CLAIMS

[Claim 1]

A method for detecting *n* target nucleic acids in a sample, comprising:

(a) detecting signals at *n* detection temperatures, while incubating with *n* compositions for detecting the target nucleic acids, a sample suspected of containing at least one of the *n* target nucleic acids in a reaction vessel;

wherein *n* is an integer of 2 or more,

wherein the incubation comprises a plurality of cycles and the detection of signals is carried out at at least one of the plurality of cycles,

wherein each of the *n* compositions for detecting the target nucleic acids provides a signal change at a corresponding detection temperature among the *n* detection temperatures in the presence of a corresponding target nucleic acid, the signal change indicating the presence of a corresponding target nucleic acid,

wherein a composition for detecting an i^{th} target nucleic acid among the *n* compositions for detecting the target nucleic acids provides a signal change at an i^{th} detection temperature among the *n* detection temperatures and provides a constant signal at the other detection temperatures in the presence of the i^{th} target nucleic acid, the signal change indicating the presence of the i^{th} target nucleic acid, and

wherein *i* represents an integer from 1 to *n*, and the i^{th} detection temperature is lower than the $(i+1)^{th}$ detection temperature; and

(b) determining the presence of the *n* target nucleic acids from the signals detected in step (a), wherein the presence of the i^{th} target nucleic acid is determined by the signal change detected at the i^{th} detection temperature.

[Claim 2]

The method of claim 1, wherein in the temperature range covering all of the *n* detection temperatures, the composition for detecting the i^{th} target nucleic acid has a

signal-changing temperature range (SChTR) in which the signal changes depending on the presence of the i^{th} target nucleic acid, and ~~a~~ one or two signal-constant temperature ranges (SCoTRs) in which the signal is constant even in the presence of the i^{th} target nucleic acid.

[Claim 3]

The method of claim 2, wherein the composition for detecting the i^{th} target nucleic acid is any one of:

(i) an Under-Signal-Change (UnderSC) composition having a characteristic that the signal-changing temperature range is lower than the signal-constant temperature range;

(ii) an Over-Signal-Change (OverSC) composition having a characteristic that the signal-changing temperature range is higher than the signal-constant temperature range; and

(iii) an Inter-Signal-Change (InterSC) composition having a characteristic that the signal-changing temperature range is higher than one of two signal-constant temperature ranges, and lower than the other of the two signal-constant temperature ranges.

[Claim 4]

The method of claim 2, wherein the i^{th} detection temperature is selected within the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid, wherein the i^{th} detection temperature is not included in the signal-changing temperature ranges of the compositions for detecting the other target nucleic acids.

[Claim 5]

The method of claim 2, wherein the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid overlaps partially with the signal-changing temperature range of a composition for detecting a target nucleic acid having an adjacent detection temperature, and does not overlap with the signal-changing

temperature range of a composition for detecting a target nucleic acid having a detection temperature that is not adjacent thereto.

[Claim 6]

The method of claim 3, wherein, (i) when n is 2, the composition for detecting the first target nucleic acid is an UnderSC composition or an InterSC composition, and the composition for detecting the second target nucleic acid is an InterSC composition or an OverSC composition~~.~~ or (ii) when n is 3 or more, the composition for detecting the first target nucleic acid is an UnderSC composition or an InterSC composition, the composition for detecting the n^{th} target nucleic acid is an InterSC composition or an OverSC composition, and each of compositions for detecting target nucleic acids other than the first target nucleic acid and the n^{th} target nucleic acid is an InterSC composition.

[Claim 7]

The method of claim 1, wherein the composition for detecting the i^{th} target nucleic acid comprises a label that provides a signal dependent on the presence of the i^{th} target nucleic acid.

[Claim 8]

The method of claim 7, wherein the label is linked to an oligonucleotide or is incorporated into an oligonucleotide during the incubation.

[Claim 9]

The method of claim 1, wherein the composition for detecting the i^{th} target nucleic acid provides a duplex providing a signal change; wherein the duplex providing the signal change comprises a label.

[Claim 10]

The method of claim 7, wherein the composition for detecting the i^{th} target nucleic

acid provides a duplex providing a signal change, and the composition for detecting the i^{th} target nucleic acid provides a signal from the label when the duplex providing the signal change is in an associated form or in a dissociated form.

[Claim 11]

The method of claim 9, wherein the duplex providing the signal change has initially been included in the composition for detecting the i^{th} target nucleic acid.

[Claim 12]

The method of claim 11, wherein the duplex providing the signal change is generated by hybridization between a label-linked oligonucleotide and an oligonucleotide hybridizable with the label-linked oligonucleotide.

