Claims

1. A bacterial identification method characterized by having the following steps (1) to (3):
(1) A step of performing a reverse transcription reaction using a first reaction system comprising a primer for reverse transcription to prepare a cDNA containing a base sequence for identification of a bacterium of interest, an RNA extracted from the bacterium in a sample, and an enzyme with RNA-dependent DNA polymerase activity to obtain a reaction mixture containing the synthesized cDNA and the primers for the reverse transcription,
(2) A step of performing PCR using a second reaction system comprising the reaction mixture, a primer pair for synthesis of a double-stranded DNA containing the base sequence for identification of the bacterium of interest, and an enzyme with DNA-dependent DNA polymerase activity, provided that in the second reaction system, the concentration of the primer for reverse transcription supplied from the reaction mixture is not less than 0.08nM and not more than 20nM, and
(3) A step of detecting the generation of the double-stranded DNA containing the base sequence for identification of the bacterium of interest from the second reaction system after PCR,
wherein, the basic sequence for identification of the bacterium of interest is a basic sequence contained in a 16S rDNA of the bacterium and the RNA includes a 16S rRNA of the bacterium in the sample.

2. The bacterial identification method according to claim 1, characterized in that the steps (1) and (2) are performed sequentially in different reaction vessels or in the same reaction vessel.

3. The bacterial identification method according to claim 1 or 2, characterized in that the primer for reverse transcription consist of a base sequence of SEQ ID NO : 1.

4. The bacterial identification method according to claim 3, characterized in that the primer pair is at least one primer pair selected from the group consisting of the following (Group A) to (Group C):

(Group A)

・ A primer pair 1a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 2 and a reverse primer consisting of a base sequence of SEQ ID NO : 3,

・ A primer pair 2a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 4 and a reverse primer consisting of a base sequence of SEQ ID NO : 5,

・ A primer pair 3a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 6 and a reverse primer consisting of a base sequence of SEQ ID NO : 7,

・ A primer pair 4a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 8 and a reverse primer consisting of a base sequence of SEQ ID NO : 9,

・ A primer pair 5a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 10 and a reverse primer consisting of a base sequence of SEQ ID NO : 11,

・ A primer Pair 6a: a combination of a forward primer consisting of a base sequence of SEQ ID NO:12 and a reverse primer consisting of a base sequence of SEQ ID NO:13,

・ A primer pair 7a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 14 and a reverse primer consisting of a base sequence of SEQ ID NO : 15

(Group B)

A primer pair in which one or two bases have been added, deleted or replaced in part of the sequence of one or both primers of each primer pair in (group A) above without impairing their function as primers, and

(Group C)

A primer pair consisting of a complementary sequence corresponding to the base sequence of one or both primers of each primer pair in (Group A) or (Group B) above.

5. The bacterial identification method according to any one of claims 1 to 5, characterized in that in the step (3), Tm value of the generated double-stranded DNA is measured, and the obtained Tm value is compared with the Tm value of the double-

stranded DNA including the sequence for identification of the bacterium of interest that has been measured beforehand, or data obtained secondarily from them, to identify the bacterium in the sample.

6. The bacterial identification method according to any one of claims 1 to 6, characterized in that, after the step (1), the reaction mixture is directly alone or diluted and used in the step (2).

7. A kit when used for identifying bacteria in a sample by PCR using as a template a cDNA contained in a reaction mixture by reverse transcription reaction of an RNA extracted from bacteria in the sample, characterized in that the kit comprising (a) to (c) below:

(a) a primer for reverse transcription to prepare a cDNA containing a base sequence for identification of the bacterium of interest for preparation of the first reaction system for reverse transcription reaction,

(b) a primer pair for synthesis of a double-stranded DNA containing the base sequence for the identification of the bacteria of interest, for the preparation of a second reaction system for the PCR, and

(c) an enzyme with RNA-dependent DNA polymerase activity,

wherein, the second reaction system comprises the reaction mixture by the reverse transfer reaction, wherein the amount of primer for reverse transcription is adjusted such that the concentration of the primer for reverse transcription when fed through the reaction mixture to the second reaction system is 0.08nM or more and 20nM or less,

wherein the basic sequence for identification of the bacterium of interest is a basic sequence contained in a 16S rDNA of the bacterium and the RNA includes a 16S rRNA of the bacterium in the sample.

8. The kit when used according to claim 7, characterized in that further comprising an instruction manual describing the reverse transcription reaction and the PCR method.

9. The kit when used according to claim 8, characterized in that the instruction manual describes the procedure so that the concentration of the primer for reverse transcription in the second reaction system is not less than 0.08 nM and not more than 20 nM.

10. The kit when used according to claim 8 or 9, characterized in that the primer for reverse transcription consist of a base sequence of SEQ ID NO : 1.

11. The kit when used according to claim 10, characterized in that the primer pair is at least one primer pair selected from the group consisting of the following (Group A) to (Group C):

(Group A)

・ A primer pair 1a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 2 and a reverse primer consisting of a base sequence of SEQ ID NO : 3,

・ A primer pair 2a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 4 and a reverse primer consisting of a base sequence of SEQ ID NO : 5,

・ A primer pair 3a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 6 and a reverse primer consisting of a base sequence of SEQ ID NO : 7,

・ A primer pair 4a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 8 and a reverse primer consisting of a base sequence of SEQ ID NO : 9,

・ A primer pair 5a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 10 and a reverse primer consisting of a base sequence of SEQ ID NO : 11,

・ A primer Pair 6a: a combination of a forward primer consisting of a base sequence of SEQ ID NO:12 and a reverse primer consisting of a base sequence of SEQ ID NO:13,

・ A primer pair 7a: a combination of a forward primer consisting of a base sequence of SEQ ID NO : 14 and a reverse primer consisting of a base sequence of SEQ ID NO : 15

(Group B)

A primer pair in which one or two bases have been added, deleted or replaced in part of the sequence of one or both primers of each primer pair in (group A) above without impairing their function as primers, and

(Group C)

A primer pair consisting of a complementary sequence corresponding to the base sequence of one or both primers of each primer pair in (Group A) or (Group B) above.