. The reaction mixture according to claim 51 or 52, wherein the second set of at least three detection probes include at least one detection probe selected from the group consisting of SEQ ID NOs:45-47 and 50.
54. The reaction mixture according to any one of claims 51-53, wherein the second set of at least three detection probes include at least two or more detection probes selected from the group consisting of SEQ ID NOs: 45-47 and 50.
55. The reaction mixture according to any one of claims 51-54, wherein the second set of at least three detection probes include at least three or more detection probes selected from the group consisting of SEQ ID NOs: 45-47 and 50.
56. The reaction mixture according to any one of claims 51-55, wherein the second set of at least three detection probes include a first detection probe that is SEQ ID NO:45 or SEQ ID NO:47, a second detection probe that is SEQ ID NO:46, and a third detection probe that is SEQ ID NO:50.
57. The reaction mixture according to any one of claims 51-56, wherein the second amplification primer pair comprises a third amplification primer and a fourth amplification primer, the third amplification primer comprises a nucleotide sequence SEQ ID NO:43, and the fourth amplification primer comprises a nucleotide sequence SEQ ID NO:44.
58. The reaction mixture according to claim 41, wherein the TGF β I gene sequence comprises nucleotides encoding amino acid residue 555 from the biological sample from the subject.
59. The reaction mixture according to claim 41 or 58, wherein the set of at least three detection probes include at least one detection probe selected from the group consisting of SEQ ID NOs:45-47 and 50.

60. The reaction mixture according to any one of claims 41 and 58-59, wherein the set of at least three detection probes include at least two or more detection probes selected from the group consisting of SEQ ID NOs: 45-47 and 50.
61. The reaction mixture according to any one of claims 41 and 58-60, wherein the set of at least three detection probes include at least three or more detection probes selected from the group consisting of SEQ ID NOs: 45-47 and 50.
62. The reaction mixture according to any one of claims 41 and 58-61, wherein the set of at least three detection probes include a first detection probe that is SEQ ID NO:45 or SEQ ID NO:47, a second detection probe that is SEQ ID NO:46, and a third detection probe that is SEQ ID NO:50.
63. A method for detecting corneal dystrophy comprising:
- (A-1) amplifying a first TGF β I gene sequence from a biological sample from a human subject using a reaction mixture comprising at least a first amplification primer pair and a set of at least three detection probes;
 - (B-1) hybridizing a first detection probe of the set of at least three detection probes to the first TGF β I gene sequence; and
 - (C-1) detecting a mutation in the first TGF β I gene sequence based on (i) the hybridization of the first detection probe of the set of at least three detection probes to the first TGF β I gene sequence and (ii) the failure of a second and a third detection probe of the set of at least three detection probes to hybridize to first TGF β I gene sequence.
64. The method according to claim 63, wherein the reaction mixture comprises a reaction mixture as recited in any one of claims 41-50 and 58-62.
65. The method according to claim 63 or 64, further comprising:
- (A-2) amplifying a second TGF β I gene sequence from the biological sample using the same reaction mixture, wherein the reaction mixture comprises at least a second amplification primer pair and a second set of at least three detection probes;
 - (B-2) hybridizing a first detection probe of the second set of at least three detection probes to the second TGF β I gene sequence; and

(C-2) detecting a mutation in the second TGF β I gene sequence based on (i) the hybridization of the first detection probe of the second set of at least three detection probes to the second TGF β I gene sequence and (ii) the failure of a second detection probe and a third detection probe of the second set of at least three detection probes to hybridize to the second TGF β I gene sequence.

66. The method according to claim 65, wherein the reaction mixture comprises a reaction mixture as recited in any one of claims 51-57.

67. The method according to claim 65 or 66, wherein the amplifying (A-1), the amplifying (A-2), the hybridizing (B-1), the hybridizing (B-2), the detecting (C-1), and the detecting (C-2) are performed with a same aliquot of the biological sample.

68. The method according to any one of claims 65-67, wherein the amplifying the first TGF β I gene sequence (A-1) and the amplifying the second TGF β I gene sequence (A-2) are performed concurrently.

69. The method according to any one of claims 65-68, wherein the hybridizing (B-1) and the hybridizing (B-2) are performed concurrently.

70. The method according to any one of claims 65-69, wherein the detecting (C-1) and the detecting (C-2) are performed concurrently.

71. Use of a reaction mixture as recited in any one of claims 7-70 for predicting the risk of complication following laser eye surgery in a subject through a detection of heterozygous corneal dystrophy in the human subject.

72. The use according to claim 71, wherein the laser eye surgery comprises one of Lasik and Excimer laser surgery.

73. A method for detecting a genomic mutation associated with corneal dystrophy in a sample from a human subject, the method comprising:

(A) providing epithelial cells of a human subject adhered to a tip of a substrate;

(B) agitating the tip of the substrate in a lysis solution that lyses cells adhered to the substrate;

(C) removing the substrate from the lysis solution upon completion of the agitating (B),
(D) incubating the lysis solution after the removing (C);
(E) isolating genomic DNA from the lysis solution to form a gDNA solution; and
(F) determining an identity of at least a nucleotide present in the TGF β I gene using at least a first primer pair, a set of at least three detection probes, and the gDNA solution by concurrently exposing the gDNA solution to the at least three detection probes, wherein:
the at least a nucleotide is located at a particular position of the TGF β I gene corresponding to a single nucleotide polymorphism (SNP) associated with corneal dystrophy, and
each of at least two detection probes of the set of the at least three detection probes is configured to detect a different mutation at the particular position of the TGF β I gene.

