

Claims

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

- (a) providing a sample comprising polynucleotides;
- (b) providing a composition comprising at least one DNA polymerase enzyme, at least one enhancer, and at least one DNA polymerase inhibitor, wherein:
 - i) the enhancer is a polynucleotide comprising a sequence that is complementary to a target polynucleotide sequence;
 - ii) the DNA polymerase inhibitor is a polynucleotide comprising a conserved region and a variable region, wherein the conserved region is recognized and bound by the DNA polymerase enzyme, and the variable region is complementary to a portion of the enhancer;
 - iii) complementary sequences of the variable region of the inhibitor and enhancer form a duplexed inhibitory DNA complex which inhibits DNA polymerase activity;
 - iv) the composition comprises an amount of inhibitory complex that has been determined to have the fastest response and/or highest signal-to-noise ratio by using first derivative of a titration curve of inhibitory complex v polymerase activity and/or by using first derivative of a titration curve of ratios of enhancer:inhibitor;
- (c) contacting the sample comprising nucleic acid with the composition of (b), wherein target polynucleotide binding to:
 - (i) the enhancer sequence region of the duplex in (b) displaces the inhibitor, thereby releasing and activating the DNA polymerase;
- (d) providing a signalling nanostructure that is reactive to active DNA polymerase enzyme from step (c);
- (e) contacting the signalling nanostructure with active DNA polymerase enzyme from step (c);
- (f) detecting signal development, wherein a change in the intensity of signal indicates the presence of target nucleic acid in the sample when using composition (b).

2. The method of claim 1, wherein the signalling nanostructure in d) comprises:

- i) a self-priming portion responsive to the DNA polymerase enzyme, whereby in the presence of labelled oligonucleotides (dNTPs) and signal development reagents, the activated DNA polymerase enzyme adds labelled oligonucleotides to the signalling nanostructure and the signal development reagents bind to the labelled oligonucleotides incorporated into the self-primed portion; or
- ii) a self-priming exonuclease dumbbell nanostructure responsive to DNA polymerase enzyme exonuclease activity, wherein activated DNA polymerase enzyme removes labelled dNTPs from the dumbbell signalling nanostructure.

3. The method of claim 1 or 2, further comprising the step of:

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

4. The method according to any one of claims 1 to 3, wherein the DNA polymerase inhibitor conserved sequence region comprises the nucleic acid sequence set forth in SEQ ID NO: 14; 5'-CAATGTACAGTATTG-3'.

5. The method according to any one of claims 1 to 4, wherein the enhancer to inhibitor ratio in the composition is less than 1:1.

6. The method according to any one of claims 1 to 5, wherein the enhancer is at least one nucleotide longer than the inhibitor duplex region.

7. The method according to claim 6, wherein the enhancer is 35 to 45 nucleotides in length.

8. The method according to any one of claims 1 to 7, wherein about half of the length of the enhancer oligonucleotide forms the inhibitor-enhancer duplex and about half forms an overhang segment.

9. The method according to any one of claims 2 to 8, wherein the the signalling nanostructure in d) comprises:

i) a self-priming portion comprising the nucleic acid sequence set forth in SEQ ID NO: 5: 5'-CGGCGTACGTAGAGCGTTGAGCAGGATGCCAACAGTCGATCAGGACGAGTGCTAACG CATTGTCGATAGCTCAGCTGTCTGAGCTATCGACAATGCGTT-3'; or

ii) a self-priming exonuclease dumbbell nanostructure comprising the nucleic acid sequence set forth in SEQ ID NO: 6: 5'-GTGCGTACATAGATCGTTATCTGTCTAACGATCTATGTAC GCACTCACTCAGCTAACGCATTGTCGATAGCTCAGCTGTCTGAGCTATCGACAATGCGT T-3'.

10. The method according to any one of claims 1 to 9, wherein the dNTP label is biotin.

11. The method according to any one of claims 1 to 10, 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, ADHP, nitrocefin, luminol, starch and iodine, wherein signals can be measured and quantified as but not limited to colour, fluorescence, luminescence or electrochemical changes.

12. The method according to any one of claims 1 to 11, 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.

13. The method according to any one of claims 1 to 12, wherein the target is at least one pathogen polynucleotide.

14. The method according to any one of claims 1 to 13, wherein the target is a SARS-CoV-2 polynucleotide.

15. The method according to claim 14, wherein the inhibitor and enhancer polynucleotides are selected from the group comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

16. The method according to any one of claims 1 to 15, performed in a multi-well format, a microfluidic device or lateral flow device.

17. The method according to any one of claims 1 to 16, wherein the steps are performed in the range from 16 °C to 40 °C, preferably at room temperature.

18. A device comprising:

- (i) composition b) comprising at least one DNA polymerase enzyme and at least one inhibitory DNA complex, of any one of claims 1 to 17, at a first location;
- (ii) signalling nanostructures attached at a second location; and
- (iii) an intermediate stage for mixing of said detection nanostructures with sample nucleic acid to release active enzyme to said second location.

19. The device of claim 18, selected from a group comprising a multi-well plate, a microfluidic device and a lateral flow device.

20. The device of claim 18 or 19, wherein the device is a microfluidic device comprising:

- (i) an inlet at a first location, to introduce test sample, positive and negative controls, and reconstitute the lyophilized reagents in the device including at least one DNA polymerase enzyme, at least one enhancer, and at least one DNA polymerase inhibitor as defined in any aspect of the invention;
- (ii) a detection chamber comprising signalling nanostructures at a second location, in fluid connection with said first location, to receive activated DNA polymerase enzyme;
- (iii) valves between said first and second locations to control flow of sample and reagents;

wherein, when the device is assembled and in use, there is fluidic flow from the sample inlet to an outlet.

21. A nucleic acid detection kit comprising;

- (a) a composition comprising at least one DNA polymerase enzyme and at least one inhibitory DNA complex, wherein the inhibitory DNA complex comprises a DNA polymerase enzyme-specific DNA inhibitor and an enhancer polynucleotide, wherein the inhibitor has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 7 nucleotides complementary to, and forms a duplex with, a portion of the enhancer polynucleotide, wherein the enhancer polynucleotide is at least one nucleotide longer than the inhibitor-enhancer duplex and has more than 7 nucleotides complementary to a target polynucleotide, wherein the composition comprises an amount of inhibitory complex that has been determined to have the fastest response and/or highest signal-to-noise ratio by using first derivative of a titration curve of inhibitory complex v polymerase activity and/or by using first derivative of a titration curve of ratios of enhancer:inhibitor; and/or
- (b) a signalling nanostructure that is reactive to active DNA polymerase enzyme; and/or
- (c) labelled nucleotides (dNTPs) and signal development reagents, wherein active DNA polymerase enzyme adds labelled nucleotides to the signalling nanostructure and the

signal development reagents bind to the labelled nucleotides incorporated into the self-primed portion.

22. The nucleic acid detection kit of claim 21, wherein said composition and said signalling nanostructure are as defined according to any one of the previous claims.

23. The nucleic acid detection kit of claim 21 or 22, configured into a device according to any one of claims 18 to 20.

24. The nucleic acid detection kit of claim 21, wherein at least one of the inhibitor polynucleotides and/or enhancer polynucleotides is structurally and/or chemically modified from its natural nucleic acid.

25. The nucleic acid detection kit of claim 24, 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.