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Patent Request: Standard Patent

We, the Applicants/Nominated Persons specified below, request we be granted a patent for the invention disclosed in the accompanying standard complete specification.

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[54] **Invention Title:**
Oligonucleotides Derived from the SOD Family

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Australian Patent Application No. 48919/93

NOTICE OF ENTITLEMENT

I, Daniel Meier

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being authorised by the Applicant(s)/Nominated Person(s) in respect of an application entitled:

Oligonucleotides derived from the SOD Family

state the following:-

1. The Applicant(s)/Nominated Person(s) has/have, for the following reasons, gained entitlement from the actual inventor(s):-

[X] The Applicant is the assignee of the invention from the inventor(s)

[ ] The inventor(s) have assigned the invention to Hoffmann-La Roche Inc., Nutley, USA, who have reassigned all their rights for Australia to the Applicant

2a.* The Applicant(s)/Nominated Person(s) is/are the applicant(s) of the basic application(s) listed* on the Patent Request/* ~~in the Declaration under Article 8 of the PCT.~~

~~2b.* The Applicant(s)/Nominated Person(s) is/are entitled to rely on the basic application(s) listed* on the Patent Request/* in the Declaration under Article 8 of the PCT as follows:~~

3.* The basic application(s) listed* on the Patent Request/* ~~in the Declaration under Article 8 of the PCT~~ is/are the applications first made in a Convention Country in respect of the invention.

DATED this 27th day of August, 1993.

Daniel Meier

Daniel Meier

* Delete if not applicable

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(57) Claim

The present invention relates to the use of the superoxide dismutase (SOD; EC 1.15.1.1) gene system as a target for the detection and differentiation of pathogenic and non-pathogenic organisms of the genus Mycobacterium. In another aspect, the present invention relates to oligonucleotides derived from the SOD gene family. More particularly, it relates to oligonucleotides capable of hybridizing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying and denaturing a part of a gene coding for a superoxide dismutase (SOD) using a polymerase chain reaction (PCR) and a pair of primers, the first primer containing the sequence.

5'-AGC TTC ACC ACA GCA AGC ACC A-3' (Seq. Id. No. 1; Z205)

as priming sequence and the second primer containing the sequence

5'-TCG $\begin{smallmatrix}G\\T\end{smallmatrix}$CC CAG TTC ACG AC$\begin{smallmatrix}A\\G\end{smallmatrix}$ TTC CA-3' (Seq. Id. No. 2; Z212)

as priming sequence.

Claim

1. A method for detecting pathogenic and non-pathogenic organisms of the genus Mycobacterium, which comprises

(a) amplifying a target region of a superoxide dismutase (SOD) gene of an organism, using a pair of primers which is specific for the genus Mycobacterium,

(b) contacting the amplified target sequence with at least one oligonucleotide probe capable of hybridizing to the target region of the SOD gene; and

(c) detecting hybrids formed between the amplified target region, if any, and the oligonucleotide probe.

The present invention relates to the use of the superoxide dismutase (SOD; EC 1.15.1.1) gene system as a target for the detection and differentiation of pathogenic and non-pathogenic organisms of the genus Mycobacterium. In another aspect, the present invention relates to oligonucleotides derived from the SOD gene family. More particularly, it relates to oligonucleotides capable of hybridizing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying and denaturing a part of a gene coding for a superoxide dismutase (SOD) using a polymerase chain reaction (PCR) and a pair of primers, the first primer containing the sequence.

5'-AGC TTC ACC ACA GCA AGC ACC A-3' (Seq. Id. No. 1; Z205)

as priming sequence and the second primer containing the sequence

5'-TCG $\begin{matrix}G\\T\end{matrix}$CC CAG TTC ACG AC$\begin{matrix}A\\G\end{matrix}$ TTC CA-3' (Seq. Id. No. 2; Z212)

as priming sequence.

These oligonucleotides may be used in the form of primers and probes to amplify and detect target sequences derived from SOD genes, especially, from SOD genes of pathogenic organisms. The primers and probes according to the present invention may also be used for amplifying and detecting target sequences derived from SOD genes of non-pathogenic organisms.

Objects of the present invention are the aforementioned oligonucleotides; primers and probes based on these oligonucleotides; methods, reagents and kits for the amplification and detection of target sequences derived from SOD genes and for the diagnosis of infections caused by procaryotic organisms; and the use of said oligonucleotides for the aforementioned purposes.

The invention therefore provides a method for detecting pathogenic and non-pathogenic organisms of the genus Mycobacterium, which comprises

(a) amplifying a target region of a superoxide dismutase (SOD) gene of an organism, using a pair of primers which is specific for the genus Mycobacterium,

(b) contacting the amplified target sequence with at least one oligonucleotide probe capable of hybridizing to the target region of the SOD gene; and

(c) detecting hybrids formed between the amplified target region, if any, and the oligonucleotide probe.

In another embodiment of the invention provides an oligonucleotide capable of hybridizing to a region of the target sequence which is substantially conserved among the SOD genes of the different species belonging to the genus Mycobacterium.

SOD is an enzyme which catalyzes the dismutation of free superoxide (O_2^-) to give molecular oxygen and hydrogen peroxide as follows:

$$2\,O_2^- + 2\,H^+ \rightarrow O_2 + H_2O_2$$

The resulting peroxide is then converted by catalases or peroxidases into H_2O. Superoxide (O_2^-) is a toxic by-product of aerobic respiration and reacts with peroxide (O_2^{2-}) to cause damage to proteins, lipids and nucleic acids. The enzyme superoxide

dismutase (SOD) counteracts the toxic effects of the radicals by conversion of superoxide to molecular oxygen and peroxide.

The term "polymerase chain reaction" or "PCR" refers to a process of amplifying one or more specific nucleic acid sequences, wherein (1) oligonucleotide primers which determine the ends of the sequences to be amplified are annealed to single-stranded nucleic acids in a test sample, (2) a nucleic acid polymerase extends the 3'-ends of the annealed primers to create a nucleic acid strand complementary in sequence to the nucleic acid to which the primers were annealed, (3) the resulting double-stranded nucleic acid is denatured to yield two single-stranded nucleic acids, and (4) the processes of primer annealing, primer extension and product denaturation are repeated enough times to generate easily identified and measured amounts of the sequences defined by the primers. Practical control of the sequential annealing, extension and denaturation steps is exerted by varying the temperature of the reaction container, normally in a repeating cyclical manner. Annealing and extension occur optimally in the 37°C - 80°C temperature range (exact value depending on primer concentrations and sequences), whereas denaturation requires temperatures in the 80°C - 100°C range (exact value depending on target sequence and concentration).

The term "oligonucleotide" refers to a single-stranded nucleic acid comprised of two or more deoxyribonucleotides, such as primers, probes, nucleic acid fragments to be detected and nucleic acid controls. The exact size of an oligonucleotide depends on many factors and the ultimate function or use of the oligonucleotide. Oligonucleotides can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis by a method such as

the phosphotriester method of Narang et al., Meth. Enzymol. 68, 90-99 (1979); the phosphodiester method of Brown et al., Meth. Enzymol. 68, 109-151 (1979); the diethylphosphoramidite method of Beaucage et al., Tetrahedron Lett. 22, 1859-1862 (1981); and the solid support method described in the U.S. Patent Specification No. 4,458,066.

The term "primer" refers to an oligonucleotide, whether natural or synthetic, capable of acting as a point of initiation of DNA synthesis under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand is induced, i.e. in the presence of the different nucleoside triphosphates and an agent for polymerization (e.g. a DNA polymerase or a reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically ranges from 15 to 40 nucleotides. Short primer molecules generally require lower temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with a template and serve to initiate DNA synthesis under the chosen reaction conditions.

The term "primer" may refer to more than one primer, particularly in the case where there is some ambiguity in the information regarding one or both ends of the target region to be amplified. If a conserved region shows significant levels of polymorphism in a population, mixtures of primers can be prepared that will amplify such sequences, or the primers can be designed to amplify even mismatched sequences. A primer can be labeled, if desired, by incorporating a label detectable by radiometric, spectroscopic, photochemical, biochemical, immunochemical or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (as commonly used in ELISAs), biotin or haptens and proteins for which antisera or monoclonal antibodies are available. A label can also be used to "capture" the primer, so as to facilitate the immobilization of either the primer or a primer extension product, such as amplified DNA, on a solid support.

The term "probe" refers to an oligonucleotide containing a hybridizing sequence complementary to a part of the target sequence which may be labeled with a tag or attached to a solid support. Depending on the assay methods utilized for detecting hybrids formed between probes and nucleic acid sequences the probes may contain additional features in addition to the

hybridizing region. In the dot blot format, for example, the probes are typically labeled. If the probe is first immobilized, as in the "reverse" dot blot format described below, the probe can also contain long stretches of poly-dT that can be fixed to a nylon support by irradiation, a technique described in more detail in PCT Patent Publication No. 89/11548. The appropriate length of a probe depends on the intended use of the probe but typically ranges from 15 to 40 nucleotides. Short probe molecules generally require lower temperatures to form sufficiently stable hybrid complexes with the target. A probe need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with the target sequence.

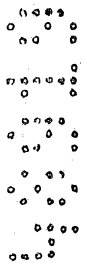
The probes of the present invention may be synthesized and labeled using the techniques described above for synthesizing oligonucleotides. For example, the probe may be labeled at the 5'-end with ^{32}P by incubating the probe with ^{32}P-ATP and kinase. A suitable non-radioactive label for probes is horseradish peroxidase (HRP). Methods for preparing and detecting probes containing this label are described in U.S. Patent Specifications Nos. 4,914,210 and 4,962,02. For additional information on the use of such labeled probes, see U.S. Patent Specification No. 4,789,630; Saiki et al., N. Eng. J. Med. 319, 537 - 541 (1988); and Bugawan et al., Bio/Technology 6, 943 - 947 (1988). Useful chromogens include red leuco dye and 3,3',5,5'-tetramethylbenzidine (TMB). Helmuth, PCR Protocols, San Diego, California, Academic Press, Inc., 1990, pp. 119 - 128, describes procedures for non-isotopic detection of PCR products.

Oligonucleotides capable of hybridizing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying a part of a gene coding for a superoxide dismutase (SOD) of a mycobacterial species using a polymerase chain reaction (PCR) and a pair of primers, the first primer containing the sequence of Seq. Id. No. 3 (Z261, cf. Table 17) as priming sequence and the second primer containing the sequence of Seq. Id. No. 2 (Z212) above as priming sequence are especially preferred. Among these, oligonucleotides capable of hybridizing to a region of the target sequence which is substantially conserved among the SOD genes of the different species belonging to the genus of mycobacteria (genus specific oligonucleotides), and oligonucleotides capable of hybridizing to a region of the target sequence which is variable to a degree allowing differentiation among the SOD genes of the different species belonging to the genus of mycobacteria (species specific oligonucleotides) are even more preferred.

In an especially preferred embodiment, the present invention relates to oligonucleotides which may be used in the form of primers and probes to amplify and detect target sequences derived from SOD genes of mycobacterial species, preferably of pathogenic mycobacterial species. The target nucleic acid is amplified by a PCR, using a pair of primers allowing amplification of a part of the SOD genes of the different mycobacterial species belonging to the genus of mycobacteria. Preferably, the pair of primers consist of a first primer comprising as priming sequence a sequence which is substantially homologous to the sequence of Seq. Id. No. 3 (Z261) and a second primer

comprising as priming sequence a sequence which is substantially homologous to the sequence of Seq. Id. No. 2 (Z212). More preferably the pair of primers consist of a first primer comprising the sequence of Seq. Id. No. 3 (Z261) and a second primer comprising the sequence of Seq. Id. No. 2 (Z212) as priming sequence.

Genus detection may then be performed by mixing the amplified nucleic acid with genus specific probes comprising an oligonucleotide



sequence of Seq.Id.No. 4 as hybridizing sequence, preferably a pool of probes comprising the oligonucleotide sequences of Z310 - Z317 as hybridizing sequences (Table 18), and detecting if hybridization occurs. The species identification may be carried out by determining the pattern of hybridization of the amplified nucleic acid to species specific probes capable of hybridizing to a region of the target sequence which is variable to a degree allowing differentiation among the SOD genes of the different species belonging to the genus of mycobacteria.

Preferred species specific probes that may be utilized in accordance with the present invention are probes which contain as a hybridizing sequence a sequence which is substantially homologous to the following sequences: 5'-CCT TCG GAT CCT TCG ACC GGT TCC GCG CGC AGT TCA G-3' (Z303, Seq. Id. No.13) for M. intracellulare; 5'-GCT AGG CAT TGT TCC GCT GCT GCT GC-3' (Z336, Seq. Id. No. 17) and 5'-ACG AAC TTC CCG CTA GGC ATT GTT CCG CTG CTG CTG C-3' (Z302, Seq. Id. No. 22) and 5'-AGT CGA CTT TGC CAA GGC GTT T-3' (Z337, Seq. Id. No. 23) for M. tuberculosis; 5'-ACG ACA GCC TGG GCG ATC GGC T-3' (Z369, Seq. Id. No. 21) for M. fortuitum; 5'-TCT GGG CGC CCG GTT GCT CAC CTT T-3' (Z366, Seq. Id. No. 15) for M. gordonae; 5'-TCC GCG ATC GTC GGG CAT GAG AAG GCC CTC GCG TTC A-3' (Z309, Seq. Id. No. 18) for M. xenopi; 5'-TGA CAC ACT CGG CAG CAG GCT GCT CAC CTT CCA GCT T-3' (Z306, Seq. Id. No. 20) for M. scrofulaceum; 5'-GTC CCC GAA CGG CGG AGA CAA GCC GAC CGG AGA TCT C-3' (Z304, Seq. Id. No. 16) for M. simiae; 5'-CCA GAC GAA CTT TCC ACT CGG A-3' (Z340, Seq. Id. No. 19) for M. kansasii; and 5'-GTC CTT CGA CAA GTT CCG AGC GCA ATT CAG CGC CGC C-3' (Z301, Seq. Id. No. 14) for M. avium/M. brunense.

Other regions within the sequences of the amplicons obtainable with the primers Z205 and Z212 may also be used to design species specific probes.

The methods provided by the present invention for the diagnosis of infections caused by pathogenic organisms comprise the steps of amplifying the target sequence if present and detecting and identifying the amplicon, if any, by means of probe hybridization assays.

An important aspect of the present invention is the amplification of a part of a gene coding for a SOD. Those practicing the present invention should note that, although the polymerase chain reaction is the preferred amplification method, amplification of target sequences in a sample may be accomplished by any known method, such as a ligase chain reaction (LCR),

transcription amplification and self-sustained sequence replication and other methods not listed here, each of which provides sufficient amplification so that the target sequence can be detected by nucleic acid hybridization to one or more appropriate probes.

Although the PCR process is well known in the art (see U.S. Patent Specifications Nos. 4,683,195; 4,683,202 and 4,965,188), some general PCR information is provided below for purposes of clarity and full understanding of the invention to those unfamiliar with the PCR process.

To amplify a target nucleic acid sequence in a sample by PCR, the sequence must be accessible to the components of the amplification system. In general, this accessibility is ensured by isolating the nucleic acids from the sample. A variety of techniques for extracting nucleic acids from biological samples are known in the art. For example, see those described in Higuchi et al., PCR Technology (Erlich ed., Stockton Press, New York, 1989). Alternatively, if the sample is fairly readily disruptable, the nucleic acid need not be purified prior to amplification by the PCR technique, i.e., if the sample is comprised of cells, particularly peripheral blood lymphocytes or aminiocytes, lysis and dispersion of the intracellular components can be accomplished merely by suspending the cells in hypotonic buffer.

