

What is eClaimed is:

1. A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

- (a) a support surface comprising one or more immobilized capture oligonucleotides;
- (b) a detection probe comprising an oligonucleotide tag, a target complement and a
detection oligonucleotide;
- (c) an amplification template;
- (d) a nucleic acid ligase;
- (e) a nucleic acid polymerase; and
- (f) a detection reagent comprising a label and a nucleic acid sequence.

2. The kit according to claim 1, further comprising an anchoring reagent comprising an
oligonucleotide tag and an anchoring oligonucleotide.

3. The kit according to claim 2, wherein the anchoring reagent is immobilized on the
support surface.

4. The kit of according to claim 2 or 3, wherein the anchoring oligonucleotide is about 10 to
about 30 nucleic acids in length.

5. The kit of according to any of claims 2 to 4, wherein the anchoring oligonucleotide
comprises 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

6. The kit according to any of claims 2 to 5, wherein the amplification template comprises a
linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal
nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of
hybridizing to the detection sequence, and an internal nucleotide sequence capable of hybridizing
to a complement of the anchoring oligonucleotide of the anchoring reagent, wherein the 5' and 3'
terminal nucleotide sequences of the amplification template do not overlap with the internal
sequence.

7. The kit according to claim 6, wherein the amplification template comprises a 5' terminal phosphate group.

8. The kit according to any of claims 1 to 7, wherein the amplification template is about 53 to about 61 nucleotides in length.

9. The kit according to any of claims 6 to 8, wherein the sum of the length of the 5' and 3' terminal sequences is about 14 to about 24 nucleotides in length.

10. The kit according to claim 9, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 to about 19 nucleotides in length.

11. The kit according to any of claims 1 to 10, wherein the amplification template comprises a 5' terminal sequence of 5'-GTTCTGTC-3' (SEQ ID NO: 1666) and 3' terminal sequence of 5'-GTGTCTA-3' (SEQ ID NO: 1667).

12. The kit according to any of claims 1 to 10, wherein the amplification template comprises a nucleotide sequence of:

a) 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668);

b) 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669);

c) 5' -

GTTCTGTCATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAA
GAGTGTCTA-3' (SEQ ID NO:1670); or

d) 5'-

GCTGTGCAATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAA
GAGCGTCGA-3' (SEQ ID NO:1671).

13. The kit according to claim 1, wherein the amplification template comprises a circular amplification template.

14. The kit according to any of claims 1 to 13, wherein the detection probe comprises a single stranded DNA oligonucleotide tag, a single stranded RNA target complement and a single stranded DNA detection oligonucleotide.
- 5 15. The kit according to claim 2, wherein the anchoring reagent comprises a single stranded DNA oligonucleotide tag and a single stranded DNA anchoring sequence.
16. The kit according to claim 14 or 15, further comprising an RNase.
- 10 17. The kit according to claim 6, wherein the detection oligonucleotide of the detection probe comprises a first sequence complementary to the 5' terminal sequence of the amplification template and an adjacent second sequence complementary to the 3' terminal sequence of the amplification template.
- 15 18. The kit according to any of claims 1 to 17, wherein the nucleic acid sequence of the detection reagent comprises
- a) a sequence with at least 90% sequence identity to 14 or 15 contiguous nucleotides of: 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672);
- b) the sequence 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672); or
- 20 c) the sequence 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).
19. The kit according to any of claims 1 to 18, wherein the label of the detection reagent comprises an electrochemiluminescent (ECL) label.
- 25 20. The kit according to any of claims 1 to 19, wherein the support surface comprises a carbon-based electrode.
21. The kit according to any of claims 1 to 20, wherein the support surface comprises a carbon ink electrode.

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22. The kit according to any of claims 1 to 21, wherein the support surface comprises a multi-well plate assay consumable, and wherein each well of the plate comprises a carbon ink electrode.

5 23. The kit according to any of claims 1 to 22, wherein a plurality of capture oligonucleotides are immobilized on a solid phase support in discrete binding domains to form an array.

