

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2006/0147964 A1 Gingeras et al.

Jul. 6, 2006 (43) Pub. Date:

(54) MYCOBACTERIAL RPOB SEQUENCES

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(21) Appl. No.: 11/313,718

(22) Filed: Dec. 22, 2005

Related U.S. Application Data

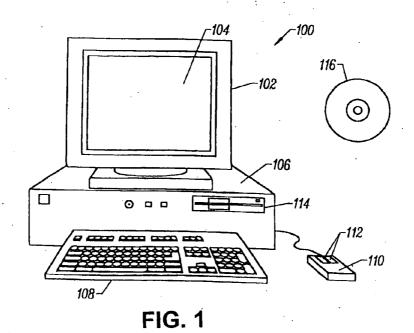
- (63) Continuation of application No. 09/285,306, filed on Apr. 2, 1999.
- (60) Provisional application No. 60/080,616, filed on Apr. 3, 1998.

Publication Classification

(51) Int. Cl. C12Q 1/68 (2006.01)C07H 21/04 (2006.01)

(57)**ABSTRACT**

This invention provides polynucleotide probes, sequences and methods for speciating and phenotyping organisms, for example, using probes based on the Mycobacterium tuberculosis rpoB gene. The groups or species to which an organism belongs may be determined by comparing hybridization patterns of target nucleic acid from the organism to hybridization patterns in a database.



~100 122-120~ 118~ 1327 1/0 SYSTEM MEMORY CENTRAL SPEAKER CONTROLLER PROCESSOR 134 -124 DISPLAY ADAPTER r-102 -126 -108 -128 ~130 REMOVABLE **MONITOR** FIXED NETWORK INTERFACE **KEYBD** DISK DISK

FIG.2

MYCOBACTERIAL RPOB SEQUENCES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application derives priority from U.S. Ser. No. 60/080,616, filed Apr. 3, 1998, and incorporated by reference. Applications U.S. Ser. No. 08/797,812, filed Feb. 7, 1997, now U.S. Pat. No. 6,228,575; U.S. Ser. No. 60/011, 339, filed Feb. Feb. 8, 1996; U.S. Ser. No. 60/012,631, filed Mar. 1, 1996; U.S. Ser. No. 08/629,031, filed Apr. 8, 1996, now abandoned; and 60/017,765, filed 15 May 15, 1996 are directed to related subject matter. These applications are specifically incorporated by reference in their entirety for all purposes.

STATEMENT OF GOVERNMENT INTEREST

[0002] The work described in this application was supported in part by grant number 1R43a140400 by the NIAID. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] This invention is directed to polymorphisms in rpoB genes of mycobacteria and use of the same in the identification and characterization of microorganisms.

[0005] 2. Background of the Invention

[0006] Multidrug resistance and human immunodeficiency virus (HIV-1) infections are factors which have had a profound impact on the tuberculosis problem. An increase in the frequency of *Mycobacterium tuberculosis* strains resistant to one or more anti-mycobacterial agents has been reported, Block, et al., (1994) *JAMA* 271:665-671. Immunocompromised HIV-1 infected patients not infected with *M. tuberculosis* are frequently infected with *M. avium* complex (MAC) or *M. avium-M. intracellulare* (MAI) complex. These mycobacteria species are often resistant to the drugs used to treat *M. tuberculosis*. These factors have re-emphasized the importance for the accurate determination of drug sensitivities and mycobacteria species identification.

[0007] In HIV-1 infected patients, the correct diagnosis of the mycobacterial disease is essential since treatment of M. tuberculosis infections differs from that called for by other mycobacteria infections, Hoffner, S. E. (1994) Eur. J. Clin. Microbiol. Inf. Dis. 13:937-941. Non-tuberculosis mycobacteria commonly associated with HIV-1 infections include M. kansasii, M. xenopi, M. fortuitum, M. avium and M. intracellulare, Wolinsky, E., (1992) Clin. Infect. Dis. 15:1-12, Shafer, R. W. and Sierra, M. F. 1992 Clin. Infect. Dis. 15:161-162. Additionally, 13% of new cases (HIV-1 infected and non-infected) of M. tuberculosis are resistant to one of the primary anti-tuberculosis drugs (isoniazid [INH], rifampin [RIF], streptomycin [STR], ethambutol [EMB] and pyrazinamide [PZA] and 3.2% are resistant to both RIF and INH, Block, et al., JAMA 271:665-671, (1994). Consequently, mycobacterial species identification and the determination of drug resistance have become central concerns during the diagnosis of mycobacterial diseases.

[0008] Methods used to detect, and to identify *Mycobacterium* species vary considerably. For detection of *Mycobacterium tuberculosis*, microscopic examination of acid-

fast stained smears and cultures are still the methods of choice in most microbiological clinical laboratories. However, culture of clinical samples is hampered by the slow growth of mycobacteria. A mean time of four weeks is required before sufficient growth is obtained to enable detection and possible identification. Recently, two more rapid methods for culture have been developed involving a radiometric, Stager, C. E. et al., (1991) J. Clin. Microbiol. 29:154-157, and a biphasic (broth/agar) system Sewell, et al., (1993) J. Clin. Microbiol. 29:2689-2472. Once grown, cultured mycobacteria can be analyzed by lipid composition. the use of species specific antibodies, species specific DNA or RNA probes and PCR-based sequence analysis of 16S rRNA gene (Schirm, et al. (1995) J. Clin. Microbiol. 33:3221-3224; Kox, et al. (1995) J. Clin. Microbiol. 33:3225-3233) and IS6110 specific repetitive sequence analysis (For a review see, e.g., Small et al., P. M. and van Embden, J. D. A. (1994) Am. Society for Microbiology, pp. 569-582). The analysis of 16S rRNA sequences (RNA and DNA) has been the most informative molecular approach to identify Mycobacteria species (Jonas, et al., J. Clin. Microbiol. 31:2410-2416 (1993)). However, to obtain drug sensitivity information for the same isolate, additional protocols (culture) or alternative gene analysis is necessary.

[0009] To determine drug sensitivity information, culture methods are still the protocols of choice. *Mycobacteria* are judged to be resistant to particular drugs by use of either the standard proportional plate method or minimal inhibitory concentration (MIC) method. However, given the inherent lengthy times required by culture methods, approaches to determine drug sensitivity based on molecular genetics have been recently developed.

[0010] Because resistance to RIF in E. coli strains was observed to arise as a result of mutations in the rpoB gene, Telenti, et al., id., identified a 69 base pair (bp) region of the M. tuberculosis rpoB gene as the locus where RIF resistant mutations were focused. Kapur, et al., (1995) Arch. Pathol. Lab. Med. 119:131-138, identified additional novel mutations in the M. tuberculosis rpoB gene which extended this core region to 81 bp. In a detailed review on antimicrobial agent resistance in mycobacteria, Musser (Clin. Microbiol. Rev., 8:496-514 (1995)), summarized all the characterized mutations and their relative frequency of occurrence in this 81 bp region of rpoB. Missense mutations comprise 88% of all known mutations while insertions (3 or 6 bp) and deletions (3, 6 and 9 bp) account for 4% and 8% of the remaining mutations, respectively. Approximately 90% of all RIF resistant tuberculosis isolates have been shown to have mutations in this 81 bp region. The remaining 10% are thought possibly to involve genes other than rpoB.

[0011] For the above reasons, it would be desirable to have simpler methods which identify and characterize microorganisms, such as *Mycobacteria*, both at the phenotypic and genotypic level. This invention fulfills that and related needs.

SUMMARY OF THE INVENTION

[0012] In one aspect, the invention provides isolated nucleic acids comprising at least 25, 50, 75, 100, or 200 contiguous bases from an rpoB sequence shown in Table 1 (SEQ ID NOS: 1-181). Some nucleic acid comprise a complete sequence shown in Table 1.

[0013] The invention further provides a set of probes perfectly complementary to and spanning such nucleic acids, preferably spanning one of the complete sequences shown in Table 1 (SEQ ID NOS: 1-181).

[0014] The invention further provides methods of classifying mycobacteria. Some such methods entail providing a sample comprising a mycobacterial rpoB target nucleic acid from a mycobacteria, determining the sequence of a segment of at least 50 contiguous bases from the target nucleic acid; comparing the determined sequence to at least one sequence shown in Table 1; and classifying the mycobacteria from the extent of similarity of the compared sequences. Preferably, at least 100 or 200 contiguous bases are determined from the target nucleic acid. Preferably, the determined sequence is compared with a plurality of sequences from Table 1, for example, 10, 20, 50 or all of the sequence from Table 1 (SEQ ID NOS: 1-181).

[0015] In other methods of classification, the identity of one or more bases in the target sequence at one or more positions corresponding to one or more of the highlighted positions in a sequence shown in Table 1 is determined. The identity of the one or more bases characterizing the species of mycobacteria that is present in the sample. In some methods, the identity of at least 10 bases in the target nucleic acid at positions corresponding to highlighted positions in a sequence shown in Table 1 is determined. In some methods, the identity of at least 20 bases in the target sequence at highlighted positions shown in Table 1 are identified. In some methods, at least 20 determined bases are compared with 20 bases occupying corresponding positions in each of at least ten sequences from Table 1.

[0016] In another aspect, the invention provides sequence-specific polynucleotide probes or primers that hybridizes to a segment of a mycobacterial rpoB sequence shown in Table 1 or its complement without hybridizing to the *M. tuberculosis* sequence designated ATCC9-Mtb in Table 1 or its complement, the segment including a highlighted nucleotide position shown in Table 1. In some such probes, a central position of the probe aligns with a highlighted nucleotide position shown in Table 1. In some such primers, the 3' end of the primer aligns with a highlighted nucleotide position shown in Table 1. Some probes and primers are between 10 and 50 bases long.

[0017] In another aspect, the invention provides a computer-readable storage medium for storing data for access by an application program being executed on a data processing system. Such a system comprises a data structure stored in the computer-readable storage medium. The data structure includes information resident in a database used by the application program and includes a plurality of records, each record comprising information identifying a polymorphism or sequence shown in Table 1. Some records have a field identifying a base occupying a polymorphic site and a field identifying location of the polymorphic site. Some records record a contiguous segment of at least 50, 100, or 200 bases from an rpoB sequence shown in Table 1. Some storage medium comprise at least ten records each recording a contiguous segment of at least 50 bases from at least ten rpoB sequences shown in Table 1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1: Computer that may be utilized to execute software embodiments of the present invention.

[0019] FIG. 2: A system block diagram of a typical computer system that may be used to execute software embodiments of the invention.

DEFINITIONS

[0020] A polynucleotide can be DNA or RNA, and single-or double-stranded. Polynucleotide can be naturally occurring or synthetic, and can be of any length. Preferred polynucleotide probes of the invention include contiguous segments of DNA, or their complements including any of the highlighted bases shown in Table 1. The segments are usually between 5 and 100 bases, and often between 5-10, 5-20, 10-20, 10-50, 20-50 or 20-100 bases. The highlighted site can occur within any position of the segment. Preferred polynucleotide probes are capable of binding in a base-specific manner to a complementary strand of nucleic acid. Such probes include peptide nucleic acids, as described in Nielsen et al., *Science* 254, 1497-1500 (1991), and probes having nonnaturally occurring bases.

