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(54) Title: **LepA/Gufl gene sequences as a diagnostic target for the identification of bacterial species.**

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Title

LepA / *GulI* gene sequences as a diagnostic target for the identification of bacterial species.

Field of the Invention

The present invention relates to nucleic acid primers and probes to detect one or more bacterial and yeast and fungal species. More specifically the invention relates to the *LepA* and *GulI* gene sequences, their corresponding RNAs, specific probes, primers and oligonucleotides related thereto and their use in diagnostic assays to detect and/ or discriminate bacterial, yeast and fungal species, i.e. microorganisms.

Background to the Invention

Microbial infections represent a major cause of morbidity and mortality worldwide, and the spectrum of microorganisms causing disease continues to increase. Microorganisms (bacteria, fungi and yeast) responsible for causing infectious diseases are traditionally detected in hospital laboratories with the aid of microbiological culture methods with poor sensitivity (25-82%), which are very time-consuming, generally taking from two to five days to complete, and up to eight days for the diagnosis of fungal infections. Definitive diagnosis is usually based on either, the recovery and identification of a specific microorganism from clinical specimens or microscopic demonstration of fungi with distinct morphological features. However, there are numerous cases where these methods fail to provide conclusive proof as to the infecting agent or microorganism. In these instances, the detection of specific host antibody responses can be used, although again this can be affected by the immune status of the patient.

Time is critical in the detection and identification of infectious microorganisms. Effective treatment depends on finding the source of infection and making appropriate decisions about antibiotics quickly and efficiently. Only after pathogens are correctly identified, can targeted therapy using a specific antibiotic begin. Many physicians would like to see the development of better in vitro amplification and direct detection diagnostic techniques for the early diagnosis of microbial infection. Recently, Roche™ launched a real time PCR based assay (Septifast™), for the detection of microbial DNA in clinical samples. Therefore, there is a clear need for the development of novel rapid diagnostic tests for clinically significant bacterial and fungal pathogens for bioanalysis applications in the clinical sector. This has led the current inventors to identify novel nucleic acid targets for application in Nucleic Acid Diagnostic (NAD) tests.

It is clear though, that development of faster, more accurate diagnostic methods are required, particularly in light of the selection pressure caused by modern anti-microbial treatments which give rise to increased populations of resistant virulent strains with mutated genome sequences. Methods that enable early diagnosis of microbial causes of infection enable the selection of a specific narrow spectrum antibiotic or antifungal to treat the infection (Datamonitor report:

Stakeholder opinion -Invasive fungal infections, options outweigh replacements 2004;

Datamonitor report: Stakeholder Opinion-Sepsis, under reaction to an overreaction, 2006).

LepA (leader peptidase A) has recently been assigned the function of ribosomal elongation factor (Qin *et al.*, 2006, Cell). *LepA* is highly conserved and is present in all bacteria and mitochondria. There are 2444 *LepA* gene sequences (~ 1.8 kb in length) available in GenBank including 2229 bacterial sequences. Using Clustal W sequence alignments, the *LepA* gene of *Bacillus*, *Listeria*, *Enterobacteriaceae*, *Mycobacteria*, *Staphylococci* and *Streptococci* were compared *in silico* to other molecular targets including *tufA* and the *ssrA* genes. In general, *LepA* seemed to have sufficient sequence heterogeneity to enable its application for microorganism species identification in nucleic acid based tests (Table 1).

	<i>LepA</i> (range of % homology between species)	<i>tufA</i> (range of % homology between species)	<i>ssrA</i> (range of % homology between species)
<i>Bacillus</i> species	72-97	81-99	62-100
<i>Listeria</i> species	89-90	99	97-99
Enterobacteriaceae (including <i>E.coli</i>)	59-99	83-99	92-99
<i>Mycobacterium</i> species	78-99	87-100	84-100
<i>Streptococcus</i> species	70-91	76-97	62-100
<i>Staphylococcus</i> species	80-83	91-95	81-99

Table 1: Percentage range of homology between *Bacillus* species, *Listeria* species, Enterobacteriaceae, *Mycobacterium* species, *Streptococcus* species and *Staphylococcus* species in the *LepA* gene compared to the *tufA* (equivalent commercialised mRNA) and *ssrA* genes (RiboSEQ technology).

GUF1, which is similar to the *E. coli* elongation factor-type GTP-binding protein *LepA*, is a gene encoding a novel evolutionarily conserved GTPase coding protein (GTPase of Unknown Function 1, Kiser GL and Weinert TA (1995) GUF1, a gene encoding a novel evolutionarily conserved GTPase in budding yeast. Yeast 11(13): 1311-6), which, was predicted to be the GTPase of the elongation factor-type class. There are 94 *Guf1* sequences available in NCBI GeneBank including 3 *Candida* and 6 *Aspergillus*.

Definitions

"Synthetic oligonucleotide" refers to molecules of nucleic acid polymers of 2 or more nucleotide bases that are not derived directly from genomic DNA or live organisms. The term synthetic

oligonucleotide is intended to encompass DNA, RNA, and DNA/RNA hybrid molecules that have been manufactured chemically, or synthesized enzymatically *in vitro*.

An "oligonucleotide" is a nucleotide polymer having two or more nucleotide subunits covalently joined together. Oligonucleotides are generally about 10 to about 100 nucleotides. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof such as OMe. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages or by non-nucleotide moieties that do not prevent hybridization of the oligonucleotide to its complementary target nucleotide sequence. Modified linkages include those in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage, a methylphosphonate linkage, or a neutral peptide linkage. Nitrogenous base analogs also may be components of oligonucleotides in accordance with the invention.

A "target nucleic acid" is a nucleic acid comprising a target nucleic acid sequence. A "target nucleic acid sequence," "target nucleotide sequence" or "target sequence" is a specific deoxyribonucleotide or ribonucleotide sequence that can be hybridized to a complementary oligonucleotide.