24. The kit according to any of claims 1 to 23, wherein the capture oligonucleotides immobilized on the support surface are selected from:

10 1. Capture set 1 comprising:

a. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-64;

b. capture oligonucleotides comprising a sequence having at least 95% identity to a sequence selected from SEQ ID Nos: 1-64;

15 c. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence having at least 95% identity to as sequence selected from SEQ ID Nos: 1-64;

d. capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-64; and

20 e. capture oligonucleotides selected from any of (a)-(d); or

2. Capture set 2 comprising:

f. capture oligonucleotides comprising a sequence having at least 20 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-10;

25 g. capture oligonucleotides comprising a sequence having at least 95% identity to a sequence selected from SEQ ID Nos: 1-10;

h. capture oligonucleotides having at least 20 consecutive nucleotides of a sequence having at least 95% identity to as sequence selected from SEQ ID Nos: 1-10;

30 i. capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-10; and

j. capture oligonucleotides selected from any of (f)-(i).

25. A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

(a) a support surface comprising immobilized capture oligonucleotide;

(b) an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide;

(c) a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample;

(d) a detection probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence, wherein the first nucleic acid sequence of the targeting probe and second nucleic acid sequence of the detection probe are complementary to adjacent sequences of the target nucleotide;

(e) a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal nucleotide sequence capable of hybridizing to a complement of the anchoring sequence of the anchoring reagent and a second internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the first and second internal sequences;

(f) a nucleic acid ligase;

(g) a nucleic acid polymerase; and

(h) a detection reagent comprising an electrochemiluminescent (ECL) label and a nucleic acid sequence.

26. A kit according to any of claims 1 to 25, further comprising a detection mixture comprising a linear amplification template and one or more additional components, selected from: ligation buffer, adenosine triphosphate (ATP), bovine serum albumin (BSA), Tween 20, T4 DNA ligase, and combinations thereof.

27. A kit according to claim 26, wherein the detection mixture comprises one or more components for rolling circle amplification selected from BSA, buffer, deoxynucleoside triphosphates (dNTP), Tween 20, Phi29 DNA polymerase, or a combination thereof.

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281. A method of detecting a target oligonucleotide comprising a target nucleic acid sequence in a sample, the method comprising:

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(a) contacting the sample with a detection probe comprising an oligonucleotide tag, a target complement and a detection oligonucleotide under conditions in which the target complement hybridizes to the target nucleic acid sequence of the target oligonucleotide to form a reaction product;

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(b) contacting a support surface on which a capture oligonucleotide is immobilized with a mixture containing the reaction product under conditions in which the oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotide to form an immobilized detection complex;

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(c) contacting the immobilized detection complex with a detection mixture comprising an amplification template;

(d) amplifying the amplification template to form an amplicon comprising one or more nucleic acid sequences comprising detection labeling sites;

(e) contacting the amplicon with a detection reagent comprising a label and a nucleic acid sequence that is complementary to the detection labeling sites under conditions in which the nucleic acid sequence of the detection reagent hybridizes to the detection labeling sites of the amplicon; and

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(f) detecting the label bound to the detection labeling sites.

~~2. The method according to claim 1, wherein the sample is contacted with an anchoring reagent and the detection probe in (a), wherein the anchoring reagent comprises an oligonucleotide tag and an anchoring sequence.~~

3. — The method according to claim 2, wherein the detection probe comprises a single stranded DNA oligonucleotide tag, a single stranded RNA target complement and a single stranded DNA detection oligonucleotide.

4. — The method according to claim 3, wherein the anchoring reagent comprises a single stranded DNA oligonucleotide tag and a single stranded DNA anchoring sequence.

5. — The method according to claim 3 or 4, comprising contacting the immobilized detection complex with a RNase to digest single stranded RNA of unbound probe before (c).

629. A method of detecting a target oligonucleotide comprising a target nucleic acid sequence in a sample, the method comprising:

(a) contacting the sample with:

(i) a detection probe comprising an oligonucleotide tag comprising a single stranded DNA sequence, a target complement comprising a single stranded RNA sequence and a detection oligonucleotide comprising a single stranded DNA sequence; and

(ii) an anchoring reagent comprising an oligonucleotide tag comprising a single stranded DNA sequence and an anchoring sequence comprising a single stranded DNA sequence,

wherein the target complement of the detection probe hybridizes to the target nucleic acid sequence of the target oligonucleotide to form a reaction product comprising the oligonucleotide tag, a double stranded RNA duplex comprising the target nucleic acid sequence of the target oligonucleotide and the target complement;

(b) contacting a support surface comprising one or more electrodes on which a plurality of capture oligonucleotides are immobilized in discrete binding domains with a mixture comprising the reaction product under conditions in which the oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotides to form a detection complex on the support surface;