Each cycle of the PCR involves the separation of the nucleic acid duplex formed by primer extension. In a preferred embodiment of the PCR process, strand separation is achieved by heating the reaction to a sufficiently high temperature and for an effective time to cause the denaturation of the duplex, but not to cause an irreversible denaturation of the polymerase (see U.S. Patent Specification No. 4,965,188). Typical heat denaturation involves temperatures ranging from about 80 °C to 105 °C for times ranging from seconds to minutes. Strand separation, however, can be accomplished by any suitable denaturing method including physical, chemical or enzymatic means. Strand separation can be induced by a helicase, for example, or an enzyme capable of exhibiting helicase activity. For example, the enzyme RecA has helicase activity in the presence of ATP. The reaction conditions suitable for strand separation by helicases are known in the art; cf. Kuhn Hoffman-Berling, CSH-Quantitative Biology 43, 63 - 67 (1978) and Radding, Ann. Rev. Genetics 16, 405 - 436 (1982).

No matter how strand separation is achieved, however, once the strands are separated, the next step in PCR involves hybridizing the separated strands with primers that flank the target sequence. The primers

are then extended to form complementary copies of the target strands. For successful PCR amplification, the primers are designed so that the position at which each primer hybridizes along a duplex sequence is such that an extension product synthesized from one primer, when separated from the template (complement), serves as a template for the extension of the other primer. The cycle of denaturation, hybridization, and extension is repeated as many times as necessary to obtain the desired amount of amplified nucleic acid.

Template-dependent extension of primers in PCR is catalyzed by a polymerizing agent in the presence of adequate amounts of deoxyribonucleoside triphosphates (usually dATP, dGTP, dCTP and dTTP; dUTP is used in place of or in addition to dTTP if the UNG sterilization system described below is incorporated) in a reaction medium comprised of the appropriate salts, metal cations, and pH buffering system. Suitable polymerizing agents are enzymes known to catalyze template-dependent DNA synthesis. Examples of polymerases suitable for use with a DNA template include E. coli DNA polymerase I or the Klenow fragment of that enzyme, T_4 DNA polymerase and Taq DNA polymerase, a heat stable DNA polymerase isolated from Thermus aquaticus. The latter enzyme is widely used in the amplification and sequencing of nucleic acids. The reaction conditions for using Taq DNA polymerases are known in the art and are described in Gelfand, PCR Technology (1989), supra. Other heat stable polymerases may also be suitable.

The PCR method can be performed in a step wise fashion, where after each step new reagents are added, or in a fashion where all of the reagents are added simultaneously, or in a partial step wise fashion, where fresh or different reagents are added after a given number of steps. For example, if strand separation is induced by heat and the polymerase is heat sensitive, then the polymerase has to be added after every round of strand separation. However, if, for example, a helicase is used for denaturation, or if a thermostable polymerase is used for extension, then all of the reagents may be added initially, or, alternatively, if molar ratios of reagents are of consequence to the reaction, the reagents may be replenished periodically as they are depleted by the synthetic reaction.

Those skilled in the art will know that the PCR process is most usually carried out as an automated process with a thermostable enzyme. In this process, the temperature of the reaction mixture is cycled through a

denaturing region, a primer annealing region and a reaction region. A machine (thermocycler) specifically adapted for use with a thermostable enzyme is commercially available.

Those skilled in the art will also be aware of the problem of contamination of a PCR by the amplified nucleic acid from previous reactions. Methods to reduce this problem allow the enzymatic degradation of any amplified DNA from previous reactions. The PCR amplification is carried out in the presence of dUTP instead of dTTP. The resulting double stranded uracil containing product is subject to degradation by uracil N-glycosylase (UNG), whereas normal thymine-containing DNA is not degraded by UNG. Adding UNG to the amplification reaction mixture before the amplification is started degrades all uracil containing DNA that might serve as target. Because the only source of uracil containing DNA is the amplified product of a previous reaction, this method effectively sterilizes the reaction mixture, eliminating the problem of contamination from previous reactions (carryover). UNG is rendered temporarily inactive by heat, so the denaturation steps in the amplification procedure also serve to inactivate the UNG. New amplification products, therefore, though incorporating uracil, are formed in an UNG-free environment and are not degraded.

Another important aspect of the present invention in successful performance of the present methods is probe hybridization. The oligonucleotide probes of the present invention hybridize specifically with a particular segment of the SOD gene of an organism to be detected and identified and have sufficient destabilizing mismatches with the sequences from different organisms in the case of genus specific probes, and other species of the same genus, in the case of species specific probes.

The probes of the invention can be used to determine if nucleic acid sequences are present in a sample by determining if the probes bind to the sequences present in the sample. Suitable assay methods for purposes of the present invention to detect hybrids formed between probes and nucleic acid sequences in a sample are known in the art. For example, the detection can be accomplished by Southern blotting and liquid hybridization using a dot blot format. In the dot blot format, the unlabeled amplified sample is bound to a solid support, such as a membrane, the membrane incubated with labeled probe under suitable hybridization conditions, the unhybridized probe removed by washing, and the filter monitored for the presence of bound probe. When multiple samples are analyzed with few probes, such as

is the case when samples are screened for the presence of nucleic acid of a particular genus using genus specific probes, the dot blot format is quite useful.

An alternate method is quite useful when large numbers of different probes are to be used. This method is a "reverse" dot blot, in which the amplified sequence contains a label, and the probe is bound to the solid support. In this format, the unlabeled probes are bound to the membrane and exposed to the labeled sample under appropriate hybridization conditions. Unhybridized labeled sample is then removed by washing under suitable conditions, and the filter is then monitored for the presence of bound sequences. Because species determination requires the use of multiple species specific probes for each amplified sample, the reverse dot blot format is the preferred test format for this step.

Alternatively, it may be desirable to use a detection method having a plurality of probe hybridization sites or wells. For example, a solid support such as a microtiter plate is particularly useful in large scale clinical applications of the present methods. Methods for hybridization/capture of PCR amplified DNA or solid supports are known. In one embodiment of those methods the amplified target DNA is labeled (e.g., with biotin) during amplification in the PCR reaction. The labeled DNA is specifically captured by hybridization of PCR product to a target-specific oligonucleotide capture probe that has been bound to the microtiter plate well. The bound product is suitab · detected according to the type of label used. For example, if biotin is used as a label, avidin HRP complex is added and is reacted with either (a) hydrogen peroxide substrate and O-phenylene diamine (OPD) chromogen or (b) hydrogen peroxide substrate and tetramethylbenzidine chromogen (TMB). A color metric signal develops, allowing for the quantitative detection of the PCR amplified DNA.

As practiced in laboratories, detection procedures using microtiter plate assays can be standardized for a wide range of targets. It may be necessary to individually determine the appropriate hybridization and stringency conditions to insure the maximum specificity taking into account the length of the probes utilized.

In another suitable assay system a labeled probe is added during the PCR amplification process. Any probe that hybridizes to target DNA during each synthesis step is degraded by the 5' to 3' exonuclease activity of the polymerase used to catalyze primer extension. The degradation product

from the probe is then detected. Thus, the presence of the degradation product indicates that the hybridization between the probe and the target DNA occurred.

The present invention also relates to sets of reagents for amplifying a part of a gene coding for a superoxide dismutase (SOD), especially an SOD of a mycobacterial species, as well as to sets of reagents for detecting a mycobacterial species and for differentiating among different species belonging to the genus of mycobacteria.

The sets of reagents for the amplification comprise the primers according to the present invention, i.e a pair of universal primers and/or a pair of primers suitable for the amplification of a part of the SOD genes of the different species belonging to a particular genus, especially a pair of primers suitable for the amplification of a part of the SOD genes of mycobacterial species. They may optionally also contain a DNA polymerase, e.g. one of the aforementioned DNA polymerases, and/or the substrate nucleoside triphosphates mentioned above and/or the appropriate buffers for PCR. In addition to the above components, the sets of reagents may also contain instructions for carrying out the amplification according to the present invention.

The sets of reagents for detecting a mycobacterial species comprise one or more genus specific probes, especially a pool of genus specific probes according to the present invention. In some cases, the probes may be fixed to an appropriate support membrane. Other optional components of the kit include, for example, means used to label and/or detect label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for hybridization reactions. In addition to the above components, the sets of reagents may also contain instructions for carrying out the detection methods according to the present invention.

The sets of reagents for differentiating among different species belonging to the genus of mycobacteria comprise one or more species specific probes according to the present invention. In some cases, the probes may be fixed to an appropriate support membrane. Other optional components of the kit include, for example, means used to label and/or detect label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for hybridization reactions. In addition to the above components, the sets of reagents may also contain

instructions for carrying out the differentiation methods according to the present invention.

The sets of reagents for detecting a mycobacterial species and for differentiating among different species belonging to the genus of mycobacteria may additionally also contain one or more of the components mentioned above for the sets of reagents for the amplification.

In another embodiment of the present invention, the sets of reagents for the amplification, detection and differentiation may also contain positive and/or negative controls. Preferably a positive control includes a nucleic acid sequence that is amplifiable using the same primer pair used to amplify the desired target nucleic acids in a test sample. Methods for using a positive control, wherein both the target that may or may not be present and the positive control use the same primer pair, are known to those skilled in the art. Preferably the positive control is designed so that the product DNA is of a discrete size readily distinguishable from the size of the target or is modified in ways known to those skilled in the art (mutations/restriction sites).

The following examples are intended to describe the present invention in more detail. They are only for ilustrative purposes and not to limit the scope of the invention in any way.

Example 1: Amplification of Mycobacterial SOD-Sequences with the Primers Z205 and Z212

The primers were sythesized on a MilliGen/Biosearch Cyclone Plus Synthesizer using the DNA synthezising kit for sequences of up to 50 nucleotides (Millipore GmbH, Eschborn FRG). The oligonucleotides were eluted from the cartidges with 25% NH_4OH, dried in vacuo, and dissolved without further purification to a final concentration of 20 µM.

About 1 ng of previously purified chromosomal DNA of the mycobacterial species listed in Table 1a and the non-mycobacterial species listed in Table 2a, were amplified by a PCR with the following cycling profile in a Thermocycler 480 (Perkin Elmer): Heat for 5 min at 98 °C (initial denaturation), cool to 55 °C before the addition of 5 U of Taq polymerase (Perkin Elmer AG, Küsnacht, Switzerland), and cycle 35 times for 30 sec at 94 °C (denaturation), 30 sec at 37 - 55 °C (annealing), 30 sec at 72 °C (extension) and hold for 10 min at 72 °C.

A standard PCR reaction consisted of: 10 µl 10X buffer (100 mM of Tris; pH=8.3 at room temperature; 500 mM of KCl; 15 mM of $MgCl_2$; 0.015 % of gelatine), 16 µl of deoxyribonucleotide-mix (1.25 µmol of each dATP; dGTP; dCTP; dTTP), 5 µl of primer Z205 (20 µM), 5 µl of primer Z212 (20 µM), 10 µl of DNA to be amplified and 53 µl of H_2O. 10 µl of the amplified mixtures were loaded on 1% agarose gels and separated at 200 V, using the 123 bp and the 1 kb ladder as size standards (Bethesda Research Laboratories, Gaithersburg). After appropriate separation, the gels were stained with ethidiumbromide and photographed. For further experimental details see: Molecular Cloning, 1989, J. Sambrook, E.F. Fritsch, T. Maniatis; (eds.); Cold Spring Harbor Laboratory Press.

The results are compiled in Tables 1a and 2a; a "+" indicates that a visible amplicon was obtained. Using the primer pair Z205 and Z212 at an annealing temperature of 37 °C, all DNA preparations from the 27 different species of Mycobacteria tested (listed in Table 1a) and all the 106 non-mycobacterial species listed in Table 2a gave visible amplicons on stained gels . The most predominant (and in most cases the only visible fragment) was a DNA fragment co-migrating with the predicted amplicon from M. tuberculosis of 489 bp spanning the region 188 to 678 of the M. tuberculosis sequence. Therefore, the primer pair Z205 and Z212 can be viewed as universal primers.

Example 2: <u>Cloning and Sequencing the Amplicons from selected bacteria obtained with primers Z205 and Z212</u>

The region corresponding to positions 188 to 666 within the SOD gene of M. tuberculosis of the 9 mycobacterial species and the 4 non-mycobacterial species listed below was amplified using the primer pair Z205 and Z212. The amplicons were subsequently cloned and sequenced as described hereinafter:

M. tuberculosis (ATCC 1400); M. avium (ATCC 25291); M. intracellulare (DSM 43223); M. scrofulaceum (ATCC 19981); M. kansasii (DSM 43224); M. fortuitum (ATCC 6841); M. simiae (ATCC 25275); M. gordonae (DSM 610); M. xenopi (ATCC 19250); Corynebacterium diphteriae (ATCC 11913); Corynebacterium pseudodiphteriticum (RC 181); Nocardia asteroides (ATCC 43005); and Actinomyces viscosus (ATCC 15987).

Details of the procedures can be found in the previously referenced laboratoy manual: "Molecular Cloning". About 1 ng of chromosomal DNA

from the 13 organisms listed above were amplified as described in Example 1. The possibly frayed ends of the PCR amplicons were filled-in using the four deoxynucleotides (dNTP) and Klenow polymerase. Unincorporated primers and dNTP's were separated from the amplicons by diluting the reaction and passing the solution over a Quiagen tip 5 following the instructions of the manufacturer (Diagen, Düsseldorf, FRG). The eluted and precipitated DNA was dissolved and phosphorylated using a 5'-endlabeling-kit (Boehringer Mannheim GmbH, Mannheim, FRG). The filled-in and kinased amplicons were separated by electrophoresis on NuSieve GTG agarose (FMC, BioProducts Europe, Vallensbaek Strand, Denmark) and the amplicons co-migrating with the 489 bp fragment of M. tuberculosis were excised. The DNA was recovered from the gel slices and purified with a Quiagen tip 5 as described above. The pellet obtained after precipitation was dissolved, quantitated and used for ligation.

The plasmid pUC 19 was digested to completion with SmaI, phenol extracted, precipitated and dissolved before being dephosphorylated with calf thymus alkaline phosphatase. The vector DNA was again phenol extracted, precipitated and diluted to about 1 ng/µl. Vector DNA and all enzymes used in the vector preparation were from Boehringer Mannheim (Mannheim, FRG).

The digested, dephosphorylated vector DNA was ligated to the purified SOD amplicons for 18 h at 22 °C using 1 U T4-ligase. DH5a competent cells (Bethesda Research Laboratories, Gaithersburg, USA) were transformed with 2 - 5 µl of the ligase mixture, following the instructions of the supplier and plated on LB-agar with X-gal, IPTG and ampicillin (for details see "Molecular Cloning").

