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(54) Titre : OLIGONUCLEOTIDES DERIVES DE LA FAMILLE DES SOD
(54) Title: OLIGONUCLEOTIDES DERIVED FROM THE SOD FAMILY

(57) **Abrégé/Abstract:**

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 hybridizing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying and enaturing 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 in the form of primers and probes are useful for the amplification and detection of target sequences derived from SOD genes, especially, from SOD genes of pathogenic and non-pathogenic organisms including bacteria (e.g. mycobacteria), fungi and protozoans.



ABSTRACT

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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 hybridizing to a single-stranded nucleic acid sequence (target sequence) which is obtainable by amplifying and enaturing 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 in the form of primers and probes are useful for the amplification and detection of target sequences derived from SOD genes, especially, from SOD genes of pathogenic and non-pathogenic organisms including bacteria (e.g. mycobacteria), fungi and protozoans.

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 including bacteria, fungi and protozoans. In another aspect, the present invention
5 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
10 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.

15 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 including bacteria, fungi and protozoans. The primers and probes according to the present invention may also be used for amplifying and detecting target sequences derived from SOD
20 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.

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



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
10 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
15 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)
20 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
25 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° - 100°C range (exact value depending on target sequence and concentration).

30 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
35 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
5 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
10 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
15 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
20 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
25 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
30 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
35 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.

Preferred oligonucleotides in accordance with the present invention are those which are capable of hybridizing to a region of the target sequence which is substantially conserved among the SOD genes of different organisms such as bacteria, fungi and protozoa (universal primers) or substantially conserved among the SOD genes of the different species belonging to a particular genus (genus specific oligonucleotides), and those which are 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 a particular genus (species specific oligonucleotides).

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.

As mentioned earlier, the oligonucleotides of the present invention may be used in the form of primers and probes to amplify and detect target sequences derived from SOD genes of pathogenic organisms including bacteria, fungi and protozoans and of non-pathogenic organisms. In a preferred aspect, 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 species belonging to a particular genus. Genus detection may then be performed by mixing the amplified nucleic acid with genus specific probes and detecting if hybridization occurs, whereas species identification is carried out by determining the pattern of hybridization of the amplified nucleic acid to species specific probes.

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

5 (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* which 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;

10 (b) contacting the amplified target sequence with at least one oligonucleotide probe sufficiently complementary to the target region of the SOD gene to reliably hybridize thereto; and

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

15 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.

5 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.

10 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).
15 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.

20 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
25 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
30 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).

35 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
5 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
10 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
15 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, *T₄* DNA polymerase and Taq DNA polymerase, a heat stable DNA polymerase isolated from *Thermus aquaticus*. The latter enzyme is widely
20 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
25 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.
30 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.

35 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.

5 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
10 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
15 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
20 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 suitably 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)
25 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
30 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
35 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.

5 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.

10 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
15 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.

20 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,
25 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.

30 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
35 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 illustrative purposes and not to limit the scope of the invention in any way.

Reference is also made to the attached figures wherein:

Figure 1 illustrates the position and orientation of primers used for sequencing cloned SOD - amplicons; and

Figure 2 illustrates an example of the specificity of the species specific probe selected for *M. intracellulare*.

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

The primers were synthesized on a MilliGen/Biosearch Cyclone Plus* Synthesizer using the DNA synthesizing kit for sequences of up to 50 nucleotides (Millipore GmbH, Eschborn FRG). The oligonucleotides were eluted from the cartridges with 25% NH₄OH, dried in vacuo, and dissolved without further purification to a final concentration of 20 µM.

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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
30 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, Küssnacht, 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.

*Trade-mark

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 MgCl₂; 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 H₂O. 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: Cloning and Sequencing the Amplicons from selected bacteria obtained with primers Z205 and Z212

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 diphtheriae* (ATCC 11913); *Corynebacterium pseudodiphtheriticum* (RC 181); *Nocardia asteroides* (ATCC 43005); and *Actinomyces viscosus* (ATCC 15987).

Details of the procedures can be found in the previously referenced laboratory 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
5 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'-endlabelling-kit (Boehringer Mannheim GmbH, Mannheim, FRG). The filled-in and kinased amplicons were separated by electrophoresis on NuSieve*GTG
10 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.

15 The plasmid pUC 19 was digested to completion with *Sma*I, 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,
20 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
25 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
30 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
35 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 S³⁵ following the instructions given by the manufacturer (Boehringer Mannheim, Mannheim, FRG).

5 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
10 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*
15 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.

20 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

25 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
30 (1984); GAP-Alignment) was used for alignment.

35 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 conserved 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. diphtheriae*, *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 disappear 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 amplifiable 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: *Edwardsiella 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 obtained 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 equimolar 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
5 pmol of an equimolar mix of the 8 probes (Z310 - Z317) were labeled at the 5'-
end to a specific activity of about $3 \times 10^7/\mu\text{g}$ 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
unincorporated label was removed using a Biospin*6 column (BioRad,
10 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
15 (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 $\mu\text{g}/\text{ml}$). Prehybridization was performed at 60 °C for 60 min
20 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 $\mu\text{g}/\text{ml}$ and $5 - 8 \times 10^6$ cpm of labeled genus
specific probes. Details of the buffer preparation can be found in the
25 "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
30 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. scrofulaceum*,
35 *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*,

*Trade-mark

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);

30 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. Buchanan + 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: *M. avium*-*M. brunense* Specific Probe

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

	B	2	M. acapulcensis	14473
	B	3	M. africanum	25420
	B	4	M. bovis BCG	1401
	B	5	M. bovis	19210
5	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
10	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
15	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
20			H ₂ O	

None of the 106 tested species of non-mycobacterial 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:

25 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
30 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.

35 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).

5 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
10 *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).

15 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*
20 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).

5 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*
10 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:

15 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
20 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.

25 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
30 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.

- 5 Table 1a: Mycobacterial species, their origin, amplification with the universal primers Z205 and Z212, amplification with the genus specific primers Z261 and Z212 and hybridization with the genus specific pool of probes Z310 - Z317.
- 10 Table 1b: Mycobacterial species, their origin and hybridization with the species specific probes Z301 (*M. avium*/*M. brunense*); Z337, Z336 and Z302 (*M. tuberculosis*); Z303 (*M. intracellulare*); Z304 (*M. simiae*); Z340 (*M. kansasii*); Z306 (*M. scrofulaceum*); Z366 (*M. gordonae*); Z369 (*M. fortuitum*); and Z309 (*M. xenopi*).
- 15 Table 2a: Non-mycobacterial species, their origin, amplification with the universal primers Z205 and Z212, amplification with the genus-specific primers Z261 and Z212 and hybridization with the genus-specific pool of probes (Z310 - Z317).
- 20 Table 2b: Non-mycobacterial species, their origin and hybridization with the species-specific probes mentioned above for Table 1b.
- Table 3: Sequence of primers used in the sequencing of the amplicons obtained with primers Z205 and Z212 after cloning in pUC 19.
- Table 4: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. tuberculosis*.
- Table 5: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. avium*.
- Table 6: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. intracellulare*.
- 25 Table 7: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. scrofulaceum*.
- Table 8: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. kansasii*.
- 30 Table 9: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *M. fortuitum*.