An "oligonucleotide probe" is an oligonucleotide having a nucleotide sequence sufficiently complementary to its target nucleic acid sequence to be able to form a detectable hybrid probe:target duplex under high stringency hybridization conditions. An oligonucleotide probe is an isolated chemical species and may include additional nucleotides outside of the targeted region as long as such nucleotides do not prevent hybridization under high stringency hybridization conditions. Non-complementary sequences, such as promoter sequences, restriction endonuclease recognition sites, or sequences that confer a desired secondary or tertiary structure such as a catalytic active site can be used to facilitate detection using the invented probes. An oligonucleotide probe optionally may be labelled with a detectable moiety such as a radioisotope, a fluorescent moiety, a chemiluminescent, a nanoparticle moiety, an enzyme or a ligand, which can be used to detect or confirm probe hybridization to its target sequence. Oligonucleotide probes are preferred to be in the size range of from about 10 to about 100 nucleotides in length, although it is possible for probes to be as much as and above about 500 nucleotides in length, or below 10 nucleotides in length.

A "hybrid" or a "duplex" is a complex formed between two single-stranded nucleic acid sequences by Watson-Crick base pairings or non-canonical base pairings between the complementary bases. "Hybridization" is the process by which two complementary strands of nucleic acid combine to form a double-stranded structure ("hybrid" or "duplex").

"Complementarity" is a property conferred by the base sequence of a single strand of DNA or RNA which may form a hybrid or double-stranded DNA:DNA, RNA:RNA or DNA:RNA

through hydrogen bonding between Watson-Crick base pairs on the respective strands. Adenine (A) ordinarily complements thymine (T) or uracil (U), while guanine (G) ordinarily complements cytosine (C).

The term "stringency" is used to describe the temperature, ionic strength and solvent composition existing during hybridization and the subsequent processing steps. Those skilled in the art will recognize that "stringency" conditions may be altered by varying those parameters either individually or together. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. Stringency conditions are chosen to maximize the difference in stability between the hybrid formed with the target and the non-target nucleic acid.

'High stringency' conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5xSSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5xDenhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1xSSPE, 1.0% SDS at 42° C. when a probe of about 500 nucleotides in length is used.

'Medium stringency' conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5xSSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5xDenhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0xSSPE, 1.0% SDS at 42° C. when a probe of about 500 nucleotides in length is used.

'Low stringency' conditions are those equivalent to binding or hybridization at 42° C. in a solution consisting of 5xSSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5xDenhardt's reagent [50xDenhardt's contains per 500ml: 5g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5xSSPE, 0.1% SDS at 42° C. when a probe of about 500 nucleotides in length is used.

In the context of nucleic acid *in-vitro* amplification based technologies, "stringency" is achieved by applying temperature conditions and ionic buffer conditions that are particular to that *in-vitro* amplification technology. For example, in the context of PCR and real-time PCR, "stringency" is achieved by applying specific temperatures and ionic buffer strength for hybridisation of the oligonucleotide primers and, with regards to real-time PCR hybridisation of the probe/s, to the target nucleic acid for *in-vitro* amplification of the target nucleic acid.

One skilled in the art will understand that substantially corresponding probes of the invention can vary from the referred-to sequence and still hybridize to the same target nucleic acid

sequence. This variation from the nucleic acid may be stated in terms of a percentage of identical bases within the sequence or the percentage of perfectly complementary bases between the probe and its target sequence. Probes of the present invention substantially correspond to a nucleic acid sequence if these percentages are from about 100% to about 80% or from 0 base mismatches in about 10 nucleotide target sequence to about 2 bases mismatched in an about 10 nucleotide target sequence. In preferred embodiments, the percentage is from about 100% to about 85%. In more preferred embodiments, this percentage is from about 90% to about 100%; in other preferred embodiments, this percentage is from about 95% to about 100%

By "sufficiently complementary" or "substantially complementary" is meant nucleic acids having a sufficient amount of contiguous complementary nucleotides to form, under high stringency hybridization conditions, a hybrid that is stable for detection.

By "nucleic acid hybrid" or "probe:target duplex" is meant a structure that is a double-stranded, hydrogen-bonded structure, preferably about 10 to about 100 nucleotides in length, more preferably 14 to 50 nucleotides in length, although this will depend to an extent on the overall length of the oligonucleotide probe. The structure is sufficiently stable to be detected by means such as chemiluminescent or fluorescent light detection, autoradiography, electrochemical analysis or gel electrophoresis. Such hybrids include RNA:RNA, RNA:DNA, or DNA:DNA duplex molecules.

"RNA and DNA equivalents" refer to RNA and DNA molecules having the same complementary base pair hybridization properties. RNA and DNA equivalents have different sugar groups (i.e., ribose versus deoxyribose), and may differ by the presence of uracil in RNA and thymine in DNA. The difference between RNA and DNA equivalents do not contribute to differences in substantially corresponding nucleic acid sequences because the equivalents have the same degree of complementarity to a particular sequence.

By "preferentially hybridize" is meant that under high stringency hybridization conditions oligonucleotide probes can hybridize their target nucleic acids to form stable probe:target hybrids (thereby indicating the presence of the target nucleic acids) without forming stable probe:non-target hybrids (that would indicate the presence of non-target nucleic acids from other organisms). Thus, the probe hybridizes to target nucleic acid to a sufficiently greater extent than to non-target nucleic acid to enable one skilled in the art to accurately detect the presence of (for example *Candida*) and distinguish these species from other organisms. Preferential hybridization can be measured using techniques known in the art and described herein.

By "theranostics" is meant the use of diagnostic testing to diagnose the disease, choose the correct treatment regime and monitor the patient response to therapy. The theranostics of the

invention may be based on the use of an NAD assay of this invention on samples, swabs or specimens collected from the patient.