74. The method according to claim 73, further comprising:

(G) determining an identity of at least a second nucleotide present in the TGF β I gene using at least a second primer pair, a second set of at least three detection probes, and the gDNA solution by concurrently exposing the gDNA solution to the set of at least three detection probes and the second set of at least three detection probes, wherein:
the at least a second nucleotide is located at a second particular position, independent of the position of the at least a nucleotide, of the TGF β I gene corresponding to a second single nucleotide polymorphism (SNP) associated with corneal dystrophy, and
at least two detection probes of the second set of the at least three detection probes are configured to detect a respective mutation at the second particular position of the TGF β I gene.

75. The method according to claim 75, wherein the determining (F) and the determining (G) are performed concurrently.

76. The method according to claim 74 or 75, wherein the determining (F) and the determining (G) are performed using the same gDNA solution.

WHAT IS CLAIMED:

1. A reaction mixture for detecting corneal dystrophy in a human subject, the reaction mixture comprising:
(A) at least a first amplification primer pair for amplifying and determining a first TGFβ1 gene sequence from a biological sample from the subject; and
(B) a first set of at least four detection probes including a first detection probe comprising the sequence of SEQ ID NO:25, a second detection probe comprising the sequence of SEQ ID NO:26, a third detection probe comprising the sequence of SEQ ID NO:48 and a fourth detection probe consisting of the sequence of SEQ ID NO:49.
2. The reaction mixture according to claim 1, further comprising a second set of at least three detection probes includes a fifth detection probe comprising a sequence selected from SEQ ID NO:45 and SEQ ID NO:47, a sixth detection probe comprising the sequence of SEQ ID NO:46, and a seventh detection probe comprising the sequence of SEQ ID NO:50, and at least a second amplification primer pair for amplifying and determining a second TGFβ1 gene sequence from the biological sample from the subject.
3. The reaction mixture according to claim 2, wherein the first amplification primer pair comprises a first amplification primer and a second amplification primer, the first amplification primer comprises nucleotide sequence SEQ ID NO:1, and the second amplification primer comprises nucleotide sequence SEQ ID NO:2.
4. The reaction mixture according to claims 2-3, wherein the first TGFβ1 gene sequence comprises nucleotides encoding amino acid residue 124 from the biological sample from the subject and the second TGFβ1 gene sequence comprises nucleotides encoding amino acid residue 555 from the biological sample from the subject.
5. The reaction mixture according to any of claims 2-4, wherein the second amplification primer pair comprises a third amplification primer and a fourth amplification primer, the third amplification primer comprises a nucleotide sequence SEQ ID NO:43, and the fourth amplification primer comprises a nucleotide sequence SEQ ID NO:44.
6. A method for detecting corneal dystrophy comprising:
(A) amplifying a first TGFβ1 gene sequence from a biological sample from a human subject using a reaction mixture comprising at least a first amplification primer pair for amplifying a TGFβ1 gene sequence and a set of at least four detection probes including a first detection probe comprising the sequence of SEQ ID NO:25, a second detection probe comprising the sequence of SEQ ID NO:26, a third detection probe comprising the sequence of SEQ ID NO: 48 and a fourth detection probe consisting of the sequence of SEQ ID NO:49;
(B) hybridizing one detection probe of the set of at least four detection probes to the first TGFβ1 gene sequence; and
(C) detecting a mutation in the first TGFβ1 gene sequence based on (i) the hybridization of the detection probe of the set of at least four detection probes that has hybridized in step (B) to the

first TGFβ1 gene sequence and the failure of the other three detection probes of the set of at least four detection probes to hybridize to the first TGFβ1 gene sequence.

7. The method according to claim 6, wherein the reaction mixture comprises a reaction mixture as recited according to any of claims 1 to 5.
8. A method for detecting a genomic mutation associated with corneal dystrophy in a sample from a human subject, the method comprising:
 - (A) using epithelial cells of a human subject adhered to a tip of a substrate;
 - (B) agitating the tip of the substrate in a lysis solution that lyses cells adhered to the substrate;
 - (C) removing the substrate from the lysis solution upon completion of the agitating (B);
 - (D) incubating the lysis solution after the removing (C);
 - (E) isolating genomic DNA from the lysis solution to form a gDNA solution; and
 - (F) determining an identity of at least a nucleotide present in the TGFβ1 gene using at least a first primer pair for amplifying a TGFβ1 gene sequence, a first set of at least four detection probes including a first detection
probe comprising the sequence of SEQ ID NO:25, a second detection probe comprising the
sequence of SEQ ID NO:26, a third detection probe comprising the sequence of SEQ ID NO:48 and
a fourth detection probe consisting of the sequence of SEQ ID NO:49, and the gDNA solution by
concurrently exposing the gDNA solution to the first set of at least four detection probes, wherein:
the at least a nucleotide is located at a particular position of the TGFβ1 gene corresponding to a
single nucleotide polymorphism (SNP) associated with corneal dystrophy.
9. The method according to claim 8, further comprising: (G) determining an identity of at least a
second nucleotide present in the TGFβ1 gene using at least a second primer pair, a second set of
at least three detection probes including a fifth detection probe comprising a sequence selected
from SEQ ID NO:45 and SEQ ID NO:47, a sixth detection probe comprising the sequence of SEQ ID
NO:46, and a seventh detection probe comprising the sequence of SEQ ID NO:50, and the gDNA
solution by concurrently exposing the gDNA solution to the first set of at least four detection
probes and the second set of at least three detection probes, wherein:
the at least second nucleotide is located at a second particular position,
independent of the position of the at least a nucleotide, of the TGFβ1 gene corresponding to a
second single nucleotide polymorphism (SNP) associated with corneal dystrophy.