From each transformation of the 13 cloned amplicons, ten recombinant (colorless) colonies were picked and grown in 200 µl of LB-broth dispensed into a well of a microtiterplate. A crude DNA preparation was performed using a boiling-freezing method and appropriate aliquots of the DNA solution were amplified without further purification by a PCR using the pUC-sequencing and reverse sequencing primers (Boehringer Mannheim; Mannheim, FRG). Two wells from each of the 13 different amplifications containing plasmids with the expected insert size of 592 bp (103 bp stemming from the multiple cloning site and 489 bp from the SOD amplicon) were used for further sequencing. The recombinant plasmid DNA from 2 independant clones from each of the 13 different organisms listed above was purified

using the Quiagen "Plasmid" maxi kit (Diagen, Düsseldorf, FRG) and sequenced by the dideoxymethod using the pUC sequencing kit with S^{35} following the instructions given by the manufacturer (Boehringer Mannheim, Mannheim, FRG).

The position of the primers used for sequencing is shown in Figure 1. Besides the two primers annealing in the multiple-cloning site of pUC 19 (pUC forward and pUC reverse), the same two primers used for cloning (Z205 and Z212) were used in the sequencing reactions. For all mycobacterial species and Nocardia asteroides, two additional forward primers (Z256 and Z243) and one reverse primer (Z244) were used. This strategy allowed perfect overlapping reading of the sequencing-gels with only about 150 bp to be read from any one reaction. The positions of the primers specific for the sequencing of the SOD-amplicons from the remaining commensalic bacteria (Z257 and Z245 for C. diphteriae, Z258 and Z247 for C. pseudodiphteriticum and Z246 for A. viscosus are also indicated in Figure 1. The sequence of all sequencing primers within the SOD gene is given in Table 3. Since primer Z212 contains 2 wobble bases, it was possible to ensure that two independant clones were sequenced from each of the 13 species.

A total of 29.1 kb were read from the 26 clones derived from 9 mycobacterial and 4 non-mycobacterial species. The consensus-sequences are shown in Tables 4 - 16.

Example 3: Comparison of SOD Sequences to determine Mycobacteria Specific PCR Primers

Each of the SOD sequences shown in Tables 4 - 16 were aligned as to allow comparison to each other in order to identify the regions of pronounced homology within the members of the genus mycobacteria and pronounced heterogeneity when compared to the corresponding regions from the non-mycobacterial species. The GCG-program (Genetics Computer Group Sequence Analysis software, Version 7.1, Dervereux et al., NAR 12, 387 - 395 (1984); GAP-Alignment) was used for alignment.

In this way, primers specific for all the 27 species of the genus mycobacteria could be derived which would allow the amplification of the part of their SOD-coding sequences flanked by the primers annealing to the conserved regions. These conserved regions within the genus mycobacteria exhibit a number of mismatches when compared to the corresponding regions of the 4 sequenced non-mycobacterial species. Therefore, primers

selected by those criteria most likely would prevent the amplification of non-mycobacterial species.

The primer sequences selected (Z261 and Z212) were one of several combinations tested for their ability to amplify all 27 species of mycobacteria (Table 1a), but none of the 93 non-mycobacterial target DNA's (Table 2a). One of the two primers (Z212) was used to amplify both the mycobacterial and the non-mycobacterial DNA's. The sequence of Z212 corresponds to positions 677 - 655 within the SOD-gene of M. tuberculosis. The discriminatory amplification of the DNA from mycobacterial and non-mycobacterial species therefore rests with the counterprimer Z261 (positions 245 - 269 within the SOD gene of M. tuberculosis). The sequence of Z261 together with the mismatches within the 9 sequenced species of the genus mycobacteria and the 4 selected non-mycobacterial species is shown in Table 17. With the introduction of two wobble bases (positions 249 and 264), a maximum of 2 mismatches remain with the 9 sequenced mycobacterial species. The last 5 nucleotides at the 3'-end of the primer are conseved among all the 9 sequenced mycobacteria.

Example 4: Amplification with Genus Specific Primers Z261 and Z212

Using DNA from M. tuberculosis, the amplicons obtained with the primer pair Z261/Z212 are 434 bp long. The annealing temperature for the genus specific amplification was optimized using DNA from M. tuberculosis, M. intracellulare, C. diphteriae, Nocardia asteroides and human DNA as representative test DNA's using the PCR standard conditions described in Example 1. The following temperatures were evaluated: 37 °C; 45 °C; 50 °C; 55 °C; 58 °C; 60 °C; 64 °C; 67 °C and 70 °C; the amplicons seen with non-mycobacterial DNA at non-stringent temperatures (37 °C and 45 °C) are starting to dissapear at higher annealing temperatures (> 58 °C). At 64 °C a series of fragments can still be seen with human DNA which are not present at 67 °C. Either 60 or 67 °C was used with the primer pair Z261 and Z212. The visible amount of amplicons was higher with 60 °C although 67 °C was clearly the more discriminatory temperature. The optimal magnesium concentration was found to be 1.5 mM. Therefore, the final cycling protocol for the genus specific primers (Z261 and Z212) was as follows: Heat for 5 min at 98 °C, cool to 55 °C, add 1 µl of Taq (5 U) and amplify for 35 cycles, 30 sec each, 94 °C, 60 or 67 °C, 72 °C followed by a 10 min incubation at 72 °C.

The results are compiled in Tables 1a and 2a; a "+" indicates that visible amplicons were obtained and a "-" indicates that no or very few amplicons were obtained. All 27 mycobacterial species were amplified with the genus specific primers Z261 and Z212 as verified by gel electrophoresis using an annealing temperature of 67 °C (Table 1a). However the signals obtained on gels with M. gadium, M. marinum and M. haemophilum were weak compared to the signals of the other 24 species. After increasing the cycling time to 1 min, visible signals were obtained also with the 3 species in question.

The primers Z261 and Z212 did not amplify 102 out of the 106 non-mycobacterial species (Table 2a) as judged by the absence of visible signals on agarose gels. To ensure that the correct amount of amplifyable material was introduced in the PCR-reaction, all DNA's were tested (and found positive) in parallel with the primer pair Z205 and Z212. Four bacterial species gave weak signals on gels with the PCR-conditions used, namely: Edwadsiella tarda, Ewingella americana, Klebsiella pneumoniae and Salmonella hautena. However, none of the amplicons from these 4 species corresponded exactly in size and intensity to the amplicons obtained with the mycobacterial DNA.

Example 5: Selection of Genus Specific Probes

The sequences otained by amplifying the 9 mycobacterial and 4 non-mycobacterial DNA's with the genus specific primers Z261 and Z212 (Tables 4 - 16) were aligned and analyzed for regions of maximum homology within the mycobacteria and maximum heterogeneity among the non-mycobacterial species. The genus specific probe finally selected covers position 382 to 421 in the M. tuberculosis sequence. The common sequence is shown in Table 18, together with the mismatches present in the sequenced non-mycobacterial species. Only 2 mismatches occur in Nocardia asteroides, but this DNA does not amplify under the conditions described with the genus specific PCR primer Z261 and Z212, due to 11 mismatches present. Instead of introducing a total of 14 wobble bases to cover all mismatches within the 9 sequenced species of mycobacteria (Table 18), we opted to synthesize each of the genus-specific oligos separately, resulting in a pool of 8 oligonucleotides (M. intracellulare and M. xenopi are grouped together) where each of the individual probes is present in eqimolar amounts. The sequence of the oligos constituting the genus specific pool of probes is shown in Table 19. Probe Z310 detects M. tuberculosis, Z311 both M. intracellulare and M. xenopi, Z312 M.

avium, Z313 M. gordonae, Z314 M. fortuitum, Z315 M. scrofulaceum, Z316 M. simiae and Z317 M. kansasii.

Example 6: Hybridization with Genus Specific Probes

The genus specific pool of probes (Table 19) was labeled as follows: (80 pmol of an equimolar mix of the 8 probes (Z310 - Z317) were labeled at the 5'-end to a specific activity of about 3 X 10^7/µg using 100 γCi of ^{32}P-γ ATP (10 mCi/ml; 5000 Ci/mmol) following the instructions given by the supplier of the 5'-labeling kit (Boehringer Mannheim, Mannheim, FRG). The unincoporated label was removed using a Biospin 6 column (BioRad, Richmond, USA). 20 µl of alkali-denatured PCR mixtures obtained with the genus specific primers Z261 and Z212 from the 27 mycobacterial species (Table 1a) and 106 non-mycobacterial species (Table 2a) were diluted with 100 µl TE-buffer and spotted on prewetted solid supports ("Gene Screen plus" filters; Du Pont de Nemours, Bad Homburg, FRG) using a 96-well manifold (Schleicher & Schuell, Dassel, FRG). The filters were crosslinked in a Stratagene UV-linker (Statagene, La Jolla, USA) and prehybridized in a 50 ml Falcon tube containing 5 ml of prehybridization solution (6 X SSC, 10 mM sodiumphosphat pH 6.8, 1 mM EDTA pH 8.0, 1% SDS, denatured salmon sperm DNA 100 µg/ml). Prehybridization was performed at 60 °C for 60 min in a rotating hybridization oven. The prehybridization fluid was discarded and replaced by 5 ml of hybridization solution (3 M tetramethylammonium chlorid, 10 mM sodiumphosphat pH 6.8, 1 mM EDTA pH 7.6, 0.5% SDS, denatured salmon sperm DNA 100 µg/ml and 5 - 8 x 10^6 cpm of labeled genus specific probes. Details of the buffer preparation can be found in the "Molecular Cloning" compendium referred to earlier. Hybridization was performed for 3 - 16 h at 65 °C. The filters were washed for 4 X 15 min in prewarmed (70 °C) 2 X SSC/0.2% SDS at 70 °C before being autoradiographed for 1 - 2 h at -70 °C using intensifying screens.

The results are compiled in Tables 1a and 2a; a "+" indicates that a hybridization signal was obtained and a "-" indicates that no hybridization signal was obtained. The genus specific pool of probes (Z310 - Z317) was used to probe all 27 species of mycobacteria (Table 1a). The pool recognized all 9 sequenced species of mycobacteria (Tables 4 - 12), specifically: M. tuberculosis, M. avium, M. intracellulare, M. fortuitum, M. scofulaceum, M. gordonae, M. simiae, M. xenopi and M. kansasii. In addition, the following 13 non-sequenced species of mycobacteria are recognized with the genus specific probe pool: M. africanum, M. acapulcensis, M. bovis, M. brunense, M. chelonai, M. flavescens, M. marinum, M. gastri, M. terrae,

M. triviale, M. nonchromaticum, M. oboense and M. smegmatis (indicated by "+" in Table 1a). Weaker signals under the hybridization conditions described were obtained with: M. haemophilum, M. gadium, M. szlugai and M. plei (indicated by "(+)" in Table 1a). No signal was obtained with M. rhodensiae (indicated by "(-)" in Table 1a).

None of the amplicons obtained with the genus-specific primers Z261 and Z212 from the non-mycobacterial species listed in Table 2a (not being visible on gels) as well as the 4 species which gave weak visible signals on gels; see Example 7) was detected with the pool of genus specific probes (Z310 - Z317). The results are summarized in Table 2a.

Example 7: Identification of Species Specific Probes

The sequences of the amplicons obtained by amplifying the 9 mycobacterial DNA's with the genus specific primers Z261 and Z212 (Tables 4 - 12) were aligned as described in Example 3 and each of the mycobacterial sequences were analyzed one by one for regions of pronounced heterogeneity when compared to the remaining mycobacterial sequences. In this way, species specific sequences could be identified and experimentally analyzed. The regions which numerically showed the highest number of mismatches (maximal heterogeneity in the alignment program used) turned out to be not always the regions which resulted in the most discriminatory hybridization results. Noteworthy is also that the number of mismatches of one sequence when compared to the others depends on the criteria used for the alignments. The positions given for the species specific probes refer to the sequences they are derived from (Tables 4 - 12), and not to the numbers attributed to them in the different alignment programs.

Example 7.1: M. tuberculosis Specific Probe

Three regions were selected with the following sequences (Table 4):

Z302: 5'-ACG AAC TTC CCG CTA GGC ATT GTT CCG CTG CTG CTG C-3'
(Seq. Id. No. 22; position 550 - 586);

Z336: 5'-GCT AGG CAT TGT TCC GCT GCT GCT GC-3'
(Seq. Id. No. 17; position 561-586); and

Z337: 5'-AGT CGA CTT TGC CAA GGC GTT T-3'
(Seq. Id. No. 23; position 633-654).

Z302, Z336 and Z337 were labeled and hybridized as described in Example 6 against filters on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. The hybridization temperature was 70 °C for Z302 and 60 °C for the remaining two probes. The hybridization was carried out for 1-16h, the washing temperature was 80 °C for Z302, 65 °C for Z337 and 60 °C for Z336. The films were exposed for 1-3 h.

The probe Z302, being 37 nucleotides long, recognized under the conditions used only M. tuberculosis and the close relatives combined in a "macrocluster" (Bergey's Manual of Determinative Bacteriology, 1974, 8th Edition, Waverly Press Inc, Baltimore, R.E. Buchanon + N.E. Gibbons (Eds.)) with M. bovis, M. bovis BCG and M. africanum. The remaining 23 mycobacterial species listed in Table 1b were not recognized by Z302 nor any of the 106 non-mycobacterial species listed in Table 2b.

Probe Z336 represents the last 26 nucleotides on the 3'-end of the original probe Z302. The truncated probe Z336 hybridized under the modified conditions described only to M. tuberculosis, M. bovis, M. bovis BCG and M. africanum. No crossreaction is seen with the remaining 23 mycobacterial species listed in Table 1b, nor with any of the 106 non-mycobacterial species listed in Table 2b.

Z337 (a probe of 22 nucleotides) positioned towards the end of the sequenced part of the SOD gene is specific for the M. tuberculosis-M. bovis-M. africanum complex and does not cross react with the remaining 23 mycobacterial species listed in Table 1b, nor with any of the 106 non-mycobacterial species listed in Table 2b.

Although the specificity of the three probes Z302, Z336 and Z337 is comparable, the probes Z336 and Z337 are preferred ones due to the lower washing temperature.

Example 7.2: <u>M.avium-M.brunense Specific Probe</u>

The region 423 - 459 (Table 5) was selected with the following sequence:

Z301: 5'-GTC CTT CGA CAA GTT CCG AGC GCA ATT CAG CGC CGC C-3'
(Seq. Id. No. 14; position 423 - 459).

Z301 was labeled and hybridized as described in Example 6 against a filter on which mycobacterial amplicons obtained with Z261 and Z212 were immobilized. Hybridization was performed for 1-16 h at a temperature of 70

°C for Z301, the washing temperature was 80 °C for Z301. The films were exposed for 3 h. With the M. avium-M. brunense specific probe Z301, the amplicons from M. avium were recognized together with the amplicons from M. brunense (n = 6; amplicons derived from the same stock DNA preparation). There are no sequencing data are available for M. brunense and thus no rational probe design was possible for this species. It can not be entirely ruled out at this point that the stock of M. brunense was in fact contaminated with M. avium amplicons. Until new stocks of M. brunense are grown, amplified and sequenced Z301 is described as a probe recognizing both M. avium and M. brunense. Z301 did not recognize any of the other mycobacterial species listed in Table 1b. Again none of the tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. avium-M. brunense specific probe.

Example 7.3: M. intracellulare Specific Probe

The region 416 - 452 (Table 6) was selected with the following sequence:

Z303: 5'- CCT TCG GAT CCT TCG ACC GGT TCC GCG CGC AGT TCA G - 3'
(Seq. Id. No. 13).

Z303 was end-labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. The hybridization was performed at 60 °C for 16h, the washing temperature was 70 °C and the films were exposed for 3h, 6h and 16h. An example of the specificity of the species specific probe selected for M. intracellulare is shown in Figure 2 (16 h exposure) and the results are summarized in Table 1b.