[0021] The term primer refers to a single-stranded polynucleotide capable of acting as a point of initiation of template-directed DNA synthesis under appropriate conditions (i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or 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 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. The term primer site refers to the area of the target DNA to which a primer hybridizes. The term primer pair means a set of primers including a 5' upstream primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3', downstream primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

[0022] A cDNA or cRNA is derived from an RNA if it produced by a process in which the RNA serves as a template for production of the cDNA or cRNA.

[0023] Hybridizations are usually performed under stringent conditions, for example, at a salt concentration of no more than 1 M and a temperature of at least 25° C. For example, conditions of 5×SSPE (750 mM NaCl, 50 mM Na Phosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30° C. are suitable for allele-specific probe hybridizations.

[0024] An isolated nucleic acid means an object species invention that is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition). Preferably, an isolated nucleic acid comprises at least about 50, 80 or 90 percent (on a molar basis) of all macromolecular species present. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods).

[0025] For sequence comparison and homology determination, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm

program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0026] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally, Ausubel et al., infra).

[0027] One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

[0028] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic

acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0029] The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which the probe nucleic acid is designed to specifically hybridize. It is the presence or expression level of the target nucleic acid that is to be detected or quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the overall sequence (e.g. gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

[0030] "Subsequence" refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

DETAILED DESCRIPTION

I. Mycobacterial Sequences of rpoB Genes

[0031] Table 1 shows a comparison of a substantial collection of mycobacterial strains of an about 700-nucleotide conserved region of an rpoB gene. The sequences shown in Table 1 are identified as follows: SEQ ID NOS: 1-56, respectively, are shown on pages 21, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61 and 65; SEQ ID NOS: 57-112, respectively, are shown on pages 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 62 and 65; SEQ ID NOS: 113-168, respectively, are shown on pages 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63 and 66; SEQ ID NOS: 169-181, respectively, are shown on pages 24, 28, 32, 36, 40, 44, 52, 56, 60, 64 and 68. The first sequence, designated as a reference sequence, is from M. tuberculosis. Nucleotides are numbered consecutively starting from the first nucleotide of the reference sequences. Other sequences are from other strains of mycobacteria. For example, the sequences designated ATCC-av, M29, M30 . . . M104 are from M. avium. Sequences designated from ATT-chelnew, M11, M13, and M17 are from M. chelonae. Sequences designated ATCC-for, M53, M55, M56, and M74 are from M. fortuitum, and so forth. Complete correspondence between strain designations and strain types is shown in Table 2. Nucleotides in a mycobacterial sequence are accorded the same number as the corresponding position of the reference sequence when the two are maximally aligned. Differences between a sequence and the reference sequences are shown in highlighted type. Many of the highlighted positions are common to all tested members of a species. Other highlighted positions vary among different isolates in a species. Both types of variation can be useful in speciation analysis.

II. Analysis of Species Variations

[0032] A. Preparation of Samples

[0033] An rpoB sequence is isolated from a sample of an unknown mycobacteria being tested. Nucleic acids can be isolated from myobacteria by standard methods as described in WO 97/29212 (incorporated by reference in its entirety for all purposes). The rpoB sequences to be analyzed can then be isolated and amplified by means of PCR. See generally PCR Technology. Principles and Applications for DNA Amplification (ed. H. A. Erlich, Freeman Press, NY, N.Y., 1992); PCR Protocols: A Guide to Methods and

Applications (eds. Innis, et al., Academic Press, San Diego, Calif., 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, 17 (1991); PCR (eds. McPherson et al., IRL Press, Oxford); and U.S. Pat. No. 4,683,202 (each of which is incorporated by reference for all purposes). Primers for PCR preferably flank the regions of interest rpoB genes, although primers to internal sites can be used if it is intended to analyze only certain sites of potential species variation. Exemplary primers are described in WO 97/29212. If necessary, additional sequences flanking the sequences shown in Table 1 can be determined using probes based on the sequences in Table 1 to isolate full-length rpoB sequences from the appropriate mycobacterial species.

[0034] B. Detection of Species-Specific Variations in Target DNA

[0035] 1. Sequence-Specific Probes

[0036] The design and use of sequence-specific probes for analyzing polymorphisms is described by e.g., Saiki et al., Nature 324, 163-166 (1986); Dattagupta, EP 235,726, Saiki, WO 89/11548. Sequence-specific probes can be designed that hybridize to a segment of target DNA in one isolate of mycobacteria that do not isolate to a corresponding isolate in another due to the presence of allelic or species variations in the respective segments from the two sequences. Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the sequences. Some probes are designed to hybridize to a segment of target DNA such that the site of potential sequence variation aligns with a central position (e.g., in a 15 mer at the 7 position; in a 16 mer, at either the 8 or 9 position) of the probe. This design of probe achieves good discrimination in hybridization between different allelic and species variants.

[0037] Sequence-specific probes are often used in pairs, one member of a pair showing a perfect match to a reference form of a target sequence and the other member showing a perfect match to a variant form. Several pairs of probes can then be immobilized on the same support for simultaneous analysis of multiple potential variations within the same target sequence.

[0038] 2. Tiling Arrays

[0039] The bases occupying sites of potential variation can also be identified by hybridization to nucleic acid arrays, some example of which are described by WO 95/11995 (incorporated by reference in its entirety for all purposes). Such arrays contain a series of overlapping probes spanning a reference sequence. Any of the rpoB sequences shown in Table 1, or contiguous segments of, for example, at least 25, 50, 100 or 200 bases thereof, can serve as a reference sequence. WO 95/11995 also describes subarrays that are optimized for detection of a variant forms of a precharacterized polymorphism. Such a subarray contains probes designed to be complementary to a second reference sequence, which is a variant of the first reference sequence. The inclusion of a second group (or further groups) can be particular useful for analyzing short subsequences of the primary reference sequence in which multiple mutations are expected to occur within a short distance commensurate with the length of the probes (i.e., two or more mutations within 9 to 21 bases).

[0040] 3. Sequence-Specific Primers

[0041] A sequence-specific primer hybridizes to a site on target DNA overlapping a polymorphism and only primes amplification of a variant form to which the primer exhibits perfect complementarily. See Gibbs, Nucleic Acid Res. 17, 2427-2448 (1989). This primer is used in conjunction with a second primer which hybridizes at a distal site. Amplification proceeds from the two primers leading to a detectable product signifying the particular variant form is present. A control is usually performed with a second pair of primers, one of which shows a single base mismatch at the site of variation and the other of which exhibits perfect complementarily to a distal site. The single-base mismatch prevents amplification and no detectable product is formed. The method works best when the mismatch is included in the 3'-most position of the primer aligned with the point of variation because this position is most destabilizing to elongation from the primer. See, e.g., WO 93/22456.

[0042] 4. Direct-Sequencing

[0043] The direct analysis of mycobacterial sequences can be accomplished using either the dideoxy chain termination method or the Maxam Gilbert method (see Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind et al., *Recombinant DNA Laboratory Manual*, (Acad. Press, 1988)).

III. Methods of Use

[0044] The sequences and polymorphisms shown in Table 1 are useful for identifying the presence of myobacteria in samples, and optionally, classifying the mycobacteria. The sample can be obtained from a patient or from a biological source, such as a food product.

[0045] The sequences shown in Table 1 can be used for design of sequence-specific probes or primers encompassing polymorphic sites as described above. These probes or primers can then be used to determine the base occupying a corresponding position in an rpoB sequence from an isolate in a sample under test. A base in one sequence corresponds with a base in another when the two bases occupy the same position when the two sequences are maximally aligned by one of the criteria described in Definitions.

[0046] Alternatively, the sequences shown in Table 1 can be used for design of tiling arrays in which one or more of the sequences serves as a reference sequence. At least one set of overlapping probes is designed spanning a segment of the reference sequence, as described in WO95/11995 or EP 717,113. Target sequences from samples under test can be hybridized to such arrays, optionally in combination with controls of known rpoB sequences. The hybridization pattern of a target sequence to such an array can be analyzed to determine the identity of bases at which the target sequence differs from the reference sequence, as described in WO 95/11995.

[0047] One or more of the above methods, or direct sequencing, can be used to identify the base occupying at least one and usually several (e.g., 5, 10, 15, 25, 50 or 100) sites of potential variation between the 16S RNA and/or rpoB gene in an unknown mycobacteria relative to bases occupying corresponding sites in one or more known strains of mycobacteria, such as those shown in Table 1. This analysis results in a profile of bases occupying particular

sites that characterizes the mycobacterial strain under test. The profile is compared with the corresponding profiles of different mycobacterial isolates shown in e.g., Table 1. In general, the unknown mycobacterium isolate is characterized as being from the same mycobacterial species as the precharacterized isolate with which it shares the greatest similarity in base profile.

[0048] In some methods, the sequence of a contiguous segment of the rpoB target nucleic acid is determined in a sample under test for comparison with one or more of the sequences shown in Table 1. The mycobacteria is classified by the extent of similarity. For example, if a target nucleic acid shows greater sequence identity to rpoB sequences from one species than any other, the sample from which the target was obtained is typically classified as arising from that species.

[0049] Alternatively, an array of tiled probes based on a reference sequence shown in Table 1 can be used for identifying and characterizing mycobacterial sequences based on comparison of hybridization patterns. Such an array is hybridized to a 16S RNA or rpoB target sequence from a sample, and the hybridization pattern compared with the hybridization pattern of one or more control sequences. The hybridization patterns of control sequences can be historic controls, stored, for example, in a computer database, or can be contemporaneous controls performed at or near the same time as the hybridization to the target sequence. Optionally, hybridization of target and reference sequence can be performed simultaneously using different labels.

[0050] Method of classifying unknown mycobacterial isolate by matching the hybridization pattern of a target sequence with those of control sequences from characterized species are described in more detail in WO 97/29212 (incorporated by reference in its entirety for all purposes). In an idealized case, the detection of a particular hybridization pattern in an isolate characterizes that isolate as belonging to a particular species. This can occur when the hybridization pattern detected in the isolate is uniquely associated with a specific species. More frequently however, such an unique one-to-one correspondence is not present. Instead, the hybridization pattern observed in an isolate does not bear a unique correspondence with a previously characterized species. However, the hybridization pattern detected is associated with a probability of the organism being screened belonging to a particular species (or not) or carrying a particular phenotypic trait (or not). As a result, analysis of an increasing number of polymorphic sites in an isolate, allows one to classify the isolated with an increasing level of confidence. Algorithms can be used to derive such composite probabilities from the comparison of multiple polymorphic forms between an isolate and references. Typically, the mathematical algorithm makes a call of the identity of the species and assign a confidence level to that call. One can determine the confidence level (>90%, >95% etc.) that one desires and the algorithm will analyze the hybridization pattern and either provide an identification or not. Occasionally, the call is that the sample may be one of two, three or more species, in which case a specific identification is not be possible. However, one of the strengths of this technique is that the rapid screening made possible by the chip-based hybridization allows one to continuously expand a database of patterns ultimately to enable the identification of species previously unidentifiable due to lack of sufficient information.