Object of the Invention

It is an object of the current invention to provide sequences and/or diagnostic assays to detect and identify one or more microorganism species (bacteria, yeast, fungi). The current inventors have made use of the LepA and GufI gene sequences to design primers and probes for use in the detection and identification of bacterial and yeast and fungal species.

Summary of the Invention

The present invention provides a diagnostic kit for detection and identification of bacterial and yeast and fungal species i.e. microorganisms, comprising at least one oligonucleotide probe capable of binding to at least a portion of the LepA gene or GufI gene or its corresponding mRNA. The oligonucleotide probe may have a sequence substantially homologous to or substantially complementary to a portion of the LepA or GufI gene or its corresponding mRNA. It will thus be capable of binding or hybridizing with a complementary DNA or RNA molecule.

The nucleic acid molecule may be synthetic.

The kit may comprise more than one such probe. In particular the kit may comprise a plurality of such probes. In addition the kit may comprise additional probes for other organisms, such as, for example, bacterial species or viruses.

The portion of the LepA gene may, for example, be equivalent to a portion of the region of the gene between base pair (bp) position 57 to bp 228 or bp position 522 to bp position 659 in *Staphylococcus aureus*. Particularly preferred are portions equivalent to a portion of the region of the gene between base pair positions 66 to 215, 66 to 81, 200 to 215 and 167 to 189 of the Group B streptococcal LepA gene, and positions 57 to 228, 57 to 74, 209 to 228 and 112 to 134 of the *S. aureus* LepA gene. The portion of the LepA gene may, for example, be equivalent to a portion of the region of the gene between bp position 66 to bp 215 in *Group B Streptococcus*. For *Mycobacteria*, the portion of *LepA* may be equivalent to a portion of the region of the gene between bp 618 to 772 and bp 1203 to bp 1817 in *M. tuberculosis* or the equivalent regions in other *Mycobacterium tuberculosis* complex (MTC) species and non-MTC mycobacteria. In *Bordetella*, the portion of the *Lep A* may be equivalent to 3 regions bp 160 to bp 612, bp 552 to bp 1081 and bp 1006 to bp 1638.

The portion of the GufI gene may be equivalent to a portion of the region of the gene from base pair position 190 to base pair position 2204 of the gene. Particularly preferred are portions equivalent to a portion of the region of the gene from base pair positions 190 to 1064, 270 to 300, 190 to 212, 466 to 491, 507 to 537, 466 to 489, 740 to 762, 828 to 858, 740 to 762 and 1043 to 1064 of the *C. albicans* GufI gene, or from base pair position 613 to 2204, 613 to 635,

820 to 839, 1339 to 1358, 1573 to 1592, 1951 to 1973 and 2183 to 2204 of the *A. fumigatus* *GufI* gene.

The oligonucleotide probe may have a sequence selected from the group comprising SEQ ID NO 11 or SEQ ID NO 12, SEQ ID NO 15, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 30, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 40, SEQ ID NO 43, SEQ ID NO 46, SEQ ID NO 49, SEQ ID NO 52 or a sequence substantially homologous to or substantially complementary to those sequences, which can also act as a probe for the *LepA* and or *GufI* genes.

The kit may comprise more than one such probe. In particular the kit may comprise a plurality of such probes. In addition the kit may comprise additional probes for other organisms, such as, for example, bacterial species or viruses.

The identified sequences are suitable not only for *in vitro* DNA/RNA amplification based detection systems but also for signal amplification based detection systems. Furthermore, the sequences of the invention identified as suitable targets provide the advantages of having significant intragenic sequence heterogeneity in some regions, which is advantageous and enables aspects of the invention to be directed towards group or species-specific targets, and also having significant sequence homogeneity in some regions, which enables aspects of the invention to be directed towards genus-specific microorganism primers and probes for use in direct nucleic acid detection technologies, signal amplification nucleic acid detection technologies, and nucleic acid *in vitro* amplification technologies for microorganism diagnostics. The *LepA* and *GufI* sequences allow for multi-test capability and automation in diagnostic assays.

One of the advantages of the sequences of the present invention is that the intragenic *LepA* and *GufI* nucleotide sequence diversity between closely related microorganism species enables specific primers and probes for use in diagnostics assays for the detection of bacteria to be designed. The *LepA* and *GufI* nucleotide sequences, both DNA and RNA can be used with direct detection, signal amplification detection and *in vitro* amplification technologies in diagnostics assays. The *LepA* and *GufI* sequences allow for multi-test capability and automation in diagnostic assays.

The kit may further comprise at least one primer for amplification of at least a portion of the *LepA* or *GufI* genes. Suitably the kit comprises a forward and a reverse primer for a portion of the *LepA* or *GufI* gene. The kit may also comprise additional primers or probes.

The primer may have a sequence selected from the group comprising SEQ ID NO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 16, 17, 18, 19, 22, 23, 24, 25, 28, 29, 31, 32, 33, 34, 38, 39, 41, 42, 44, 45, 47, 48, 50, 51, 53, 54 or a sequence substantially homologous to or substantially complementary to those sequences, which can also act as a primers for the *LepA* or *GufI* genes.

The kit may comprise at least one forward *in vitro* amplification primer and/ or at least one reverse *in vitro* amplification primer, the forward amplification primer having a sequence selected from the group consisting of SEQ ID NO 1,3 5,7, 9, 13, 16, 18, 22, 24, 28, 31, 33, 38, 41, 44, 47, 50, 53, or a sequence being substantially homologous or complementary thereto

5 which can also act as a forward amplification primer for the *LepA* or *GufI* gene, and the reverse amplification primer having a sequence selected from the group consisting of SEQ ID NO 2, 4, 6, 8, 10, 14, 17, 19, 23, 25, 29, 32, 34, 39, 42, 45, 48, 51, 54, or a sequence being substantially homologous or complementary thereto which can also act as a reverse amplification primer for the *LepA* or *GufI* gene.