- (c) contacting the support surface with a RNase to digest single stranded RNA of unbound detection probe;
- (d) contacting the immobilized detection complex with a detection mixture comprising a rolling circle amplification (RCA) template and a polymerase;
- 5 (e) amplifying the template by RCA to form an extended sequence attached to the detection complex, wherein the extended sequence comprises multiple nucleic acid sequences comprising detection labeling sites;
- (f) contacting the extended sequence with a detection reagent comprising an electrochemiluminescent (ECL) label and a nucleic acid sequence that is
10 complementary to the detection labeling sites of the extended sequence under conditions in which the nucleic acid sequence of the detection reagent hybridizes to the detection labeling sites; and
- (g) detecting the ECL label bound to the extended sequence by contacting the ECL label with an ECL read buffer comprising an ECL co-reactant, and applying an electrical
15 potential to the electrodes.

307. A method of detecting a target nucleotide sequence in a sample, the method comprising:

- (a) contacting the sample with a mixture comprising:
 - 20 (i) a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample; and
 - (ii) a detecting probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence,
25 wherein the first nucleic acid sequence of the targeting probe and second nucleic acid sequence of the detecting probe are complementary to adjacent nucleic acid sequences of the target oligonucleotide;
- (b) incubating the mixture comprising the target oligonucleotide, targeting probe and detecting probe in the presence of a nucleic acid ligase under conditions in which the
30 targeting probe and the detecting probe bind to their corresponding nucleotide sequences of the target oligonucleotide and the nucleic acid ligase ligates the

- targeting and detecting probes to form a reaction product comprising the
oligonucleotide tag and detection oligonucleotide;
- (c) contacting a support surface on which a capture oligonucleotide is immobilized with
the mixture comprising the reaction product under conditions in which the
oligonucleotide tag of the reaction product hybridizes to the capture oligonucleotide
to form an immobilized detection complex;
- (d) contacting the immobilized detection complex with a detection mixture comprising an
amplification template;
- (e) amplifying the amplification template to form an amplicon comprising one or more
nucleic acid sequences comprising detection labeling sites;
- (f) contacting the amplicon with a detection reagent comprising a label and a nucleic
acid sequence is that is complementary to the detection labeling sites under conditions
in which the nucleic acid sequence of the detection reagent hybridizes to the detection
labeling sites; and
- (g) detecting the label bound to the support surface.

~~8. The method according to claim 7, wherein the detecting probe has a 5' end that hybridizes
to a target nucleotide sequence adjacent to a 3' end of the targeting probe.~~

~~9. The method according to claim 7, comprising exposing the reaction product formed in (b)
to denaturing conditions to dissociate the reaction product from the target oligonucleotide.~~

~~10. The method according to any of the preceding claims, wherein the amplification template
is amplified by polymerase chain reaction (PCR).~~

~~11. The method according to any of the preceding claims, wherein the amplification template
is amplified by rolling circle amplification (RCA).~~

~~12. The method according to claim 11, wherein the amplification template comprises a
circular amplification template.~~

13. —The method according to claim 11 or 12, wherein the amplicon generated by RCA comprises an extended sequence attached to the immobilized detection complex.

14. —The method according to any of the preceding claims, wherein the amplicon comprises multiple detection labeling sites.

15. —The method according to claim 11, wherein the amplification template comprises a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, and an internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the internal sequence.

16. —The method according to claim 11, wherein the amplification template comprises a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal sequence capable of hybridizing to a complement of the anchoring oligonucleotide sequence and a second internal sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the first and second internal sequences.

17. —The method according to claim 16, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 to about 24 nucleotides in length.

18. —The method according to claim 17, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 or about 15 nucleotides in length.

19. —The method according to any of the preceding claims, wherein the amplification template comprises a 5' terminal sequence of 5'-GTTCTGTC-3' (SEQ ID NO: 1666) and 3' terminal sequence of 5'-GTGTCTA-3' (SEQ ID NO: 1667).

20. — The method according to any of the preceding claims, wherein the detection oligonucleotide comprises a first sequence complementary to the 5' terminal sequence of the amplification template and an adjacent second sequence complementary to the 3' terminal sequence of the amplification template.

21. — The method according to claims 19 or 20, wherein the amplification template comprises a nucleotide sequence of 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).

