

CLAIMS

1. A primer-dependent amplification and detection method that is capable of amplifying and detecting in a sample as few as ten copies of at least one rare DNA intended target sequence ("rare target sequence") in a mixture containing, for each rare target sequence, 10,000 copies of a closely related unintended target sequence ("closely related sequence" or "unintended target sequence") that differs from the rare target sequence by as little as one or two base pairs, comprising:
 - (a) preparing a primer-dependent amplification reaction mixture that includes the sample, a DNA polymerase, deoxyribonucleoside triphosphates, an amplification buffer, homogeneous fluorescence detection means for detecting amplification products, and for each rare target sequence a pair of a first primer and a second primer that are specific for the rare target sequence but mismatched to the closely related sequence,
 - (b) repeatedly cycling the primer-dependent amplification reaction mixture by said primer-dependent amplification method to amplify each rare target sequence present in the sample, and
 - (c) detecting that rare target sequence by measuring the intensity of fluorescence from the homogeneous fluorescence detection means;
- wherein (i) the first primer is an allele-discriminating multi-part primer comprising from the 5' end to the 3' end a first anchor sequence, a first bridge sequence, and a first foot sequence that is mismatched to the closely related sequence by at least its 3'-terminal or 3'-penultimate nucleotide, and (ii) the second primer is an allele-discriminating primer.
2. The method according to claim 1 wherein each first primer is a SuperSelective primer.
3. The method of claim 1 or claim 2 wherein the second primer is a SuperSelective primer that is mismatched to the closely related sequence by at least its 3'-terminal or 3'-penultimate nucleotide.
4. The method according to any of claims 1-3 wherein each primer contains a 3'-terminal interrogating nucleotide that is complementary to the rare target sequence but mismatched to the unintended target sequence.

5. The method according to any of claims 1-4 wherein said cycling comprises temperature cycling in a non-symmetric polymerase chain reaction (PCR) method.
6. The method according to claim 5 wherein said detecting comprises real-time detection.
7. The method according to claim 5 wherein the PCR method is a digital PCR method,
5 and said detecting comprises end-point detection.
8. The method according to any of claims 1-7 wherein the at least one rare target sequence in the sample includes at least two different rare target sequences, and the homogeneous fluorescence detection means comprises at least two different homogeneous fluorescence detection probes for the at least two different rare target
10 sequences respectively.
9. The method according to claims 8 wherein the at least two rare target sequences include a group of rare target sequences, and the probe for the rare target sequences in the group is labeled with the same color.
10. The method according to claim 8, wherein the probes are color-coded.
- 15 11. The method according to any of claims 1-10 wherein each different unintended target sequence differs from its corresponding rare target sequence by a single base pair, and both the first and the second primers are mismatched to that single base pair.
12. The method according to claim 11 wherein each second primer is a multi-part primer comprising from the 5' end to the 3' end a second anchor sequence, a second bridge
20 sequence, and a second foot sequence.
13. The method according to claim 11 or claim 12, wherein the homogeneous fluorescence detection means comprises a probe for each rare target sequence.
14. The method according to claim 13, wherein the first or second primer for each rare target sequence contains a 5'-tag sequence, and wherein the complement of each
25 5'-tag sequence is the target of the probe.
15. The method according to claim 8 or 13, wherein each probe comprises a sequence that is complementary to the complement of the first bridge sequence or of the second bridge sequence.
- 30 16. The method according to claim 15, wherein the probe is a shared-stem molecular beacon.

17. The method according to claim 8 wherein the primer-dependent amplification reaction mixture includes an effective amount of a selectivity-enhancing reagent.
18. The method according to claim 17 wherein the selectivity-enhancing reagent is a Hofmeister salt.
- 5 19. The method according to any of claims 1-10 wherein the at least one rare target sequence differs from its corresponding unintended target sequence by a first base pair and a second base pair that occur in *cis*, and wherein the first primer is complementary to the first base pair and the second primer is complementary to the second base pair.
- 10 20. The method of claim 19 wherein the at least one rare target sequence in the sample includes two or more rare target sequences, and the homogeneous fluorescence detection means comprises at least one homogeneous fluorescence detection probe for each rare target sequence.
- 15 21. The method according to claim 19 or claim 20 wherein the homogeneous fluorescence detection means for each rare target sequence comprises an interprimer-specific molecular beacon probe.
22. The method according to any of claims 19-21 wherein the primer-dependent amplification reaction mixture includes an effective amount of a selectivity-enhancing reagent.
- 20 23. The method according to claim 22 wherein the selectivity-enhancing reagent is a Hofmeister salt.
24. The method of claim 18 or claim 23, wherein the Hofmeister salt comprises tetramethylammonium chloride (TMAC).
25. A kit of reagents for performing the method of any of claims 1-24.
- 25 26. The kit of claim 25, wherein the homogeneous fluorescence detection means comprises a molecular beacon probe for each rare target sequence.