10 The diagnostic kit may be based on direct nucleic acid detection technologies, signal amplification nucleic acid detection technologies, and nucleic acid *in vitro* amplification technologies is selected from one or more of Polymerase Chain Reaction (PCR), Ligase Chain Reaction (LCR), Nucleic Acids Sequence Based Amplification (NASBA), Strand Displacement Amplification (SDA), Transcription Mediated Amplification (TMA), Branched DNA
15 technology (bDNA) and Rolling Circle Amplification Technology (RCAT)), or other *in vitro* enzymatic amplification technologies.

The invention also provides a nucleic acid molecule selected from the group consisting of SEQ ID NO.1 to SEQ ID NO. 178 and sequences substantially homologous thereto, or substantially complementary to a portion thereof and having a function in diagnostics based on the *LepA*
20 and/or *GufI* genes. The nucleic acid molecule may comprise an oligonucleotide having a sequence substantially homologous to or substantially complementary to a portion of a nucleic acid molecule of SEQ ID NO.1 to SEQ ID NO. 178. The invention also provides a method of detecting a target organism in a test sample comprising the steps of:

- (i) mixing the test sample with at least one oligonucleotide probe as defined above
25 under appropriate conditions; and
- (ii) hybridizing under high stringency conditions any nucleic acid that may be present in the test sample with the oligonucleotide to form a probe:target duplex; and
- (iii) determining whether a probe:target duplex is present; the presence of the duplex positively identifying the presence of the target organism in the test sample.

30 The nucleic acid molecule and kits of the present invention may be used in a diagnostic assay to detect the presence of one or more bacterial species, to measure microorganism titres in a patient or in a method of assessing the efficacy of a treatment regime designed to reduce microorganism titre in a patient or to measure microorganism contamination in an environment. The environment may be a hospital, or it may be a food sample, an environmental sample e.g.
35 water, an industrial sample such as an in-process sample or an end product requiring bioburden or quality assessment.

The kits and the nucleic acid molecule of the invention may be used in the identification and/or characterization of one or more disruptive agents that can be used to disrupt the LepA or GufI gene function. The disruptive agent may be selected from the group consisting of antisense RNA, PNA, and siRNA.

- 5 In some embodiments of the invention, a nucleic acid molecule comprising a species-specific probe can be used to discriminate between species of the same genus.

The oligonucleotides of the invention may be provided in a composition for detecting the nucleic acids of microorganism target organisms. Such a composition may also comprise buffers, enzymes, detergents, salts and so on, as appropriate to the intended use of the
10 compositions. It is also envisioned that the compositions, kits and methods of the invention, while described herein as comprising at least one synthetic oligonucleotide, may also comprise natural oligonucleotides with substantially the same sequences as the synthetic nucleotide fragments in place of, or alongside synthetic oligonucleotides.

The invention also provides for an *in vitro* amplification diagnostic kit for a target
15 microorganism comprising at least one forward *in vitro* amplification primer and at least one reverse *in vitro* amplification primer, the forward amplification primer being selected from the group consisting of one or more of a sequence being substantially homologous or complementary thereto which can also act as a forward amplification primer, and the reverse amplification primer being selected from the group consisting of one or more of a sequence
20 being substantially homologous or complementary thereto which can also act as a reverse amplification primer.

The invention also provides for a diagnostic kit for detecting the presence of candidate microorganism species, comprising one or more DNA probes comprising a sequence substantially complementary to, or substantially homologous to the sequence of the LepA or
25 GufI gene of the candidate microorganism species. The present invention also provides for one or more synthetic oligonucleotides having a nucleotide sequence substantially homologous to or substantially complementary to one or more of the group consisting of the LepA or GufI gene or mRNA transcript thereof, the microorganism LepA gene or mRNA transcript thereof, one or more of SEQ ID NO 1-SEQ ID NO 178.

30 The nucleotide may comprise DNA. The nucleotide may comprise RNA. The nucleotide may comprise a mixture of DNA, RNA and PNA. The nucleotide may comprise synthetic nucleotides. The sequences of the invention (and the sequences relating to the methods, kits compositions and assays of the invention) may be selected to be substantially homologous to a portion of the coding region of the LepA or GufI gene. The gene may be a gene from a target
35 microorganism. The sequences of the invention are preferably sufficient so as to be able form a probe:target duplex to the portion of the sequence.

The invention also provides for a diagnostic kit for a target microorganism comprising an oligonucleotide probe substantially homologous to or substantially complementary to an oligonucleotide of the invention (which may be synthetic). It will be appreciated that sequences suitable for use as *in vitro* amplification primers may also be suitable for use as oligonucleotide probes: while it is preferable that amplification primers may have a complementary portion of
 5 between about 15 nucleotides and about 30 nucleotides (more preferably about 15-about 23, most preferably about 20 to about 23), oligonucleotide probes of the invention may be any suitable length. The skilled person will appreciate that different hybridization and or annealing conditions will be required depending on the length, nature & structure (eg. Hybridization probe pairs for LightCycler, Taqman 5' exonuclease probes, hairpin loop structures etc. and sequence
 10 of the oligonucleotide probe selected.

Kits and assays of the invention may also be provided wherein the oligonucleotide probe is immobilized on a surface. Such a surface may be a bead, a membrane, a column, dipstick, a nanoparticle, the interior surface of a reaction chamber such as the well of a diagnostic plate or
 15 inside of a reaction tube, capillary or vessel or the like.

