CLAIMS

1. A method for determining the presence or absence of a first target in a sample, the method comprising:

(a) preparing a mixture for a reaction by contacting the sample or a derivative thereof putatively comprising the target with:

- a multi-component temperature-controlled probe comprising a first oligonucleotide component and a second oligonucleotide component,

wherein the first oligonucleotide component comprises a first capture region capable of hybridisation to the second oligonucleotide component by complementary base pairing to form a first double-stranded portion,

wherein the first oligonucleotide component further comprises a sensor region capable of serving as a substrate for an enzyme,

wherein the first oligonucleotide component is connected to a first detection moiety and the second oligonucleotide component is connected to a second detection moiety;

- a first enzyme capable of modifying the sensor region of the first oligonucleotide component only when the first target is present in the sample;

(b) treating the mixture under conditions suitable for the first enzyme to modify the sensor region of the first oligonucleotide component to thereby generate a first fragment comprising the first capture region and a second fragment connected to the first detection moiety, thereby enabling the first and second detection moieties to spatially separate and generate a first detectable signal,

(c) measuring a level of background signal or detectable signal generated at a defined temperature at or below which the first capture region is hybridised to the second oligonucleotide component; and

(d) determining the presence or absence of the first target based upon the level of detectable signal measured at the defined temperature, wherein a detectable signal at the defined temperature is indicative of the presence of the first target in the sample.

2. The method of claim 1 wherein the method comprises:

(i) measuring a level of background signal or detectable signal generated by the first and second detection moieties in the mixture at the defined temperature

- at a timepoint prior to or during said treating the mixture, and

- at one or more subsequent timepoint(s) during or following said treating the mixture; and

(ii) determining a presence of or a change in the level of detectable signal which differs from the background signal and is indicative of the presence of the first target in the sample.

3. The method of claim 1 or claim 2 wherein the first target is a nucleic acid and at least a portion of the sensor region hybridises to a complementary sequence in the first target to thereby form a duplex between the sensor region and the first target, optionally wherein:

(a)

the first enzyme is an endonuclease that recognises a sequence in the duplex, wherein the endonuclease digests at least one strand of the duplex to thereby form the first and second fragments, optionally wherein the endonuclease is a nicking endonuclease that digests the sensor region of the first oligonucleotide component after formation of the duplex to thereby form the first and second fragments; or

(b)

the first enzyme is an exonuclease that hydrolyses the sensor region of the first oligonucleotide component after formation of the duplex to thereby form the first and second fragments, optionally wherein the exonuclease is a polymerase with exonuclease activity, optionally wherein

- the first target is a nucleic acid,
- at least a portion of the sensor region hybridises to a complementary sequence in the first target to thereby form a duplex between the sensor region and the first target,
- said mixture further comprises a target primer capable of binding to the first target at a region upstream of said complementary sequence,
- said treating the mixture comprises:

hybridisation of the target primer to the first target by complementary base pairing,

extending the primer using the polymerase with exonuclease activity and using the nucleic acid target as a template,

wherein the polymerase comprising exonuclease activity digests the sensor region of the first oligonucleotide component after formation of the duplex to thereby form the first and second fragments.

4. The method of claim 1 or claim 2 wherein the first target is a nucleic acid and the sensor region of the first oligonucleotide component is not complementary to the target.

5. The method of any one of claims 1, 2 or 4 wherein the first target is a nucleic acid and the first enzyme is a multi-component nucleic acid enzyme (MNAzyme) comprising two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the first target, optionally wherein said treating comprises:

hybridising sensor arms of the MNAzyme to the first target by complementary base pairing, and

hybridising substrate arms of the MNAzyme to at least a portion of the sensor region of the first oligonucleotide component by complementary base pairing to facilitate cleavage of the first oligonucleotide component and generation of the first and second fragments.

6. The method of any one of claims 1 to 5 wherein the first target is a nucleic acid or an amplicon of a nucleic acid, optionally wherein the amplicon is produced by an amplification reaction selected from the group consisting of polymerase chain reaction (PCR), strand displacement amplification (SDA), nicking enzyme amplification reaction (NEAR), helicase dependent amplification (HDA), Recombinase Polymerase Amplification (RPA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence based amplification (NASBA), Ligase Chain Reaction (LCR) or Ramification Amplification Method (RAM) and reverse transcription polymerase chain reaction (RT-PCR).