22. — The method according to claims 19 or 20, wherein the amplification template comprises a nucleotide sequence of 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

23. — The method according to claims 19 or 20, wherein the amplification template comprises a sequence consisting of 5'-

GTTCTGTCATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAAGAGTG
TCTA-3' (SEQ ID NO:1670).

24. — The method according to claims 19 or 20, wherein the amplification template comprises a nucleotide sequence of 5'-

GCTGTGCAATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAAGAGC
GTCGA-3' (SEQ ID NO:1671).

25. — The method according to claim 23 or 24, wherein the detection oligonucleotide comprises 14 or 15 contiguous nucleotides of 5'-GACAGAACTAGACAC-3' (SEQ ID NO: 1664).

26. — The method according to any of the preceding claims, wherein the amplification template comprises a non-naturally occurring oligonucleotide sequence of about 50 to about 78 nucleotides in length.

27. — The method according to claim 26, wherein the non-naturally occurring oligonucleotide sequence of the amplification template is about 53 to about 76 nucleotides, about 50 to about 70 nucleotides, about 53 to about 61 nucleotides, or about 54 to about 61 nucleotides in length.

28. — The method according to claim 26 or 27, wherein the non-naturally occurring oligonucleotide sequence of the amplification template is about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, or about 76 nucleotides in length.

29. — The method according to any of claims 26 to 28, wherein the non-naturally occurring oligonucleotide sequence of the amplification template is about 61 nucleotides in length.

30. — The method according to any of the preceding claims, wherein the nucleic acid sequence of the detection reagent comprises a nucleic acid sequence with at least 90% sequence identity to 14 or 15 contiguous nucleotides of: 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672).

31. — The method according to any of the preceding claims, wherein the nucleic acid sequence of the detection reagent comprises 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672).

32. — The method according to any of the preceding claims, wherein the nucleic acid sequence of the detection reagent comprises 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).

33. — The method according to any of claims 2 to 32, wherein the anchoring sequence of the anchoring reagent comprises an oligonucleotide from about 10 to about 30 nucleic acids in length.

34. — The method according to any of claims 2 to 33, wherein the anchoring sequence of the anchoring reagent comprises an oligonucleotide of about 17 or about 25 oligonucleotides in length.

35. — The method according to any of claims 2 to 33, wherein the anchoring sequence of the anchoring reagent comprises 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

5 36. — The method according to any of claims 2 to 33, wherein the anchoring sequence of the anchoring reagent consists of 5'-AAGAGAGTAGTACAGCAGCCGTCAA-3' (SEQ ID NO:1665).

10 37. — The method of any of the preceding claims, wherein the support surface comprises one or more carbon-based electrodes.

38. — The method of any of the preceding claims, wherein the support surface comprises a multi-well plate comprising one or more carbon-based electrodes.

15 39. — The method of claim 38 or 39, wherein the electrode comprises a carbon ink electrode.

20 40. — The method of according to any of the preceding claims, wherein the support surface comprises a multi-well plate comprising one or more carbon-based electrodes, and wherein a plurality of capture oligonucleotides are immobilized on the carbon-based electrodes in discrete domains.

41. — The method of claim 40, wherein the plurality of a capture oligonucleotides are immobilized on the support surface in discrete binding domains in an array.

25 42. — The method of any of the preceding claims, wherein the label comprises an electrochemiluminescent (ECL) label.

30 43. — The method of claim 42, comprising a step of generating an assay signal by contacting the electrodes with an electrochemiluminescence read buffer comprising an electrochemiluminescence co-reactant, and applying an electrical potential to the electrodes.

44. —The method according to any of the preceding claims, wherein the capture oligonucleotides immobilized on the support surface are selected from a set of non-cross-reactive oligonucleotides that meet one or more of the following requirements:

- (a) — GC content between about 40% and about 50%;
- (b) — AG content between about 30 and about 70%;
- (c) — CT content between about 30% and about 70%;
- (d) — a maximum string of base repeats in a sequence of no more than three;
- (e) — no undesired oligonucleotide-oligonucleotide interactions with strings of more than 7 complementary base pair matches in a row;
- (f) — no undesired oligonucleotide-oligonucleotide interactions with a string of 18 consecutive bases or less where:
 - (i) — the terminal bases at each end are complementary matches; and
 - (ii) — the sum of the complementary base pair matches minus the sum of the mismatches is greater than 7;
- (g) — no strings of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs or longer that match a sequence or complement of a sequence or both in a genome or in nature;
- (h) — differences in the free energy of hybridization for the sequences with their complements is less than about 1 kCal/mol, about 2 kCal/mol, about 3 kCal/mol or about 4 kCal/mol;
- (i) — no predicted hairpin loops with 4 or more consecutive matches in the stem; and
- (j) — no predicted hairpin loops with 4 or more consecutive matches in the stem and loop sizes greater than 6 bases.