The target microorganism may be selected from the group consisting of *Streptococcus*, *Enterococcus*, *Mycobacterium*, *Bacillus*, *Listeria*, *Enterobacteriaceae*, *Neisseria*, *Chlamydia*, *Mycoplasma*, *Haemophilus*, *Clostridia*, *Bordetella* and *Staphylococci*, *Gardnerella*, *Candida*, *Aspergillus*
 20 Under these circumstances, the amplification primers and oligonucleotide probes of the invention may be designed to a gene specific or genus specific region so as to be able to identify one or more, or most, or substantially all of the desired organisms of the target organism grouping.

The test sample may comprise cells of the target microorganism. The method may also comprise
 25 a step for releasing nucleic acid from any cells of the target organism that may be present in said test sample. Ideally, the test sample is a biological sample obtained from a patient (such as a swab, or blood, urine, saliva, a bronchial lavage dental specimen, skin specimen, scalp specimen, transplant organ biopsy, stool, mucus, or discharge sample). The test samples may be a food sample, a water sample an environmental sample, an end product, end product or in-
 30 process industrial sample.

The invention also provides for the use of any one of SEQ ID NO.1 to SEQ ID NO. 178 in a diagnostic assay for the presence of one or more microorganism species. The species may be selected from the group consisting of *Streptococcus*, *Enterococcus*, *Mycobacterium*, *Bacillus*, *Listeria*, *Enterobacteriaceae*, *Neisseria*, *Chlamydia*, *Mycoplasma*, *Haemophilus*, *Clostridia*,
 35 *Bordetella* and *Staphylococci*, *Gardnerella*, *Candida*, *Aspergillus*

The invention also provides for kits for use in clinical diagnostics, theranostics, food safety diagnostics, industrial microbiology diagnostics, environmental monitoring, veterinary diagnostics, bio-terrorism diagnostics comprising one or more of the synthetic oligonucleotides of the invention. The kits may also comprise one or more articles selected from the group consisting of appropriate sample collecting instruments, reagent containers, buffers, labelling moieties, solutions, detergents and supplementary solutions. The invention also provides for use of the sequences, compositions, nucleotide fragments, assays, and kits of the invention in theranostics, Food safety diagnostics, Industrial microbiology diagnostics, Environmental monitoring, Veterinary diagnostics, Bio-terrorism diagnostics.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic nucleic acid based assay for the detection of microorganism species.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic assay to measure microorganism titres in a patient. The titres may be measured *in vitro*.

The nucleic acid molecules, composition, kits or methods may be used in a method of assessing the efficacy of a treatment regime designed to reduce microorganism titre in a patient comprising assessing the microorganism titre in the patient (by *in vivo* methods or *in vitro* methods) at one or more key stages of the treatment regime. Suitable key stages may include before treatment, during treatment and after treatment. The treatment regime may comprise an anti- microbial or anti-fungal agent, such as a pharmaceutical drug.

The nucleic acid molecules, composition, kits or methods may be used in a diagnostic assay to measure potential microorganism contamination, for example, in a hospital.

The nucleic acid molecules, composition, kits or methods may be used in the identification and/or characterization of one or more disruptive agents that can be used to disrupt the *LepA* and *Gul I* gene functions. Suitable disruptive agents may be selected from the group consisting of antisense RNA, PNA, siRNA.

Brief Description of the Drawings

Figure 1: Real-time PCR amplification assay based on *LepA* for *S. aureus* demonstrating the inclusivity of the *S. aureus LepA* real-time PCR assay.

Figure 2: Real-time PCR amplification assay based on *LepA* for *S. aureus* demonstrating the sensitivity of the *S. aureus LepA* real-time PCR assay.

Figure 3: Real-time PCR amplification assay based on *LepA* for *B. pertussis* demonstrating the inclusivity of the *B. pertussis LepA* real-time PCR assay.

Figure 4: Real-time PCR amplification assay based on *LepA* for *B. pertussis* demonstrating the sensitivity of the *B. pertussis LepA* real-time PCR assay.

Figure 5: Real-time PCR amplification assay based on *LepA* for *Mycobacterium tuberculosis* complex demonstrating the inclusivity of the *Mycobacterium tuberculosis* complex *LepA* real-time PCR assay.

Figure 6: Real-time PCR amplification assay based on *LepA* for *Mycobacterium tuberculosis* complex demonstrating the exclusivity of the *Mycobacterium tuberculosis* complex *LepA* real-time PCR assay.

Figure 7: Real-time PCR amplification assay based on *LepA* for *Mycobacterium tuberculosis* complex demonstrating the sensitivity of the *Mycobacterium tuberculosis* complex *LepA* real-time PCR assay.

Detailed Description of the Invention

Materials and Methods

Bacterial strains:

DNA stocks for *Mycobacteria* spp. used in this study were obtained from an independent laboratory. Other bacterial species were grown in either Tryptone Soya broth, Luria broth, nutrient broth or nutrient agar overnight at 37°C

DNA Extraction

DNA was isolated from bacterial cells using the MagNA Pure System (Roche Molecular Systems) in combination with the MagNA pure Yeast and Bacterial isolation kit III or using the Edge Biosystems PurElute™ Bacterial Genomic Kit.