IV. Modified Polypeptides and Gene Sequences

[0051] The invention further provides variant forms of nucleic acids and corresponding proteins. The nucleic acids comprise one of the sequences described in Table 1. Some nucleic acid encode full-length variant forms of proteins. Variant proteins have the prototypical amino acid sequences of encoded by nucleic acid sequence shown in Table 1 (read so as to be in-frame with the full-length coding sequence of which it is a component).

[0052] Variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is a eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage promoters, glycolytic enzyme promoters and tRNA promoters, depends on the host selected. Commercially available expression vectors can be used. Vectors can include host-recognized replication systems, amplifiable genes, selectable markers, host sequences useful for insertion into the host genome, and the like.

[0053] The means of introducing the expression construct into a host cell varies depending upon the particular construction and the target host. Suitable means include fusion, conjugation, transfection, transduction, electroporation or injection, as described in Sambrook, supra. A wide variety of host cells can be employed for expression of the variant gene, both prokaryotic and eukaryotic. Suitable host cells include bacteria such as *E. coli*, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell lines and derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide. Processing includes glycosylation, ubiquitination, disulfide bond formation, general post-translational modification, and the like.

[0054] The protein may be isolated by conventional means of protein biochemistry and purification to obtain a substantially pure product, i.e., 80, 95 or 99% free of cell component contaminants, as described in Jacoby, *Methods in Enzymology* Volume 104, Academic Press, New York (1984); Scopes, *Protein Purification, Principles and Practice*, 2nd Edition, Springer-Verlag, New York (1987); and Deutscher (ed), *Guide to Protein Purification, Methods in Enzymology*, Vol. 182 (1990). If the protein is secreted, it can be isolated from the supernatant in which the host cell is grown. If not secreted, the protein can be isolated from a lysate of the host cells.

[0055] In addition to substantially full-length polypeptides expressed by variant genes, the present invention includes biologically active fragments of the polypeptides, or analogs thereof, including organic molecules which simulate the interactions of the peptides. Biologically active fragments include any portion of the full-length polypeptide which confers a biological function on the variant gene product, including ligand binding, and antibody binding. Ligand binding includes binding by nucleic acids, proteins or polypeptides, small biologically active molecules, or large cellular structures.

[0056] Polyclonal and/or monoclonal antibodies that specifically bind to variant gene products but not to corresponding prototypical gene products are also provided. Antibodies can be made by injecting mice or other animals with the variant gene product or synthetic peptide fragments thereof. Monoclonal antibodies are screened as are described, for example, in Harlow & Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Press, New York (1988); Goding, Monoclonal antibodies, Principles and Practice (2d ed.) Academic Press, New York (1986). Monoclonal antibodies are tested for specific immunoreactivity with a variant gene product and lack of immunoreactivity to the corresponding prototypical gene product. These antibodies are useful in diagnostic assays for detection of the variant form, or as an active ingredient in a pharmaceutical composition.

V. Kits

[0057] The invention further provides kits comprising at least one sequence-specific probe as described above. Often, the kits contain one or more pairs of sequence-specific probes hybridizing to different forms of a polymorphism. In some kits, the sequence-specific probes are provided immobilized to a substrate. For example, the same substrate can comprise sequence-specific probes for detecting at least 10, 100 or all of the variations shown in Table 1. Optional additional components of the kit include, for example, restriction enzymes, reverse-transcriptase or polymerase, the substrate nucleoside triphosphates, means used to label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for reverse transcription, PCR, or hybridization reactions. Usually, the kit also contains instructions for carrying out the methods.

VI. Computer Databases

[0058] FIG. 1 illustrates an example of a computer system that can be used to store records relating to polymorphisms of the invention and perform algorithms comparing polymorphic profiles and to classify species. FIG. 2 shows a computer system 100 which includes a monitor 102, screen 104, cabinet 106, keyboard 108, and mouse 110. Mouse 110 may have one or more buttons such as mouse buttons 112. Cabinet 106 houses a CD-ROM drive 114, a system memory and a hard drive (see FIG. 2) which can be utilized to store and retrieve software programs incorporating code that implements the present invention, data for use with the present invention, and the like. Although a CD-ROM 116 is shown as an exemplary computer readable storage medium, other computer readable storage media including floppy disks, tape, flash memory, system memory, and hard drives may be utilized. Cabinet 106 also houses familiar computer components such as a central processor, system memory, hard disk, and the like.

[0059] FIG. 2 shows a system block diagram of computer system 100 that may be used to execute software embodiments of the present invention. As in FIG. 1, computer system 100 includes monitor 102 and keyboard 108. Computer system 100 further includes subsystems such as a central processor 102, system memory 120, I/O controller 122, display adapter 124, removable disk 126 (e.g., CD-ROM drive), fixed disk 128 (e.g., hard drive), network interface 130, and speaker 132. Other computer systems suitable for use with the present invention may include additional or fewer subsystems. For example, another computer system can include more than one processor 102 (i.e., a multi-processor system) or a cache memory.

[0060] Arrows such as 134 represent the system bus architecture of computer system 100. However, these arrows are illustrative of any interconnection scheme serving to link the subsystems. For example, a local bus can be utilized to connect the central processor to the system memory and display adapter. Computer system 100 shown in FIG. 1 is but an example of a computer system suitable for use with the present invention.

[0061] The computer stores records relating to the polymorphisms of the record. Some such records record a polymorphism by reference to the position of a polymorphic site and the identity of base(s) occupying that site in one or more species. Some databases include records for at least ten polymorphic sites in at least ten of the sequences shown in Table 1. Some databases include records for all of the polymorphic sites in at least one of the sequences shown in Table 1. Some databases includes records for at least 100, 1000, or 2000 polymorphic sites shown in Table 1. Some databases include records for all of the polymorphic sites shown in Table 1.

[0062] The foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

[0063] All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

T CCCAGGACGTGGAGGCGATCACACCGCAGACBET CCCAGGACGTGGAGGCGATCACACCGCAGAGG CCCAGGACGTGGAGGCGATCAMAACGGGCGAGAC Table 1 BASE NOS 1-60 00A00C0ATCACACCGGGGATCACACCGGGGATCACACCCGGGATCACACCGCCCAGGAATCACACCCCCCAGGAACGATCACCCCCCCAGGAACGATCACCCCCCCAGGAACGATCACACCCC GACGT CCCAGGACGT CCCAGG \$5C (10 NO 2)
\$5C (10 NO 4)
\$5C (10 NO 6)
\$5C (10 NO 7)

ATCC9-Hb, txt AGC01-av, txt H30. txt H30. txt H31. txt H31. txt H31. txt H31. txt H34. txt H64 (1av2), txt H64 (1av2), txt H69 (1av2), txt H60 (1av2), txt H60 (1av2), txt H10 (1av2), txt H11 (1av2), txt H12 (1av2), txt H12 (1av2), txt H13 (1av2), txt H14 (1av2), txt H15 (1av2), txt H15 (1av2), txt H16 (1av2), txt H17 (1av2), txt H18 (1av

Table 1
BASE NOS 1-60

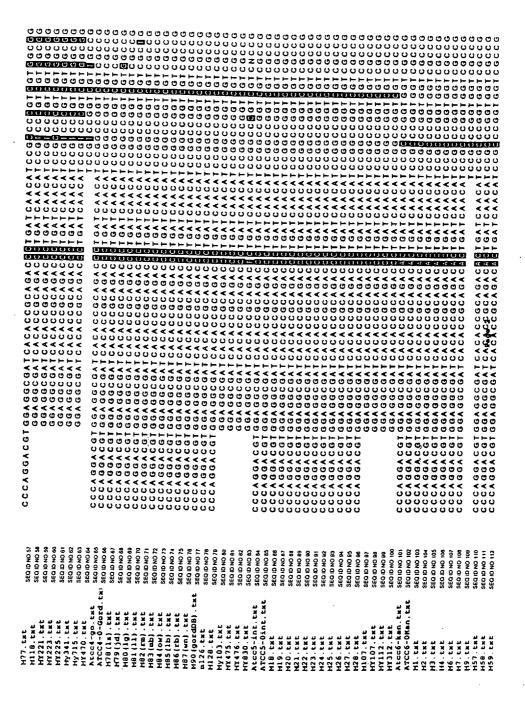


Table 1
BASE NOS 1-60

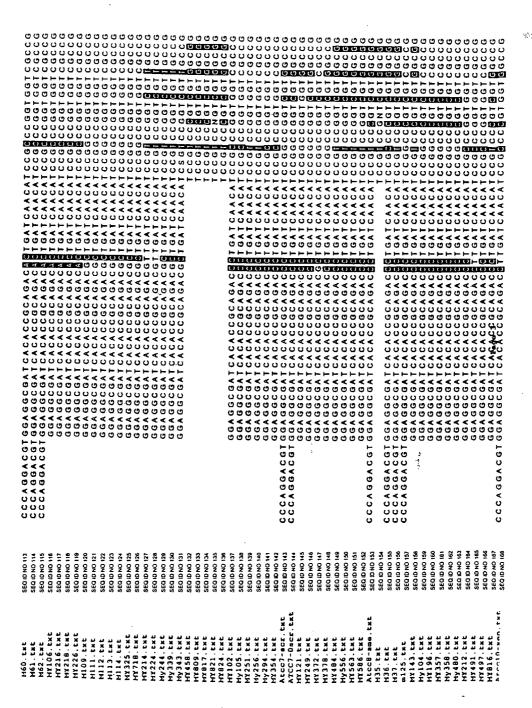
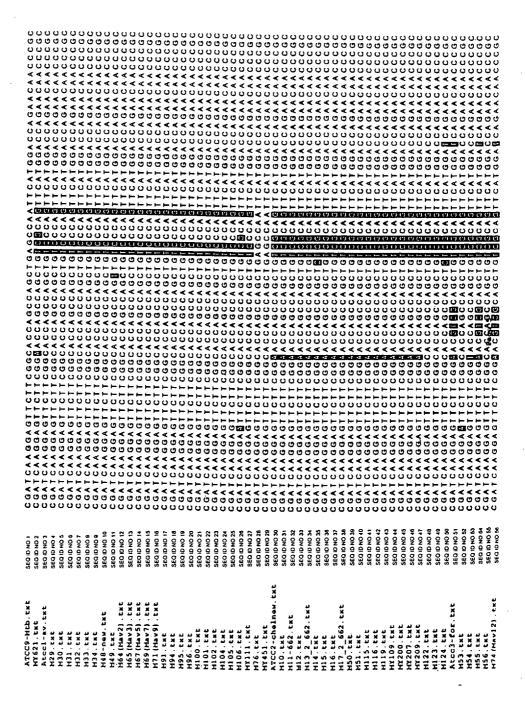


Table 1
BASE NOS 1-60

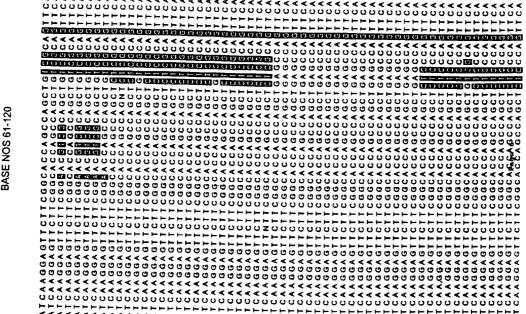
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SEO D NO 179
SEO D NO 179
SEO D NO 171
SEO D NO 173
             . txt
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Table 1



