

OBJECTS AND SUMMARY OF THE INVENTION

[0016]

In view of the above, an object of the present invention is to provide a kit for detecting multiple target nucleic acids capable of simultaneously amplifying and detecting multiple genes by means of one reaction vessel containing one kind of reaction solution and one label.

[0017]

A first aspect of the present invention provides a method for together detecting multiple target nucleic acids differing from each other, the multiple target nucleic acids including a first target nucleic acid and a second target nucleic acid, the method comprising: using solution being capable of containing the first target nucleic acid and the second target nucleic acid therein, at a denaturation temperature T0, first double-stranded hydrogen bond of the first target nucleic acid being cut off to be dissociate into first two single strands, second double-stranded hydrogen bond of the second target nucleic acid being cut off to be dissociate into second two single strands, respectively, the solution further containing therein: a first target's primer at an annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated; a second target's primer at the annealing temperature T1 specifically bonding with either of the second two single strands into which the second target nucleic acid has been dissociated; a first target's probe at the annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated, the first target's probe including a first labeling substance changing first fluorescent signals thereof when the first target's probe specifically bonds with either of the first two single strands; DNA polymerase; deoxyribonucleoside triphosphate at an elongation temperature T2 bonding by action of the DNA polymerase with both of the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into which the

second target nucleic acid has been dissociated; and a second target's probe at the annealing temperature T1 bonding with neither the first two single strands into which the first target nucleic acid has been dissociated nor the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe at a second target detection temperature T3 bonding with the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe including a second labeling substance changing second fluorescent signals thereof when the second target's probe specifically bonds with either of the second two single strands, wherein:

T0 is defined as the denaturation temperature; T1 is defined as the annealing temperature; T2 is defined as the elongation temperature; and T3 is defined as the second target detection temperature, T0 through T3 being set up such that a condition that: $T0 > T2 \geq T1 > T3$ is satisfied; and

the first labeling substance is equal to the second labeling substance so that the first fluorescent signals are also equal to the second fluorescent signals, characterized in that: difference caused by changes of the first and second fluorescent signals according to the second labeling substance is captured to judge existence or non-existence of the second target nucleic acid..

In one example, there is provided a detection method, comprising: using the kit for together detecting multiple target nucleic acids differing from each other as defined in the first aspect; a dissociating step of increasing temperature of the solution to the denaturation temperature T0 so as to dissociate the first target nucleic acid into the first two single strands, and to dissociate the second target nucleic acid into the second two single strands respectively; a first target nucleic acid detecting step of decreasing the temperature of the solution to the annealing temperature T1 so as to detect whether or not the first target nucleic acid is contained in the solution based on the first fluorescent signals shown by the first target's probe; an amplification step of

CLAIMS:

1. A method for together detecting multiple target nucleic acids differing from each other, the multiple target nucleic acids including a first target nucleic acid and a second target nucleic acid, the method comprising:

5 using solution being capable of containing the first target nucleic acid and the second target nucleic acid therein, at a denaturation temperature T0, first double-stranded hydrogen bond of the first target nucleic acid being cut off to be dissociate into first two single strands, second double-stranded hydrogen bond of the second target nucleic acid being cut off to be dissociate into second two single strands,
10 respectively,

the solution further containing therein:

a first target's primer at an annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated;

15 a second target's primer at the annealing temperature T1 specifically bonding with either of the second two single strands into which the second target nucleic acid has been dissociated;

a first target's probe at the annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been
20 dissociated, the first target's probe including a first labeling substance changing first fluorescent signals thereof when the first target's probe specifically bonds with either of the first two single strands;

DNA polymerase;

deoxyribonucleoside triphosphate at an elongation temperature T2 bonding by
25 action of the DNA polymerase with both of the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into which the second target nucleic acid has been dissociated; and

a second target's probe at the annealing temperature T1 bonding with neither the first two single strands into which the first target nucleic acid has been dissociated nor the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe at a second target detection temperature T3
5 bonding with the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe including a second labeling substance changing second fluorescent signals thereof when the second target's probe specifically bonds with either of the second two single strands,

wherein:

10 T0 is defined as the denaturation temperature; T1 is defined as the annealing temperature; T2 is defined as the elongation temperature; and T3 is defined as the second target detection temperature, T0 through T3 being set up such that a condition that: $T0 > T2 \geq T1 > T3$ is satisfied; and

the first labeling substance is equal to the second labeling substance so that the
15 first fluorescent signals are also equal to the second fluorescent signals,

characterized in that:

difference caused by changes of the first and second fluorescent signals according to the second labeling substance is captured to judge existence or non-existence of the second target nucleic acid.

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2. The method for together detecting multiple target nucleic acids differing from each other as defined in claim 1, wherein the fluorescent signals are shown by quenching light when the annealing occurs.

25 3. The method for together detecting multiple target nucleic acids differing from each other as defined in claim 1, wherein the fluorescent signals are shown by emitting light when the annealing occurs.

4. The method for together detecting multiple target nucleic acids differing from each other as defined in any one of claim 1 to 3, wherein the first target nucleic acid is a *Mycoplasma pneumoniae* P1 gene and the second target nucleic acid is internal control composition.

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5. The method for together detecting multiple target nucleic acids differing from each other as defined in any one of claim 1 to 3, wherein the first target nucleic acid is a *Chlamydia trachomatis* endogeneous plasmid gene and the second target nucleic acid is a *Nisseria gonorrhoeae* CMT gene.

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