[Claim 13]

The method of claim 9, wherein the duplex providing the signal change is generated in incubating.

[Claim 14]

The method of claim 13, wherein the duplex providing the signal change is generated by (i) hybridization between a label-linked oligonucleotide and the target nucleic acid or (ii) a cleavage reaction dependent on the presence of a target nucleic acid.

[Claim 15]

The method of claim 14, wherein the composition for detecting the target nucleic acid comprises a tagging oligonucleotide that hybridizes to the target nucleic acid, and the cleavage reaction dependent on the presence of the target nucleic acid involves cleavage of the tagging oligonucleotide.

[Claim 16]

The method of claim 9, wherein the duplex providing the signal change is a single-typed duplex or plural-typed duplexes.

[Claim 17]

The method of claim 16, wherein, (i) when the duplex providing the signal change is the single-typed duplex, the amount of the single-typed duplex changes depending on the presence of the target nucleic acid, thereby changing the signal or (ii) when the duplex providing the signal change is the plural-typed duplexes, the amount ratio between the plural-typed duplexes changes depending on the presence of the target nucleic acid, thereby changing the signal.

[Claim 18]

The method of claim 16, wherein, when the duplex is the plural-typed duplexes, the Tm values of the duplexes are different from each other.

[Claim 19]

The method of claim 4, wherein the composition for detecting the i^{th} target nucleic acid provides a duplex providing a signal change, and the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid is determined depending on the length and/or sequence of the duplex.

[Claim 20]

The method of claim 1, wherein the detection of signals is carried out at at least two of the plurality of cycles.

[Claim 21]

The method of claim 20, wherein the signal change is measured using the signals detected at the at least two of the plurality of cycles.

[Claim 22]

The method of claim 1, wherein the signal change at the i^{th} detection temperature is measured using a signal detected at the at least one of the plurality of cycles and a reference signal value.

[Claim 23]

The method of claim 22, wherein the reference signal value is obtained from a reaction in the absence of the i^{th} target nucleic acid.

[Claim 24]

The method of claim 1, wherein the detection of a signal at each of the *n* detection temperatures is carried out using a single type of detector.

[Claim 25]

The method of claim 24, wherein the signals detected at the *n* detection temperatures are not differentiated from each other by the single type of detector.

[Claim 26]

The method of claim 1, wherein the incubation comprises a nucleic acid amplification reaction.

[Claim 27]

A method for detecting two target nucleic acids in a sample, comprising:

(a) detecting signals at a first detection temperature and a second detection temperature, while incubating the sample suspected of containing at least one of the two target nucleic acids with a composition for detecting a first target nucleic acid and a composition for detecting a second target nucleic acid in a reaction vessel;

wherein the incubation comprises a plurality of cycles, and the detection of signals is carried out at at least one of the plurality of cycles,

wherein the composition for detecting the first target nucleic acid provides a signal change at the first detection temperature and provides a constant signal at the second detection temperature in the presence of the first target nucleic acid, the signal change indicating the presence of the first target nucleic acid; and the composition for detecting the second target nucleic acid provides a signal change at the second detection and provides a constant signal at the first detection temperature in the presence of the second target nucleic acid, the signal change indicating the presence of the second target nucleic acid, and

wherein the first detection temperature is lower than the second detection temperature, and

(b) determining the presence of the two target nucleic acids from the signals detected in step (a); wherein the presence of the first target nucleic acid is determined by the signal change detected at the first detection temperature, and the presence of the second target nucleic acid is determined by the signal change detected at the second detection temperature.

[Claim 28]

A method for detecting three target nucleic acids in a sample, comprising:

(a) detecting signals at a first detection temperature, a second detection temperature, and a third detection temperature, while incubating the sample suspected of containing at least one of the three target nucleic acids with a composition for detecting a first target nucleic acid, a composition for detecting a second target nucleic acid, and a composition for detecting a third target nucleic acid in a reaction vessel;

wherein the incubation comprises a plurality of cycles, and the detection of signals is carried out at at least one of the plurality of cycles,

wherein the composition for detecting the first target nucleic acid provides a signal change at the first detection temperature and provides a constant signal at the second detection temperature and the third detection temperature in the presence of the first target nucleic acid, the signal change indicating the presence of the first target nucleic

acid; the composition for detecting the second target nucleic acid provides a signal change at the second detection temperature and provides a constant signal at the first detection temperature and the third detection temperature in the presence of the second target nucleic acid, the signal change indicating the presence of the second target nucleic acid; and the composition for detecting the third target nucleic acid provides a signal change at the third detection temperature and provides a constant signal at the first detection temperature and the second detection temperature in the presence of the third target nucleic acid, the signal change indicating the presence of the third target nucleic acid, and

wherein the first detection temperature is lower than the second detection temperature, and the second detection temperature is lower than the third detection temperature, and

(b) determining the presence of the three target nucleic acids from the signals detected in step (a),

wherein the presence of the first target nucleic acid is determined by the signal change detected at the first detection temperature, the presence of the second target nucleic acid is determined by the signal change detected at the second detection temperature, and the presence of the third target nucleic acid is determined by the signal change detected at the third detection temperature.