- 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*.
- 5 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 diphtheriae*.
- 10 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*.
- 15 Table 16: DNA-sequence of the amplicons obtained with primers Z205 and Z212 being part of the SOD-gene of *Actinomyces viscosus*.
- 20 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.
- 25 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	+	+	+
5	M. africanum	25420	+	+	+
	M. avium	25291	+	+	+
	M. bovis	19210	+	+	+
	M. bovis BCG ***	1401	+	+	+
	M. brunense	23434	+	+	+
10	M. chelonae	35752	+	+	+
	M. flavescens	DSM43219*	+	+	+
	M. fortuitum	6841	+	+	+
	M. gadium	27726	+	+	(+)
	M. gastri	15754	+	+	+
15	M. gordonae	DSM610*	+	+	+
	M. haemophilum	29548	+	+	(+)
	M. intracellulare	DSM 43223*	+	+	+
	M. kansasii	DSM43224*	+	+	+
	M. marinum	927	+	+	+
20	M. nonchromogenicum	19530	+	+	+
	M. obuense	27023	+	+	+
	M. phlei	DSM750*	+	+	(+)
	M. rhodesiae	27024	+	+	-
	M. scrofulaceum	19981	+	+	+
25	M. simiae	25275	+	+	+
	M. smegmatis	14468	+	+	+
	M. szulgai	35799	+	+	(+)
	M. terrae	15755	+	+	+
	M. triviale	23292	+	+	+
30	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

35 3) Hybridization with genus specific pool of probes Z310 - Z317

*** Serum- und Impfinstitut, Bern, Switzerland

** clinical isolate

* DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

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

35 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

40 ** clinical isolate

* DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

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

Table 2a (continued)

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

Table 2a (continued)

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

35 * clinical isolate

** DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

*** RC (Roche collection)

**** RKI (Robert Koch Institute, Berlin, FRG)

[illegible]

Table 2b (continued)

[illegible]

Table 2b (continued)

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

35 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

40 ** DSM (Deutsche Sammlung von Mikroorganismen, Göttingen, FRG)

*** RC (Roche collection)

****RKI (Robert Koch Institute, Berlin, FRG)

Sequencing Primers

5	All Mycobacteria									Seq. Id.	Position	
	N. asteroides									No.		
	Z205:	5'-AGC	TTC	ACC	ACA	GCA	AGC	ACC	A-3'	1	188 - 209	
	Z256:	5'-CAC	^T _A ^G _C	ATC	TGG	TGG	AAG	AAC	CT-3'	24	337 - 359	
10	Z243:	5'-ACG	^C _T ^G _G	^C _T ^G _G	ACA	AGC	CGA	CCG	G-3'	25	368 - 389	
	Z244:	5'-GTC	^G _C ^T _G	TGG	TCG	^G _A ^T _A	^G _C ^A _C	TGG	A-3'	26	551 - 539	
	Z212:	5'-TCG	^G _T ^C _C	CAG	TTC	ACG	^A _G ^C _A	TTC	CA-3'	2	678 - 655	
	C. diphtheriae											
	Z205:	see above										
15	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	
20	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										
25	Z212:	see above										

Mycobacterium tuberculosis SOD gene for super oxide dismutase

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACCTACG	TAAAGGGCGC	CAATGACGCC	GTCGCCAAAC
251	TCGAAGAGGC	GCGCGCCAAG	GAAGATCACT	CAGCGATCTT	GCTGAACGAA
301	AAGAATCTAG	CTTTCAACCT	CGCCGGCCAC	GTCAATCACA	CCATCTGGTG
10 351	GAAGAACCTG	TCGCCTAACG	GTGGTGACAA	GCCCACCGGC	GAAGTCGCCC
401	CAGCCATCGC	CGACGCGTTC	GGTTCGTTCG	ACAAGTTCCG	TGCGCAGTTC
451	CACGCGGCCG	CTACCACCGT	GCAGGGGTCG	GGCTGGGCGG	CACTGGGCTG
501	GGACACACTC	GGCAACAAGC	TGCTGATATT	CCAGGTTTAC	GACCACCAGA
551	CGAACTTCCC	GCTAGGCATT	GTTCCGCTGC	TGCTGCTCGA	CATGTGGGAA
15 601	CACGCCTTCT	ACCTGCAGTA	CAAGAACGTC	AAAGTCGACT	TTGCCAAGGC
651	GTTTTGGAAC	GTCGTGAACT	GGGCCGAT..		
701					
751					

20

Table 5**Mycobacterium avium SOD gene for super oxide dismutase**

1					
51					
25 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACCTACG	TCAAAGGCGT	GAACGACGCT	CTTGCCAAGC
251	TCGAAGAGGC	CCGCGCCAAC	GAGGACCACG	CTGCGATCTT	CCTGAACGAA
301	AAGAACCTCG	CCTTCCACCT	GGGCGGCCAC	GTCAACCACT	CGATCTGGTG
30 351	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
35 601	CACGCGTTCT	ACCTGCAGTA	CAAGAACGTC	AAGGCGGACT	ACGTCAAGGC
651	GTTCTGGAAC	GTCGTGAACT	GGGCCGAT..		
701					
751					

Mycobacterium intracellulare SOD gene for super oxide dismutase

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACCTACG	TCAAAGGCGT	GAACGACGCT	CTGTCCAAGC
251	TCGAAGAGGC	CCGTGCCAAC	GAAGATCACG	CTGCGATCTT	CCTGAACGAA
301	AAGAACCTGG	CCTTTCACCT	GGGCGGCCAC	GTCAACCACT	CCATCTGGTG
10 351	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
15 601	CACGCTTTCT	ACCTGCAGTA	CAAGAACGTC	AAGGCGGACT	ACGTCAAGGC
651	GTTCTGGAAC	GTCGTGAACT	GGGACGAT..		
701					
751					

20

Table 7**Mycobacterium scrofulaceum SOD gene for super oxide dismutase**

1					
51					
25 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACGTACG	TCAAGGGCGT	GAACGACGCC	GTCGCCAAAC
251	TCCAAGAGGC	ACGCGCCAAT	GACGACCACG	CCGCGATCTT	CCTGAACGAA
301	AAGAACCTGG	CGTTCCACCT	CGGCGGCCAC	GTGAACCACT	CGATCTGGTG
30 351	GAAGAACCTC	TCGCCGGACG	GCGGCGACAA	GCCGACCGGA	GAAGTGGCCG
401	CCGCGATCGA	TGACGCGTTC	GGATCGTTCG	ACAAATTCCG	CGCCCAGTTC
451	AGTGCGGCCG	CCAACGGCCT	GCAGGGTTCG	GGCTGGGCGG	TGCTGGGCTA
501	TGACACACTC	GGCAGCAGGC	TGCTCACCTT	CCAGCTTTAC	GACCAGCAGG
551	CCAACGTCCC	GCTCGGCATC	ATTCCGCTGC	TGCAGGTCGA	CATGTGGGAG
35 601	CACGCCTTTT	ACTTGCAGTA	CAAGAACGTC	AAGGCCGACT	ACGTCAAGGC
651	CTTCTGGAAT	GTCGTGAACT	GGGCCGAG..		
701					
751					

