Description

Title of Invention:

ENZYME PREPARATION CONTAINING THERMOSTABLE DNA POLYMERASE, METHOD FOR PRODUCING SAME, AND METHOD FOR DETECTING SUBJECT ORGANISM TO BE DETECTED

Technical Field

[0001]

The present invention relates to an enzyme preparation containing a thermostable DNA polymerase, a method for producing the same, and a method for detecting a subject organism to be detected.

Background Art

[0002]

In recent years, a polymerase chain reaction (PCR) intended for detecting specific fungi, bacteria, viruses, or other organisms has enhanced its popularity in the medical, veterinary, food, and other fields because the analysis results can be known in a short period of time such as about 2 hours. However, a technology for detecting unspecified fungi, bacteria, viruses or other organisms in a short period of time has yet been established.

[0003]

Very great merits are expectable when a trace of an unspecific organism can be detected, identified, and quantified from a place which should be in a sterile environment in nature. For example, blood, cerebrospinal fluid, amniotic fluid, urea, or the like can be used as a sample

Claims

[Claim 1]

A thermostable DNA polymerase preparation, wherein:

(1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 2]

A production method for a thermostable DNA polymerase preparation, comprising the steps of:

(1) culturing transformant eukaryotic cells carrying a gene encoding the thermostable DNA polymerase,

(2) obtaining an extract containing the thermostable DNA polymerase from the cultured transformant eukaryotic cells, followed by subjecting the extract to heat treatment, or

subjecting the cultured transformant eukaryotic cells to heat treatment, followed by obtaining an extract containing the thermostable DNA polymerase from the heat-treated transformant cells.

[Claim 3]

The production method according to claim 2, wherein the heat treatment is performed at a temperature of 50°C or more.

[Claim 4]

The production method according to claim 2 or 3, wherein the gene encoding the thermostable DNA polymerase used for the transformation is derived from a thermophilic bacterium or a hyperthermophilic bacterium.

[Claim 5]

The production method according to any of claims 2 to 4, wherein the gene encoding the thermostable DNA polymerase comprises DNA consisting of the base sequence of SEQ NO: 1.

[Claim 6]

The production method according to any of claims 2 to 5, wherein the eukaryotic cells are selected from the group consisting of fungi, plant cells, insect cells, and animal cells.

[Claim 7]

The production method according to any of claims 2 to 6, wherein the preparation is that in which:

(1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 8]

A thermostable DNA polymerase preparation comprising the extract obtained by the production method according to any of claims 2 to 7 or a purified preparation thereof.

[Claim 9]

A detection method for a subject organism to be detected in a sample, comprising:

(1) an amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation; and

(2) a detection step of detecting an amplification product of the intended gene in amplification products in the amplification step,

wherein the thermostable DNA polymerase preparation is any one of the following (a) and (b):

(a) a thermostable DNA polymerase preparation produced using eukaryotic cells as a host;

(b) a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 10]

The detection method according to claim 9, wherein the amplification products of the amplification step are quantified in the detection step and the subject organism to be detected in the sample is quantified from the quantification results obtained.

[Claim 11]

A quantification and/or identification method for a subject organism to be detected in a sample, comprising:

(1) a first amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers (B) and (M), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation,

(2) a first quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the first amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

(3) a second amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers (F), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation produced using a bacterium as a host, and

(4) a second quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the second amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to quantify the amplification products in the first quantification and/or identification step of quantifying and/or identifying the subject organism to be detected and the second amplification step to perform the quantification and/or identification of the subject organism to be detected in the sample from the quantification results obtained,

wherein the primers (B), (F) and (M) are:

(B) a primer set capable of amplifying a plurality of regions of the 16S rRNA genes of all bacteria and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a primer set capable of amplifying a plurality of regions of the 18S rRNA genes of all fungi and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance

wherein the thermostable DNA polymerase preparation in the first amplification step is any one of the following (a) and (b):

(a) a thermostable DNA polymerase preparation produced using eukaryotic cells as a host;

(b) a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using

primers capable of amplifying only the bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase.