[Claim 29]

A kit comprising *n* compositions for detecting *n* target nucleic acids in a sample,

wherein *n* is an integer of 2 or more,

wherein each of the *n* compositions for detecting the *n* target nucleic acids provides a signal change at a corresponding detection temperature among *n* detection temperatures, the signal change indicating the presence of a corresponding target nucleic acid,

wherein a composition for detecting an i^{th} target nucleic acid among the *n* target nucleic acids provides a signal change at an i^{th} detection temperature among the *n* detection temperatures and provides a constant signal at the other detection temperatures

in the presence of the i^{th} target nucleic acid, and

wherein i represents an integer from 1 to n, and the i^{th} detection temperature is lower than the $(i+1)^{th}$ detection temperature.

[Claim 30]

The kit of claim 29, wherein in the temperature range covering all of the n detection temperatures, the composition for detecting the i^{th} target nucleic acid has a signal-changing temperature range (SChTR) in which the signal changes depending on the presence of the i^{th} target nucleic acid, and ~~a~~ one or two signal-constant temperature range (SCoTR) in which the signal is constant even in the presence of the i^{th} target nucleic acid.

[Claim 31]

The kit of claim 30, wherein the composition for detecting the i^{th} target nucleic acid is any one of:

(i) an Under-Signal-Change (UnderSC) composition having a characteristic that the signal-changing temperature range is lower than the signal-constant temperature range;

(ii) an Over-Signal-Change (OverSC) composition having a characteristic that the signal-changing temperature range is higher than the signal-constant temperature range; and

(iii) an Inter-Signal-Change (InterSC) composition having a characteristic that the signal-changing temperature range is higher than one of two signal-constant temperature ranges, and lower than the other of the two signal-constant temperature ranges.

[Claim 32]

The kit of claim 30, wherein the i^{th} detection temperature is selected within the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid, wherein the i^{th} detection temperature is not included in the signal-changing

temperature ranges of the compositions for detecting the other target nucleic acids.

[Claim 33]

The kit of claim 30, wherein the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid overlaps partially with the signal-changing temperature range of a composition for detecting a target nucleic acid having an adjacent detection temperature, and does not overlap with the signal-changing temperature range of a composition for detecting a target nucleic acid having a detection temperature that is not adjacent thereto.

[Claim 34]

The kit of claim 31, wherein, (i) when n is 2, the composition for detecting the first target nucleic acid is an UnderSC composition or an InterSC composition, and the composition for detecting the second target nucleic acid is an InterSC composition or an OverSC composition, or (ii) when n is 3 or more, the composition for detecting the first target nucleic acid is an UnderSC composition or an InterSC composition, the composition for detecting the n^{th} target nucleic acid is an InterSC composition or an OverSC composition, and each of compositions for detecting target nucleic acids other than the first target nucleic acid and the n^{th} target nucleic acid is an InterSC composition.

[Claim 35]

The kit of claim 29, wherein the composition for detecting the i^{th} target nucleic acid comprises a label that provides a signal dependent on the presence of the i^{th} target nucleic acid.

[Claim 36]

The kit of claim 35, wherein the label is linked to an oligonucleotide or is incorporated into an oligonucleotide during an incubation of the kit and the sample.

[Claim 37]

The kit of claim 29, wherein the composition for detecting the i^{th} target nucleic acid provides a duplex providing a signal change.

[Claim 38]

The kit of claim 35, wherein the composition for detecting the i^{th} target nucleic acid provides a duplex providing a signal change, and the composition for detecting the i^{th} target nucleic acid provides a signal from the label when the duplex providing the signal change is in an associated form or in a dissociated form.

[Claim 39]

The kit of claim 30, wherein the composition for detecting the i^{th} target nucleic acid provides a duplex providing a signal change, and the signal-changing temperature range of the composition for detecting the i^{th} target nucleic acid is determined depending on the length and/or sequence of the duplex.

[Claim 40]

The kit of claim 29, wherein signals provided by the n compositions for detecting the n target nucleic acids are not distinguished from each other by a single type of detector.