Table 8**Mycobacterium kansasii SOD gene for super oxide dismutase**

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACCTACG	TCAAGGGCGC	CAACGATGCG	GTCGCCAAAC
251	TCGAAGAGGC	GCGCGCCAAG	GAAGACCACT	CGGCGATCTT	GCTGAACGAG
301	AAGAACTTGG	CCTTCAACCT	CGCCGGCCAC	GTCAACCACA	CGATCTGGTG
10 351	GAAGAACCTT	TCTCCCAACG	GAGGCGACAA	GCCGACCGGC	GAATCTGCCG
401	CGGCCATCGA	CGAGGCGTTC	GGGTCCTTCG	ACAAGTTTCG	TGCCCAATTC
451	CACGCCGCCG	CCACCACGGT	GCAGGGGTCG	GGCTGGGCGG	CGCTGGGCTG
501	GGACACTCTC	GGCAACAAGC	TGCTGATATT	CCAGGTCTAC	GACCACCAGA
551	CGAACTTTCC	ACTCGGAATC	ATTCCGTTAC	TGCTGCTCGA	CATGTGGGAA
15 601	CACGCTTTCT	ACCTCCAGTA	CAAGAATGTC	AAGGTCGACT	TCGCCAAAGC
651	ATTCTGGAAC	GTCGTGAACT	GGGACGAT..		
701					
751					

20

Table 9**Mycobacterium fortuitum SOD gene for super oxide dismutase**

1					
51					
25 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCGCGTACG	TCAAGGGCGT	CAACGACGCC	GTGGCCAAGC
251	TCGATGAGGC	GCGGGCCAAC	GGTGACCACG	CGGCGATCTT	CCTCAACGAG
301	AAGAACCTGG	CGTTCCATCT	CGGCGGCCAC	GTGAACCACT	CGATCTGGTG
30 351	GAAGAACCTG	TCCCCCAACG	GTGGTGACAA	GCCGACGGGC	GATCTGGCCG
401	CGGCGATCGA	CGATCAGTTC	GGCTCGTTCG	ACAAGTTCCA	GGCGCAGTTC
451	ACCGCCGCCG	CCAACGGGCT	GCAGGGGCTCG	GGCTGGGCCC	TGCTCGGCTA
501	CGACAGCCTG	GGCGATCGGC	TGCTGACCTT	CCAGCTCTAC	GACCAGCAGG
551	CCAACGTGCC	GCTCGGCATC	ATCCCGCTGC	TCCAGGTCGA	CATGTGGGAG
35 601	CACGCCTTCT	ACCTGCAGTA	CAAGAACGTC	AAGGCCGACT	ACGTCAAGGC
651	GTTCTGGAAA	GTCGTGAACT	GGGACGAT..		
701					
751					

Mycobacterium simiae SOD gene for super oxide dismutase

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAT	GCGACGTACG	TCAAGGGTTT	GAACGACGCC	ATTGCCAAGC
251	TTGAAGAGGC	GCGGGCCAAC	GACGACCATG	CCGCGATCTT	CTTGAACGAG
301	AAGAATCTGG	CATTCCACCT	CGGTGGCCAC	GTCAACCACT	CCATCTGGTG
10 351	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
15 601	CACGCCTTCT	ACCTGCAGTA	CAAGAACGTC	AAGGCGGACT	ACGTCAAGGC
651	GTTCTGGAAC	GTCGTGAACT	GGGACGAT..		
701					
751					

20

Table 11*Mycobacterium gordonae* SOD gene for super oxide dismutase

1					
51					
25 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCCACCTACG	TCAAAGGCGT	CAACGACGCG	GTCGCCAAGC
251	TGGAAGAAGC	GCGCGCCAAA	GGCGACCACT	CGGCCATCTT	TTTGAACGAG
301	AAGAACCTGG	CCTTCCACCT	GGGCGGTCAC	GTCAACCACT	CCATCTGGTG
30 351	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
35 601	CACGCCTTCT	ACCTGCAGTA	CAAGAACGTC	AAGGCCGACT	ACGTCAAGGC
651	CTTCTGGAAC	GTCGTGAACT	GGGACGAC..		
701					
751					

Mycobacterium xenopi SOD gene for super oxide dismutase

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCGACGTACG	TCAAAGGCGC	CAACGACGCG	CTCGCCAAGC
251	TGGAGGAGGC	GCGCGCCAAA	GACGATCATT	CCGCGATCGT	CGGGCATGAG
301	AAGGCCCTCG	CGTTCAACCT	GGCCGGCCAT	GTCAATCACT	GCCTGTGGTG
10 351	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
15 601	CACGCCTTCT	ACCTGCAGTA	CAAGAATGTC	AAGGCTGACT	TCGCCAAAGC
651	ATTCTGGAAC	GTCGTGAACT	GGGCCGAT..		
701					
751					

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Table 13*Corynebacterium diptheriae* SOD gene for super oxide dismutase

1					
51					
25 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	GCTAACTACG	TGAACGGTGC	AAATACTGCT	CTTGAGAAGC
251	TGCAAAAGGC	TCGCGAGAAC	GGTGAGATCG	GTGCTGTTGT	CACCGCTTTG
301	TCCAAGGATT	TGGCTTTCAA	CTTGGGTGGC	CACACCAACC	ACTCCATCTT
30 351	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	CTGACCCAC	TTCTTATGCT	CGATATGTGG
35 601	GAGCACGCTT	TCTACCTTCA	GTACAAGAAC	GTGAAGGCTG	ACTACGTCAA
651	GGCTGTGTGG	AACGTCGTGA	ACTGGGACGA	TG.....	
701					
751					

C. pseudodiphtheriticum SOD gene for super oxide dismutase

1					
51					
5 101					
151				AGC	TTCACCACAG
201	CAAGCACCAC	AACACTTACG	TGCAGGGTGC	TAACGCAGCT	TTGGACGCTC
251	TGGAAGAAGA	GCGCAACGGC	GAAGCCAACC	CAGACCGCAT	CCGTGCGCTG
301	TCCAAGAACT	TGGCTTTCCA	ACCTGGCCAC	ACCAACCACT	CCATCTTCTG
10 351	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	CGTGAACCTC	ACCCCACTGC	TGATGCTGGA	TATGTGGGAG
15 601	CACGCTTTCT	ACCTGCAGTA	CAAGAACGTC	AAGCCTGATT	ACGTCAAGGC
651	TGTCTGGAAC	GTCGTGAACT	GGGACGAT..		
701					

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Table 15

Nocardia asteroides SOD gene for super oxide dismutase

1					
51					
101					
25 151				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
30 401	CGGCCATCGA	CGACCAGTTC	GGTTCGTTTCG	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
35 651	TTGTGGAACG	TCGTGCACTG	GGCCGAT...		
701					
751					