SEC DNO 53
SEC DNO 56
SEC DNO 66
SEC DNO 66
SEC DNO 67
SEC DNO 68
SEC DNO 67
SEC DNO 70

H19. EXE H123. EXE H723. EXE H723. EXE H723. EXE H775. EXE H776. EXE H



BASE NOS 61-120

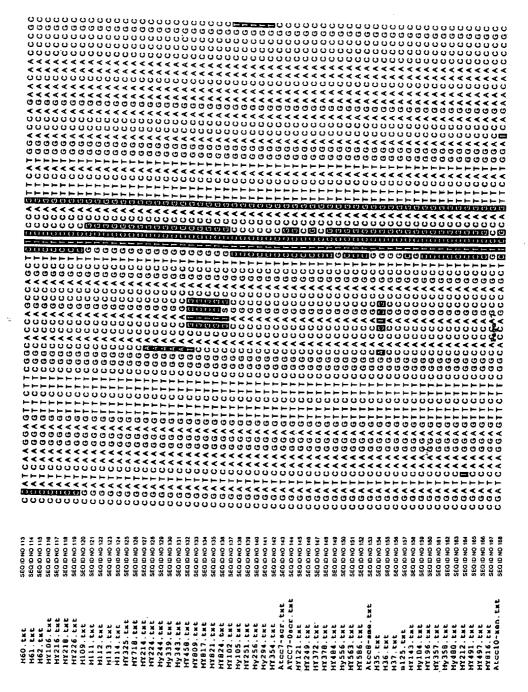
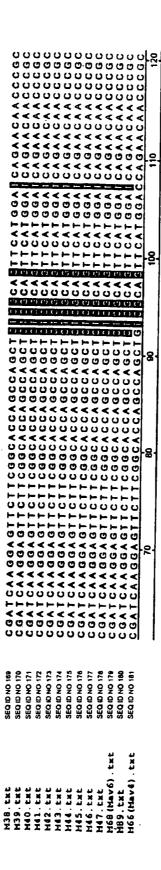


Table 1
BASE NOS 61-1

Table 1

BASE NOS 61-120



Fable 1 ASE NOS 121-180

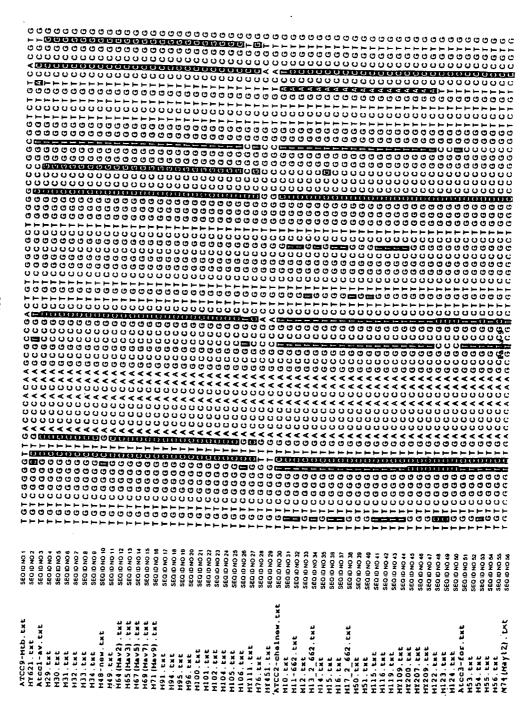


Table 1

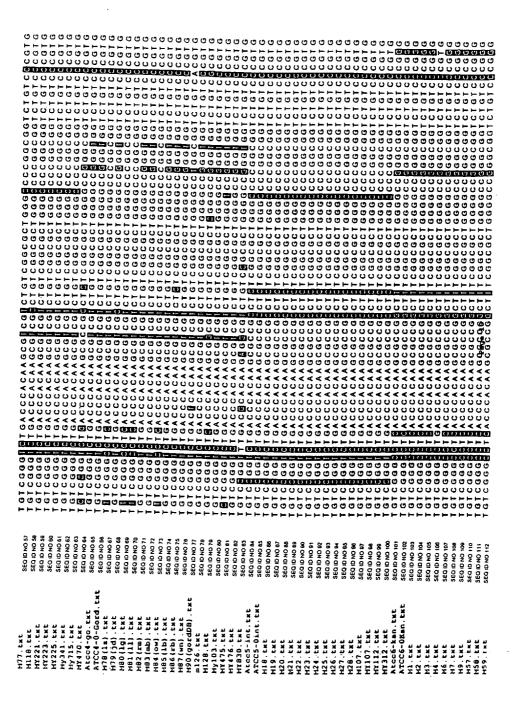
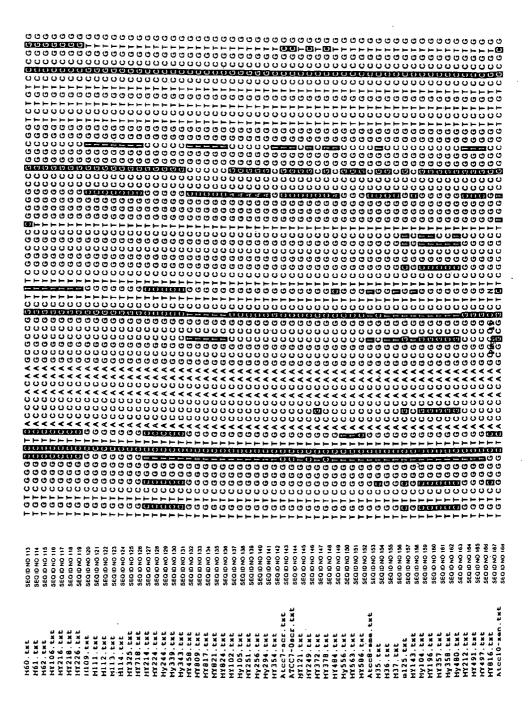


Table 1 3ASE NOS 121-180



l able 1 BASE NOS 121-18

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M38.txt M40.txt M41.txt M42.txt M42.txt M43.txt M46.txt M46.txt M46.txt M47.txt M89.txt H89.txt H66(Mav4).txt	

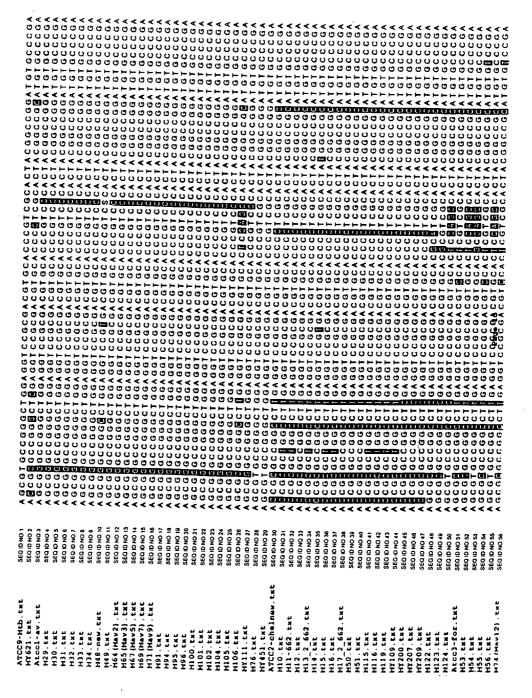


Table 1 BASE NOS 181-240 Table 1 BASE NOS 181-240

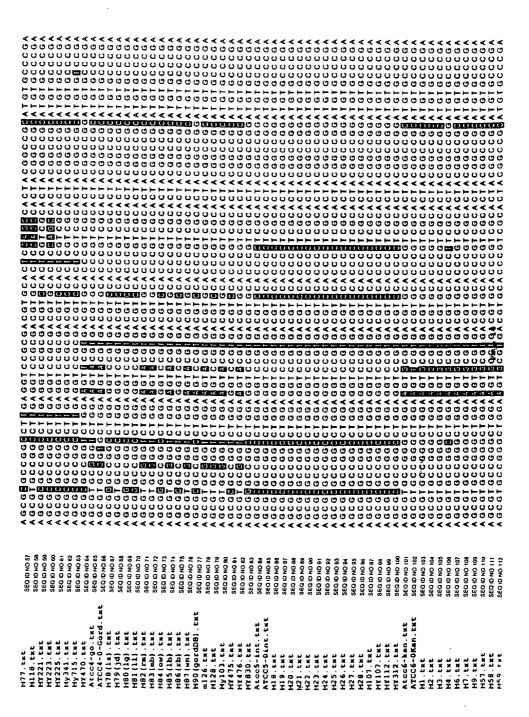
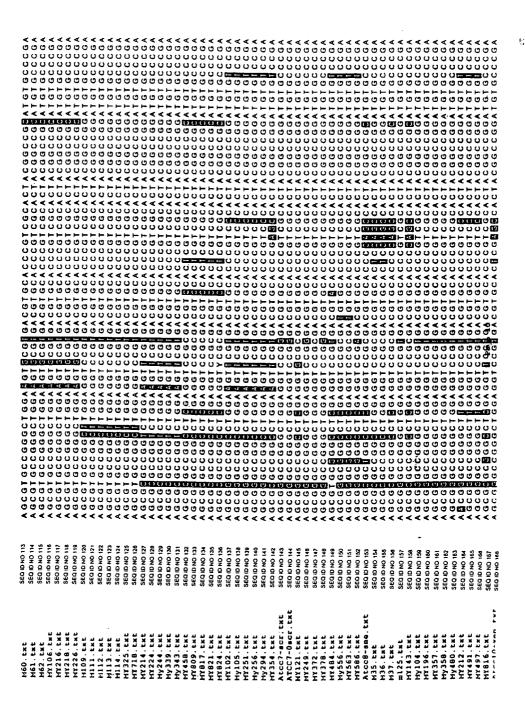