45. —The method according to claim 44, wherein the capture oligonucleotides immobilized on the support surface are selected from:

- (a) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-64;
- (b) — capture oligonucleotides comprising a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-64;

(c) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-64;

5 (d) — capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-64; and

(e) — capture oligonucleotides selected from any of (a)-(d).

10 46. — The method according to claim 44, wherein the capture oligonucleotides immobilized on the support surface are selected from:

(a) — capture oligonucleotides comprising a sequence having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-10;

15 (b) — capture oligonucleotides comprising a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-10;

(c) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-10;

20 (d) — capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-10; and

(e) — capture oligonucleotides selected from any of (a)-(d).

47. — A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

25 (a) — a support surface comprising one or more immobilized capture oligonucleotides;

(b) — a detection probe comprising an oligonucleotide tag, a target complement and a detection oligonucleotide;

(c) — an amplification template;

(d) — a nucleic acid ligase;

30 (e) — a nucleic acid polymerase; and

(f) — a detection reagent comprising a label and a nucleic acid sequence.

48. — The kit according to claim 47, further comprising an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide.

5 49. — The kit according to claim 48, wherein the anchoring reagent is immobilized on the support surface.

50. — The kit of according to claims 48 or 49, wherein the anchoring oligonucleotide is about 10 to about 30 nucleic acids in length.

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51. — The kit according to claim 50, wherein the anchoring oligonucleotide is 17 or 25 oligonucleotides in length.

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52. — The kit of according to any of claims 48 to 50, wherein the anchoring oligonucleotide comprises 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

53. — The kit according to any of claims 48 to 50, wherein the anchoring oligonucleotide consists of 5'-AAGAGAGTAGTACAGCAGCCGTCAA-3' (SEQ ID NO:1665).

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54. — The kit according to any of claims 47 to 53, wherein the amplification template comprises a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, and an internal nucleotide sequence capable of hybridizing to a complement of the anchoring sequence of the anchoring reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the internal sequence.

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55. — The kit according to any of claims 47 to 53, wherein the amplification template comprises a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal nucleotide sequence capable of hybridizing

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to a complement of the anchoring sequence of the anchoring reagent and a second internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the first and second internal sequences.

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56. — The kit according to claim 54 or 55, wherein the amplification template comprises a 5' terminal phosphate group.

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57. — The kit according to any of claims 47 to 57, wherein the amplification template is about 53 to about 61 nucleotides in length.

58. — The kit according to any of claims 54 to 57, wherein the sum of the length of the 5' and 3' terminal sequences is about 14 to about 24 nucleotides in length.

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59. — The kit according to claim 58, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 to about 19 nucleotides in length.

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60. — The kit according to claim 58, wherein the sum of the length of the 3' and 5' terminal sequences is about 14 or about 15 nucleotides in length.

61. — The kit according to any of claims 47 to 60, wherein the amplification template comprises a 5' terminal sequence of 5'-GTTCTGTC-3' (SEQ ID NO: 1666) and 3' terminal sequence of 5'-GTGTCTA-3' (SEQ ID NO: 1667).

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62. — The kit according to any of claims 47 to 60, wherein the amplification template comprises a nucleotide sequence of 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).

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63. — The kit according to any of claims 47 to 60, wherein the amplification template comprises a nucleotide sequence of 5'-AAGAGAGTAGTACAGCA-3' (SEQ ID NO:1669).

64. — The kit according to any of claims 47 to 60, wherein the amplification template comprises a sequence consisting of 5'-
GTTCTGTCATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAAGAGTG
TCTA 3' (SEQ ID NO:1670).

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65. — The kit according to any of claims 47 to 60, wherein the amplification template comprises a nucleotide sequence of 5'-
GCTGTGCAATATTTTCAGTGAATGCGAGTCCGTCTAAGAGAGTAGTACAGCAAGAGC
GTCGA 3' (SEQ ID NO:1671).

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66. — The kit according to claim 47, wherein the amplification template comprises a circular amplification template.