The arrangements of DNA on the filter was as follows:

A 2	M. tuberculosis (clinical isolate 1400)	
A 3	M. tuberculosis	27294
A 4	M. avium	25291
A 5	M. fortuitum	6841
A 6	M. gordonae	DSM 610
A 7	M. kansasii	DSM 43224
A 8	M. scrofulaceum	19981
A 9	M. simiae	25275
A 10	M. xenopi	19250
A 11	M. intracellulare	DSM 43223

B	2	M. acapulcensis	14473
B	3	M. africanum	25420
B	4	M. bovis BCG	1401
B	5	M. bovis	19210
B	6	M. brunense	23434
B	7	M. chelonai	35752
B	8	M. flavescens	DSM 43219
B	9	M. gastri	15754
B	10	M. smegmatis	14468
B	11	M. terrae	15755
C	2	M. triviale	23292
C	3	M. marinum	927
C	4	M. haemophilum	29548
C	5	M. nonchromogenicum	19530
C	6	M. obuense	27023
C	7	M. phlei	DSM 750
C	8	M. rhodesiae	27024
C	9	M. szulgai	35799
C	10	M. gadium	27726
		H_2O	

None of the 106 tested species of non-mycobaterial organisms did react under the same conditions as described above for the mycobacterial species.

Example 7.4: M.scrofulaceum Specific Probe

The region 501 - 537 (Table 7) was selected with the following sequence:

Z306: 5'-TGA CAC ACT CGG CAG CAG GCT GCT CAC CTT CCA GCT T-3' (Seq. Id. No. 20).

Z306 was labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 70 °C for 1-16 h, the washing temperature was 80 °C and the films were exposed for 3 h, 6 h and 16 h. Z306 hybridized only to M. scrofulaceum DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the the M. scrofulaceum specific probe.

Example 7.5: M. kansasii Specific Probe

The region 546 - 567 (Table 8) was selected with the following sequence:

Z340: 5'-CCA GAC GAA CTT TCC ACT CGG A-3'
(Seq. Id. No. 19).

Z340 was labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 60 °C for 1-16 h, the washing temperature was 65 °C and the films were exposed for 3 h, 6 h and 16 h. Z340 hybridized only to M. kansasii DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. kansasii specific probe.

Example 7.6: M. fortuitum Specific Probe

The region 500 - 521 (Table 9) was selected with the following sequence:

Z369: 5'-ACG ACA GCC TGG GCG ATC GGC T-3'
(Seq. Id. No. 21).

Z369 was end-labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 60 °C for 1-16 h, the washing temperature was 70 °C and the films were exposed for 3 h, 6 h and 16 h. Z369 hybridized only to M. fortuitum DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. fortuitum specific probe.

Example 7.7: M. simiae Specific Probe

The region 360 - 396 (Table 10) was selected with the following sequence:

Z304: 5'-GTC CCC GAA CGG CGG AGA CAA GCC GAC CGG AGA TCT C-3'
(Seq. Id. No. 16).

Z304 was labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 70 °C for 1-16 h, the washing temperature was 80 °C and the films were exposed for 3 h, 6 h and 16 h. Z304 hybridized only to M. simiae DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. simiae specific probe.

Example 7.8: M. gordonae Specific Probe

The region 507 - 531 (Table 11) was selected with the following sequence:

Z366: 5'-TCT GGG CGG CCG GTT GCT CAC CTT T-3'
(Seq. Id. No. 15).

Z366 was end-labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 60 °C for 1-16 h, the washing temperature was 70 °C and the films were exposed for 3 h, 6 h and 16 h. Z366 hybridized only to M. gordonae DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. gordonae specific probe.

Example 7.9: M. xenopi Specific Probe

The region 280 - 316 (Table 12) was selected with the following sequence:

Z309: 5'-TCC GCG ATC GTC GGG CAT GAG AAG GCC CTC GCG TTC A-3'
(Seq. Id. No. 18).

Z309 was end-labeled and hybridized as described in Example 6 against a filter on which 28 mycobacterial amplicons (26 different species) obtained with Z261 and Z212 were immobilized. Hybridization was performed at a temperature of 70 °C for 1-16 h, the washing temperature was 80 °C and the films were exposed for 3 h, 6 h and 16 h. Z309 hybridized only to M. xenopi

DNA but not to any of the other mycobacteria listed in Table 1b. None of the 106 tested non-mycobacterial DNA's listed in Table 2b cross-reacted with the M. xenopi specific probe.

Brief description of the figures:

Fig.1: Position of the sequencing primers within the SOD-gene.

Fig.2: Dot-blot with immobilized amplicons obtained with the genus-specific primers Z 261 and Z212 hybridized against the probe Z 303, specific for M.intracellulare.

List of Tables

Table 10: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of M. simiae.

Table 11: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of M. gordonae.

Table 12: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of M. xenopi.

Table 13: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of Corynebacterium diphteriae.

Table 14: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of Corynebacterium pseudodiphtheriticum.

Table 15: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of Nocardia asteroides.

Table 16: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of Actinomyces viscosus.

Table 17: Sequence of the consensus-primer Z261 and the mismatches within the 9 sequenced species of the genus mycobacteria, the 4 sequenced non-mycobacterial organisms as well as the corresponding part sequence derived from E. coli- and the human SOD-gene.

Table 18: Consensus sequence of the genus-specific probe for mycobacteria, the mismatches within the 4 sequenced non-mycobacterial organisms as well as the corresponding part of the sequence derived from E. coli- and the human SOD-gene. The sequence of the individual oligonucleotides the genus-specific pool is composed of are also listed.

Table 1a

Mycobacteria	ATCC	Z205/Z212 [1]	Z261/Z212 [2]	Z310 - Z317 [3]
M. acapulcensis	14473	+	+	+
M. africanum	25420	+	+	+
M. avium	25291	+	+	+
M. bovis	19210	+	+	+
M. bovis BCG ***	1401	+	+	+
M. brunense	23434	+	+	+
M. chelonae	35752	+	+	+
M. flavescens	DSM43219*	+	+	+
M. fortuitum	6841	+	+	+
M. gadium	27726	+	+	(+)
M. gastri	15754	+	+	+
M. gordonae	DSM610*	+	+	+
M. haemophilum	29548	+	+	(+)
M. intracellulare	DSM 43223*	+	+	+
M. kansasii	DSM43224*	+	+	+
M. marinum	927	+	+	+
M. nonchromogenicum	19530	+	+	+
M. obuense	27023	+	+	+
M. phlei	DSM750*	+	+	(+)
M. rhodesiae	27024	+	+	-
M. scrofulaceum	19981	+	+	+
M. simiae	25275	+	+	+
M. smegmatis	14468	+	+	+
M. szulgai	35799	+	+	(+)
M. terrae	15755	+	+	+
M. triviale	23292	+	+	+
M. tuberculosis **	1400	+	+	+
M. tuberculosis	27294	+	+	+
M. xenopi	19250	+	+	+

1) Amplification with universal primer pair Z205/Z212
2) Amplification with genus specific primer pair Z261/Z212
3) Hybridization with genus specific pool of probes Z310 - Z317

*** Serum- und Impfinstitut, Bern, Switzerland
** clinical isolate
* DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

Table 1b

Mycobacteria	ATCC	Hybridization with Species Specific Probes								
		A	B	C	D	E	F	G	H	I
M. acapulcensis	14473	-	-	-	-	-	-	-	-	-
M. africanum	25420	-	+	-	-	-	-	-	-	-
M. avium	25291	+	-	-	-	-	-	-	-	-
M. bovis	19210	-	+	-	-	-	-	-	-	-
M. bovis BCG ***	1401	-	+	-	-	-	-	-	-	-
M. brunense	23434	+	-	-	-	-	-	-	-	-
M. chelonai	35752	-	-	-	-	-	-	-	-	-
M. flavescens	DSM43219*	-	-	-	-	-	-	-	-	-
M. fortuitum	6841	-	-	-	-	-	-	-	+	-
M. gadium	27726	-	-	-	-	-	-	-	-	-
M. gastri	15754	-	-	-	-	-	-	-	-	-
M. gordonae	DSM610*	-	-	-	-	-	-	+	-	-
M. haemophilum	29548	-	-	-	-	-	-	-	-	-
M. intracellulare	DSM43223*	-	-	+	-	-	-	-	-	-
M. kansasii	DSM43224*	-	-	-	-	+	-	-	-	-
M. marinum	927	-	-	-	-	-	-	-	-	-
M. nonchromogenicum	19530	-	-	-	-	-	-	-	-	-
M. obuense	27023	-	-	-	-	-	-	-	-	-
M. phlei	DSM750*	-	-	-	-	-	-	-	-	-
M. rhodesiae	27024	-	-	-	-	-	-	-	-	-
M. scrofulaceum	19981	-	-	-	-	-	+	-	-	-
M. simiae	25275	-	-	-	+	-	-	-	-	-
M. smegmatis	14468	-	-	-	-	-	-	-	-	-
M. szulgai	35799	-	-	-	-	-	-	-	-	-
M. terrae	15755	-	-	-	-	-	-	-	-	-
M. triviale	23292	-	-	-	-	-	-	-	-	-
M. tuberculosis **	1400	-	+	-	-	-	-	-	-	-
M. tuberculosis	27294	-	+	-	-	-	-	-	-	-
M. xenopi	19250	-	-	-	-	-	-	-	-	+

A: M. avium probe Z301
B: MTB probes Z337/Z336/Z302
C: M. intracellulare probe Z303
D: M. simiae probe Z304
E: M. kansasii probe Z340
F: M. scrofulaceum probe Z306
G: M. gordonae probe Z366
H: M. fortuitum probe Z369
I: M. xenopi probe Z309

*** Serum- und Impfinstitut, Bern, Switzerland
** clinical isolate
* DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

Table 2a

	Non-mycobacterial Organisms	ATCC	Z205/Z212 [1]	Z261/Z212 [2]	Z310 - Z317 [3]
	Acinetobacter calcoaceticus	17945	+	-	-
	A. calcoaceticus	23055	+	-	-
	Acinetobacter lwoffii	15309	+	-	-
	Actinomyces bovis	DSM43014**	+	-	-
	Actinomyces israelii	12102	+	-	-
	Actinomyces meyeri	35568	+	-	-
	Actinomyces odontolyticus	17929	+	-	-
	Actinomyces viscosus	15987	+	-	-
	Aeromonas hydrophila	7966	+	-	-
	Agrobact. tumefaciens	23308	+	-	-
	Alcaligenes faecalis	8750	+	-	-
	Arthrobacter globiformis	DSM20124**	+	-	-
	Aspergillus flavus	RKI****	+	-	-
	Aspergillus fumigatus	RKI****	+	-	-
	Bacillus cereus	27348	+	-	-
	Bacillus fragilis	RKI****	+	-	-
	Bacillus subtilis	6633	+	-	-
	Bacteroides hetaitaomicron	RKI****	+	-	-
	Bifidobacterium dentium	DSM20084**	+	-	-
	Bordatella parapertussis	DSM4922**	+	-	-
	Branhamella catarrhalis	8176	+	-	-
	Brevibact. epidermidis	DSM20660**	+	-	-
	Campylobacter jejuni	33560	+	-	-
	Campylobacter pylori	*	+	-	-
	Candida albicans	10231	+	-	-
	Candida glabrata	RKI****	+	-	-
	Citrobacter diversus	DSM4570**	+	-	-
	Citrobacter freundii	8090	+	-	-
	Clostridium difficile	RKI****	+	-	-
	Corynebact. diphteriae	11913	+	-	-
	Corynebacter flavescens	10340	+	-	-
	Corynebact. minutissimum	23348	+	-	-
	C. pseudodiphtheriticum	RC181***	+	-	-
	C. pseudogenitalium	33035	+	-	-
	Corynebacter striatum	6940	+	-	-
	Corynebacter variabilis	15753	+	-	-
	Corynebacter xerosis	373	+	-	-
	E. coli	25922	+	-	-
	Edwardsiella tarda	15947	+	(+)	-
	Enterococcus avium	14025	+	-	-
	Enterococcus durans	19432	+	-	-

Table 2a (continued)

Non-mycobacterial Organisms	ATCC	Z205/Z212 [1]	Z261/Z212 [2]	Z310 - Z317 [3]
Enterococcus faecalis	19433	+	-	-
Enterobacter cloacae	13047	+	-	-
Ewingella americana	DSM4580**	+	(+)	-
Flavobacterium odoratum	4651	+	-	-
Haemophilus influenzae	9333	+	-	-
Klebsiella pneumoniae	13883	+	(+)	-
Lactobacillus acidophilus	DSM20079**	+	-	-
Leishmania mexicana	RKI****	+	-	-
Micrococcus luteus	RC9346***	+	-	-
Neisseria lactamica	23970	+	-	-
Neisseria sicca	9913	+	-	-
Nocardia argentinensis	31306	+	-	-
Nocardia asteroides	43005	+	-	-
Nocardia brevicatena	15333	+	-	-
Peptostreptococcus anaerobius	RKI****	+	-	-
Peptostreptococcus asaccharolyticus	RKI****	+	-	-
Plesiomonas shigelloides	14029	+	-	-
Plsasmodium falciparum 3D7	RC***	+	-	-
Prevotella livia	RKI****	+	-	-
Prevotella intermedia	RKI****	+	-	-
Propionbacterium acnes	DSM1897**	+	-	-
Proteus mirabilis	12453	+	-	-
Proteus vulgaris	6380	+	-	-
Pseudomonas acidovorans	RC15858***	+	-	-
Pseudomonas aeruginosa	10145	+	-	-
Pseudomonas alcaligenes	14909	+	-	-
Pseudomonas fluorescens	13525	+	-	-
Pseudomonas putida	12633	+	-	-
Pseudomonas putrefaciens	DSM50426**	+	-	-
Rhodococcus equi	6939	+	-	-
Rhodococcus sputi	29627	+	-	-
Saccharomyces cerevisiae	RKI****	+	-	-
Salmonella bongori	RC5951***	+	-	-
Salmonella hautenea	RC5507***	+	(+)	-
Salmonella salamae	RC5554***	+	-	-
Salmonella typhimurium	13311	+	-	-
Serratia marcescens	8100	+	-	-
Shigella sonnei	11060	+	-	-

Table 2a (continued)

Non-mycobacterial Organisms	ATCC	Z205/Z212 [1]	Z261/Z212 [2]	Z310 - Z317 [3]
Staphylococcus aureus	29213	+	-	-
Staphylococcus capitis	27840	+	-	-
Staphylococcus cohnii	29974	+	-	-
Staphylococcus epidermidis	12228	+	-	-
S. haemolyticus	29970	+	-	-
Staphylococcus hominis	27844	+	-	-
S. saprophyticus	15305	+	-	-
Staphylococcus simulans	27848	+	-	-
Staphylococcus warneri	27838	+	-	-
Staphylococcus xylosus	29971	+	-	-
Streptococcus agalactiae	13813	+	-	-
Streptococcus constellatus	27823	+	-	-
Streptococcus equi	33398	+	-	-
Streptococcus group A	RC17A4***	+	-	-
Streptococcus mitis	33399	+	-	-
Streptococcus morbillorum	27824	+	-	-
Streptococcus mutans	25175	+	-	-
Streptococcus pneumoniae	6301	+	-	-
Streptococcus pneumoniae	6303	+	-	-
Streptococcus pyogenes	19615	+	-	-
Streptococcus salivarius	7073	+	-	-
Streptococcus sanguis	10556	+	-	-
Trypanosoma cruzei	RKI****	+	-	-
Veillonella dispar	DSM20735**	+	-	-
Vibrio alginolyticus	DSM2171**	+	-	-
Yersinia enterocolitica	9610	+	-	-
Y. pseudotuberculosis	907	+	-	-