[Claim 12]

A quantification and/or identification method for a subject organism to be detected in a sample, comprising:

(1) a first amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers (B), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation,

(2) a first quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the first amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

(3) a second amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers (F), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation produced using a bacterium as a host,

(4) a second quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the second amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to quantify the amplification products in the first quantification and/or identification step of quantifying and/or identifying the subject organism to be detected and the second amplification step to perform the quantification and/or identification of the subject organism to be detected in the sample from the quantification results obtained,

(5) a third amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (M), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation, and

(6) a third quantification and/or identification step of analyzing melting temperatures (Tm values) of amplification products in the third amplification step

based on melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

wherein the primers (B), (F) and (M) are:

(B) a primer set capable of amplifying a plurality of regions of the 16S rRNA genes of all bacteria and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a primer set capable of amplifying a plurality of regions of the 18S rRNA genes of all fungi and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance

wherein the thermostable DNA polymerase preparations in the first and third amplification steps are independently the following (a) or (b):

(a) a thermostable DNA polymerase preparation produced using eukaryotic cells as a host; and

(b) a thermostable DNA polymerase preparation, wherein:

(b1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase.

[Claim 13]

The quantification and/or identification method according to claim 11 or 12, wherein amplification regions of the bacterial 16S rRNA genes are 3 to 10 regions.

[Claim 14]

The quantification and/or identification method according to claim 11 or 12, wherein amplification regions of the fungal 18S rRNA genes are 3 to 10 regions.

[Claim 15]

The quantification and/or identification method according to any of claims 11 to 14, further comprising the step of correcting the measurement errors of the Tm values of the amplification products by using a constant concentration of DNA of a standard strain of *Escherichia coli* as a template for a standard control to measure a standard Tm value each time using any one of a primer set capable of amplifying a plurality of regions of the 16S rRNA genes of all bacteria and a primer set containing all or part of each of the base sequences of the primers.

[Claim 16]

The quantification and/or identification method according to any of claims 11 to 15, wherein the combination of melting temperatures ($Tm^{M1}$ to $Tm^{MN}$: N = 3 to 10) of the amplification products based on the amplification regions are analyzed on the basis of the combination of melting temperatures ($Tm^{S1}$ to $Tm^{Sn}$: n = 3 to 10) specific for the subject organism to be detected to quantify and/or identify the subject organism to be detected.

[Claim 17]

The quantification and/or identification method according to claim 16, further comprising the step of minimizing the influence of the measurement errors by identifying the subject organism to be detected using not only combinations of the Tm values but also combinations of differences between the Tm values for an algorithm for identifying the organism.

[Claim 18]

A set for quantifying and/or identifying a subject organism to be detected contained in a sample, comprising a thermostable DNA polymerase preparation for amplifying nucleic acid prepared from the sample and primers for amplifying an intended gene specific for the subject organism to be detected,

wherein the thermostable DNA polymerase preparation is any one of the following (a) and (a):

(b) a thermostable DNA polymerase preparation produced using eukaryotic cells as a host; and

(b) a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase.

[Claim 19]

A set for quantifying and/or identifying a subject organism to be detected contained in a sample, comprising:

any one of the following (a) and (b):

(a) a thermostable DNA polymerase preparation produced using eukaryotic cells as a host;

(b) a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase,

for amplifying nucleic acid prepared from the sample,

a thermostable DNA polymerase preparation for amplifying nucleic acid prepared from the sample, produced using bacterial cells as a host, and

primers for amplifying an intended gene specific for the subject organism to be detected.

[Claim 20]

The set according to claim 19, wherein the primers are the following primers (B), (F) and (M):

(B) a primer set capable of amplifying a plurality of regions of the 16S rRNA gene of all bacteria and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a primer set capable of amplifying a plurality of regions of the 18S rRNA gene of all fungi and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance.

Description

Title of Invention:

ENZYME PREPARATION CONTAINING THERMOSTABLE DNA POLYMERASE, METHOD FOR PRODUCING SAME, AND METHOD FOR DETECTING SUBJECT ORGANISM TO BE DETECTED

Technical Field

[0001]

The present invention relates to an enzyme preparation containing a thermostable DNA polymerase, a method for producing the same, and a method for detecting a subject organism to be detected.