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67. — The kit according to any of claims 47 to 66, wherein the detection probe comprises a single stranded DNA oligonucleotide tag, a single stranded RNA target complement and a single stranded DNA detection oligonucleotide.

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68. — The kit according to claim 67, wherein the anchoring reagent comprises a single stranded DNA oligonucleotide tag and a single stranded DNA anchoring sequence.

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69. — The kit according to claim 67 or 68, comprising an RNase.

70. — The kit according to claim 54 or 55, wherein the detection oligonucleotide of the detection probe comprises a first sequence complementary to the 5' terminal sequence of the amplification template and an adjacent second sequence complementary to the 3' terminal sequence of the amplification template.

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71. — The kit according to any of claims 47 to 70, wherein the nucleic acid sequence of the detection reagent comprises a sequence with at least 90% sequence identity to 14 or 15 contiguous nucleotides of: 5' CAGTGAATGCGAGTCCGTCT 3' (SEQ ID NO:1672).

72. — The kit according to any of claims 47 to 70, wherein the nucleic acid sequence of the detection reagent comprises the sequence 5'-CAGTGAATGCGAGTCCGTCT-3' (SEQ ID NO:1672).

5 73. — The kit according to any of claims 47 to 70, wherein the nucleic acid sequence of the detection reagent comprises the sequence 5'-CAGTGAATGCGAGTCCGTCTAAG-3' (SEQ ID NO:1668).

10 74. — The kit according to any of claims 47 to 73, wherein the label of the detection reagent comprises an electrochemiluminescent (ECL) label.

75. — The kit according to any of claims 47 to 74, wherein the support surface comprises a carbon-based support surface.

15 76. — The kit according to any of claims 47 to 75, wherein the support surface comprises a carbon-based electrode.

77. — The kit according to any of claims 47 to 76, wherein the support surface comprises a carbon ink electrode.

20 78. — The kit according to any of claims 47 to 77, wherein the support surface comprises a multi-well plate assay consumable, and each well of the plate comprises a carbon ink electrode.

79. — The kit according to claim 47, wherein the support surface comprises a bead.

25 80. — The kit according to any of claims 47 to 78, wherein a plurality of capture oligonucleotides are immobilized on the solid phase support in discrete binding domains to form an array.

30 81. — The kit according to claim 80, wherein a plurality capture oligonucleotides and at least one anchoring reagent are immobilized on the solid phase support in discrete binding domains to

form an array, wherein each binding domain comprises one of the plurality of capture oligonucleotides and at least one anchoring reagent.

82. — The kit according to any of claims 47 to 81, wherein the capture oligonucleotides immobilized on the support surface are selected from a set of non-cross-reactive oligonucleotides that meet one or more of the following requirements:

- (a) — GC content between about 40% and about 50%;
- (b) — AG content between about 30 and about 70%;
- (c) — CT content between about 30% and about 70%;
- (d) — a maximum string of base repeats in a sequence of no more than three;
- (e) — no undesired oligonucleotide-oligonucleotide interactions with strings of more than 7 complementary base pair matches in a row;
- (f) — no undesired oligonucleotide-oligonucleotide interactions with a string of 18 consecutive bases or less where:
 - (i) — the terminal bases at each end are complementary matches; and
 - (ii) — the sum of the complementary base pair matches minus the sum of the mismatches is greater than 7;
- (g) — no strings of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs or longer that match a sequence or complement of a sequence or both in a genome or in nature;
- (h) — differences in the free energy of hybridization for the sequences with their complements is less than about 1 kCal/mol, about 2 kCal/mol, about 3 kCal/mol or about 4 kCal/mol;
- (i) — no predicted hairpin loops with 4 or more consecutive matches in the stem; and
- (j) — no predicted hairpin loops with 4 or more consecutive matches in the stem and loop sizes greater than 6 bases.

83. — The kit according to claim 82, wherein the capture oligonucleotides immobilized on the support surface are selected from:

(a) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-64;

(b) — capture oligonucleotides comprising a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-64;

(c) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-64;

(d) — capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-64; and

(e) — capture oligonucleotides selected from any of (a)-(d).