1) Amplification with universal primer pair Z205/Z212
2) Amplification with genus specific primer pair Z261/Z212
3) Hybridization with genus specific pool of probes Z310 - Z317

* clinical isolate
** DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG
*** RC (Roche collection)
**** RKI (Robert Koch Institute, Berlin, FRG)

Table 2b

Non-mycobacterial Organisms	ATCC	Hybridization with Species Specific Probes A	B	C	D	E	F	G	H	I
Acinetobact. calc.	17945	-	-	-	-	-	-	-	-	-
A. calcoaceticus	23055	-	-	-	-	-	-	-	-	-
Acinetobacter lwoffii	15309	-	-	-	-	-	-	-	-	-
Actinomyces bovi	DSM43014**	-	-	-	-	-	-	-	-	-
Actinomyces israelii	12102	-	-	-	-	-	-	-	-	-
Actinomyces meyeri	35568	-	-	-	-	-	-	-	-	-
Actinomyces odont.	17929	-	-	-	-	-	-	-	-	-
Actinomyces viscosus	15987	-	-	-	-	-	-	-	-	-
Aeromonas hydrophila	7966	-	-	-	-	-	-	-	-	-
Agrobact. tumefaciens	23308	-	-	-	-	-	-	-	-	-
Alcaligenes faecalis	8750	-	-	-	-	-	-	-	-	-
Arthrobact. globiformis	DSM20124**	-	-	-	-	-	-	-	-	-
Aspergillus flavus	RKI****	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	RKI****	-	-	-	-	-	-	-	-	-
Bacillus cereus	27348	-	-	-	-	-	-	-	-	-
Bacillus fragilis	RKI****	-	-	-	-	-	-	-	-	-
Bacillus subtilis	6633	-	-	-	-	-	-	-	-	-
Bacteroides hetaitaomicron	RKI****	-	-	-	-	-	-	-	-	-
Bifidobact. dentium	DSM20084**	-	-	-	-	-	-	-	-	-
Bordatella parapert.	DSM4922**	-	-	-	-	-	-	-	-	-
Branhamella catarrh.	8176	-	-	-	-	-	-	-	-	-
Brevibact. epidermidis	DSM20660**	-	-	-	-	-	-	-	-	-
Campylobacter jejuni	33560	-	-	-	-	-	-	-	-	-
Campylobacter pylori	*	-	-	-	-	-	-	-	-	-
Candida albicans	10231	-	-	-	-	-	-	-	-	-
Candida glabrata	RKI****	-	-	-	-	-	-	-	-	-
Citrobacter diversus	DSM4570**	-	-	-	-	-	-	-	-	-
Citrobacter freundii	8090	-	-	-	-	-	-	-	-	-
Clostridium difficile	RKI****	-	-	-	-	-	-	-	-	-
Corynebact. diphteriae	11913	-	-	-	-	-	-	-	-	-
Corynebact. flavescens	10340	-	-	-	-	-	-	-	-	-
Corynebact. minutiss.	23348	-	-	-	-	-	-	-	-	-
C. pseudodipht.	RC181***	-	-	-	-	-	-	-	-	-
C. pseudogenitalium	33035	-	-	-	-	-	-	-	-	-
Corynebacter striatum	6940	-	-	-	-	-	-	-	-	-
Corynebacter variabilis	15753	-	-	-	-	-	-	-	-	-
Corynebacter xerosis	373	-	-	-	-	-	-	-	-	-
E. coli	25922	-	-	-	-	-	-	-	-	-
Edwardsiella tarda	15947	-	-	-	-	-	-	-	-	-
Enterococcus avium	14025	-	-	-	-	-	-	-	-	-

Table 2b (continued)

Non-mycobacterial Organisms	ATCC	Hybridization with Species Specific Probes A	B	C	D	E	F	G	H	I
Enterococcus durans	19432	-	-	-	-	-	-	-	-	-
Enterococcus faecalis	19433	-	-	-	-	-	-	-	-	-
Enterobacter cloacae	13047	-	-	-	-	-	-	-	-	-
Ewingella americana	DSM4580**	-	-	-	-	-	-	-	-	-
Flavobact. odoratum	4651	-	-	-	-	-	-	-	-	-
Haemophilus infl.	9333	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	13883	-	-	-	-	-	-	-	-	-
Lactobacillus acidoph.	DSM20079**	-	-	-	-	-	-	-	-	-
Leishmania mexicana	RKI****	-	-	-	-	-	-	-	-	-
Micrococcus luteus	RC9346***	-	-	-	-	-	-	-	-	-
Neisseria lactamica	23970	-	-	-	-	-	-	-	-	-
Neisseria sicca	9913	-	-	-	-	-	-	-	-	-
Nocardia argent.	31306	-	-	-	-	-	-	-	-	-
Nocardia asteroides	43005	-	-	-	-	-	-	-	-	-
Nocardia brevicatena	15333	-	-	-	-	-	-	-	-	-
Peptostreptococcus anaerobius	RKI****	-	-	-	-	-	-	-	-	-
Peptostreptococcus asaccharolyticus	RKI****	-	-	-	-	-	-	-	-	-
Plesiomonas shigell.	14029	-	-	-	-	-	-	-	-	-
Plasmodium falciparum 3D7	RC***	-	-	-	-	-	-	-	-	-
Prevotella livia	RKI****	-	-	-	-	-	-	-	-	-
Prevotella intermedia	RKI****	-	-	-	-	-	-	-	-	-
Propionbact. acnes	DSM1897**	-	-	-	-	-	-	-	-	-
Proteus mirabilis	12453	-	-	-	-	-	-	-	-	-
Proteus vulgaris	6380	-	-	-	-	-	-	-	-	-
Pseudom. acidovorans	RC15858***	-	-	-	-	-	-	-	-	-
Pseudom. aeruginosa	10145	-	-	-	-	-	-	-	-	-
Pseudom. alcaligenes	14909	-	-	-	-	-	-	-	-	-
Pseudom. fluorescens	13525	-	-	-	-	-	-	-	-	-
Pseudomonas putida	12633	-	-	-	-	-	-	-	-	-
Pseudom. putrefac.	DSM50426**	-	-	-	-	-	-	-	-	-
Rhodococcus equi	6939	-	-	-	-	-	-	-	-	-
Rhodococcus sputi	29627	-	-	-	-	-	-	-	-	-
Saccharomyces cervisiae	RKI****	-	-	-	-	-	-	-	-	-
Salmonella bongori	RC5951***	-	-	-	-	-	-	-	-	-
Salmonella hautenea	RC5507***	-	-	-	-	-	-	-	-	-
Salmonella salamae	RC5554***	-	-	-	-	-	-	-	-	-
Salmonella typh.	13311	-	-	-	-	-	-	-	-	-

Table 2b (continued)

Non-mycobacterial Organisms	ATCC	Hybridization with Species Specific Probes A	B	C	D	E	F	G	H	I
Serratia marcescens	8100	-	-	-	-	-	-	-	-	-
Shigella sonnei	11060	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	29213	-	-	-	-	-	-	-	-	-
Staphylococcus capitis	27840	-	-	-	-	-	-	-	-	-
Staphylococcus cohnii	29974	-	-	-	-	-	-	-	-	-
S. epidermidis	12228	-	-	-	-	-	-	-	-	-
S. haemolyticus	29970	-	-	-	-	-	-	-	-	-
S. hominis	27844	-	-	-	-	-	-	-	-	-
S. saprophyticus	15305	-	-	-	-	-	-	-	-	-
S. simulans	27848	-	-	-	-	-	-	-	-	-
S. warneri	27838	-	-	-	-	-	-	-	-	-
Staph. xylosus	29971	-	-	-	-	-	-	-	-	-
Streptococcus agalact.	13813	-	-	-	-	-	-	-	-	-
Streptococcus constel.	27823	-	-	-	-	-	-	-	-	-
Streptococcus equi	33398	-	-	-	-	-	-	-	-	-
Streptococcus group A	RC17A4***	-	-	-	-	-	-	-	-	-
Streptococcus mitis	33399	-	-	-	-	-	-	-	-	-
Streptococcus morbil.	27824	-	-	-	-	-	-	-	-	-
Streptococcus mutans	25175	-	-	-	-	-	-	-	-	-
Streptococcus pneum.	6301	-	-	-	-	-	-	-	-	-
Streptococcus pneum.	6303	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	19615	-	-	-	-	-	-	-	-	-
Streptococcus saliv.	7073	-	-	-	-	-	-	-	-	-
Streptococcus sanguis	10556	-	-	-	-	-	-	-	-	-
Trypanosoma Cruzei	RKI****	-	-	-	-	-	-	-	-	-
Veillonella dispar	DSM20735**	-	-	-	-	-	-	-	-	-
Vibrio alginolyticus	DSM2171**	-	-	-	-	-	-	-	-	-
Yersinia enterocolitica	9610	-	-	-	-	-	-	-	-	-
Y. pseudotuberculosis	907	-	-	-	-	-	-	-	-	-

A: M. avium probe Z301
B: MTB probes Z337/Z336/Z302
C: M. intracellulare probe Z303
D: M. simiae probe Z304
E: M. kansasii probe Z340
F: M. scrofulaceum probe Z306
G: M. gordonae probe Z366
H: M. fortuitum probe Z369
I: M. xenopi probe Z309

* clinical isolate
** DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG
*** RC (Roche collection)
****RKI (Robert Koch Institute, Berlin, FRG)

Table 3

Sequencing Primers

		Seq. Id. No.	Position
All Mycobacteria			
N. asteroides			
Z205:	5'-AGC TTC ACC ACA GCA AGC ACC A-3'	1	188 - 209
Z256:	5'-CAC $^{T}_{A}C^{G}_{C}$ ATC TGG TGG AAG AAC CT-3'	24	337 - 359
Z243:	5'-ACG $G^{C}_{T}G$ $G^{C}_{T}G$ ACA AGC CGA CCG G-3'	25	368 - 389
Z244:	5'-GTC TG^{G}_{C} TGG TCG TA^{G}_{A} $A^{G}_{C}C$ TGG A-3'	26	551 - 539
Z212:	5'-TCG $^{G}_{T}CC$ CAG TTC ACG AC^{A}_{G} TTC CA-3'	2	678 - 655
C. diphteriae			
Z205:	see above		
Z257:	5'-GGA TTT GGC TTT CAA CTT GGG-3'	27	306 - 326
Z245:	5'-GGC GAG CCA ACC GGC GCT TTG G-3'	28	376 - 397
Z212:	see above		
C. pseudo-diphtheriticum			
Z258:	5'-GAA GAA CCT GAG CCT TAA CGG T-3'	29	351 - 372
Z247:	5'-GAG CCA ACC GGT GAG CTA GCC-3'	30	379 - 399
Z212:	see above		
A. viscosus			
Z246:	5'-GAA GAA CCT CTC CCC CAA CGG C-3'	31	351 - 372
Z244:	see above		
Z212:	see above		

Table 4

Mycobacterium tuberculosis SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACCTACG TAAAGGGCGC CAATGACGCC GTCGCCAAAC
    251  TCGAAGAGGC GCGCGCCAAG GAAGATCACT CAGCGATCTT GCTGAACGAA
    301  AAGAATCTAG CTTTCAACCT CGCCGGCCAC GTCAATCACA CCATCTGGTG
    351  GAAGAACCTG TCGCCTAACG GTGGTGACAA GCCCACCGGC GAACTCGCCG
    401  CAGCCATCGC CGACGCGTTC GGTTCGTTCG ACAAGTTCCG TGCGCAGTTC
    451  CACGCGGCCG CTACCACCGT GCAGGGGTCG GGCTGGGCGG CACTGGGCTG
    501  GGACACACTC GGCAACAAGC TGCTGATATT CCAGGTTTAC GACCACCAGA
    551  CGAACTTCCC GCTAGGCATT GTTCCGCTGC TGCTGCTCGA CATGTGGGAA
    601  CACGCCTTCT ACCTGCAGTA CAAGAACGTC AAAGTCGACT TTGCCAAGGC
    651  GTTTTGGAAC GTCGTGAACT GGGCCGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 5

Mycobacterium avium SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACCTACG TCAAAGGCGT GAACGACGCT CTTGCCAAGC
    251  TCGAAGAGGC CCGCGCCAAC GAGGACCACG CTGCGATCTT CCTGAACGAA
    301  AAGAACCTCG CCTTCCACCT GGGCGGCCAC GTCAACCACT CGATCTGGTG
    351  GAAGAACCTG TCGCCGGACG GCGGTGACAA GCCCACCGGT GAGCTGGCCG
    401  CCGCGATCGA CGACGCGTTC GGGTCCTTCG ACAAGTTCCG AGCGCAATTC
    451  AGCGCCGCCG CCAACGGCCT GCAGGGCTCC GGCTGGGCGG TGCTGGGCTA
    501  TGACACCCTG GGCAGCCGGT TGCTGACCTT CCAGCTCTAC GACCAGCGGG
    551  CCAACGTCCC GCTGGGCATC ATCCCGCTTC TGCAGGTCGA CATGTGGGAG
    601  CACGCGTTCT ACCTGCAGTA CAAGAACGTC AAGGCGGACT ACGTCAAGGC
    651  GTTCTGGAAC GTCGTGAACT GGGCCGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 6

Mycobacterium intracellulare SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACCTACG TCAAAGGCGT GAACGACGCT CTGTCCAAGC
    251  TCGAAGAGGC CCGTGCCAAC GAAGATCACG CTGCGATCTT CCTGAACGAA
    301  AAGAACCTGG CCTTTCACCT GGGCGGCCAC GTCAACCACT CCATCTGGTG
    351  GAAGAACCTG TCGCCGGACG GCGGCGACAA GCCGACCGGC GAATTGGCCG
    401  CCGCGATCGA CGACGCCTTC GGATCCTTCG ACCGGTTCCG CGCGCAGTTC
    451  AGCGCGGCCG CCAACGGCCT GCAGGGGTCG GGCTGGGCGG TGCTGGGCTA
    501  CGACACCCTC GGCAACCGGC TGCTGACCTT CCAGCTCTAC GACCAGCAGG
    551  CCAACGTGCC GCTGGGCATC ATTCCGCTGC TGCAGGTCGA CATGTGGGAG
    601  CACGCTTTCT ACCTGCAGTA CAAGAACGTC AAGGCGGACT ACGTCAAGGC
    651  GTTCTGGAAC GTCGTGAACT GGGACGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 7

Mycobacterium scrofulaceum SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACGTACG TCAAGGGCGT GAACGACGCC GTCGCCAAAC
    251  TCCAAGAGGC ACGCGCCAAT GACGACCACG CCGCGATCTT CCTGAACGAA
    301  AAGAACCTGG CGTTCCACCT CGGCGGCCAC GTGAACCACT CGATCTGGTG
    351  GAAGAACCTC TCGCCGGACG GCGGCGACAA GCCGACCGGA GAACTGGCCG
    401  CCGCGATCGA TGACGCGTTC GGATCGTTCG ACAAATTCCG CGCCCAGTTC
    451  AGTGCGGCCG CCAACGGCCT GCAGGGTTCG GGCTGGGCGG TGCTGGGCTA
    501  TGACACACTC GGCAGCAGGC TGCTCACCTT CCAGCTTTAC GACCAGCAGG
    551  CCAACGTCCC GCTCGGCATC ATTCCGCTGC TGCAGGTCGA CATGTGGGAG
    601  CACGCCTTTT ACTTGCAGTA CAAGAACGTC AAGGCCGACT ACGTCAAGGC
    651  CTTCTGGAAT GTCGTGAACT GGGCCGAG.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 8