7. The method of any one of claims 1 to 6 wherein:

the multi-component temperature-controlled probe does not comprise more than two detection moieties;

the first oligonucleotide component is not directly labelled with more than one detection moiety;

the first oligonucleotide component is not connected to more than one detection moiety;

the second oligonucleotide component is not directly labelled with more than one detection moiety; or

the second oligonucleotide component is not connected to more than one detection moiety.

8. The method of any one of claims 1 to 7, wherein the method comprises determining the presence or absence of the first target and a second target in the sample, wherein:

said preparing in step (a) comprises contacting the sample or a derivative thereof putatively comprising the first and/or second target with:

- the multi-component temperature-controlled probe for detection of the first target,

- a second nucleic acid probe for detection of the second target, the second nucleic acid probe comprising third and fourth detection moieties,

wherein the first and second detection moieties are capable of generating the first detectable signal, and the third and fourth detection moieties are capable of generating a second detectable signal, and

- a first enzyme capable of modifying the sensor region of the first oligonucleotide component only when the first target is present in the sample;

said treating in step (b) comprises treating the mixture under conditions suitable for:

- the first enzyme to modify the sensor region of the first oligonucleotide component to thereby generate a first fragment comprising the first capture region and a second fragment connected to the first detection moiety, thereby enabling the first and second detection moieties to spatially separate and generate the first detectable signal,

- the second target to induce a modification of the second nucleic acid probe, thereby enabling the third and fourth detection moieties to spatially separate and generate a second detectable signal;

said measuring in step (c) comprises measuring a level of background or detectable signal:

- at said defined temperature which is a first temperature at or below which the first capture region is hybridised to the second oligonucleotide component,

- at a second temperature at or above which the first capture region is not hybridised to the second oligonucleotide component,

said determining in step (d) comprises determining whether at one or more timepoints during or after said treating:

- the first detectable signal is generated at the first temperature at or below which the first capture region is hybridised to the second oligonucleotide component,

- the second detectable signal arising from said modification of the second nucleic acid probe is generated at the second temperature, wherein the second detectable signal is indicative of the presence of the second target in the sample.

9. The method of claim 8 wherein at the second temperature, dissociation of the second oligonucleotide component from the capture region of either the first oligonucleotide component present in the absence of the first target, or the first fragment generated by modification of the first oligonucleotide component in the presence of the first target generate an equal, similar or equivalent background signal.

10. The method of claim 8 or claim 9 wherein:

at the first temperature the third and fourth detection moieties do not generate a signal which differs from the background signal; or

at the second temperature the first and second detection moieties do not generate a signal which differs from the background signal.

11. The method of any one of claims 8 to 10 wherein the first and second detectable signals are detectable by a single detector, optionally wherein the first and second detectable signals are detectable in the same fluorescent channel.

12. The method of any one of claims 8 to 11 wherein:

- the second nucleic acid probe is a substrate for a second target multi-component nucleic acid enzyme (MNAzyme) the second target MNAzyme comprising two partzyme oligonucleotides capable of self-assembling to form the second target MNAzyme only in the presence of the second target;

- the mixture further comprises:

the second target MNAzyme is capable of cleaving the second nucleic acid probe only when the second target is present in the sample;

- said treating further comprises:

hybridising sensor arms of the second target MNAzyme to the second target by complementary base pairing, and

hybridising substrate arms of the second target MNAzyme to the second nucleic acid probe by complementary base pairing to facilitate

cleavage of the second nucleic acid probe thereby providing said modification to the second nucleic acid probe and enabling the third and fourth detection moieties to spatially separate and generate the second detectable signal,

optionally wherein the second nucleic acid probe is a stem-loop oligonucleotide comprising a double-stranded stem portion of hybridised nucleotides opposing strands of which are linked by an unbroken single-stranded loop portion of unhybridised nucleotides of which all or a portion is complementary to the substrate arms of the second target MNAzyme, optionally wherein the stem-loop oligonucleotide is an intact stem-loop oligonucleotide and the said modification comprises cleavage of the loop portion and the formation of a split stem-loop oligonucleotide.