84. — The kit according to claim 82, wherein the capture oligonucleotides immobilized on the support surface are selected from:

(a) — capture oligonucleotides comprising a sequence having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence selected from SEQ ID Nos: 1-10;

(b) — capture oligonucleotides comprising a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-10;

(c) — capture oligonucleotides having at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 or 36 consecutive nucleotides of a sequence having at least 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from SEQ ID Nos: 1-10;

(d) — capture oligonucleotides comprising a sequence selected from SEQ ID Nos: 1-10; and

(e) — capture oligonucleotides selected from any of (a)-(d).

85. — A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

(a) — a support surface comprising one or more immobilized capture oligonucleotides;

- (b) — an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide;
- (c) — a detection probe comprising an oligonucleotide tag, a target complement and a single stranded DNA detection oligonucleotide;
- 5 (d) — a detection reagent comprising an electrochemiluminescent (ECL) label and a nucleic acid sequence.
- (e) — a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal nucleotide sequence capable of hybridizing to a complement of the anchoring sequence of the anchoring reagent and a second internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the first and second internal sequences;
- 10 (f) — a nucleic acid ligase; and
- 15 (g) — a nucleic acid polymerase.

86. — The kit according to claim 85, wherein the anchoring reagent is immobilized on the support surface.

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87. — The kit according to claim 85 or 86, wherein the anchoring reagent comprises a single stranded DNA oligonucleotide tag and a single stranded DNA anchoring oligonucleotide; and the detection probe comprises a single stranded DNA oligonucleotide tag, a single stranded RNA target complement and a single stranded DNA detection oligonucleotide; and wherein the kit further comprises an RNase.

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88. — A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

- (a) — a support surface comprising immobilized capture oligonucleotide;

- (b) — a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample;
- (c) — a detecting probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence, wherein the first nucleic acid sequence of the targeting probe and second nucleic acid sequence of the detecting probe are complementary to adjacent sequences of the target nucleotide;
- (d) — an amplification template;
- (e) — a nucleic acid ligase;
- (f) — a nucleic acid polymerase; and
- (g) — a detection reagent comprising a label and a nucleic acid sequence.

89. — The kit according to claim 88, wherein the targeting probe has a terminal 3' nucleotide complementary to a region of the target nucleotide sequence adjacent to the region to which the 5' terminal nucleotide of the detecting probe is complementary.

90. — The kit according to claim 89, wherein the terminal 3' nucleotide of the targeting probe is complementary to a polymorphic nucleotide of the target nucleotide sequence.

91. — A kit for detecting a target nucleotide sequence in a sample, the kit comprising:

- (a) — a support surface comprising immobilized capture oligonucleotide;
- (b) — an anchoring reagent comprising an oligonucleotide tag and an anchoring oligonucleotide;
- (c) — a targeting probe comprising a single stranded oligonucleotide tag and a first nucleic acid sequence that is complementary to a first region of the target nucleotide sequence in the sample;
- (d) — a detecting probe comprising a detection oligonucleotide and a second nucleic acid sequence that is complementary to a second region of the target nucleotide sequence, wherein the first nucleic acid sequence of the targeting probe and

second nucleic acid sequence of the detecting probe are complementary to adjacent sequences of the target nucleotide;

(e) — a linear amplification template comprising a 5' terminal nucleotide sequence and a 3' terminal nucleotide sequence, wherein the 5' and 3' terminal nucleotide sequences are capable of hybridizing to the detection sequence, a first internal nucleotide sequence capable of hybridizing to a complement of the anchoring sequence of the anchoring reagent and a second internal nucleotide sequence capable of hybridizing to a complement of the nucleic acid sequence of the detection reagent, wherein the 5' and 3' terminal nucleotide sequences of the amplification template do not overlap with the first and second internal sequences;

(f) — a nucleic acid ligase;

(g) — a nucleic acid polymerase; and

(h) — a detection reagent comprising an electrochemiluminescent (ECL) label and a nucleic acid sequence.

92. — A kit according to any of claims 47 to 91, further comprising a detection mixture comprising a linear amplification template and one or more additional components, selected from: ligation buffer, adenosine triphosphate (ATP), bovine serum albumin (BSA), Tween 20, T4 DNA ligase, and combinations thereof.

93. — A kit according to claim 92, wherein the detection mixture comprises one or more components for rolling circle amplification selected from BSA, buffer, deoxynucleoside triphosphates (dNTP), Tween 20, Phi29 DNA polymerase, or a combination thereof.

94. — A kit according to claim 92 or 93, wherein the detection mixture comprises acetyl BSA.

95. — A kit according to any of claims 47 to 91, further comprising an ECL read buffer.