CLAIMS

1. A method for detecting a variation of a reference sequence in a target nucleic acid region,

wherein the target nucleic acid region in a nucleic acid to be examined for a variation of the reference sequence has sequences identical to upstream and downstream sequences of the reference sequence of a nucleic acid having the reference sequence,

the method comprising the steps of:

performing a template-dependent nucleic acid amplification reaction for amplifying a region containing the reference sequence in the target nucleic acid region using, as a template, the nucleic acid to be examined for a variation of the reference sequence, in the presence of a 10 to 200 nucleotide single-stranded nucleic acid capable of hybridizing with the reference sequence, and

examining the presence or absence of an amplified product,

wherein the single-stranded nucleic acid is RNA or a chimeric nucleic acid composed of RNA and one or more different nucleic acids,

wherein the single-stranded nucleic acid contains a sequence complementary to the reference sequence, and

wherein the single-stranded nucleic acid has a higher complementarity to the reference sequence than to a variant sequence having a variation of the reference sequence.

2. The detection method according to claim 1, wherein the target nucleic acid region in the nucleic acid having the reference sequence shares the same locus as the target nucleic acid region in the nucleic acid to be examined for a variation of the reference sequence.

3. The detection method according to claim 1 or 2, wherein the single-stranded nucleic acid has a complementarity of 100% to the reference sequence.

4. The detection method according to any one of claims 1 to 3, wherein the variation of the reference sequence is deletion mutation, insertion mutation, or substitution mutation in the reference sequence, or methylation of a base in the reference sequence.

5. The detection method according to any one of claims 1 to 4, wherein the template-dependent nucleic acid amplification reaction is any one selected from the group consisting of PCR, RT-PCR, LAMP, ICAN, NASBA, LCR, SDA, TRC method, TMA, and RPA.

6. The detection method according to claim 5, wherein the template-dependent nucleic acid amplification reaction is PCR.

7. The detection method according to claim 6, wherein the PCR contains a denaturation step, an annealing step, and an elongation step in a cycle.

8. The detection method according to claim 7, wherein the annealing step and the elongation step are performed at the same temperature.

9. The detection method according to any one of claims 1 to 8, wherein the single-stranded nucleic acid is 15 to 30 nucleotides in length.

10. The detection method according to any one of claims 1 to 9, wherein the single-stranded nucleic acid is a single-stranded RNA.

11. The detection method according to any one of claims 1 to 10, wherein the nucleic acid containing the target nucleic acid region is a nucleic acid obtained from a clinical sample of a subject.

12. A method for screening for a cell having a variation of a reference sequence in a target nucleic acid region, the method comprising the steps of:

preparing a nucleic acid from a subject cell;

carrying out the detection method according to any one of claims 1 to 11 using the obtained nucleic acid as a template and determining the presence or absence of an amplified product; and

identifying the cell as having a variation of the reference sequence when the presence of the amplified product has been determined.

13. A kit for use in the detection method according to any one of claims 1 to 11, the kit comprising a single-stranded nucleic acid, wherein the single-stranded nucleic acid is RNA or a chimeric nucleic acid composed of RNA and one or more different nucleic acids, and wherein the single-stranded nucleic acid contains a sequence complementary to a reference sequence in a target nucleic acid region.

14. A detection reagent for use in the detection method according to any one of claims 1 to 11, the detection reagent comprising a single-stranded nucleic acid, wherein the single-stranded nucleic acid is RNA or a chimeric nucleic acid composed of RNA and one or more different nucleic acids, and wherein the single-stranded nucleic acid contains a sequence complementary to a reference sequence in a target nucleic acid region.