Table 1
BASE NOS 181-240



lable 1 BASE NOS 181-240

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(Mav6).
H39.
H41.
H42.
H42.
H42.
H42.
H42.
H46.
H69.
```

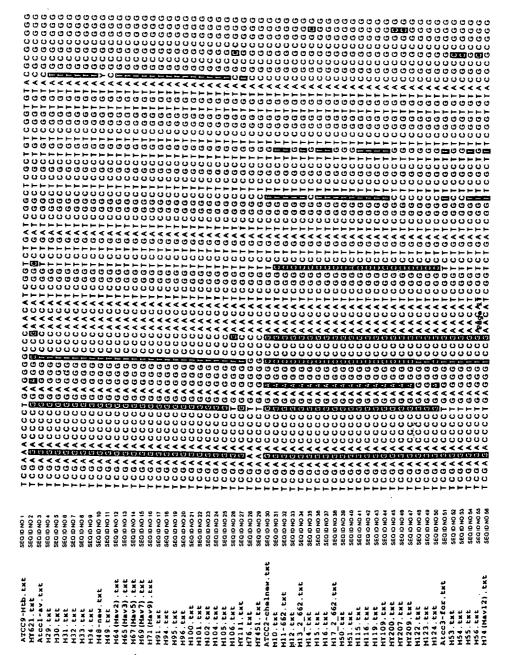


Table 1
BASE NOS 241-300

SEC ID NO 93
SEC ID NO 63
SEC ID NO 64
SEC ID NO 74
SEC ID NO 75
SEC I t x M17. tkt M1221. tkt M1221. tkt M1223. tkt M1223. tkt M1225. tkt M470. tkt M470. tkt M9715. tkt M9716. tkt M9717. tkt M9717. tkt M9717. tkt M9717. tkt M9718. tkt

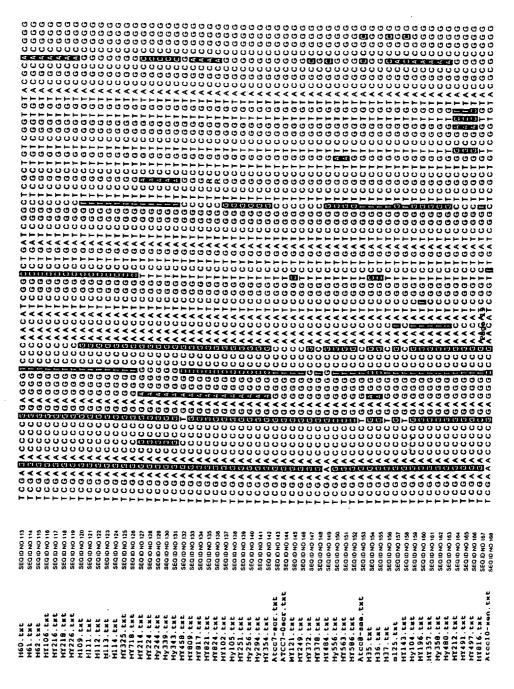


Table 1 BASE NOS 241-300

| **able 1** BASE NOS 241-300

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(Kav 6).
M38.
M40.
H41.
H42.
H42.
H42.
H44.
H46.
H66.
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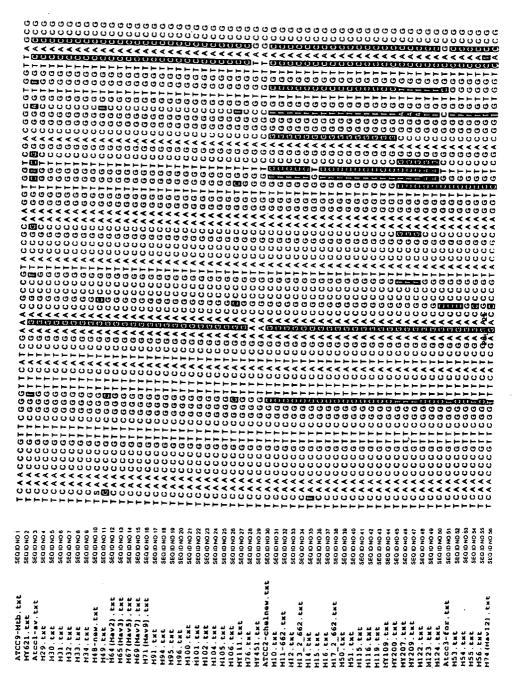


Table 1
BASE NOS 301-360

H118. tax H118. tax H118. tax H1223. tax H1223. tax H1223. tax H1223. tax H1236. tax H1240. tax H126. tax H127. tax H137. tax H147. tax H147. tax H157. tax H156. tax H177. tax

Table 1 3ASE NOS 301-360

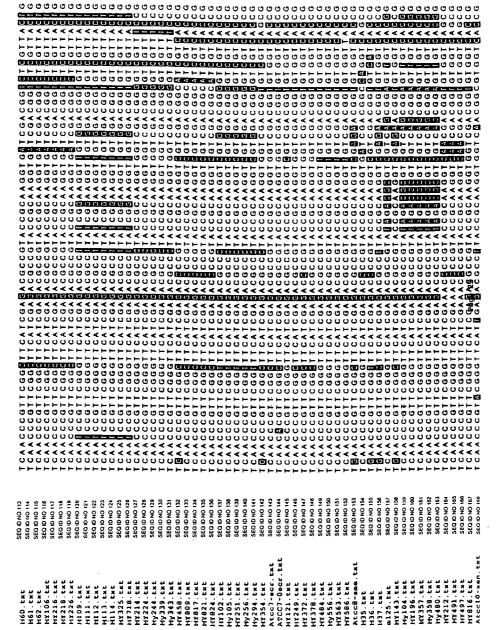


Table 1 3ASE NOS 301-36

Table 1 BASE NOS 301-360

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130 + ct	SEO ID NO 436	1、0110000日本のののです。				
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4-4	2000000					
140. CAL	SEC ID NO 1/1			A C C G C A A G G T A G T C M A C A A	TO TOOL O	
M41.txt	SEO ID NO 172	THE CHAPTER OF THE PROPERTY OF THE				
3						
M42. txt	SEQ ID NO 173	A C C C G T E C G G G T T C A T		COCCA TOOLOGY ACCOC		
474 671	SEO ID NO 131					
747.546			ロンコランマロマラ	ACCGCAAGGTGGTCBACGG		
M44. txt	SEO ID NO 175	TACTTOOCCE TOCCOA	0000			
			コンションくりくり	© © ∪ < 12 0 0 0 0 0 0 0 < < > > > > <		
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340. CKC	SEC ID NO 177	· A C C C G T B C G G G T T C A T		COUNTRACTOR A COUNTRACTOR		
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H6B (Mav6) . txt	SEQ 10 NO 179	TACT TO COCK TO COCK				
			コンション・ロくう	0004E0-00-054409004		
36V. CXC	SEC 10 NO 180	ACCCGTECGGGTTCAT		COCK BOT FOOT OF A COCK		
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HOD (MAV4) . CXC	354 ID 40 18)	ACCCGIICGGGTTCAT	CGABACGCCGT	TACCGCAAGGTGGTCGACGG	GTGGTANAGCG	
•						
				79C UP&	196	

Table 1
BASE NOS 361-420

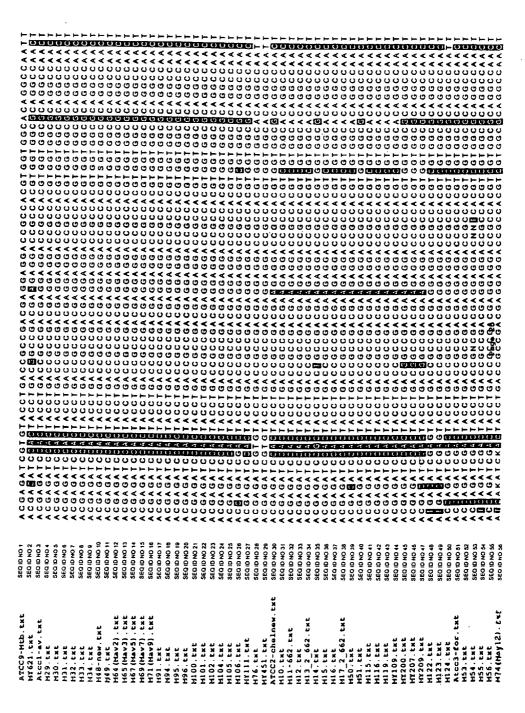


Table 1
BASE NOS 361-420

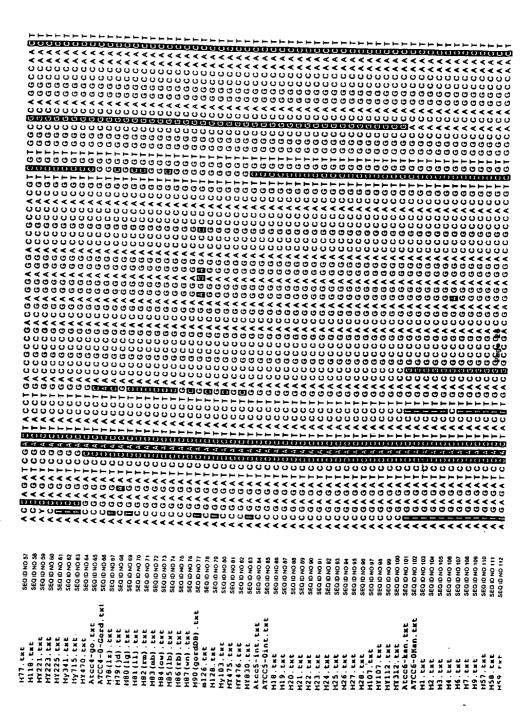


Table 1 BASE NOS 361-420

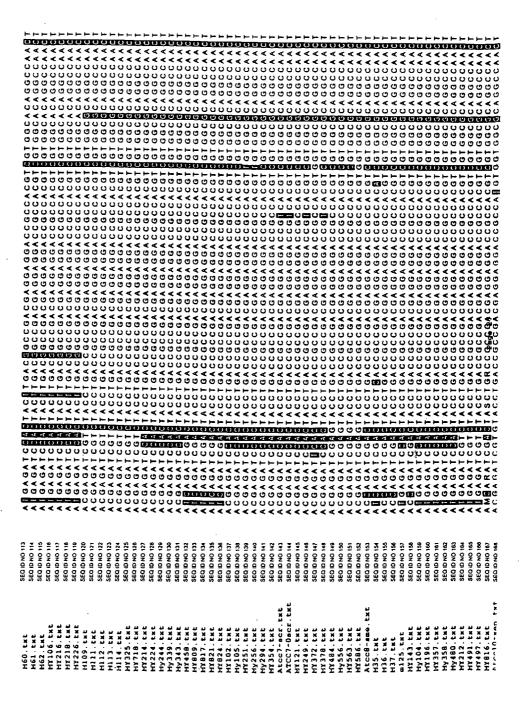


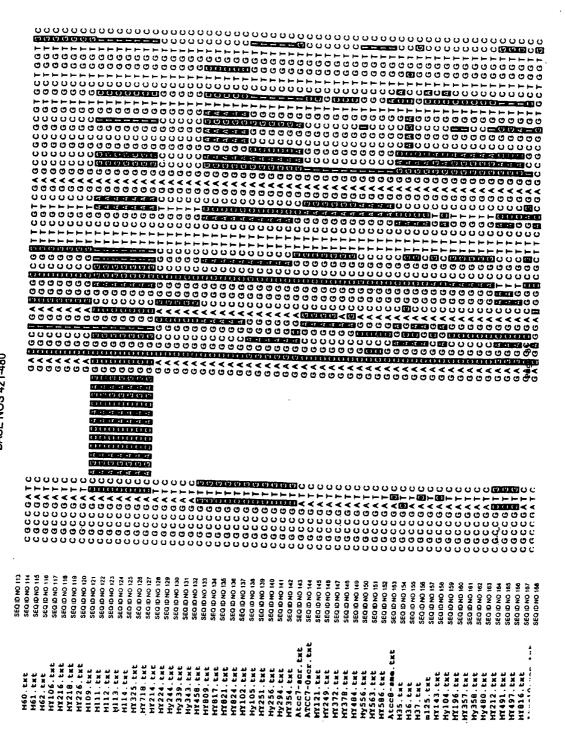
Table 1
BASE NOS 361-420

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 1 6 A C C G C C G A C G A C G A C G C C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G A C G 
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SEO ID NO 177
SEO ID NO 177
SEO ID NO 178
  H38.
H400.
H610.
H62.
H66.
H66.
H66.
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Table 1
BASE NOS 421-480

Table 1 BASE NOS 421-480

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H77. Ext
H77. Ext
H77. Ext
H7223. Lax
SEODNOS
H7325. Lax
SEODNOS
H7315. Lax
H7315. Lax
SEODNOS
H7315. Lax
SEODNOS
H7315. Lax
SEODNOS
H73113. Lax
SEODNOS
H7315. Lax
SEODNOS
H7
```