13. The method of any one of claims 8 to 11 wherein:

(a)

- the second target is a nucleic acid,
- the second nucleic acid probe is a stem-loop oligonucleotide comprising a double-stranded stem portion of hybridised nucleotides opposing strands of which are linked by an unbroken single-stranded loop portion of unhybridised nucleotides of which all or a portion is complementary to the second target,
- the mixture further comprises a polymerase with exonuclease activity,
- said treating the mixture comprises using conditions suitable for:

hybridisation of the second target to the single-stranded loop portion of the stem-loop oligonucleotide by complementary base pairing to form a first double-stranded sequence comprising a portion of the second target,

hybridisation of a primer to the second target to form a second double-stranded sequence located upstream relative to the first double-stranded sequence comprising the portion of the second target,

extending the primer using the polymerase with exonuclease activity and using the second target as a template,

wherein the polymerase comprising exonuclease activity digests the single-stranded loop portion of the first double-stranded sequence and thereby forms a split stem-loop oligonucleotide; or

(b)

- the second target is a nucleic acid,

- the second nucleic acid probe is a stem-loop oligonucleotide comprising a double-stranded stem portion of hybridised nucleotides opposing strands of which are linked by an unbroken single-stranded loop portion of unhybridised nucleotides of which all or a portion is complementary to the second target,

- the mixture further comprises an endonuclease, and

- said treating the mixture comprises using conditions suitable for:

hybridisation of the second target to the single-stranded loop portion of the stem-loop oligonucleotide by complementary base pairing to form a double-stranded sequence comprising a portion of the second target,

association of the endonuclease with the double-stranded sequence comprising a portion of the second target, and

catalytic activity of endonuclease allowing it to digest the single-stranded loop portion of the double-stranded sequence and thereby form a split stem-loop oligonucleotide.

14. The method of claim 12 or claim 13 wherein:

- the stem portion of the intact stem-loop oligonucleotide has a melting temperature (Tm) that is above the Tm of the stem portion of the split stem-loop oligonucleotide;

- the first temperature is below the Tm of the stem portion of the intact stem-loop oligonucleotide, and the stem portion of the split stem-loop oligonucleotide;

- the second temperature is below the Tm of the stem portion of the intact stem-loop oligonucleotide, and is above the Tm of the stem portion of the split stem-loop oligonucleotide; and

- the first temperature is below the second temperature,

optionally wherein the Tm of the stem portion of the split stem-loop oligonucleotide is above the first temperature, and optionally wherein the Tm of the stem portion of the intact and split stem-loop oligonucleotide(s) is above the Tm of the first double-stranded portion of the multi-component temperature-controlled probe.

15. The method of any one of claims 8 to 11 wherein:

the second nucleic acid probe is a stem-loop oligonucleotide comprising a double-stranded stem portion of hybridised nucleotides opposing strands of which are linked by an

unbroken single-stranded loop portion of unhybridized nucleotides of which all or a portion is complementary to the second target, and wherein

the modification of the second nucleic acid probe is a conformational change arising from hybridisation of the second target to the single-stranded loop portion by complementary base pairing that causes spatial separation of the third and fourth detection moieties, optionally wherein the conformational change is dissociation of the opposing strands in the double-stranded stem portion of the second nucleic acid probe.

16. The method of any one of claims 8 to 11, wherein:

- the second target is a nucleic acid,
- the mixture further comprises:

a primer complementary to a first sequence in the second target,

a pitcher oligonucleotide comprising a region complementary to a second sequence in the second target that differs from the first sequence, and a tag portion that is not complementary to the second target,

a first polymerase comprising exonuclease activity, and

optionally a second polymerase, and

- said treating the mixture comprises:

suitable conditions to hybridise the primer and the pitcher oligonucleotide to the second target,

extending the primer using the first or second polymerase and the second target as a template to thereby cleave off the tag portion,

hybridising the cleaved tag portion to the second nucleic acid probe by complementary base pairing,

and extending the tag portion using the polymerase and the second nucleic acid probe as a template to generate a double-stranded catcher sequence comprising the second nucleic acid probe thereby providing said modification to the second nucleic acid probe and enabling the third and fourth detection moieties to provide the second detectable signal.

17. A composition comprising:

a multi-component temperature-controlled probe comprising a first oligonucleotide component and a second oligonucleotide component,

wherein the first oligonucleotide component comprises a first capture region capable of hybridisation to the second oligonucleotide component by complementary base pairing to form a first double-stranded portion,

wherein the first oligonucleotide component is connected to a first detection moiety and the second oligonucleotide component is connected to a second detection moiety,

wherein:

the first oligonucleotide component further comprises a sensor region capable of serving as a substrate for an enzyme,

wherein digestion of the sensor region by the enzyme generates a first fragment and a second fragment,

and wherein the first fragment comprises the first capture region, and the second fragment is connected to the first detection moiety;

and wherein:

the multi-component temperature-controlled probe does not comprise more than two detection moieties;

the first oligonucleotide component is not connected to more than one detection moiety; or

the first fragment is not directly labelled with a detection moiety.