***LepA* gene sequence analysis for diagnostics assay design**

The publicly available sequences of the *LepA* genes for *Mycobacteria* and *Bordetella* spp. were acquired from the Genbank database and aligned using Clustal W. PCR primers (Table 2) were designed to amplify regions of the *LepA* gene in a range of *Mycobacterial* and *Bordetella* species. For *Bordetella*, PCR primers BR1F/BR1R amplify a region equivalent to 160bp to 612 bp of the *B. pertussis* Tohama-a gene. PCR primers BR2F/BR2R amplify a region equivalent to 552 bp to 1081 bp of the *B. pertussis* Tohama-a gene. Primer set BR3F/BR3R amplify from bp position 1006 to bp position 1638 bp of *B. pertussis* Tohama-a gene. Primer set MycobSF3/MycobSR2 amplify a region equivalent to bp 1203 to 1817 bp in the *Mycobacterium tuberculosis* *LepA* gene. Conventional PCR amplification of these sequence regions in *Bordetella* and *Mycobacteria* species was performed using Sigma SuperPak™ reagents on the MWG Biotech Primus using the thermocycling conditions outlined in table 3. PCR products were purified for DNA sequencing using either the High Pure PCR Product Purification Kit from Roche or the ExoSAP-IT purification kit according to manufacturers instructions. Purified PCR products were sent to the external sequence service provider for sequencing. DNA sequence information for *LepA* was generated for regions 1-3 for *B. pertussis*, *B. avium*, *B. petrii* and *B. holmesei* for regions 1-2 for *B. parapertussis* and *B. bronchiseptica*, *B. hinzii* and

region 2 of *B. trematum*. DNA sequence information was generated for Mycobacterial species *Lep A* region equivalent to 1203 bp to 1817 bp in *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. canettii*, *M. caprae*, *M. microti*, *M. pinipedii*, *M. tuberculosis*, *M. smegmatis*, *M. celatum*, *M. fortuitum*, *M. intracellulare*, *M. malmmonense*, *M. paratuberculosis* and *M. scrofulaceum*.

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Oligonucleotide Name	5'-3' sequence
BR1F	AGCGCCTTGACGTTCTC
BR1R	AAGATYGCCGAHATCCGC
BR2F	RTAYTCCTGSGGCATGAA
BR2R	CGTGTTACGCCCAART
BR3F	TTGATGCCSGCRATGA
BR3R	ACSATCAAGGCSCAGAC
MycobSF3	CACTCCGCGGTAGATGTC
MycobSR2	AAGTTCCTAATCTGCGCCG

Table 2: Sequencing primers for *Bordetella* and *Mycobacterial* spp.

Step		Temperature	Time	Cycles
1	Denaturation	95°C	3-4 min	1
2	Amplification			
	a. Denaturation	95°C	30 sec	30-35
	b. Annealing	50°C or 55°C	30 sec or 1 min	30-35
	c. Elongation	72°C	30 sec	30-35
3	Final Elongation	72°C	7 min	1
4	d. Hold	8°C		1

Table 3: Thermocycling conditions used for amplification of *LepA* gene regions in *Bordetella* and *Mycobacteria* spp.

15

PCR primer and DNA probe design for *Lep A* and *Guf A* targets.

LepA sequences available in GenBank for Staphylococci and closely related species were aligned and PCR primer sets SAF1/R1 and SAF2/R2 and oligonucleotide probes SAP1 and

SAP2 were designed. Similarly, PCR primers GBSF1/R1 and oligonucleotide probe GBSP1 were designed based on *in silico* analysis of published *LepA* sequences for *Streptococci* and closely related species. Sequence information generated for *LepA* regions 1-3 in *Bordetella* spp. was aligned with available *LepA* sequences for *Bordetella* and closely related species in GenBank and 5 primer sets, 2 for region 1, 2 for region 3 and 1 for region 2 were designed in addition to 2 oligonucleotide probes each for regions 1 and 3 and 1 probe for region 2 for *B. pertussis* specific identification. Sequence information in GenBank and sequence information generated in this study for Mycobacterial species was analysed to design Primer set MTC F/R and oligonucleotide probe MTCP for the detection of the MTC complex species. Additionally oligonucleotide probe MSP was designed for *M. smegmatis* *LepA* identification (Table 4). *In silico* analysis of GenBank *Guf 1* sequences for *Candida* and *Aspergillus* identified 3 gene regions suitable for oligonucleotide primer and probe design. A selection of primers and probes were designed from these regions for the identification of *C. albicans* and *Aspergillus fumigatus*.

15

Oligonucleotide name	Oligonucleotide sequence 5'-3'
<i>S. aureus</i>	
SAF1	TACCAACTGCTTTCATCT
SAR1	CAATTTGAAGTACCTGTACA
SAF2	TTTACGTTGACATAATTCCA
SAR2	CAGAAGTGACGGTTGATA
SAP1	TTTACG GCT TAT GTCACCGCCAT
SAP2	TAGTTGCACGAACATATGGCTC
<i>Group B Streptococci</i>	
GBSF1	AACCAATTGCTTTCAT
GBSR1	CAGCTATTGGACAAAA
GBSP1	CGTAGTGCTTTTATATCAGAACG
<i>Bordetella pertussis</i>	
BP1A	ACGAACTCGTAGTCCATCGAC
BPR1A	GCGCTTGTGTTGCACAGT
BP1B	GTCGAAGAAATCGAGCAC
BPR1B	GTATTCCTGGGGCATGAA
BP1PA	CTGGCGCCCGTGGTAGCTC
BP1PB	TTGTTGCACAGTGTCATCACCG
BP2F1	CGCTGTTTCGACCTCATAG
BP2R1	CAGCTGGTCGTATTCCGA
BP2P	GACACTTCGGGCTCGAACATCA
BP3F1	TCCATCTTGTGAGCAC
BP3R1	AACGACTGACTTCGTAC
BP3F2	TGCCGATCACGTCTT
BP3R2	GGTGGTTCGACGCTTC
BP3P1	ACTTCCATGCCCAGTTCCG
BP3P2	CGAACTGGGCATGGAAG
<i>Mycobacteria</i>	
MTCF1	AGACCGTGCGGATCTTG

MTCR1	CATGGAGATCACCCGTGA
MTCP	TCGTCTTTGTGCACCCGATAC
MSP	ACGACCTTCTCGGAACCGT

Table 4: Oligonucleotide primers and probes based on *LepA* for bacterial species identification

5

Demonstration of *LepA* as a target for bacterial species identification in real-time PCR assays.