Table 16

Actinomyces viscosus SOD gene for super oxide dismutase

1						
51						
5 101						
151					AGC	TTCACCACAG
201	CAAGCACCAC	GCCGCCTACG	TCGCTGGCGC	CAACGCCGCC	CTGGAGGCCC	
251	TCGCCGCCGC	CCGCGAGGAC	GGCGACCTGG	GTGCGATCAA	CCTGTGGGAG	
301	AAGAACCTCG	CCTTCAACCT	GGGCGGCCAC	ACCAACCACT	CCGTGTTCTG	
10 351	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	
15 601	CACGCATTCT	ACCTCGACTA	CCTCAACGTC	AAGGCCGACT	ACGTCAAGGC	
651	CATCTGGAAC	GTCGTGAACT	GGGACGAT..			
701						
751						

20

Table 17

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

Z261	5'-CCA A _G ^A C TCG AAG AGG CGC G _G ^C G CCA A-3'	X
M. avium	5'-... ..C. ... -3'	1
25 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
30 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
35 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

40 EMBL Data Bank, Heidelberg, FRG, and GenBank, Los Alamos, USA

Table 18

Consensus sequence:

5		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 C
		A
	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'
10	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:

15	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'
20	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 GCG ATC GAC G-3'
	Z317	M. kans.	5'-GAC AAG CCG ACC GGC GAA CTC GCC GCG GCC ATC GAC G-3'

(1) GENERAL INFORMATION:

5

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10

15

(ii) TITLE OF INVENTION: Oligonucleotides derived from the SOD Gene Family

(iii) NUMBER OF SEQUENCES: 44

20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

25

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: EP 92810780.4
(B) FILING DATE: 13-OCT-1992

30

(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

35

40

(ii) MOLECULE TYPE: other nucleic acid

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

AGCTTCACCA CAGCAAGCAC CA

45

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

50

55

(ii) MOLECULE TYPE: other nucleic acid

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

TCGKCCCACT TCACGA~~CTT~~ CCA

60

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

5

(ii) MOLECULE TYPE: other nucleic acid

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

10 CCAARCTCGA AGAGGCGCGS GCCAA

25

(2) INFORMATION FOR SEQ ID NO: 4:

15

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

20

(ii) MOLECULE TYPE: other nucleic acid

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

25 GACAAGCCSA CSGGHGANYT SGCCGCVGCS ATCGMYG

36

(2) INFORMATION FOR SEQ ID NO: 5:

30

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

35

(ii) MOLECULE TYPE: other nucleic acid

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

40 GACAAGCCCA CCGGCGAACT CGCCGCAGCC ATCGCCG

37

(2) INFORMATION FOR SEQ ID NO: 6:

45

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

50

(ii) MOLECULE TYPE: other nucleic acid

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

55 GACAAGCCGA CCGGCGAATT GGCCGCCGCS ATCGACG

37

(2) INFORMATION FOR SEQ ID NO: 7:

60

(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:

5

GACAAGCCCA CCGGTGAGCT GGCCGCCGCG ATCGACG

37

(2) INFORMATION FOR SEQ ID NO: 8:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

20

GACAAGCCGA CCGGTGACCT GGCCGCCGCG ATCGACG

37

(2) INFORMATION FOR SEQ ID NO: 9:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

35

GACAAGCCGA CGGGCGATCT GGCCGCGGCG ATCGACG

37

(2) INFORMATION FOR SEQ ID NO: 10:

40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

45

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

50

GACAAGCCGA CCGGAGAACT GGCCGCCGCG ATCGATG

37

(2) INFORMATION FOR SEQ ID NO: 11:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

60

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

GACAAGCCGA CCGGAGATCT CGCCGCCGCC ATCGACG

37

5 (2) INFORMATION FOR SEQ ID NO: 12:

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

(ii) MOLECULE TYPE: other nucleic acid

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

GACAAGCCGA CCGGCGAACT CGCCGCCGCC ATCGACG

37

20 (2) INFORMATION FOR SEQ ID NO: 13:

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

(ii) MOLECULE TYPE: other nucleic acid

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

CCTTCGGATC CTTGACCGG TTCCGCGCGC AGTTCAG

37

35 (2) INFORMATION FOR SEQ ID NO: 14:

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

(ii) MOLECULE TYPE: other nucleic acid

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

GTCCTTCGAC AAGTTCCGAG CGCAATTCAG CGCCGCC

37

50 (2) INFORMATION FOR SEQ ID NO: 15:

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

(ii) MOLECULE TYPE: other nucleic acid

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

TCTGGGCGGC CGGTTGCTCA CCTTT

25

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

10

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

GTCCCCGAAC GGCGGAGACA AGCCGACCGG AGATCTC

37

15

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

25

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

GCTAGGCATT GTTCCGCTGC TGCTGC

26

30

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

40

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

TCCGCGATCG TCGGGCATGA GAAGGCCCTC GCGTTCA

37

45

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

55

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

CCAGACGAAC TTTCCACTCG GA

22

60

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs

2108247

-50-

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

5 (ii) MOLECULE TYPE: other nucleic acid

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

10 TGACACACTC GGCAGCAGGC TGCTCACCTT CCAGCTT 37

(2) INFORMATION FOR SEQ ID NO: 21:

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

20 (ii) MOLECULE TYPE: other nucleic acid

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

25 ACGACAGCCT GGGCGATCGG CT 22

(2) INFORMATION FOR SEQ ID NO: 22:

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

35 (ii) MOLECULE TYPE: other nucleic acid

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

40 ACGAACTTCC CGCTAGGCAT TGTTCCGCTG CTGCTGC 37

(2) INFORMATION FOR SEQ ID NO: 23:

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

50 (ii) MOLECULE TYPE: other nucleic acid

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

55 AGTCGACTTT GCCAAGGCGT TT 22

(2) INFORMATION FOR SEQ ID NO: 24:

60 (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:

5 CACWCSATCT GGTGGAAGAA CCT

23

(2) INFORMATION FOR SEQ ID NO: 25:

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

15

(ii) MOLECULE TYPE: other nucleic acid

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

20 ACGGYGGYGA CAAGCCGACC GG

22

(2) INFORMATION FOR SEQ ID NO: 26:

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

30

(ii) MOLECULE TYPE: other nucleic acid

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

35 GTCTGSTGGT CGTARASCTG GA

22

(2) INFORMATION FOR SEQ ID NO: 27:

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

45

(ii) MOLECULE TYPE: other nucleic acid

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

50 GGATTTGGCT TTCAACTTG G

21

(2) INFORMATION FOR SEQ ID NO: 28:

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

60

(ii) MOLECULE TYPE: other nucleic acid

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

GGCGAGCCAA CCGGCGCTTT GG

22

(2) INFORMATION FOR SEQ ID NO: 29:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

15

GAAGAACCTG AGCCTTAACG GT

22

(2) INFORMATION FOR SEQ ID NO: 30:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

