

CLAIMS

1. A method of detecting target nucleic acids in a sample, comprising the steps of:

(a) providing a sample comprising nucleic acid;

(b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid in the sample; or

(c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid in the sample;

(d) contacting the sample comprising nucleic acid with the composition of (b) or (c), wherein target nucleic acid binding to:

(i) the variable sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

(ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a signaling nanostructure that is reactive to active DNA polymerase enzyme from step (d), wherein the signaling nanostructure comprises a self-priming portion responsive to the DNA polymerase enzyme;

(f) contacting the signaling nanostructure with active DNA polymerase enzyme from step (d) in the presence of labelled oligodeoxynucleotides (dNTPs) and signal development reagents, wherein the activated DNA polymerase enzyme adds labelled oligonucleotides to the signaling nanostructure and the signal development reagents bind to the labelled oligonucleotides incorporated into the self-priming portion;

(g) detecting signal development, wherein the intensity of signal indicates;

(i) absence of target nucleic acid in the sample when using composition (b); or

(ii) the presence of target nucleic acid in the sample when using composition (c).

2. A method of diagnosing a disease in a subject, comprising the steps of:

(a) providing a sample comprising nucleic acid from the subject;

(b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid in the sample; or

(c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid in the sample;

(d) contacting the sample comprising nucleic acid with the composition of (b) or (c), wherein target nucleic acid binding to:

(i) the variable sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

(ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a signaling nanostructure that is reactive to active DNA polymerase enzyme from step (d), wherein the signaling nanostructure comprises a self-priming portion responsive to the DNA polymerase enzyme;

(f) contacting the signaling nanostructure with active DNA polymerase enzyme from step (d) in the presence of labelled ~~oligodeoxynucleotides~~ (dNTPs) and signal development reagents, wherein the activated DNA polymerase enzyme adds labelled oligonucleotides to the signaling nanostructure and the signal development reagents bind to the labelled oligonucleotides incorporated into the self-priming ~~ed~~ portion;

(g) detecting signal development, wherein the intensity of signal indicates;

(i) absence of target nucleic acid in the sample when using composition (b); or

(ii) the presence of target nucleic acid in the sample when using composition (c);

(h) diagnosing the patient with the disease when presence of target nucleic acid in the sample is detected.

3. The method according to claim 1 or 2, wherein the DNA aptamer conserved sequence region comprises the nucleic acid sequence set forth in 5'-CAATGTACAGTATTG-3' (SEQ ID NO: 153).

4. The method according to any one of claims 1 to 3, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer duplex region.

5. The method according to claim 4, wherein the inverter oligonucleotide is about 35 to 45, preferably about 40, nucleotides in length.

6. The method according to any one of claims 1 to 5, wherein about half of the length of the inverter oligonucleotide forms the aptamer-inverter duplex and about half forms an overhang segment.

7. The method according to any one of the preceding claims, further comprising providing one or more additional recognition nanostructures complementary to one or more target nucleic acids different from the target nucleic acid of a first recognition nanostructure in the sample, for multiplex detection.

8. The method according to claim 7, wherein the each of the recognition nanostructures comprises a combination of DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme in a ratio to form a logic gate selected from a group comprising AND, OR, NOT, NAND and NOR.

9. The method according to claim 8, wherein the combination of DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme ratio of each recognition nanostructure are selected from the group (i) to (v) comprising:

(i) two nanostructures each having 1: 1: 0.5 DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme ratio to form a AND logic gate;

(ii) two nanostructures each having 1: 1: 1 DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme ratio to form a OR logic gate;

(iii) one nanostructure having 1: 0: 1 to form a NOT logic gate;

(iv) two nanostructures each having 1: 0: 1 DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme ratio to form a NAND gate; and

(v) two nanostructures each having 1: 0: 0.5 DNA aptamer: inverter oligonucleotide: DNA polymerase enzyme ratio to form a NOR gate.

10. The method according to any one of the previous claims, wherein the self-priming portion of the signaling nanostructure comprises the nucleic acid sequence set forth in SEQ ID NO: 5.