- 10 To demonstrate the application of *LepA* as a target for bacterial spp. identification real-time PCR assays were worked up for *S. aureus*, GBS, Mycobacterium complex species(MTC) and *Bordetella pertussis*.

***S. aureus LepA* real-time PCR assay:**

- The *S. aureus LepA* real-time PCR assay was demonstrated using PCR primer set SAF2/R2 (0.5mM final concentration) and 5' exonuclease probe SAP2 (0.2mM final concentration) on the LightCycler 1.5 using the LightCycler Fast Start DNA Master HybProbe Kit and thermocycling conditions (Table 5). The panel of *S. aureus* strains listed in table 6 were tested for inclusivity and all were detected in the *S. aureus LepA*(Figure 1) while the other staphylococci species and related species (Table 6) were not detected in the test. The limit of detection of the *S. aureus LepA* real-time PCR test was established to be 2-20 *S. aureus* cell equivalents (Figure 2).

GBS *LepA* real-time PCR assay:

- The GBS *LepA* real-time PCR assay was demonstrated using PCR primer set GBSF1/R1 (0.5mM final concentration) and 5' exonuclease probe GBSP12 (0.2mM final concentration) on the LightCycler 1.5 using the LightCycler Fast Start DNA Master HybProbe Kit and thermocycling conditions (Table 5). The panel of *GBS* strains listed in table 6 were tested for inclusivity and all were detected in the assay while the other streptococci species and related species (Table 7) were not detected in the test.

***B. pertussis LepA* real-time PCR assay:**

- 30 The *B. pertussis LepA* real-time PCR assay was demonstrated using PCR primer set BP3F2/R2 (0.5mM final concentration) and 5' exonuclease probe BP3P2 (0.2mM final concentration) on the LightCycler 1.5 using the LightCycler Fast Start DNA Master HybProbe Kit and thermocycling conditions (Table 5). Inclusivity testing detected 4 of 4 *B. pertussis* strains tested and did not detect other *Bordetella* spp. strains (Figure 3). The limit of detection based on

amplification of serial dilutions of *B. pertussis* genomic DNA in the *B. pertussis* *LepA* real-time PCR assay was established as 20 *B. pertussis* cell equivalents (Figure 4).

MTC *LepA* real-time PCR assay:

A biplex real-time PCR assay for the detection of MTC *LepA* and *M. smegmatis* *LepA* has been configured on the LightCycler 2.0 instrument incorporating PCR primers MTCF1/R1(0.5mM) for the co-amplification of MTC species and *M. smegmatis* and 5'exonuclease probes MTCP(0.2mM) labelled with HEX/BHQ1 dye quencher combination and probe MSP(0.2mM) labelled with CY5/BHQ2 dye quencher moieties. Thermocycling is performed as described in table 5. Inclusivity testing for the MTC assay showed detection of all members of the MTC while non-MTC species were not detected Figures 5, 6. The LOD of the MTC assay for *M. tb* DNA was approximately 3 cell equivalents (Figure 7).

Step		Temperature	Time	Cycles
1	Denaturation	95°C	10 min	1
2	Amplification			
	Denaturation	95°C	10 sec	50
	Annealing/elongation	60°C	30 sec	50
3	Cooling	40°C	30 sec	1

Table 5: Real-time PCR conditions for the *S. aureus* *LepA* real-time PCR

15

Species panel	Source/Strain
<i>S. aureus</i>	DSM12463
<i>S. aureus</i>	9518
<i>S. aureus</i>	DSM346
<i>S. aureus</i>	8325.4
<i>S. aureus</i>	252
<i>S. aureus</i>	ATCC 9144
<i>S. aureus</i>	DSM 15676
<i>S. aureus</i>	col
<i>S. aureus</i>	NCTC 11963
<i>E.coli</i>	DSM 301
<i>K. aerogens</i>	ATCC 43086
<i>K. oxytoca</i>	NCTC 9528
<i>L. monocytogenes</i>	Serovar 7
<i>L. monocytogenes</i>	Food isolate
<i>S. algalactiae</i>	DSM 2134
<i>S. epidermidis</i>	DSM 20044
<i>S. epidermidis</i>	untyped
<i>S. haemolyticus</i>	DSM 20263
<i>S. saprophyticus</i>	ATCC 15305
<i>P. mirabilis</i>	DSM 4479
<i>B. cereus</i>	DSM 31
<i>S. chromogenes</i>	DSM 20454

<i>M. caesolyticus</i>	DSM 20597
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Table 6: *S. aureus* strains, Staphylococci and other species tested in the *S. aureus* LepA real-time PCR assay.

5

Species panel
GBS n=10
<i>Streptococcus dysgalactiae</i>
<i>Streptococcus pneumoniae</i>
<i>Streptococcus parasanguinis</i>
<i>Streptococcus intermedius</i>
<i>Streptococcus uberis</i>
<i>Streptococcus mitis</i>
<i>Enterococcus faecalis</i>
<i>Enterococcus faecium</i>
<i>Streptococcus mutans</i>
<i>Streptococcus pyogenes</i>
<i>Streptococcus sanguis</i>
<i>Streptococcus porcinus</i>
<i>Streptococcus bovis</i>
<i>Staphylococcus aureus</i>
<i>Bacillus cereus</i>
<i>Enterococcus faecalis</i>
<i>Enterococcus faecium</i>
<i>Staphylococcus epidermidis</i>
<i>Staphylococcus haemolyticus</i>
<i>Staphylococcus saprophyticus</i>

10 Table 7: GBS strains, Streptococci species and related species tested in the LepA GBS real-time PCR assay.

15 PCR primers and TaqMan probes were designed from *LepA* sequence information for *S. aureus* and *S. agalactiae* (Table 2). Real-time PCR assays incorporating these primers and probes were demonstrated on the LightCycler. Specificity testing was performed using a selection of the relevant closely species listed in Table 3. The *S. aureus* assay was 100% specific for *S. aureus* and the *S. agalactiae* assays detected all *S. agalactiae* strains and did not cross-react with any closely related Streptococcal species.