Background Art

[0002]

In recent years, a polymerase chain reaction (PCR) intended for detecting specific fungi, bacteria, viruses, or other organisms has enhanced its popularity in the medical, veterinary, food, and other fields because the analysis results can be known in a short period of time such as about 2 hours. However, a technology for detecting unspecified fungi, bacteria, viruses or other organisms in a short period of time has yet been established.

[0003]

Very great merits are expectable when a trace of an unspecific organism can be detected, identified, and quantified from a place which should be in a sterile environment in nature. For example, blood, cerebrospinal fluid, amniotic fluid, urea, or the like can be used as a sample

Claims

[Claim 1]

A thermostable DNA polymerase preparation, wherein:

(1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 2]

A production method for a thermostable DNA polymerase preparation, comprising the steps of:

(1) culturing transformant eukaryotic cells carrying a gene encoding the thermostable DNA polymerase,

(2) obtaining an extract containing the thermostable DNA polymerase from the cultured transformant eukaryotic cells, followed by subjecting the extract to heat treatment, or

subjecting the cultured transformant eukaryotic cells to heat treatment, followed by obtaining an extract containing the thermostable DNA polymerase from the heat-treated transformant cells,

wherein the preparation is that in which:

(i) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(ii) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 3]

The production method according to claim 2, wherein the heat treatment is performed at a temperature of 50°C or more.

[Claim 4]

The production method according to claim 2 or 3, wherein the gene encoding the thermostable DNA polymerase used for the transformation is derived from a thermophilic bacterium or a hyperthermophilic bacterium.

[Claim 5]

The production method according to any of claims 2 to 4, wherein the gene encoding the thermostable DNA polymerase comprises DNA consisting of the base sequence of SEQ NO: 1.

[Claim 6]

The production method according to any of claims 2 to 5, wherein the eukaryotic cells are selected from the group consisting of fungi, plant cells, insect cells, and animal cells.

[Claim 7]

A thermostable DNA polymerase preparation comprising the extract obtained by the production method according to any of claims 2 to 6 or a purified preparation thereof.

[Claim 8]

A detection method for a subject organism to be detected in a sample, comprising:

(1) an amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation; and

(2) a detection step of detecting an amplification product of the intended gene in amplification products in the amplification step,

wherein the thermostable DNA polymerase preparation is a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or

less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 9]

The detection method according to claim 8, wherein the amplification products of the amplification step are quantified in the detection step and the subject organism to be detected in the sample is quantified from the quantification results obtained.

[Claim 10]

A quantification and/or identification method for a subject organism to be detected in a sample, comprising:

(1) a first amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (B) and (M), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation,

(2) a first quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the first amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

(3) a second amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (F), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation produced using a bacterium as a host, and

(4) a second quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the second amplification step based on a combination

of melting temperatures (Tm values) specific for amplification products of the intended gene to quantify the amplification products in the first quantification and/or identification step of quantifying and/or identifying the subject organism to be detected and the second amplification step to perform the quantification and/or identification of the subject organism to be detected in the sample from the quantification results obtained,

wherein the primers (B), (F), and (M) are:

(B) a universal primer set capable of amplifying a plurality of regions of a gene of a bacterium and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a universal primer set capable of amplifying a plurality of regions of a gene of a fungus and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance

wherein the thermostable DNA polymerase preparation in the first amplification step is a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 11]

A quantification and/or identification method for a subject organism to be detected in a sample, comprising:

(1) a first amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (B), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation,

(2) a first quantification and/or identification step of analyzing a

combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the first amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

(3) a second amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (F), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation produced using a bacterium as a host,

(4) a second quantification and/or identification step of analyzing a combination of melting temperatures (Tm values) of a plurality of (3 to 10) amplification products in the second amplification step based on a combination of melting temperatures (Tm values) specific for amplification products of the intended gene to quantify the amplification products in the first quantification and/or identification step of quantifying and/or identifying the subject organism to be detected and the second amplification step to perform the quantification and/or identification of the subject organism to be detected in the sample from the quantification results obtained,