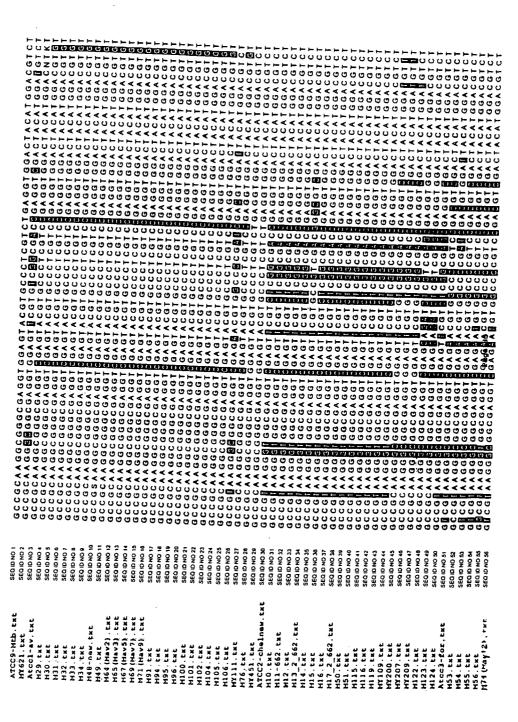


T**able 1** BASE NOS 421-480

Table 1
BASE NOS 421-480

AGCCGCGGGGTGCTGGTR	A G C G C G G G T G C T G G T B	AGCCGCGGGTGCTGGT	AGCCGCGGGTGCTGGT	AGCCGCGGGTGCTGGT	AGCCGCGGGGTGCTGGTF	TO TOUT TOUT OUT OUT OF THE	A ACCACACACACACACACACACACACACACACACACAC	A GCC GC G G G T G C T G G G T G G G T G G G T G G T G G T G G G T G G T G G T G G T G G T G G T G G T G G G T G G G T G	A GCC GC G G G T G C T G G G G G G G G G G G G G G G G G G G G	AGCGGGGGTGCTGGTG	A GCCGCGGGGTGCTGGT	A GERECOCO	470
U	C	U	C	C	Ü	ď	ď	ď	ú	ú	ú	90L	و
A MGMGGAMGGMCGCTTCM	GEGGA GGACGCTTCE	A HOHOGA HOGOCOCTTCH	A SIGBOOM GOOCGCTTCB	A B G B G G A I G G G C G C T T C B	ABGEGGAFIGGECGCTTCE	A B G G G G G G G G G G G G G G G G G G				A GOOGA HOGO COCTTOR	A DGBGGA BGG CGCTTCB	A B G C B G A C G G G C G B T T C G	46
CCGAT	COCCOATC	CCBAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	CCGAT	057
SEQ ID NO 169	SEQ ID NO 170	SEQ ID NO 171	SEQ ID NO 172	SEQ ID NO 173	SEO ID NO 174	SEO ID NO 175	SEQ ID NO 176	SEQ ID NO 177	SEQ 4D NO 178	SEG 1D NO 179	SEO ID NO 180	SEO ID NO 181	
M38.txt	M39. txt	M40. txt	H41.txt	H42.txt	M43.txt	M44.txt	M45.txt	M46.txt	M47.txt	M68 (Mav6) . txt	M89.txt	H66 (Mav4) . txt	

Table 13ASE NOS 481-540



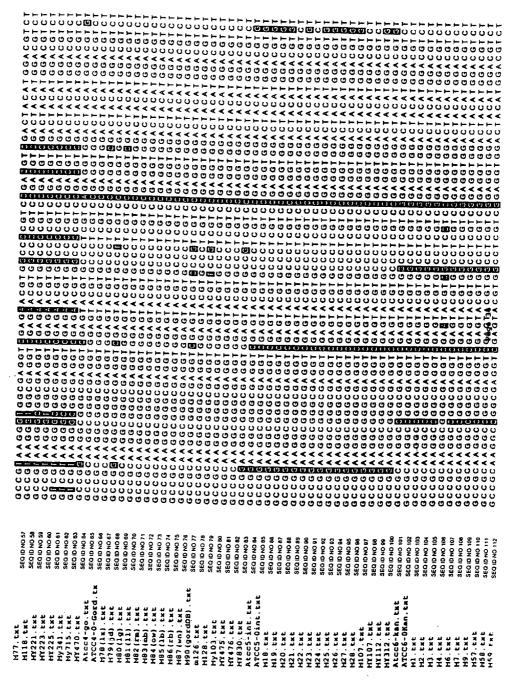


Table 1 BASE NOS 481-540 Table 1

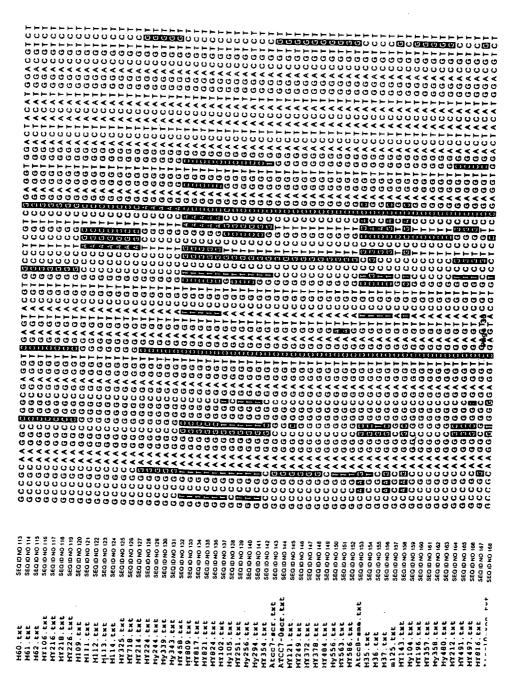


Table 1
BASE NOS 481-540

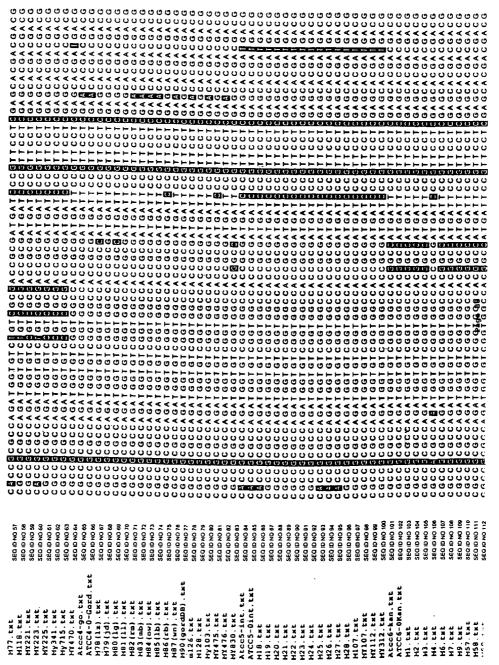
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SEQ ID NO 11
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(Mav6).
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Table 1

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SEQ B NO 2
SEQ B NO 3
SEQ B NO 6
SEQ B NO 7

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ATOCO-
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| able 1 |ASE NOS 541-600 Table 1 BASE NOS 541-600

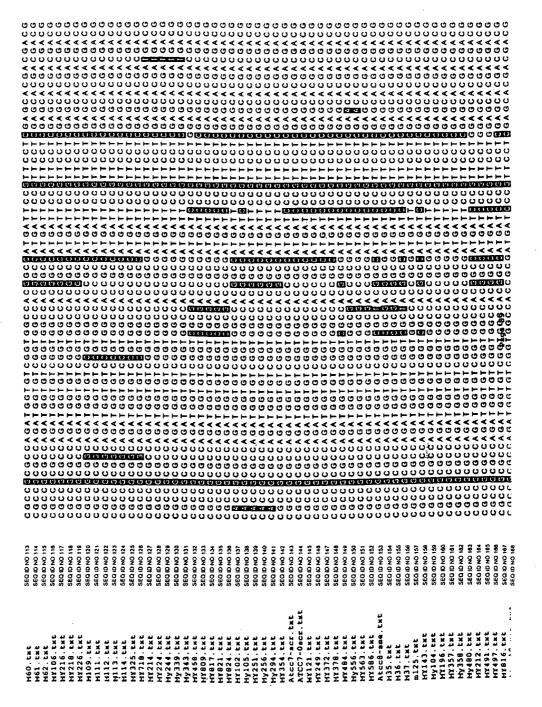


Table 1
BASE NOS 541-600

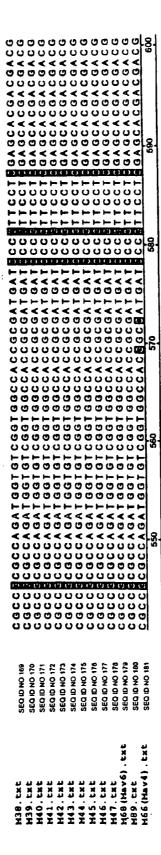


Table 1 BASE NOS 600-660

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SECIENCO 3
SECIENCO 3
SECIENCO 3
SECIENCO 10
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  MYGCS-WED. EXE

MYGCS.1. EXE

H30. EXE

H31. EXE

H32. EXE

H33. EXE

H33. EXE

H33. EXE

H34. EXE

H35. EXE

H35. EXE

H35. EXE

H35. EXE

H37. E
```

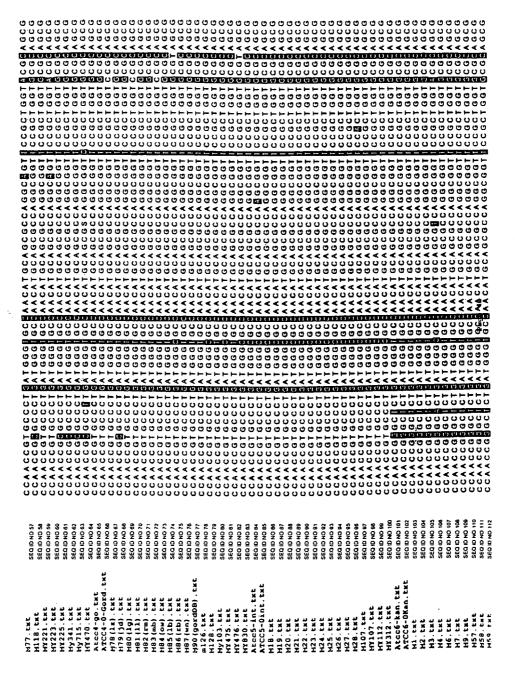


Table 1
BASE NOS 600-660



```
SEO DNO 113
SEO DNO 114
SEO DNO 115
SEO DNO 118
SEO DNO 118
SEO DNO 118
SEO DNO 118
SEO DNO 122
SEO DNO 123
SEO DNO 124
SEO DNO 125
SEO DN
                                                                                                                           i t
   H60. tat