30

GAGCCAACCG GTGAGCTAGC C

21

(2) INFORMATION FOR SEQ ID NO: 31:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

40

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

45

GAAGAACCTC TCCCCCAACG GC

22

(2) INFORMATION FOR SEQ ID NO: 32:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

60

(A) ORGANISM: Mycobacterium tuberculosis/SOD gene

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

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTAAAGGG CGCCAATGAC GCCGTCGCCA

60

AACTCGAAGA GGC GCGCGCC AAGGAAGATC ACTCAGCGAT CTTGCTGAAC GAAAAGAATC 120
 TAGCTTTCAA CCTCGCCGGC CACGTCAATC ACACCATCTG GTGGAAGAAC CTGTCGCCTA 180
 5 ACGGTGGTGA CAAGCCCACC GGC GAACTCG CCGCAGCCAT CGCCGACGCG TTCGGTTCGT 240
 TCGACAAGTT CCGTGCGCAG TTCCACGCGG CCGCTACCAC CGTGCAGGGG TCGGGCTGGG 300
 10 CGGCACTGGG CTGGGACACA CTCGGCAACA AGCTGCTGAT ATTCCAGGTT TACGACCACC 360
 AGACGAACTT CCCGCTAGGC ATTGTTCCGC TGCTGCTGCT CGACATGTGG GAACACGCCT 420
 TCTACCTGCA GTACAAGAAC GTCAAAGTCG ACTTTGCCAA GGC GTTTTGG AACGTCGTGA 480
 15 ACTGGGCCGA T 491

(2) INFORMATION FOR SEQ ID NO: 33:

20

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

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

30

(A) ORGANISM: Mycobacterium avium/SOD gene

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

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTGAACGAC GCTCTTGCCA 60
 35 AGCTCGAAGA GGCCCGCGCC AACGAGGACC ACGCTGCGAT CTTCTGAAC GAAAAGAACC 120
 TCGCCTTCCA CCTGGGCGGC CACGTCAACC ACTCGATCTG GTGGAAGAAC CTGTCGCCGG 180
 40 ACGGCGGTGA CAAGCCCACC GGTGAGCTGG CCGCCGCGAT CGACGACGCG TTCGGGTCCT 240
 TCGACAAGTT CCGAGCGCAA TTCAGCGCCG CCGCCAACGG CCTGCAGGGC TCCGGCTGGG 300
 CGGTGCTGGG CTATGACACC CTGGGCAGCC GGTTGCTGAC CTTCCAGCTC TACGACCAGC 360
 45 GGGCCAACGT CCCGCTGGGC ATCATCCCGC TTCTGCAGGT CGACATGTGG GAGCACGCGT 420
 TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GGC GTTCTGG AACGTCGTGA 480
 50 ACTGGGCCGA T 491

(2) INFORMATION FOR SEQ ID NO: 34:

55

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

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mycobacterium intracellulare/SOD gene

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

5 AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTGAACGAC GCTCTGTCCA 60
 AGCTCGAAGA GGCCCGTGCC AACGAAGATC ACGCTGCGAT CTTCTGAAC GAAAAGAACC 120
 TGGCCTTTCA CCTGGGCGGC CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCGCCGG 180
 10 ACGGCGGCGA CAAGCCGACC GGCGAATTGG CCGCCGCGAT CGACGACGCC TTCGGATCCT 240
 TCGACCGGTT CCGCGCGCAG TTCAGCGCGG CCGCCAACGG CCTGCAGGGG TCGGGCTGGG 300
 CGGTGCTGGG CTACGACACC CTCGGCAACC GGCTGCTGAC CTTCCAGCTC TACGACCAGC 360
 15 AGGCCAACGT GCCGCTGGGC ATCATTCGCG TGCTGCAGGT CGACATGTGG GAGCACGCTT 420
 TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GCGTTCTGG AACGTCGTGA 480
 20 ACTGGGACGA T 491

(2) INFORMATION FOR SEQ ID NO: 35:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 491 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (ii) MOLECULE TYPE: cDNA
 (vi) ORIGINAL SOURCE:
 (B) STRAIN: *Mycobacterium scrofulaceum*/SOD gene
 35

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

AGCTTCACCA CAGCAAGCAC CACGCCACGT ACGTCAAGGG CGTGAACGAC GCCGTCGCCA 60
 40 AACTCCAAGA GGCACGCGCC AATGACGACC ACGCCGCGAT CTTCTGAAC GAAAAGAACC 120
 TGGCGTTCCA CCTCGGCGGC CACGTGAACC ACTCGATCTG GTGGAAGAAC CTCTCGCCGG 180
 ACGGCGGCGA CAAGCCGACC GGAGAACTGG CCGCCGCGAT CGATGACGCG TTCGGATCGT 240
 45 TCGACAAATT CCGCGCCCAG TTCAGTGCGG CCGCCAACGG CCTGCAGGGT TCGGGCTGGG 300
 CGGTGCTGGG CTATGACACA CTCGGCAGCA GGCTGCTCAC CTTCCAGCTT TACGACCAGC 360
 50 AGGCCAACGT CCCGCTCGGC ATCATTCGCG TGCTGCAGGT CGACATGTGG GAGCACGCCT 420
 TTTACTTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GGCCTTCTGG AATGTCGTGA 480
 ACTGGGCCGA G 491
 55

(2) INFORMATION FOR SEQ ID NO: 36:

60 (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

5

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

	AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAGGG CGCCAACGAT GCGGTCGCCA	60
10	AACTCGAAGA GGCGCGCGCC AAGGAAGACC ACTCGGCGAT CTTGCTGAAC GAGAAGAAGT	120
	TGGCCTTCAA CCTCGCCGGC CACGTCAACC ACACGATCTG GTGGAAGAAC CTTTCTCCCA	180
	ACGGAGGCGA CAAGCCGACC GGCCTAATCG CCGCGGCCAT CGACGAGGCG TTCGGGTCCT	240
15	TCGACAAGTT TCGTGCCCAA TTCCACGCCG CCGCCACCAC GGTGCAGGGG TCGGGCTGGG	300
	CGGCGCTGGG CTGGGACACT CTCGGCAACA AGCTGCTGAT ATTCCAGGTC TACGACCACC	360
20	AGACGAACTT TCCACTCGGA ATCATTCGGT TACTGCTGCT CGACATGTGG GAACACGCTT	420
	TCTACCTCCA GTACAAGAAT GTCAAGGTCG ACTTCGCCAA AGCATTCTGG AACGTCGTGA	480
	ACTGGGACGA T	491

25

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Mycobacterium fortuitum*/SOD gene