11. The method according to any one of the previous claims, wherein the dNTP label is biotin.

12. The method according to any one of the previous claims, wherein the signal development reagents comprise a fusion protein comprising avidin or a derivative thereof and an enzyme, selected from a group comprising but not limited to HRP, beta-lactamase, amylase, beta-galactosidase, and respective substrates selected from a group comprising but not limited to DAB, TMB, ABTS, nitrocefin, luminol, starch and iodine, wherein signals can be measured and quantified as but not limited to colour, fluorescence, luminescence or electrochemical changes.

13. The method according to any one of the previous claims, wherein the target is at least one nucleic acid associated with a non-human or human disease, genetic variants, forensic, strain identification, environmental and/or food contamination.

14. The method according to any one of the previous claims, wherein the target is at least one pathogen nucleic acid.

15. The method according to any one of the previous claims, wherein step (a) is preceded by amplification of the target nucleic acid in the sample.

16. The method according to claim 15, wherein the amplification of the target nucleic acid in the sample is by nested asymmetric PCR or isothermal amplification methods.

17. The method according to any one of the previous claims, wherein the detection and signaling steps are physically separated.

18. The method according to any one of the previous claims, wherein the detection and signaling nanostructures are attached to a substrate.

19. The method according to claim 18, wherein the signaling nanostructures are attached to beads.

20. The method according to claim 18 or 19, wherein the detection and signaling nanostructures are attached to a microfluidic device or lateral flow device.

21. The method according to any one of the previous claims, wherein the steps d) to g), preferably steps a) to g), are performed at a temperature in the range from 16°C to 40°C, preferably at ambient temperature.

22. The method according to any one of the previous claims, wherein the target nucleic acid is a HPV nucleic acid.

23. The method according to claim 22, wherein the aptamer and inverter oligonucleotides are selected from those listed in Table 2, Table 4 or Table 5.

24. A device comprising:

(i) composition b) or composition c) comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, as defined in any one of the previous claims, at a 1st location;

(ii) signaling nanostructures comprising a self-priming portion responsive to active DNA polymerase enzyme, as defined in any one of the previous claims, attached at a 2nd location; and

(iii) an intermediate stage for mixing of said detection nanostructures with sample nucleic acid to release active enzyme to said 2nd location.

25. The device of claim 24, selected from a group comprising a microfluidic device and a lateral flow device.

26. The device of claim 24 or 25, wherein the device is a microfluidic device comprising:

(i) a common signaling cartridge configured to receive one or more assay cassettes, wherein the cartridge comprises a base with membranes embedded to immobilize signaling nanostructures, and a common outlet which makes fluid connection with said 2nd location in each of the one or more assay cassettes;

(ii) one or more assay cassettes each comprising, at a 1st location, an inlet and at least one DNA polymerase enzyme and at least one recognition nanostructure; an intermediate stage microchannel in fluid connection between the 1st and 2nd locations,

for mixing of said detection nanostructures with sample nucleic acid to release active enzyme to said 2nd location;

wherein, when the device is assembled and in use, there is fluidic flow from the sample inlet to the common outlet, actuated by a withdrawal septum.

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27. A nucleic acid detection kit comprising;

(a) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a
10 variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid; and/or

(b) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the
15 aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a
20 target nucleic acid; optionally

(c) a signaling nanostructure that is reactive to active DNA polymerase enzyme, wherein the signaling nanostructure comprises a self-priming portion responsive to the DNA polymerase enzyme; optionally

(d) labelled nucleotides (dNTPs) and signal development reagents, wherein active DNA
25 polymerase enzyme adds labelled nucleotides to the signaling nanostructure and the signal development reagents bind to the labelled nucleotides incorporated into the self-primed portion.

28. The nucleic acid detection kit of claim 27, wherein (a) to (d) are as defined according to
30 any one of the previous claim statements.

29. The nucleic acid detection kit of claim 27 or 28, configured into a device according to any one of claims 24 to 26.

35 30. The nucleic acid detection kit of claim 27, wherein at least one of the aptamer and/or inverter oligonucleotides is structurally and/or chemically modified from its natural nucleic acid.

31. The nucleic acid detection kit of claim 30, wherein said structural and/or chemical modification is selected from the group comprising the addition of tags, such as fluorescent tags, radioactive tags, biotin, a 5' tail, the addition of phosphorothioate (PS) bonds, 2'-O-Methyl modifications and/or phosphoramidite C3 Spacers during synthesis.
32. Use of a nucleic acid detection kit of any one of claims 27 to 31 in a method according to any one of claims 1 to 23.