Mycobacterium kansasii SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACCTACG TCAAGGGCGC CAACGATGCG GTCGCCAAAC
    251  TCGAAGAGGC GCGCGCCAAG GAAGACCACT CGGCGATCTT GCTGAACGAG
    301  AAGAACTTGG CCTTCAACCT CGCCGGCCAC GTCAACCACA CGATCTGGTG
    351  GAAGAACCTT TCTCCCAACG GAGGCGACAA GCCGACCGGC GAACTCGCCG
    401  CGGCCATCGA CGAGGCGTTC GGGTCCTTCG ACAAGTTTCG TGCCCAATTC
    451  CACGCCGCCG CCACCACGGT GCAGGGGTCG GGCTGGGCGG CGCTGGGCTG
    501  GGACACTCTC GGCAACAAGC TGCTGATATT CCAGGTCTAC GACCACCAGA
    551  CGAACTTTCC ACTCGGAATC ATTCCGTTAC TGCTGCTCGA CATGTGGGAA
    601  CACGCTTTCT ACCTCCAGTA CAAGAATGTC AAGGTCGACT TCGCCAAAGC
    651  ATTCTGGAAC GTCGTGAACT GGGACGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .........  .......... .......... ..........
```

Table 9

Mycobacterium fortuitum SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCGCGTACG TCAAGGGCGT CAACGACGCC TGGCCAAGC
    251  TCGATGAGGC GCGGGCCAAC GGTGACCACG CGGCGATCTT CCTCAACGAG
    301  AAGAACCTGG CGTTCCATCT CGGCGGCCAC GTGAACCACT CGATCTGGTG
    351  GAAGAACCTG TCCCCCAACG GTGGTGACAA GCCGACGGGC GATCTGGCCG
    401  CGGCGATCGA CGATCAGTTC GGCTCGTTCG ACAAGTTCCA GGCGCAGTTC
    451  ACCGCCGCCG CCAACGGGCT GCAGGGCTCG GGCTGGGCCG TGCTCGGCTA
    501  CGACAGCCTG GGCGATCGGC TGCTGACCTT CCAGCTCTAC GACCAGCAGG
    551  CCAACGTGCC GCTCGGCATC ATCCCGCTGC TCCAGGTCGA CATGTGGGAG
    601  CACGCCTTCT ACCTGCAGTA CAAGAACGTC AAGGCCGACT ACGTCAAGGC
    651  GTTCTGGAAA GTCGTGAACT GGGACGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .. ........ .......... .......... ..........
```

Table 10

Mycobacterium simiae SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAT GCGACGTACG TCAAGGGTTT GAACGACGCC ATTGCCAAGC
    251  TTGAAGAGGC GCGGGCCAAC GACGACCATG CCGCGATCTT CTTGAACGAG
    301  AAGAATCTGG CATTCCACCT CGGTGGCCAC GTCAACCACT CCATCTGGTG
    351  GAAAAACCTG TCCCCGAACG GCGGAGACAA GCCGACCGGA GATCTCGCCG
    401  CCGCCATCGA CGACGCCTTC GGTTCGTTCG ACAAGTTCCG CGCACAGTTC
    451  AGCGCCGCCG CCAACGGCTT GCAGGGCTCG GGCTGGGCGG TACTCGGCTA
    501  CGACACCCGG GGCGACCGAC TGCTGACCTT CCAGCTTTAC GACCAGCAGG
    551  CCAACGTCCC GCTGGGCATC ATCCCGCTGC TGCAGGTCGA CATGTGGGAG
    601  CACGCCTTCT ACCTGCAGTA CAAGAACGTC AAGGCGGACT ACGTCAAGGC
    651  GTTCTGGAAC GTCGTGAACT GGGACGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 11

Mycobacterium gordonae SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCACCTACG TCAAAGGCGT CAACGACCCG GTCGCCAAGC
    251  TGGAAGAAGC GCGCGCCAAA GGCGACCACT CGGCCATCTT TTTGAACGAG
    301  AAGAACCTGG CCTTCCACCT GGGCGGTCAC GTCAACCACT CCATCTGGTG
    351  GAAGAACCTG TCGCCGGACG GCGGCGACAA GCCGACCGGT GACCTGGCCG
    401  CCGCGATCGA CGACCAGTTC GGCTCGTTCG ACAAGTTCCA GGCTCAGTTC
    451  AGCGCCGCCG CAAACGGCCT ACAGGGCTCG GGCTGGGCGG TGCTCGGCTA
    501  CGACACTCTG GGCGGCCGGT TGCTCACCTT TCAGCTCTAC GACCAGCAGG
    551  CCAATGTCCC GCTCGGTGTC ATTCCGCTGT TGCAGGTCGA CATGTGGGAG
    601  CACGCCTTCT ACCTGCAGTA CAAGAACGTC AAGGCCGACT ACGTCAAGGC
    651  CTTCTGGAAC GTCGTGAACT GGGACGAC.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 12

Mycobacterium xenopi SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCGACGTACG TCAAAGGCGC CAACGACGCG CTCGCCAAGC
    251  TGGAGGAGGC GCGCGCCAAA GACGATCATT CCGCGATCGT CGGGCATGAG
    301  AAGGCCCTCG CGTTCAACCT GGCCGGCCAT GTCAATCACT GCCTGTGGTG
    351  GAAGAACCTG TCCCCCAACG GCGGTGACAA GCCGACCGGC GAATTGGCCG
    401  CCGCCATCGA CGACGCGTTC GGCTCGTTCG ACAAGTTCCG CGCCCAGTTC
    451  ACCGCGGCCG CCACGACCGT GCAGGGGTCG GGCTGGGCGG CACTCGGCTG
    501  GGACAGCCTG GGTGGCAAGC TCCTGGTGTT CCAGGTCTAC GACCACCAGT
    551  CCAACTTCCC GCTCGGGATC GTCCCCCTGC TGGTGCTCGA CATGTGGGAG
    601  CACGCCTTCT ACCTGCAGTA CAAGAATGTC AAGGCTGACT TCGCCAAAGC
    651  ATTCTGGAAC GTCGTGAACT GGGCCGAT.. .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 13

Corynebacterium diphteriae SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCTAACTACG TGAACGGTGC AAATACTGCT CTTGAGAAGC
    251  TGCAAAAGGC TCGCGAGAAC GGTGAGATCG GTGCTGTTGT CACCGCTTTG
    301  TCCAAGGATT TGGCTTTCAA CTTGGGTGGC CACACCAACC ACTCCATCTT
    351  CTGGAAGAAC CTCTCCCCTA ACGGTGGCGG CGAGCCAACC GGCGCTTTGG
    401  CTGAGGCAAT TGCCAAGGAG TTCGGTTCTT TTGAGAAGTT CAAGGATCAC
    451  TTCTCTGCTG CGGCTCTTGG TCTGCAGGGT TCCGGCTGGG CTGTTCTCGG
    501  CTACGATCAC ATCGGTGGCC GTCTGGTTAT CGAGCAGCTC ACTGACCAGC
    551  AGGGCAACAT CTCCGCTAAC CTGACCCCAC TTCTTATGCT CGATATGTGG
    601  GAGCACGCTT TCTACCTTCA GTACAAGAAC GTGAAGGCTG ACTACGTCAA
    651  GGCTGTGTGG AACGTCGTGA ACTGGGACGA TG........ ..........
    701  .......... .......... ........   .......... ..........
    751  .......... .......... .......... ..........
```

Table 14

C. pseudodiphteriticum SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC AACACTTACG TGCAGGGTGC TAACGCAGCT TTGGACGCTC
    251  TGGAAGAAGA GCGCAACGGC GAAGCCAACC CAGACCGCAT CCGTGCGCTG
    301  TCCAAGAACT TGGCTTTCCA ACCTGGCCAC ACCAACCACT CCATCTTCTG
    351  GAAGAACCTG AGCCCTAACG GTGGCGGCGA GCCAACCGGT GAGCTAGCAG
    401  AGGCTATCGA CCGCGACTTT GGTTCCTTCG AGAAGTTCAA GGCGCACTTC
    451  TCCGCAGCAG CACTCGGCCT GCAGGGTTCC GGCTGGGCCG TGCTGGGTTA
    501  CGACCACATT GCTGGTCGCC TGCTCGTTGA GCAGCTGACC GACCAGCAGG
    551  GCAACACTTC CGTGAACTTC ACCCCACTGC TGATGCTGGA TATGTGGGAG
    601  CACGCTTTCT ACCTGCAGTA CAAGAACGTC AAGCCTGATT ACGTCAAGGC
    651  TGTCTGGAAC GTCGTGAACT GGGACGAT.. .......... ..........
    701  .......... .......... .......... ..........
```

Table 15

Nocardia asteroides SOD gene for super oxide dismutase

```
      1  .......... .......... .......... .......... ..........
     51  .......... .......... .......... .......... ..........
    101  .......... .......... .......... .......... ..........
    151  .......... .......... .......... .......AGC TTCACCACAG
    201  CAAGCACCAC GCCGCCTACG TCGCCGGTGC CAACACGGCA CTGGAGAAGC
    251  TGGAAGCCGC CCGTGAGGCC GGCGATCACA GCGCGATCTT CCTGCACGAG
    301  AAGAACCTCG CGTTCCACCT CGGCGGACAC GTCAACCACT CCATCTGGTG
    351  GAAGAACCTG TCCCCCAACG GTGGCGACAA GCCGGTCGGC GAGCTGGCCG
    401  CGGCCATCGA CGACCAGTTC GGTTCGTTCG ACAAGTTCCG CGCGCAGTTC
    451  ACCGCCGCGC CAACGGCCTG CAGGGCTCGG GCTGGGCGGT GCTCGGTTAC
    501  GACACCCTCG GCCAGAAGCT GCTGACCTTC CAGCTCTACG ACCAGCAGGC
    551  CAACGTGCCG CTGGGCATCA TCCCGCTGCT CCAAGTCGAC ATGTGGGAGC
    601  ACGCCTTCTA CCTGCAGTAC AAGAACGTCA AGGCCGACTA CGTGACCGCG
    651  TTGTGGAACG TCGTGCACTG GGCCGAT... .......... ..........
    701  .......... .......... .......... .......... ..........
    751  .......... .......... .......... ..........
```

Table 16

Actinomyces viscosus SOD gene for super oxide dismutase

```
   1  ..........  ..........  ..........  ..........  ..........
  51  ..........  ..........  ..........  ..........  ..........
 101  ..........  ..........  ..........  ..........  ..........
 151  ..........  ..........  ..........  .......AGC  TTCACCACAG
 201  CAAGCACCAC  GCCGCCTACG  TCGCTGGCGC  CAACGCCGCC  CTGGAGGCCC
 251  TCGCCGCCGC  CCGCGAGGAC  GGCGACCTGG  GTGCGATCAA  CCTGTGGGAG
 301  AAGAACCTCG  CCTTCAACCT  GGGCGGCCAC  ACCAACCACT  CCGTGTTCTG
 351  GAAGAACCTC  TCCCCCAACG  GCGGCGGCCA  GCCCGAGGGC  GAGCTCGCCG
 401  AGGCCATCAA  GGACTCCTTC  GGCTCCTTCG  AGAAGTTCCA  GGCGCAGTTC
 451  ACCGCCACCG  CCCTGGGCAT  CCAGGGCTCG  GGCTGGGCCG  TGCTCGCCTA
 501  CGACTCAATC  TCCGGCAAGC  TGCTGATCTT  CCAGCTCTTC  GACCAGCAGG
 551  CCAACGTGCC  CGTGGGCACG  ACCCCGCTGT  TCATGGTGGA  CATGTGGGAG
 601  CACGCATTCT  ACCTCGACTA  CCTCAACGTC  AAGGCCGACT  ACGTCAAGGC
 651  CATCTGGAAC  GTCGTGAACT  GGGACGAT..  ..........  ..........
 701  ..........  ..........  ..........  ..........  ..........
 751  ..........  ..........  ..........  ..........
```

Table 17

Mismatchanalysis for PCR Primer Z261 (X = number of mismatches)

	Z261	5'-CCA A^{A}_{G}C TCG AAG AGG CGC G^{C}_{G}G CCA A-3'	X
	M. avium	5'-... ... ... .C. ... .-3'	1
	M. fortuitum	5'-... ... ... .T. ... ...-3'	1
	M. gordonae	5'-... ... .G. ... .A. ... ...-3'	2
	M. intrac.	5'-... ... ... .C. .T. ... .-3'	2
	M. kansasii	5'-... ... ...-3'	0
	M. scroful.	5'-... ... ..C ... ... A.. ... .-3'	2
	M. simiae	5'-... ... .T. ... ...-3'	1
	M. tuberculosis	5'-... ... ...-3'	0
	M. xenopi	5'-... ... .G. .G. ... ...-3'	2
	A. viscosus	5'-AGG CC. ... CC. CC. .C. ... AGG .-3'	13
	C. diphth.	5'-AG. ... .GC ..A ... .T. ... AG. .-3'	8
	C. ps. dipht.	5'-A.G CT. .G. ... .A. A.. ..A A.G G-3'	11
	Nocardia	5'-AG. ... .G. ... CC. .C. .T. AGG C-3'	11
	Human *	5'-GG. ... ATT ..A G.A .TG A.T GA. G-3'	15
	E. coli **	5'-AA. GC. .GC C.. .AT TTG C.A A.C T-3'	17

* taken from sequence AC M123267 / ** taken from sequence AC X03951
EMBL Data Bank, Heidelberg, FRG, and GenBank, Los Alamos, USA

Table 18

Consensus sequence:

		5'-GAC AAG CCC ACG GGT GAG CTG GCC GCG GCC ATC GAT G-3'
		G C C T T C A G CC
		A C A C
	A. viscosus	5'-.G. C.. ... GA. .. .. .A. ... ... A.G .-3'
	C. diphth.	5'-.G. GGC GA. C.A AC. .G. GCT TTG ..T .A. GCA AT. .-3'
	C. ps. dipht.	5'-.G. G.. ..A ... ...A ..A .A. ..T ... ... C-3'
	N. asteroides	5'-... ... ... GT. ... ...-3'
	E. coli	5'-AC. .CC .T. CA. ... ... AAA ... ..T ... ..A C-3'
	Human	5'-.TG ... GT. TG. ... AG. A.T AAA .G. CT. .CT ..A .-3'

Individual oligonucleotides constituting the pool of genus-specific probes:

Z310	M. tuberc.	5'-GAC AAG CCC ACC GGC GAA CTC GCC GCA GCC ATC GCC G-3'
Z311	M. intrac.	5'-GAC AAG CCG ACC GGC GAA TTG GCC GCC GCG ATC GAC G-3'
	M. xenopi	C
Z312	M. avium	5'-GAC AAG CCC ACC GGT GAG CTG GCC GCC GCG ATC GAC G-3'
Z313	M. gord.	5'-GAC AAG CCG ACC GGT GAC CTG GCC GCC GCG ATC GAC G-3'
Z314	M. fort.	5'-GAC AAG CCG ACG GGC GAT CTG GCC GCG GCG ATC GAC G-3'
Z315	M. scroful.	5'-GAC AAG CCG ACC GGA GAA CTG GCC GCC GCG ATC GAT G-3'
Z316	M. simiae	5'-GAC AAG CCG ACC GGA GAT CTC GCC GCC GCC ATC GAC G-3'
Z317	M. kans.	5'-GAC AAG CCG ACC GGC GAA CTC GCC GCG GCC ATC GAC G-3'

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGCTTCACCA CAGCAAGCAC CA 22

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TCGKCCCAGT TCACGACRTT CCA 23

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCAARCTCGA AGAGGCGCGS GCCAA 25

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GACAAGCCSA CSGGHGANYT SGCCGCVGCS ATCGMYG 36

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GACAAGCCCA CCGGCGAACT CGCCGCAGCC ATCGCCG 37

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GACAAGCCGA CCGGCGAATT GGCCGCCGCS ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GACAAGCCCA CCGGTGAGCT GGCCGCCGCG ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GACAAGCCGA CCGGTGACCT GGCCGCCGCG ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACAAGCCGA CGGGCGATCT GGCCGCGGCG ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GACAAGCCGA CCGGAGAACT GGCCGCCGCG ATCGATG 37

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GACAAGCCGA CCGGAGATCT CGCCGCCGCC ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GACAAGCCGA CCGGCGAACT CGCCGCGGCC ATCGACG 37

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCTTCGGATC CTTCGACCGG TTCCGCGCGC AGTTCAG 37

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GTCCTTCGAC AAGTTCCGAG CGCAATTCAG CGCCGCC 37

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TCTGGGCGGC CGGTTGCTCA CCTTT 25

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTCCCCGAAC GGCGGAGACA AGCCGACCGG AGATCTC 37

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCTAGGCATT GTTCCGCTGC TGCTGC 26

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TCCGCGATCG TCGGGCATGA GAAGGCCCTC GCGTTCA 37

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CCAGACGAAC TTTCCACTCG GA 22

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGACACACTC GGCAGCAGGC TGCTCACCTT CCAGCTT 37

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

ACGACAGCCT GGGCGATCGG CT 22

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ACGAACTTCC CGCTAGGCAT TGTTCCGCTG CTGCTGC 37

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGTCGACTTT GCCAAGGCGT TT 22

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CACWCSATCT GGTGGAAGAA CCT 23

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACGGYGGYGA CAAGCCGACC GG 22

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GTCTGSTGGT CGTARASCTG GA 22

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GGATTTGGCT TTCAACTTGG G 21

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGCGAGCCAA CCGGCGCTTT GG 22

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAAGAACCTG AGCCTTAACG GT 22

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GAGCCAACCG GTGAGCTAGC C 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GAAGAACCTC TCCCCCAACG GC 22

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTAAAGGG CGCCAATGAC GCCGTCGCCA 60

AACTCGAAGA GGCGCGCGCC AAGGAAGATC ACTCAGCGAT CTTGCTGAAC GAAAAGAATC 120

TAGCTTTCAA CCTCGCCGGC CACGTCAATC ACACCATCTG GTGGAAGAAC CTGTCGCCTA 180

ACGGTGGTGA CAAGCCCACC GGCGAACTCG CCGCAGCCAT CGCCGACGCG TTCGGTTCGT 240

TCGACAAGTT CCGTGCGCAG TTCCACGCGG CCGCTACCAC CGTGCAGGGG TCGGGCTGGG 300

CGGCACTGGG CTGGGACACA CTCGGCAACA AGCTGCTGAT ATTCCAGGTT TACGACCACC 360

AGACGAACTT CCCGCTAGGC ATTGTTCCGC TGCTGCTGCT CGACATGTGG GAACACGCCT 420

TCTACCTGCA GTACAAGAAC GTCAAAGTCG ACTTTGCCAA GGCGTTTTGG AACGTCGTGA 480

ACTGGGCCGA T 491

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium avium/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTGAACGAC GCTCTTGCCA 60

AGCTCGAAGA GGCCCGCGCC AACGAGGACC ACGCTGCGAT CTTCCTGAAC GAAAAGAACC 120

TCGCCTTCCA CCTGGGCGGC CACGTCAACC ACTCGATCTG GTGGAAGAAC CTGTCGCCGG 180

ACGGCGGTGA CAAGCCCACC GGTGAGCTGG CCGCCGCGAT CGACGACGCG TTCGGGTCCT 240

TCGACAAGTT CCGAGCGCAA TTCAGCGCCG CCGCCAACGG CCTGCAGGGC TCCGGCTGGG 300

CGGTGCTGGG CTATGACACC CTGGGCAGCC GGTTGCTGAC CTTCCAGCTC TACGACCAGC 360

GGGCCAACGT CCCGCTGGGC ATCATCCCGC TTCTGCAGGT CGACATGTGG GAGCACGCGT 420

TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GGCGTTCTGG AACGTCGTGA 480

ACTGGGCCGA T 491

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium intracellulare/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTGAACGAC GCTCTGTCCA 60

AGCTCGAAGA GGCCCGTGCC AACGAAGATC ACGCTGCGAT CTTCCTGAAC GAAAAGAACC 120

TGGCCTTTCA CCTGGGCGGC CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCGCCGG 180

ACGGCGGCGA CAAGCCGACC GGCGAATTGG CCGCCGCGAT CGACGACGCC TTCGGATCCT 240

TCGACCGGTT CCGCGCGCAG TTCAGCGCGG CCGCCAACGG CCTGCAGGGG TCGGGCTGGG 300

CGGTGCTGGG CTACGACACC CTCGGCAACC GGCTGCTGAC CTTCCAGCTC TACGACCAGC 360

AGGCCAACGT GCCGCTGGGC ATCATTCCGC TGCTGCAGGT CGACATGTGG GAGCACGCTT 420

TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GGCGTTCTGG AACGTCGTGA 480

ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Mycobacterium scrofulaceum/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

AGCTTCACCA CAGCAAGCAC CACGCCACGT ACGTCAAGGG CGTGAACGAC GCCGTCGCCA 60

AACTCCAAGA GGCACGCGCC AATGACGACC ACGCCGCGAT CTTCCTGAAC GAAAAGAACC 120

TGGCGTTCCA CCTCGGCGGC CACGTGAACC ACTCGATCTG GTGGAAGAAC CTCTCGCCGG 180

ACGGCGGCGA CAAGCCGACC GGAGAACTGG CCGCCGCGAT CGATGACGCG TTCGGATCGT 240

TCGACAAATT CCGCGCCCAG TTCAGTGCGG CCGCCAACGG CCTGCAGGGT TCGGGCTGGG 300

CGGTGCTGGG CTATGACACA CTCGGCAGCA GGCTGCTCAC CTTCCAGCTT TACGACCAGC 360

AGGCCAACGT CCCGCTCGGC ATCATTCCGC TGCTGCAGGT CGACATGTGG GAGCACGCCT 420

TTTACTTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GGCCTTCTGG AATGTCGTGA 480

ACTGGGCCGA G 491

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium kansasii/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAGGG CGCCAACGAT GCGGTCGCCA 60

AACTCGAAGA GGCGCGCGCC AAGGAAGACC ACTCGGCGAT CTTGCTGAAC GAGAAGAACT 120

TGGCCTTCAA CCTCGCCGGC CACGTCAACC ACACGATCTG GTGGAAGAAC CTTTCTCCCA 180

ACGGAGGCGA CAAGCCGACC GGCGAACTCG CCGCGGCCAT CGACGAGGCG TTCGGGTCCT 240

TCGACAAGTT TCGTGCCCAA TTCCACGCCG CCGCCACCAC GGTGCAGGGG TCGGGCTGGG 300

CGGCGCTGGG CTGGGACACT CTCGGCAACA AGCTGCTGAT ATTCCAGGTC TACGACCACC 360

AGACGAACTT TCCACTCGGA ATCATTCCGT TACTGCTGCT CGACATGTGG GAACACGCTT 420

TCTACCTCCA GTACAAGAAT GTCAAGGTCG ACTTCGCCAA AGCATTCTGG AACGTCGTGA 480

ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium fortuitum/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

AGCTTCACCA CAGCAAGCAC CACGCCGCGT ACGTCAAGGG CGTCAACGAC GCCGTGGCCA 60

AGCTCGATGA GGCGCGGGCC AACGGTGACC ACGCGGCGAT CTTCCTCAAC GAGAAGAACC 120

TGGCGTTCCA TCTCGGCGGC CACGTGAACC ACTCGATCTG GTGGAAGAAC CTGTCCCCCA 180

ACGGTGGTGA CAAGCCGACG GGCGATCTGG CCGCGGCGAT CGACGATCAG TTCGGCTCGT 240

TCGACAAGTT CCAGGCGCAG TTCACCGCCG CCGCCAACGG GCTGCAGGGC TCGGGCTGGG 300

CCGTGCTCGG CTACGACAGC CTGGGCGATC GGCTGCTGAC CTTCCAGCTC TACGACCAGC 360

AGGCCAACGT GCCGCTCGGC ATCATCCCGC TGCTCCAGGT CGACATGTGG GAGCACGCCT 420

TCTACCTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GGCGTTCTGG AAAGTCGTGA 480

ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium simiae/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

AGCTTCACCA CAGCAAGCAC CATGCGACGT ACGTCAAGGG TTTGAACGAC GCCATTGCCA 60

AGCTTGAAGA GGCGCGGGCC AACGACGACC ATGCCGCGAT CTTCTTGAAC GAGAAGAATC 120

TGGCATTCCA CCTCGGTGGC CACGTCAACC ACTCCATCTG GTGGAAAAAC CTGTCCCCGA 180

ACGGCGGAGA CAAGCCGACC GGAGATCTCG CCGCCGCCAT CGACGACGCC TTCGGTTCGT 240

TCGACAAGTT CCGCGCACAG TTCAGCGCCG CCGCCAACGG CTTGCAGGGC TCGGGCTGGG 300

CGGTACTCGG CTACGACACC CGGGGCGACC GACTGCTGAC CTTCCAGCTT TACGACCAGC 360

AGGCCAACGT CCCGCTGGGC ATCATCCCGC TGCTGCAGGT CGACATGTGG GAGCACGCCT 420

TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GGCGTTCTGG AACGTCGTGA 480

ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium gordonae/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTCAACGAC GCGGTCGCCA 60

AGCTGGAAGA AGCGCGCGCC AAAGGCGACC ACTCGGCCAT CTTTTTGAAC GAGAAGAACC 120

TGGCCTTCCA CCTGGGCGGT CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCGCCGG 180

ACGGCGGCGA CAAGCCGACC GGTGACCTGG CCGCCGCGAT CGACGACCAG TTCGGCTCGT 240

TCGACAAGTT CCAGGCTCAG TTCAGCGCCG CCGCAAACGG CCTACAGGGC TCGGGCTGGG 300

CGGTGCTCGG CTACGACACT CTGGGCGGCC GGTTGCTCAC CTTTCAGCTC TACGACCAGC 360

AGGCCAATGT CCCGCTCGGT GTCATTCCGC TGTTGCAGGT CGACATGTGG GAGCACGCCT 420

TCTACCTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GGCCTTCTGG AACGTCGTGA 480

ACTGGGACGA C 491

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium xenopi/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

AGCTTCACCA CAGCAAGCAC CACGCGACGT ACGTCAAAGG CGCCAACGAC GCGCTCGCCA 60

AGCTGGAGGA GGCGCGCGCC AAAGACGATC ATTCCGCGAT CGTCGGGCAT GAGAAGGCCC 120

TCGCGTTCAA CCTGGCCGGC CATGTCAATC ACTGCCTGTG GTGGAAGAAC CTGTCCCCCA 180

ACGGCGGTGA CAAGCCGACC GGCGAATTGG CCGCCGCCAT CGACGACGCG TTCGGCTCGT 240

TCGACAAGTT CCGCGCCCAG TTCACCGCGG CCGCCACGAC CGTGCAGGGG TCGGGCTGGG 300

CGGCACTCGG CTGGGACAGC CTGGGTGGCA AGCTCCTGGT GTTCCAGGTC TACGACCACC 360

AGTCCAACTT CCCGCTCGGG ATCGTCCCCC TGCTGGTGCT CGACATGTGG GAGCACGCCT 420

TCTACCTGCA GTACAAGAAT GTCAAGGCTG ACTTCGCCAA AGCATTCTGG AACGTCGTGA 480

ACTGGGCCGA T 491

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Corynebacterium diphteriae/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

AGCTTCACCA CAGCAAGCAC CACGCTAACT ACGTGAACGG TGCAAATACT GCTCTTGAGA 60

AGCTGCAAAA GGCTCGCGAG AACGGTGAGA TCGGTGCTGT TGTCACCGCT TTGTCCAAGG 120

ATTTGGCTTT CAACTTGGGT GGCCACACCA ACCACTCCAT CTTCTGGAAG AACCTCTCCC 180

CTAACGGTGG CGGCGAGCCA ACCGGCGCTT TGGCTGAGGC AATTGCCAAG GAGTTCGGTT 240

CTTTTGAGAA GTTCAAGGAT CACTTCTCTG CTGCGGCTCT TGGTCTGCAG GGTTCCGGCT 300

GGGCTGTTCT CGGCTACGAT CACATCGGTG GCCGTCTGGT TATCGAGCAG CTCACTGACC 360

AGCAGGGCAA CATCTCCGCT AACCTGACCC CACTTCTTAT GCTCGATATG TGGGAGCACG 420

CTTTCTACCT TCAGTACAAG AACGTGAAGG CTGACTACGT CAAGGCTGTG TGGAACGTCG 480

TGAACTGGGA CGATG 495

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: C. pseudodiphteriticum/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

AGCTTCACCA CAGCAAGCAC CACAACACTT ACGTGCAGGG TGCTAACGCA GCTTTGGACG 60

CTCTGGAAGA AGAGCGCAAC GGCGAAGCCA ACCCAGACCG CATCCGTGCG CTGTCCAAGA 120

ACTTGGCTTT CCAACCTGGC CACACCAACC ACTCCATCTT CTGGAAGAAC CTGAGCCCTA 180

ACGGTGGCGG CGAGCCAACC GGTGAGCTAG CAGAGGCTAT CGACCGCGAC TTTGGTTCCT 240

TCGAGAAGTT CAAGGCGCAC TTCTCCGCAG CAGCACTCGG CCTGCAGGGT TCCGGCTGGG 300

CCGTGCTGGG TTACGACCAC ATTGCTGGTC GCCTGCTCGT TGAGCAGCTG ACCGACCAGC 360

AGGGCAACAC TTCCGTGAAC TTCACCCCAC TGCTGATGCT GGATATGTGG GAGCACGCTT 420

TCTACCTGCA GTACAAGAAC GTCAAGCCTG ATTACGTCAA GGCTGTCTGG AACGTCGTGA 480

ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 490 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nocardia asteroides/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

AGCTTCACCA CAGCAAGCAC CACGCCGCCT ACGTCGCCGG TGCCAACACG GCACTGGAGA 60

AGCTGGAAGC CGCCCGTGAG GCCGGCGATC ACAGCGCGAT CTTCCTGCAC GAGAAGAACC 120

TCGCGTTCCA CCTCGGCGGA CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCCCCCA 180