H61. tat

H62. tat

H7106. tat

H7106. tat

H7106. tat

H7109. tat

H7111. tat

H7113. tat

H7105. tat

H7105. tat

H7214. tat

H7224. tat

H7224. tat

H7224. tat

H7224. tat

H7236. tat

H7236. tat

H7237. tat

H7207. tat
```

Table 1

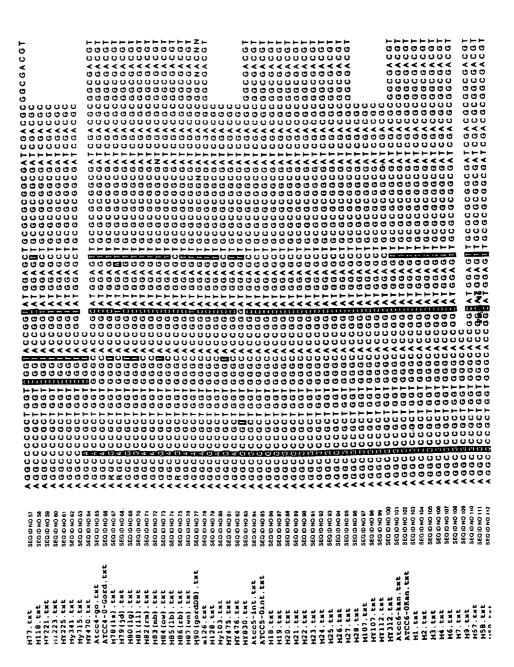
BASE NOS 600-660

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SEC ID NO 178
SEC ID NO 170
SEC ID NO 171
SEC ID NO 173
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SEC ID NO 188
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able 1	NOS 660-720
-	ASE

GA T GGA GCT GCGC GC GC GA T C GA C GC G G C G A C G T		. 6 C 6 C 6 C 6 C 6 A C 6 C 6 C 6 C 6 A C 6 C	00 40 00 00 00 A C 00 00 00 00 00 00 00 00 00 00 00 00 0	CACACACAA TCAACACACACAACA	CGCGCGGCGATCGACGCGGCGACG	GCGCGCGGCGATCGACGCGGCGACGT	CGCGCGGCGATCGACGCGGCGACG	COCOCOOCOATCOACOCOGCOACO	CGCGCGGCGATCGACGCGGCGACG	CGCGCGGGATNGACGCGGCGACG	CGCGGGGATCGACGCGGGGACG	CGCGCGGCGATCGACGCGGCGACG	GCGCGGCGATCGACGCGGCGAC	0 4 9 0 9 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0			COCOCOGCOATCOACG	CGCGCGGCGATCGACG	CGCGCGGCGATCGACG	CGCGCGCGATCGACG	CGCGCGATCGACGC		90 490 + 490 990 990 990		9049014909909090	C G C G G G G G A C G C C C C C C C C C			g			GCGCGCGCGATCGACGCGGCGACGI	-										SC G C G G C G A T C G A C G	TGCGCGGCGATCGACGC	BCGCGGCGATCGACGC	BCGCGGATCGACG	GC GC GC GAT C GA C GC G G C GA C G	0 7 4 5 7 5 5 7 5 7 4 5 7 ± 4 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	60.60.60.60.60.40.10.60.60.60.60.60.60.60.60.60.60.60.60.60		
GGCACCGG	000	LOS ASSTATISMOS COL			TOO YOU TAR	GGATGGAGCT	GGATGGAGCT	GGGATGGAGCT	GGEATGGAGCT	GGEATGGAGCT	GGEATGGAGCT	GGEATGGAGCT	GGEATGGAG				TOU VOUL V	GGEATGGAGCT	GGEATGGAGCT	GGEATGGAGCT	GGEATGGAGCT	GGENTGGAGCT	GG WAT GG A GC						GGHATGGAGCT		GGHATGGA	GGBATGGAGCT			TEGGIACCGGEATGGAG) () () () () () () () () () (7000	T 1 6 6 6 A C C	TOGGIACC	1 0 0 1 1 C C	10 C C C C C C C C C C C C C C C C C C C	A S A S A S A S B	66 A T G G A G	GONATGGABC	GGEATGGAGC	GGHATGGAGI		0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		;;:a
TOBOCCOGO !				000				0000	AGGCACCG	AGGCGCCG	A GGC GC CG	A G G C G C C G	A G G C E C C G	1900 9 0994	AGGCGGCCG			000000	0000	A G G C A C C G	A G G C E C C G	₩ 660 B CC BC	A G G C S C C G	A 6 6 C C C C G	000000V	38333388 V	99222299 ¥	19333398V	000000V		000000	A G G C C C G	AGGCCCCG	¥ G G C C C C G I	¥ 66CCCC6	000	000000	000000V	AGGCCCG	900000V	A G G C II C C G	000	5000000	90000			800000V	AGGCCCG	9000	000000 4	,,,,,,,,,,,,,,,,,,
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Table 1
BASE NOS 660-720



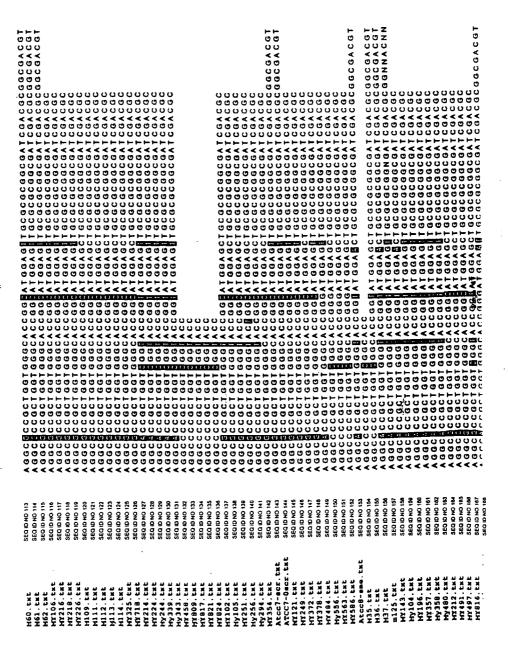


Table 1 BASE NOS 660-720

Table 1
BASE NOS 660-720

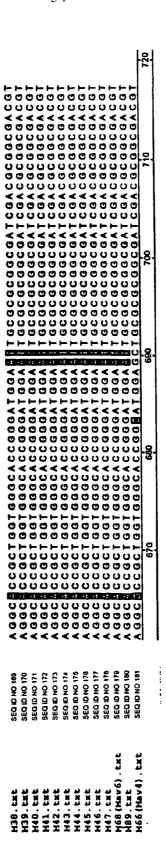


TABLE 2

TABLE 2-continued

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Affy#	SAMPLE ID#	Alt. ID	SPECIES	Affy#	SAMPLE ID#	Alt. ID	SPECIES
MY621		ATCC	M. abscessus	MY341			M. fortuitum
ATCC1	25291	ATCC-av	M. avium	MY715			M. fortuitum
M100	60300	MAC	M. avium	MY470			M. genevese
M101	60112	MAC	M. avium	ATCC4	14470	ATCC-go	M. gordonae
M102	60268	MAC	M. avium	ATCC4-0		gord.	M. gordonae
M103	60270	MAC	M. avium	M125	60068		M. gordonae
M104	60272	MAC	M. avium	M126	60182		M. gordonae
M105	60293	MAC	M. avium	M127	60214		M. gordonae
M106	60313	MAC	M. avium	M128	60283	1 7 7	M. gordonae
M107	60345	MAC	M. avium	M78	92-942	gord. LZ	M. gordonae
M29 M30	95-1764 95-1766		M. avium M. avium	M79 M80	93-692 94-94	gord. JD	M. gordonae
M31	95-1768		M. avium M. avium		93-1231	gord. LG	M. gordonae
M32	95-1770		M. avium M. avium	M81 M82	93-1231	gord. LL gord. RM	M. gordonae M. gordonae
M33	95-1775		M. avium M. avium	M83	92-1219	gord. MB	M. gordonae M. gordonae
M34	95-1776		M. avium M. avium	M84	91-1131	gord. OW	M. gordonae
M48	95-1765		M. avium	M85	91-1478	gord. LB	M. gordonae
M49	95-1769		M. avium	M86	92-642	gord. RB	M. gordonae
M63	MAC #1	MAC	M. avium	M87	93-1180	gord. WN	M. gordonae
M64	MAC #2	MAC	M. avium	M90	DB	gord. DB	M. gordonae
M65	MAC #3	MAC	M. avium	MY103		8	M. gordonae
M67	MAC #5	MAC	M. avium	MY475			M. gordonae
M69	MAC #7	MAC	M. avium	MY476			M. gordonae
M70	MAC #8	MAC	M. avium	My746			M. gordonae
M71	MAC #9	MAC	M. avium	MY830			M. gordonae
M72	MAC #10	MAC	M. avium	ATCC5		ATCC-int	M. intracellulare
M91	FM	avium-intracell.	M. avium	ATCC5-0		intra	M. intracellulare
		FM(MAC)		M18	95-1778		M. intracellulare
M92	60040	MAC	M. avium	M19	95-1780		M. intracellulare
M93	60042	MAC	M. avium	M20	94-1781		M. intracellulare
M94	60049	MAC	M. avium	M21	95-1782		M. intracellulare
M95	60051	MAC	M. avium	M22	95-1790		M. intracellulare
M96	60110	MAC	M. avium	M23	95-1794		M. intracellulare
M97	60116	MAC	M. avium	M24	95-1796		M. intracellulare
M98	60123	MAC	M. avium	M25	95-1777		M. intracellulare
M99	60176	MAC	M. avium	M26	95-1779		M. intracellulare
M78	92-773		M. bovis	M27	95-1760		M. intracellulare
MY451	25752	,moo 1 1/	M. bovis	M28	95-1761	AMMO 1	M. intracellulare
ATCC2	35752	ATCC-chel(new)	M. chelonae	ATCC6	12478	ATTC-kan	M. kansasii
M10	95A9151		M. chelonae M. chelonae	ATCC6-0	05 4 52 75	kans.	M. kansasii
M11 M115	95 A 0477 60121		M. chelonae	M1 M2	95A5375 95A10299		M. kansasii M. kansasii
M116	52942		M. chelonae	M3	96A0020		M. kansasii
M117	43192		M. chelonae	M4	95A3977		M. kansasii
M118	53180		M. chelonae	M5	95A4739		M. kansasii
M119	53131		M. chelonae	M52	95A5381		M. kansasii
M12	95A4883		M. chelonae	M57	60163		M. kansasii
M120	52923		M. chelonae	M58	60180		M. kansasii
M121	52919		M. chelonae	M59	60207		M. kansasii
M13	95A2611		M. chelonae	M59	95A2695		M. kansasii
M14	95A0779		M. chelonae	M60	60294		M. kansasii
M15	95A8654		M. chelonae	M61	60308		M. kansasii
M16	95A8882		M. chelonae	M62	60314		M. kansasii
M17	95A8881		M. chelonae	M7	95A2694		M. kansasii
M50	95A11814		M. chelonae	M73	#11	MAC#11	M. kansasii
M51	95A1102		M. chelonae	M8	94A9042		M. kansasii
M75	#13	MAC#13	M. chelonae	M9	95A1275		M. kansasii
MY109			M. chelonae	MY106			M. kansasii
MY200			M. chelonae	MY141			M. kansasii
MY207			M. chelonae	MY216			M. kansasii
MY209			M. chelonae	MY218			M. kansasii
M122	60025		M. flavescens	MY226			M. kansasii
M123	60078		M. flavescens	M108	60044		M. malmoense
M124	60252	ATTO C	M. flavescens	M109	60149		M. malmoense
ATCC3	6841	ATTC-for	M. fortuitum	M110	60211		M. malmoense
M53	60305		M. fortuitum	M111	60202		M. malmoense
M54	60344		M. fortuitum	M112	60085		M. malmoense
M55	60435		M. fortuitum	M113	60047		M. malmoense
M56	60447	MAC#12	M. fortuitum	M114	60185	ATCC	M. malmoense
M74	#12	MAC#12	M. fortuitum	MY325		ATCC	M. malmoense
M88	CH	font. CH	M. fortuitum	MY718		maimo	M. malmoense
MY221 MY223			M. fortuitum M. fortuitum	MY214 MY224			M. marinum M. marinum
MY223 MY225				MY224 MY244			
MY225			M. fortuitum	MY244			M. marinum

TABLE 2-continued

TABLE 2-continued

	IAL	ole z-conum	aca	TABLE 2-continued							
Affy#	SAMPLE ID#	Alt. ID	SPECIES	Affy#	SAMPLE ID#	Alt. ID	SPECIES				
MY339			M. marinum	TB13	3553		M. tuberculosis				
MY343			M. marinum	TB14	3468		M. tuberculosis				
MY458		ATCC	M. mucogenicum	TB15	2163		M. tuberculosis				
MY809			M. mucogenicum	TB16	DW	DW	M. tuberculosis				
MY817			M. mucogenicum	TB17	CB	CB	M. tuberculosis				
MY821			M. mucogenicum	TB18	PB	PB	M. tuberculosis				