40

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

	AGCTTCACCA CAGCAAGCAC CACGCCGCGT ACGTCAAGGG CGTCAACGAC GCCGTGGCCA	60
	AGCTCGATGA GGCGCGGGCC AACGGTGACC ACGCGGCGAT CTTCTTCAAC GAGAAGAACC	120
45	TGGCGTTCCA TCTCGGCGGC CACGTGAACC ACTCGATCTG GTGGAAGAAC CTGTCCCCCA	180
	ACGGTGGTGA CAAGCCGACG GGCATCTGG CCGCGGCGAT CGACGATCAG TTCGGCTCGT	240
50	TCGACAAGTT CCAGGCGCAG TTCACCGCCG CCGCCAACGG GCTGCAGGGC TCGGGCTGGG	300
	CCGTGCTCGG CTACGACAGC CTGGGCGATC GGCTGCTGAC CTTCCAGCTC TACGACCAGC	360
	AGGCCAACGT GCCGCTCGGC ATCATCCCGC TGCTCCAGGT CGACATGTGG GAGCACGCCT	420
55	TCTACCTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GCGTTCTGG AAAGTCGTGA	480
	ACTGGGACGA T	491

60

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs

2108247

-56-

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

5 (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mycobacterium simiae/SOD gene

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

AGCTTCACCA CAGCAAGCAC CATGCGACGT ACGTCAAGGG TTTGAACGAC GCCATTGCCA 60
AGCTTGAAGA GGC GCGGGCC AACGACGACC ATGCCGCGAT CTTCTTGAAC GAGAAGAATC 120
15 TGGCATTCCA CCTCGGTGGC CACGTCAACC ACTCCATCTG GTGGAAAAAC CTGTCCCCGA 180
ACGGCGGAGA CAAGCCGACC GGAGATCTCG CCGCCGCCAT CGACGACGCC TTCGGTTCGT 240
20 TCGACAAGTT CCGCGCACAG TTCAGCGCCG CCGCAAACGG CTTGCAGGGC TCGGGCTGGG 300
CGGTACTCGG CTACGACACC CGGGGCGACC GACTGCTGAC CTTCCAGCTT TACGACCAGC 360
AGGCCAACGT CCCGCTGGGC ATCATCCCGC TGCTGCAGGT CGACATGTGG GAGCACGCCT 420
25 TCTACCTGCA GTACAAGAAC GTCAAGGCGG ACTACGTCAA GCGTTCTGG AACGTCGTGA 480
ACTGGGACGA T 491

30

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

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

(ii) MOLECULE TYPE: cDNA

40

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mycobacterium gordonae/SOD gene

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

45

AGCTTCACCA CAGCAAGCAC CACGCCACCT ACGTCAAAGG CGTCAACGAC GCGGTCGCCA 60
AGCTGGAAGA AGCGCGCGCC AAAGGCGACC ACTCGGCCAT CTTTTTGAAC GAGAAGAACC 120
50 TGGCCTTCCA CCTGGGCGGT CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCGCCGG 180
ACGGCGGCGA CAAGCCGACC GGTGACCTGG CCGCCGCGAT CGACGACCAG TTCGGCTCGT 240
TCGACAAGTT CCAGGCTCAG TTCAGCGCCG CCGCAAACGG CCTACAGGGC TCGGGCTGGG 300
55 CGGTGCTCGG CTACGACACT CTGGGCGGCC GGTGCTCAC CTTTCAGCTC TACGACCAGC 360
AGGCCAATGT CCCGCTCGGT GTCATTCCGC TGTTGCAGGT CGACATGTGG GAGCACGCCT 420
60 TCTACCTGCA GTACAAGAAC GTCAAGGCCG ACTACGTCAA GGCCTTCTGG AACGTCGTGA 480
ACTGGGACGA C 491

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mycobacterium xenopi/SOD gene

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

15

AGCTTCACCA CAGCAAGCAC CACGCGACGT ACGTCAAAGG CGCCAACGAC GCGCTCGCCA 60

AGCTGGAGGA GGCGCGCGCC AAAGACGATC ATTCCGCGAT CGTCGGGCAT GAGAAGGCCC 120

20 TCGCGTTCAA CCTGGCCGGC CATGTCAATC ACTGCCTGTG GTGGAAGAAC CTGTCCCCCA 180

ACGGCGGTGA CAAGCCGACC GGCGAATTGG CCGCCGCCAT CGACGACGCG TTCGGCTCGT 240

TCGACAAGTT CCGCGCCCAG TTCACCGCGG CCGCCACGAC CGTGCAGGGG TCGGGCTGGG 300

25

CGGCACTCGG CTGGGACAGC CTGGGTGGCA AGCTCCTGGT GTTCCAGGTC TACGACCACC 360

AGTCCAACCT CCCGCTCGGG ATCGTCCCCC TGCTGGTGCT CGACATGTGG GAGCACGCCT 420

30 TCTACCTGCA GTACAAGAAT GTCAAGGCTG ACTTCGCCAA AGCATTCTGG AACGTCGTGA 480

ACTGGGCCGA T 491

35 (2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Corynebacterium diphtheriae/SOD gene

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

50 AGCTTCACCA CAGCAAGCAC CACGCTAACT ACGTGAACGG TGCAAATACT GCTCTTGAGA 60

AGCTGCAAAA GGCTCGCGAG AACGGTGAGA TCGGTGCTGT TGTCACCGCT TTGTCCAAGG 120

ATTTGGCTTT CAACTTGGGT GGCCACACCA ACCACTCCAT CTTCTGGAAG AACCTCTCCC 180

55

CTAACGGTGG CGGCGAGCCA ACCGGCGCTT TGGCTGAGGC AATTGCCAAG GAGTTCGGTT 240

CTTTTGAGAA GTTCAAGGAT CACTTCTCTG CTGCGGCTCT TGGTCTGCAG GGTTCGGCT 300

60 GGGCTGTTCT CGGCTACGAT CACATCGGTG GCCGTCTGGT TATCGAGCAG CTCACTGACC 360

AGCAGGGCAA CATCTCCGCT AACCTGACCC CACTTCTTAT GCTCGATATG TGGGAGCACG 420

CTTTCTACCT TCAGTACAAG AACGTGAAGG CTGACTACGT CAAGGCTGTG TGGAACGTCG 480

TGAAGTGGGA CGATG

495

5 (2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *C. pseudodiphtheriticum*/SOD gene

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

20 AGCTTCACCA CAGCAAGCAC CACAACACTT ACGTGCAGGG TGCTAACGCA GCTTTGGACG 60
CTCTGGAAGA AGAGCGCAAC GGCGAAGCCA ACCCAGACCG CATCCGTGCG CTGTCCAAGA 120
ACTTGGCTTT CCAACCTGGC CACACCAACC ACTCCATCTT CTGGAAGAAC CTGAGCCCTA 180
25 ACGGTGGCGG CGAGCCAACC GGTGAGCTAG CAGAGGCTAT CGACCGCGAC TTTGGTTCCT 240
TCGAGAAGTT CAAGGCGCAC TTCTCCGCAG CAGCACTCGG CCTGCAGGGT TCCGGCTGGG 300
30 CCGTGCTGGG TTACGACCAC ATTGCTGGTC GCCTGCTCGT TGAGCAGCTG ACCGACCAGC 360
AGGGCAACAC TTCCGTGAAC TTCACCCAC TGCTGATGCT GGATATGTGG GAGCACGCTT 420
TCTACCTGCA GTACAAGAAC GTCAAGCCTG ATTACGTCAA GGCTGTCTGG AACGTCGTGA 480
35 ACTGGGACGA T 491