ACGGTGGCGA CAAGCCGGTC GGCGAGCTGG CCGCGGCCAT CGACGACCAG TTCGGTTCGT 240

TCGACAAGTT CCGCGCGCAG TTCACCGCCG CGCCAACGGC CTGCAGGGCT CGGGCTGGGC 300

GGTGCTCGGT TACGACACCC TCGGCCAGAA GCTGCTGACC TTCCAGCTCT ACGACCAGCA 360

GGCCAACGTG CCGCTGGGCA TCATCCCGCT GCTCCAAGTC GACATGTGGG AGCACGCCTT 420

CTACCTGCAG TACAAGAACG TCAAGGCCGA CTACGTGACC GCGTTGTGGA ACGTCGTGCA 480

CTGGGCCGAT 490

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Actinomyces viscosus/SOD gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

AGCTTCACCA CAGCAAGCAC CACGCCGCCT ACGTCGCTGG CGCCAACGCC GCCCTGGAGG 60

CCCTCGCCGC CGCCCGCGAG GACGGCGACC TGGGTGCGAT CAACCTGTGG GAGAAGAACC 120

TCGCCTTCAA CCTGGGCGGC CACACCAACC ACTCCGTGTT CTGGAAGAAC CTCTCCCCCA 180

ACGGCGGCGG CCAGCCCGAG GGCGAGCTCG CCGAGGCCAT CAAGGACTCC TTCGGCTCCT 240

TCGAGAAGTT CCAGGCGCAG TTCACCGCCA CCGCCCTGGG CATCCAGGGC TCGGGCTGGG 300

CCGTGCTCGC CTACGACTCA ATCTCCGGCA AGCTGCTGAT CTTCCAGCTC TTCGACCAGC 360

AGGCCAACGT GCCCGTGGGC ACGACCCCGC TGTTCATGGT GGACATGTGG GAGCACGCAT 420

TCTACCTCGA CTACCTCAAC GTCAAGGCCG ACTACGTCAA GGCCATCTGG AACGTCGTGA 480

ACTGGGACGA T 491

The claims defining the invention are as follows:

1. A method for detecting pathogenic and non-pathogenic organisms of the genus Mycobacterium, which comprises

(a) amplifying a target region of a superoxide dismutase (SOD) gene of an organism, using a pair of primers which is specific for the genus Mycobacterium,

(b) contacting the amplified target sequence with at least one oligonucleotide probe capable of hybridizing to the target region of the SOD gene; and

(c) detecting hybrids formed between the amplified target region, if any, and the oligonucleotide probe.

2. The method of claim 1, wherein the target region is amplified by means of the polymerase chain reaction (PCR).

3. The method of claim 1 or claim 2, wherein the pair of primers which is specific for the genus Mycobacterium consists of a first primer containing the sequence of Seq. Id. No. 3 (Z261) as priming sequence and a second primer containing the sequence of Seq. Id. No. 2 (Z212) as priming sequence.

4. The method of any of claims 1 to 3, wherein the oligonucleotide probe is capable of hybridizing to a region of the target sequence which is substantially conserved among the SOD genes of the different species within the genus Mycobacterium.

5. The method of claim 4, wherein said probe contains the sequence of Seq. Id. No. 4 or a sequence complementary thereto as hybridizing sequence.

6. The method of claim 4, wherein said probe contains a sequence selected from the group consisting of sequences of Seq. Id. No. 5 (Z310), Seq. Id. No. 6 (Z311), Seq. Id. No. 7 (Z312), Seq. Id. No. 8 (Z313), Seq. Id. No. 9 (Z314), Seq. Id. No. 10 (Z315), Seq. Id. No. 11 (Z316), Seq. Id. No. 12 (Z317) and the sequences complementary to each of the foregoing sequences, as hybridizing sequence.

7. The method of any of claims 1 to 3, wherein the oligonucleotide probe is capable of hybridizing to a region of the target sequence which is variable to a degree allowing differentiation among the SOD genes of the different species within the genus Mycobacterium.

8. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. intracellulare.

9. The method of claim 8, wherein said probe contains the sequence of Seq. Id. No. 13 (Z303) or a sequence complementary thereto.

10. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. avium and M. brunense.

11. The method of claim 10, wherein said probe contains the sequence of Seq. Id. No. 14 (Z301) or a sequence complementary thereto.

12. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. gordonae.

13. The method of claim 12, wherein said probe contains the sequence of Seq. Id. No. 15 (Z366) or a sequence complementary thereto.

14. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. simiae.

15. The method of claim 14, wherein said probe contains the sequence of Seq. Id. No. 16 (Z304) or a sequence complementary thereto.

16. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. tuberculosis.

17. The method of claim 16, wherein said probe contains the sequence of Seq. Id. No. 17 (Z336), Seq. Id. No. 23 (Z337) or Seq. Id. No. 22 (Z302) or a sequence complementary thereto.

18. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. xenopi.

19. The method of claim 18, wherein said probe contains the sequence of Seq. Id. No. 18 (Z309) or a sequence complementary thereto.

20. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. kansasii.

21. The method of claim 20, wherein said probe contains the sequence of Seq. Id. No. 19 (Z340) or a sequence complementary thereto.

22. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. scrofulaceum.

23. The method of claim 22, wherein said probe contains the sequence of Seq. Id. No. 20 (Z306) or a sequence complementary thereto.

24. The method of claim 7, wherein said probe is capable of hybridizing to a species-specific region of the SOD gene of the species M. fortuitum.

25. The method of claim 24, wherein said probe contains the sequence of Seq. Id. No. 21 (Z369) or a sequence complementary thereto.

26. An oligonucleotide capable of hybridizing to the amplified target sequence obtainable at the end of step a) of the method of claim 3, which is capable of hybridizing to a region of the target sequence which is substantially conserved among the SOD genes of the different species belonging to the genus Mycobacterium.

27. An oligonucleotide according to claim 26 with the sequence of Seq. Id. No. 4, or a sequence complementary thereto.

28. An oligonucleotide according to claim 27 selected from the group consisting of sequences of Seq. Id. No. 5 (Z310), Seq. Id. No. 6 (Z311), Seq. Id. No. 7 (Z312), Seq. Id. No. 8 (Z313), Seq. Id. No. 9 (Z314), Seq. Id. No. 10 (Z315), Seq. Id. No. 11 (Z316) and Seq. Id. No. 12 (Z317) and the sequences complementary to each of the foregoing sequences.

29. An oligonucleotide capable of hybridizing to the amplified target sequence obtainable at the end of step a) of the method of claim 3, which is capable of hybridizing

to a region of the target sequence which is variable to a degree allowing differentiation among the SOD genes of the different species belonging to the genus Mycobacterium.

30. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. intracellulare.

31. An oligonucleotide according to claim 30, containing the sequence of Seq. Id. No. 13 (Z303) or a sequence complementary thereto.

32. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. avium and M. brunense.

33. An oligonucleotide according to claim 32, containing the sequence of Seq. Id. No. 14 (Z301) or a sequence complementary thereto.

34. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. gordonae.

35. An oligonucleotide according to claim 34, containing the sequence of Seq. Id. No. 15 (Z366) or a sequence complementary thereto.

36. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. simiae.

37. An oligonucleotide according to claim 36, containing the sequence of Seq. Id. No. 16 (Z304) or a sequence complementary thereto.

38. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. tuberculosis.

39. An oligonucleotide according to claim 38, containing the sequence of Seq. Id. No. 17 (Z336), Seq. Id. No. 23 (Z337) or Seq. Id. No. 22 (Z302) or a sequence complementary thereto.

40. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. xenopi.

41. An oligonucleotide according to claim 40, containing the sequence of Seq. Id. No. 18 (Z309) or a sequence complementary thereto.

42. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. kansasii.

43. An oligonucleotide according to claim 42, containing the sequence of Seq. Id. No. 19 (Z340) or a sequence complementary thereto.

44. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. scrofulaceum.

45. An oligonucleotide according to claim 44, containing the sequence of Seq. Id. No. 20 (Z306) or a sequence complementary thereto.

46. An oligonucleotide according to claim 29, which is capable of hybridizing to a species-specific region of the SOD gene of the species M. fortuitum.

47. An oligonucleotide according to claim 46, containing the sequence of Seq. Id. No. 21 (Z369) or a sequence complementary thereto.

48. A probe comprising an oligonucleotide according to any one of claims 26-28 as hybridizing sequence.

49. A pool of probes comprising two or more oligonucleotides according to claim 28 as hybridizing sequences.

50. A probe comprising an oligonucleotide according to any one of claims 29-47 as hybridizing sequences.

51. A primer comprising as priming sequence a sequence which is substantially homologous to the sequence of Seq. Id. No. 1 (Z205).

52. A primer comprising as priming sequence a sequence which is substantially homologous to the sequence Seq. Id. No. 2 (Z212).

53. A pair of primers, the first primer comprising the sequence of Seq. Id. No. 1 (Z205) as priming sequence and the second primer containing the sequence of Seq. Id. 2 (Z212) as priming sequence.

54. A primer comprising as priming sequence a sequence which is substantially homologous to the sequence of Seq. Id. No. 3 (Z261).

55. A pair of primers, the first primer comprising the sequence of Seq. Id. No. 3 (Z261) as priming sequence and the second primer containing the sequence of Seq. Id. 2 (Z212) as priming sequence.

56. A set of reagents for amplifying a part of a gene coding for a superoxide dismutase (SOD) comprising a primer according to claim 51 and/or 54 and a primer according to claim 52.

57. A set of reagents for detecting a mycobacterial species comprising a probe according to claim 48 or 49.

58. A set of reagents for differentiating among different species belonging to the genus Mycobacterium comprising a probe according to claim 50.

59. A set of reagents according to claim 57 or 58 further comprising a primer according to claim 51 and/or 54 and a primer according to claim 52.

60. A method for detecting pathogenic and non-pathogenic organisms of the genus Mycobacterium, substantially as hereinbefore described with reference to any one of the Examples.

61. An oligonucleotide capable of hybridizing to the amplified target sequence, substantially as hereinbefore described with reference to any one of the Examples.

62. A probe specific to a Mycobacterium species, substantially as hereinbefore described with reference to any one of the Examples.

63. A Mycobacterium specific primer, substantially as hereinbefore described with reference to any one of the Examples.

Dated 10 July, 1995

F.Hoffmann-La Roche AG

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

Oligonucleotides Derived from the SOD Family

Abstract

RAN 4095/095

The use of the superoxide dismutase (SOD; EC 1.15.1.1) gene system as a target for the detection and differentiation of pathogenic and non-pathogenic organisms including bacteria, fungi and protozoans is described. More particularly, there are described oligonucleotides derived from the SOD gene family which oligonucleotides are capable of hybridyzing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying and denaturing a part of a gene coding for a superoxide dismutase (SOD) using a polymerase chain reaction (PCR) and a pair of primers, the first primer containing the sequence 5'-AGC TTC ACC ACA GCA AGC ACC A-3' (Seq. Id. No. 1; Z205) as priming sequence and the second primer containing the sequence 5'-TCG G(T)CC CAG TTC ACG ACG(A,T) TTC CA-3' (Seq. Id. No. 2; Z212) as priming sequence.

These oligonucleotides may be used in the form of primers and probes to amplify and detect target sequences derived from SOD genes, especially, from SOD genes of pathogenic and non-pathogenic organisms including bacteria (e.g. mycobacteria), fungi and protozoans.

Fig. 1

POSITION AND ORIENTATION OF PRIMERS USED FOR SEQUENCING CLONED SOD - AMPLICONS

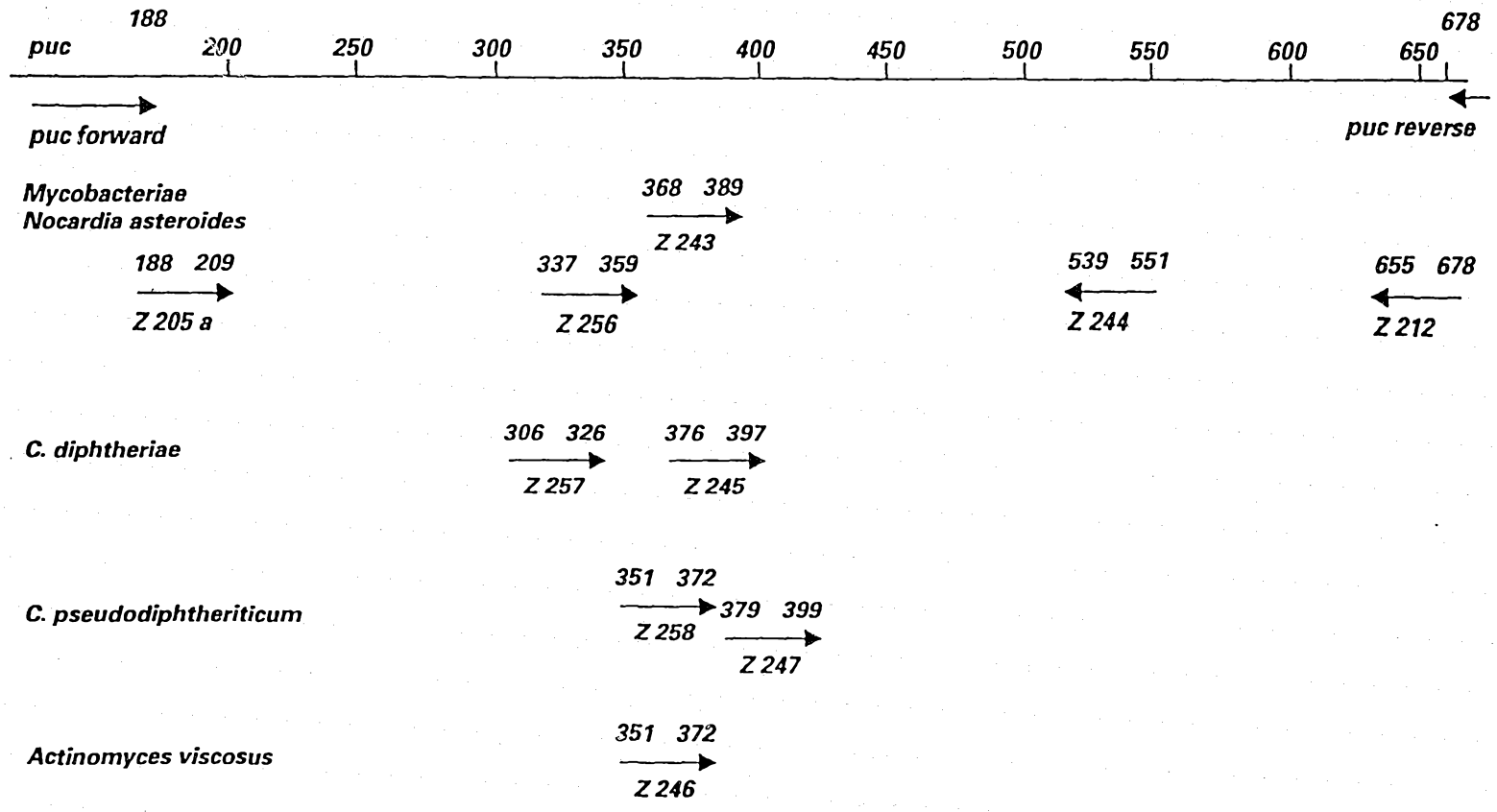


Fig. 2

Hybridization with Probe specific for M. intracellulare