(5) a third amplification step of performing a nucleic acid amplification reaction using nucleic acid prepared from the sample, primers, (M), for amplifying an intended gene specific for the subject organism to be detected, and a thermostable DNA polymerase preparation, and

(6) a third quantification and/or identification step of analyzing melting temperatures (Tm values) of amplification products in the third amplification step based on melting temperatures (Tm values) specific for amplification products of the intended gene to perform the quantification and/or identification of the subject organism to be detected in the sample,

wherein the primers (B), (F), and (M) are:

(B) a universal primer set capable of amplifying a plurality of regions of a gene of a bacterium and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a universal primer set capable of amplifying a plurality of regions of a gene of a fungus and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene

reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance

wherein the thermostable DNA polymerase preparations in the first and third amplification steps are a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 12]

The quantification and/or identification method according to claim 10 or 11, wherein amplification regions of the bacterial 16S rRNA genes are 3 to 10 regions.

[Claim 13]

The quantification and/or identification method according to claim 10 or 11, wherein amplification regions of the fungal 18S rRNA genes are 3 to 10 regions.

[Claim 14]

The quantification and/or identification method according to any of claims 10 to 13, further comprising the step of correcting the measurement errors of the Tm values of the amplification products by using a constant concentration of DNA of a standard strain of *Escherichia coli* as a template for a standard control to measure a standard Tm value each time using any one of a universal primer set capable of amplifying a plurality of regions of a gene of a bacterium and a primer set containing all or part of each of the base sequences of the primers.

[Claim 15]

The quantification and/or identification method according to any of claims 10 to 14, wherein the combination of melting temperatures ($Tm^{M1}$ to $Tm^{MN}$: N = 3 to 10) of the amplification products based on the amplification regions are analyzed on the basis of the combination of melting temperatures ($Tm^{S1}$ to $Tm^{Sn}$: n = 3 to 10) specific for the subject organism to be detected to quantify and/or identify the subject organism to be detected.

[Claim 16]

The quantification and/or identification method according to claim 15, further comprising the step of minimizing the influence of the measurement errors by identifying the subject organism to be detected using not only combinations of the Tm values but also combinations of differences between the Tm values for an algorithm for identifying the organism.

[Claim 17]

A set for quantifying and/or identifying a subject organism to be detected contained in a sample, comprising a thermostable DNA polymerase preparation for amplifying nucleic acid prepared from the sample and primers for amplifying an intended gene specific for the subject organism to be detected,

wherein the thermostable DNA polymerase preparation is a thermostable DNA polymerase preparation, wherein:

(b-1) the thermostable DNA polymerase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase.

[Claim 18]

A set for quantifying and/or identifying a subject organism to be detected contained in a sample, comprising:

a thermostable DNA polymerase preparation for amplifying nucleic acid prepared from the sample, wherein:

(b-1) the thermostable DNA polym erase has contamination with 10 fg or less of bacterially-derived nucleic acid other than a gene encoding the thermostable DNA polymerase based on 1 unit of the thermostable DNA polymerase, and

(b-2) no amplification products of the bacterially-derived nucleic acid are detected even when 32 cycles or more of gene amplification reaction are performed in the preparation under conditions containing no template using primers capable of amplifying only the bacterially-derived nucleic acid other than the gene encoding the thermostable DNA polymerase,

a thermostable DNA polymerase preparation for amplifying nucleic acid prepared from the sample, produced using bacterial cells as a host, and

primers for amplifying an intended gene specific for the subject organism to be detected.

[Claim 19]

The set according to claim 18, wherein the primers are the following primers (B), (F) and (M):

(B) a universal primer set capable of amplifying a plurality of regions of a gene of a bacterium and primers containing all or 1/3 or more of each of the base sequences of the primers,

(F) a universal primer set capable of amplifying a plurality of regions of a gene of a fungus and primers containing all or 1/3 or more of each of the base sequences of the primers, and

(M) a primer set specifically amplifying an antibiotic resistance gene reflecting an epidemic of the time such as a mec A gene exhibiting methicillin resistance.