40 (2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 490 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Nocardia asteroides*/SOD gene

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

55 AGCTTCACCA CAGCAAGCAC CACGCCGCCT ACGTCGCCGG TGCCAACACG GCACTGGAGA 60
AGCTGGAAGC CGCCCGTGAG GCCGGCGATC ACAGCGCGAT CTCCTGCAC GAGAAGAACC 120
TCGCGTTCCA CCTCGGCGGA CACGTCAACC ACTCCATCTG GTGGAAGAAC CTGTCCCCCA 180
60 ACGGTGGCGA CAAGCCGGTC GGCAGCTGG 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
5 CTGGGCCCGAT 490

(2) INFORMATION FOR SEQ ID NO: 44:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

20

(A) ORGANISM: *Actinomyces viscosus*/SOD gene

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

AGCTTCACCA CAGCAAGCAC CACGCCGCCT ACGTCGCTGG CGCCAACGCC GCCCTGGAGG 60
25 CCCTCGCCGC CGCCCGCGAG GACGGCGACC TGGGTGCGAT CAACCTGTGG GAGAAGAACC 120
TCGCCTTCAA CCTGGGCGGC CACACCAACC ACTCCGTGTT CTGGAAGAAC CTCTCCCCCA 180
30 ACGGCGGCGG CCAGCCCGAG GCGAGCTCG CCGAGGCCAT CAAGGACTCC TTCGGCTCCT 240
TCGAGAAGTT CCAGGCGCAG TTCACCGCCA CCGCCCTGGG CATCCAGGGC TCGGGCTGGG 300
CCGTGCTCGC CTACGACTCA ATCTCCGGCA AGCTGCTGAT CTTCCAGCTC TTCGACCAGC 360
35 AGGCCAACGT GCCCGTGGGC ACGACCCCGC TGTTCATGGT GGACATGTGG GAGCACGCAT 420
TCTACCTCGA CTACCTCAAC GTCAAGGCCG ACTACGTCAA GGCCATCTGG AACGTCGTGA 480
40 ACTGGGACGA T 491

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED 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* which 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;
 - (b) contacting the amplified target sequence with at least one oligonucleotide probe sufficiently complementary to the target region of the SOD gene to reliably hybridize thereto; 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 in step (a) by means of the polymerase chain reaction (PCR).
3. The method of claim 1 or claim 2, 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*.
4. The method of claim 3, wherein said probe has an hybridizing sequence the sequence of Seq. Id. No. 4 or a sequence complementary thereto.
5. The method of claim 3, wherein said probe has as hybridizing sequence a sequence selected from the group consisting 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 respectively complementary thereto.

6. The method of claim 1 or 2, wherein the oligonucleotide probe is sufficiently complementary to a variable region of the target sequence to reliably hybridize thereto thereby allowing differentiation among the SOD genes of the different species within the genus *Mycobacterium*.

7. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. intracellulare* to reliably hybridize to the species-specific region.

8. The method of claim 7, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 13 (Z303) or a sequence complementary thereto.

9. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. avium* and *M. brunense* to reliably hybridize to the species-specific region.

10. The method of claim 9, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 14 (Z301) or a sequence complementary thereto.

11. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. gordonae* to reliably hybridize to the species-specific region.

12. The method of claim 11, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 15 (Z366) or a sequence complementary thereto.

13. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. simiae* to reliably hybridize to the species-specific region.
14. The method of claim 13, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 16 (Z304) or a sequence complementary thereto.
15. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. tuberculosis* to reliably hybridize to the species-specific region.
16. The method of claim 15, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 17 (Z336), Seq. Id. No. 23 (Z337) or Seq. Id. No. 22 (Z302) or a sequence complementary thereto.
17. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. xenopi* to reliably hybridize to the species-specific region.
18. The method of claim 17, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 18 (Z309) or a sequence complementary thereto.
19. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. kansasii* to reliably hybridize to the species-specific region.
20. The method of claim 19, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 19 (Z340) or a sequence complementary thereto.

21. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. scrofulaceum* to reliably hybridize to the species-specific region.
22. The method of claim 21, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 20 (Z306) or a sequence complementary thereto.
23. The method of claim 6, wherein said probe is sufficiently complementary to a species-specific region of the SOD gene of the species *M. fortuitum* to reliably hybridize to the species-specific region.
24. The method of claim 23, wherein said probe has as the hybridizing sequence the sequence of Seq. Id. No. 21 (Z369) or a sequence complementary thereto.
25. An oligonucleotide of the sequence of Seq. Id. No. 4, or a sequence complementary thereto.
26. An oligonucleotide capable of hybridizing to a region of the target sequence which is substantially conserved among the superoxide dismutase gene of different species within the genus *Mycobacterium* 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.
27. An oligonucleotide of the sequence of Seq. Id. No. 13 (Z303) or a sequence complementary thereto.
28. An oligonucleotide of the sequence of Seq. Id. No. 14 (Z301) or a sequence complementary thereto.

29. An oligonucleotide of the sequence of Seq. Id. No. 15 (Z366) or a sequence complementary thereto.
30. An oligonucleotide of the sequence of Seq. Id. No. 16 (Z304) or a sequence complementary thereto.
31. An oligonucleotide capable of hybridizing to a species-specific region of the superoxide dismutase gene selected from the group consisting of the sequence of Seq. Id. No. 17 (Z336), Seq. Id. No. 23 (Z337) or Seq. Id. No. 22 (Z302) or a sequence complementary thereto.
32. An oligonucleotide of the sequence of Seq. Id. No. 18 (Z309) or a sequence complementary thereto.
33. An oligonucleotide of the sequence of Seq. Id. No. 19 (Z340) or a sequence complementary thereto.
34. An oligonucleotide of the sequence of Seq. Id. No. 20 (Z306) or a sequence complementary thereto.
35. An oligonucleotide of the sequence of Seq. Id. No. 21 (Z369) or a sequence complementary thereto.
36. A probe sufficiently complementary to a target region of a superoxide dismutase gene of an organism which is substantially conserved among the superoxide dismutase gene of different species within the genus *Mycobacterium* and comprising an oligonucleotide according to claim 25 or 26 as hybridizing sequence.
37. A pool of probes sufficiently complementary to a target region of a superoxide dismutase gene of an organism to reliably hybridize thereto and comprising two or more oligonucleotides according to claim 26 as hybridizing sequences.

38. A probe sufficiently complementary to a target region which is variable among the superoxide dismutase gene of different species within the genus *Mycobacterium* and comprising an oligonucleotide according to any one of claims 27 to 35 as hybridizing sequence.

39. A primer comprising as priming sequence the sequence of Seq. Id. No. 1 (Z205).

40. A primer comprising as priming sequence the sequence Seq. Id. No. 2 (Z212).

41. 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. No. 2 (Z212) as priming sequence.