MY824			M. mucogenicum	TB19	AA Mododa	AA	M. tuberculosis				
MY102 MY105			M. nonchromagenicum	TB2 TB20	M0404A 3492		M. tuberculosis M. tuberculosis				
MY251			M. nonchromagenicum M. nonchromagenicum	TB21	1435		M. tuberculosis M. tuberculosis				
MY256			M. nonchromagenicum	TB22	896		M. tuberculosis M. tuberculosis				
MY294			M. nonchromagenicum	TB23	2268		M. tuberculosis				
ATCC7	19981	ATCC-scr	M. scrofulaceum	TB24	3455		M. tuberculosis				
ATCC7-0		scrof.	M. scrofulaceum	TB25	37		M. tuberculosis				
MY121			M. scrofulaceum	TB26	173		M. tuberculosis				
MY249			M. scrofulaceum	TB27	230		M. tuberculosis				
MY372			M. scrofulaceum	TB28	2519		M. tuberculosis				
MY387			M. scrofulaceum	TB29	T29233		M. tuberculosis				
MY484			M. sinniae	TB3	1231		M. tuberculosis				
MY556			M. sinniae	TB30	SP	SP	M. tuberculosis				
MY583			M. sinniae	TB31	3201		M. tuberculosis				
MY586			M. sinniae	TB32	3219		M. tuberculosis				
ATCC8	19420	ATCC-sme	M. smegmatis	TB33	80		M. tuberculosis				
M35	95A1072		M. smegmatis	TB34	3442		M. tuberculosis				
M36	95A8183		M. smegmatis	TB35	3502		M. tuberculosis				
M37	95A4990		M. smegmatis	TB36	3759		M. tuberculosis				
M77	92-144	smeg. JL	M. smegmatis	TB37	1295		M. tuberculosis				
MY143		ATCC	M. smegmatis	TB38	337		M. tuberculosis				
MY104			M. szulgai	TB39	394		M. tuberculosis				
MY198			M. szulgai	TB4 TB40	914 499		M. tuberculosis M. tuberculosis				
MY357 MY358			M. szulgai M. szulgai	TB41	535		M. tuberculosis M. tuberculosis				
MY480			M. szulgai M. szulgai	TB41	607		M. tuberculosis M. tuberculosis				
VI 1460 ГВ74	C.17.96.5		M. tab M160 DR	TB43	707		M. tuberculosis				
MY387	0.17.50.5		M. tb	TB44	692		M. tuberculosis				
MY418			M. tb	TB45	2408		M. tuberculosis				
MY437			M. tb	TB46	1069		M. tuberculosis				
MY462			M. tb	TB47	M3282A		M. tuberculosis				
ГВ59	C.18.96.1		M. tb H37rv DR	TB48	1338		M. tuberculosis				
ГВ57	C.16.96.1		M. tb H37rv DR	TB49	1368		M. tuberculosis				
ГВ73	C.17.96.1		M. tb H37rv DR	TB5	1145		M. tuberculosis				
Γ B 60	C.18.96.2		M. tb J35 DR	TB50	65		M. tuberculosis				
ГВ65	C.22.96.9		M. tb M101 DR	TB51	727		M. tuberculosis				
ГВ82	C.18.96.4		M. tb M104 DR	TB52	3455		M. tuberculosis				
ГВ89	C.16.96.3		M. tb M104 DR	TB53	3506		M. tuberculosis				
ГВ72	C.16.98.7		M. tb M104 DR	TB54	9500387		M. tuberculosis				
ГВ66	C.22.96.10		M. tb M112 DR	TB55	9600173		M. tuberculosis				
ΓB63	C.18.96.5		M. tb M140 DR M. tb M160 DR	TB56	9503471		M. tuberculosis M. tuberculosis				
ΓΒ64 ΓΒ70	C.18.96.6 C.18.96.4		M. tb M160 DR M. tb M160 DR	TB57 TB58	9600309 9600230		M. tuberculosis M. tuberculosis				
ГВ61	C.18.96.3		M. tb Mf00 DR	TB6	1417		M. tuberculosis M. tuberculosis				
ГВ68	C.18.96.2		M. tb M60 DR	TB7	SM2341		M. tuberculosis M. tuberculosis				
ГВ71	C.18.96.5		M. tb M60 DR	TB75	2098		M. tuberculosis				
MY212			M. terrae	TB76	173/1		M. tuberculosis				
MY354			M. terrae	TB77	1122/1		M. tuberculosis				
MY491			M. terrae	TB78	1417/1		M. tuberculosis				
MY497			M. terrae	TB8	1587		M. tuberculosis				
AY816			M. triplex	TB9	M7032A		M. tuberculosis				
ATCC9	27294	Mtb	M. tuberculosis	ATCC10	19250	ATTC-xen	M. xenopi				
ATCC9-0		TB2020	M. tuberculosis	M129	60133		M. xenopi				
√A	93-1071		M. tuberculosis	M130	60200		M. xenopi				
√A	93-336		M. tuberculosis	M131	60365		M. xenopi				
V/A	92-852		M. tuberculosis	M132	60387		M. xenopi				
N/A	92-1005		M. tuberculosis	M38	95A5208		M. xenopi				
N/A	92-243		M. tuberculosis	M39	95A5399		M. xenopi				
N/A J/A	92-304		M. tuberculosis	M40 M41	95A3938		M. xenopi M. xenopi				
N/A	92-199 92-197		M. tuberculosis M. tuberculosis	M41	95A8782 95A0933		M. xenopi M. xenopi				
N/A N/A	92-197 92-484		M. tuberculosis M. tuberculosis	M42 M43	95A0933 95A4320		4				
N/A N/A	92-484 94-577		M. tuberculosis M. tuberculosis	M43 M44	95A4320 95A478		M. xenopi M. xenopi				
N/A ГВ1	936		M. tuberculosis M. tuberculosis	M45	95A2997		M. xenopi M. xenopi				
ГВ10	1122		M. tuberculosis M. tuberculosis	M46	95A8383		M. xenopi M. xenopi				
	****		THE PROCESSIONS	111.0	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		aras mossops				
TB11	3407		M. tuberculosis	M47	95A4319		M. xenopi				

TABLE 2-continued

TABLE 2-continued

Affy#	SAMPLE ID#	Alt. ID	SPECIES
MY219			М. хепорі
MY250			M. xenopi
MY252			M. xenopi
MY254			M. xenopi
MY255			M. xenopi
MY107			MAC
MY111			MAC
MY112			MAC

Affy#	SAMPLE ID#	Alt. ID	SPECIES
MY312 M66	MAC #4	MAC #4	MAC unique

480

[0064]

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1-19. (canceled)

- **20**. A polynucleotide probe or primer that hybridizes under stringent hybridization conditions to a mycobacterial rpoB sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6 and 9 or its complement without hybridizing to the *M. tuberculosis* sequence of SEQ ID NO: 1 or its complement, wherein said stringent hybridization conditions comprise 5×SSPE and a temperature of 25-30° C.
 - 21. The polynucleotide of claim 20 that is a probe.
- 22. The polynucleotide of claim 21, wherein a central position of the probe aligns with one or more bases of a sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6 and 9 which differ from the corresponding one or more bases in SEQ ID NO: 1 when the sequences are maximally aligned.
- 23. The sequence-specific polynucleotide of claim 20 that is a primer.

- **24**. The polynucleotide of claim 23, wherein the 3' end of the primer aligns with one or more bases of a sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6 and 9 which differ from the corresponding one or more bases in SEQ ID NO: 1 when the sequences are maximally aligned.
- 25. The polynucleotide of claim 20 that hybridizes under stringent hybridization conditions to at least 100 contiguous bases of a mycobacterial rpoB sequence selected from the group consisting of SEQ ID NOS: 2, 3, 4, 5, 6 and 9 or its complement without hybridizing to the *M. tuberculosis* sequence of SEQ ID NO:1 or its complement, wherein said stringent hybridization conditions comprise 5×SSPE and a temperature of 25-30° C.
- **26**. A polynucleotide probe or primer that hybridizes under stringent hybridization conditions to at least 100 contiguous bases of a mycobacterial rpoB sequence selected

from the group consisting of SEQ ID NOS: 8 and 10 or its complement without hybridizing to the M. tuberculosis sequence of SEQ ID NO:1 or its complement, wherein said stringent hybridization conditions comprise 5×SSPE and a temperature of 25-30° C.

27. The polynucleotide of claim 26, wherein the polynucleotide is a probe, and wherein a central position of the probe aligns with one or more bases of a sequence selected from the group consisting of SEQ ID NOS: 8 and 10 which

differ from the corresponding one or more bases in SEQ ID NO: 1 when the sequences are maximally aligned.

28. The polynucleotide of claim 26, wherein the polynucleotide is a primer, and wherein the 3' end of the primer aligns with one or more bases of a sequence selected from the group consisting of SEQ ID NOS: 8 and 10 which differ from the corresponding one or more bases in SEQ ID NO: 1 when the sequences are maximally aligned.

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