42. A primer comprising as priming sequence the sequence of Seq. Id. No. 3 (Z261).

43. 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. No. 2 (Z212) as priming sequence.

44. A kit for amplifying a part of a gene coding for a superoxide dismutase (SOD) comprising a first primer according to claim 39 or 42 and a second primer having as priming sequence the sequence of Seq. Id. No. 2 (Z212).

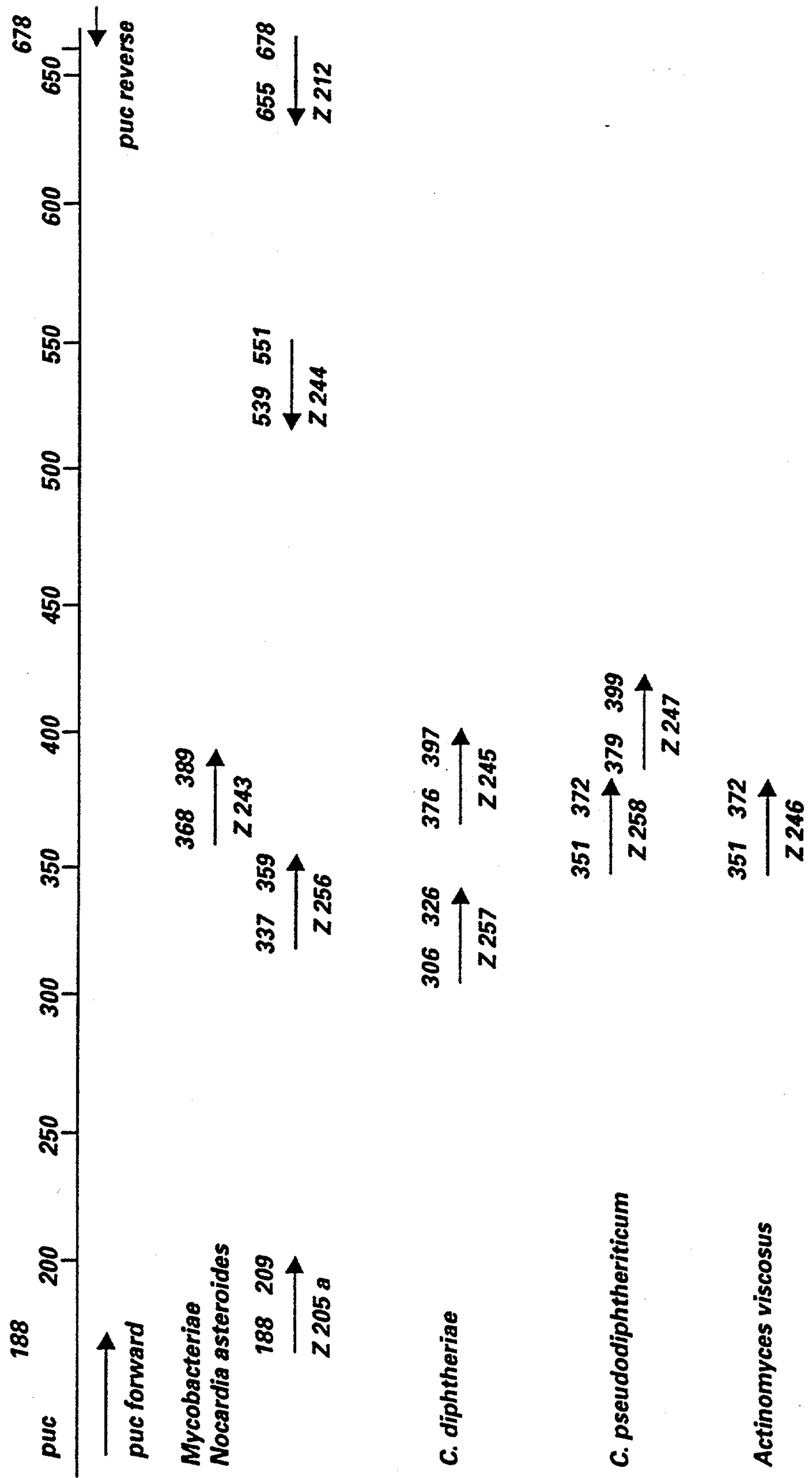
45. A kit for detecting a mycobacterial species including a probe according claim 36.

46. A kit for detecting a mycobacterial species including a probe according claim 37.

47. A kit for differentiating among different species belonging to the genus *Mycobacterium* including a probe according to claim 38.
48. A kit according to claim 45 further comprising a first primer having as primary sequence a sequence selected from the group of Seq. Id. No. 1 (Z205) and Seq. Id. No. 3 (Z261), and a second primer having as priming sequence the sequence Seq. Id. No. 2 (Z212).
49. A kit according to claim 47 further comprising a first primer having as primary sequence a sequence selected from the group of Seq. Id. No. 1 (Z205) and Seq. Id. No. 3 (Z261), and a second primer having as priming sequence the sequence Seq. Id. No. 2 (Z212).

Fig. 1

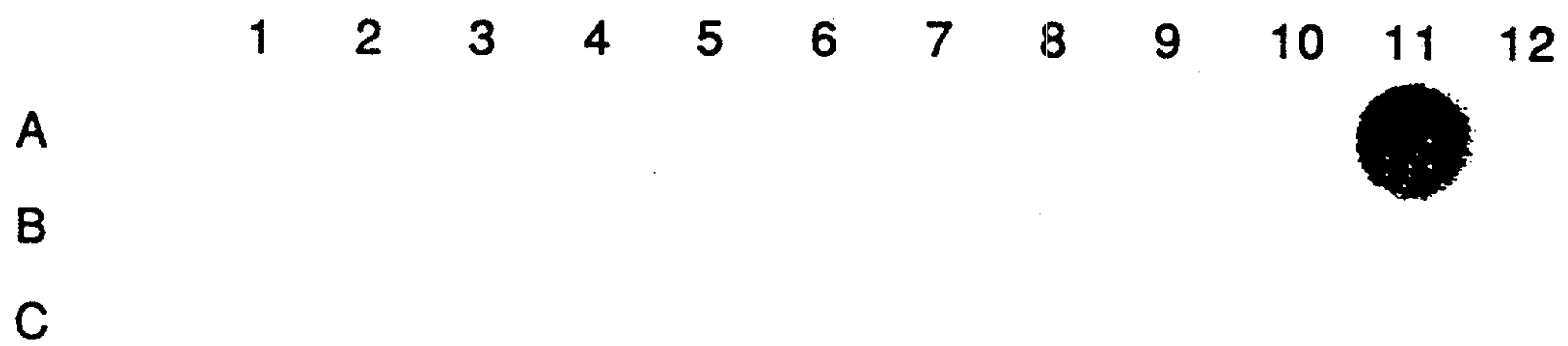
POSITION AND ORIENTATION OF PRIMERS
USED FOR SEQUENCING CLONED
SOD - AMPLICONS



2108247

Fig. 2

Hybridization with Probe specific for *M. intracellulare*



Mycobacteria