The words "comprises/comprising" and the words "having/including" when used herein with reference to the present invention are used to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

5

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

10

Sequence listing

SEQ IDs

Sites of probes, oligonucleotides etc. are shown in bold and underlined.

N or x = any nucleotide; w=a/t, m=a/c, r=a/g, k=g/t, s=c/g, y=c/t, h=a/t/c, v=a/g/c, d=a/g/t.

- 5 b=g/t/c. In some cases, specific degeneracy options are indicated in parenthesis: e.g.: (a/g) is either A or G.

Bordetella spp. primers and probes:

SEQ ID NO 1:

- 10 **BR1F**: 5'-AGCGCCTTGACGTTCTC-3'

SEQ ID NO 2:

BR1R: 5'-AAGATYGCCGAHATCCGC-3'

SEQ ID NO 3:

BR2F: 5'-RTAYTCCTGSGGCATGAA-3'

- 15 SEQ ID NO 4:

BR2R: 5'-CGTGTTCACGCCCAART-3'

SEQ ID NO 5:

BR3F: 5'-TTGATGCCSGCRATGA-3'

SEQ ID NO 6:

- 20 **BR3R**: 5'-ACSATCAAGGCSCAGAC-3'

SEQ ID NO 7:

BPF1A: 5'-ACGAACTCGTAGTCCATCGAC-3'

SEQ ID NO 8:

BPR1A: 5'-GCGCTTGTTGTTGCACAGT-3'

- 25 SEQ ID NO 9:

BPF1B: 5'-GTCGAAGAAATCGAGCAC-3'

SEQ ID NO 10:

BPR1B: 5'-GTATTCCTGGGGCATGAA-3'

SEQ ID NO 11:

- 30 **BP1PA**: 5'-CTGGCGCCCGTGGTAGCTC-3'

SEQ ID NO 12:

BP1PB: 5'-TTGTTGCACAGTGTCATCACCG-3'

SEQ ID NO 13:

BP2F1: 5'-CGCTGTTCGACCTCATAG-3'

- 35 SEQ ID NO 14:

BP2R1: 5'-CAGCTGGTCGTATTCGGA-3'

SEQ ID NO 15:

BP2P: 5'-GACACTTCGGGCTCGAACATCA-3'

SEQ ID NO 16:

BP3F1: 5'-TCCATCTTGTGAGCAC-3'

5 SEQ ID NO 17:

BP3R1: 5'-AACGACTGACTTCGTAC-3'

SEQ ID NO 18:

BP3F2: 5'-TGCCGATCACGTCTT-3'

SEQ ID NO 19:

10 BP3R2: 5'-GGTGGTTCGACGCTTC-3'

SEQ ID NO 20:

BP3P1: 5'-ACTTCCATGCCCAGTTCG-3'

SEQ ID NO 21:

BP3P2: 5'-CGA ACT GGG CAT GGA AG-3'

15 *S. aureus* primers and probes:

SEQ ID NO 22:

SAF1: 5'-TACCAACTGCTTTCATCT-3'

SEQ ID NO 23:

SAR1: 5'-CAATTTGAAGTACCTGTACA-3'

20 SEQ ID NO 24:

SAF2: 5'-TTTACGTTGACATAATTCCA-3'

SEQ ID NO 25:

SAR2: 5'-CAGAAAGTGACGGTTGATA-3'

SEQ ID NO 26:

25 SAP1: 5'-TTTACGGCTTATGTCACCGCCAT-3'

SEQ ID NO 27:

SAP2: 5'-TAGTTGCACGAACATATGGCTC-3'

GBS primers and probes;

SEQ ID NO 28:

30 GBSF1: 5'-AACCAATTGCTTTCAT-3'

SEQ ID NO 29:

GBSR1: 5'-CAGCTATTGGACAAAA-3'

SEQ ID NO 30:

GBSP1: 5'-CGTAGTGCTTTTATATCAGAACG-3'

35 *Mycobacterium* primers and probes:

SEQ ID NO 31:

MycobSF3: 5'-CACTCCGCGGTAGATGTC-3'

SEQ ID NO 32:

MycobSR2: 5'-AAGTTCCTAATCTGCGCCG-3'

SEQ ID NO 33:

5 **MTCF1:** 5'-AGACCGTGCGGATCTTG-3'

SEQ ID NO 34:

MTCR1: 5'-CATGGAGATCACCCGTGA-3'

SEQ ID NO 35:

MTCP: 5'-TCGTCTTTGTGCACCCGATAC-3'

10 **SEQ ID NO 36:**

MSP: 5'-ACGACCTTCTCGGAACCGT-3'

***Candida albicans* primers and probes:**

SEQ ID NO 37: GufI Region 1 Probe P1-CalbiGufI: 5' – CGA GAG GGA AAG AGG AAT
TAC AGT GAA AGC C – 3' GufI gene nucleotide base position from 270 to 300

15 **SEQ ID NO 38:** GufI Region 1 CanGufI-1f Forward primer: 5'- ATT GTG GCA CAC GTT
GAC CAT GG- 3' GufI gene nucleotide base position from 190 to 212

SEQ ID NO 39: GufI Region 1 CanGufI-1r Reverse primer: 5' –TGT GCT TGA ACT CCT
TGA GAT GCA TC- 3' GufI gene nucleotide base position from 466 to 491

20 **SEQ ID NO 40:** GufI Region 2 Probe P2-CalbiGufI: 5' – CTA CTT GGC ATA CAG CAT
GGG ATT GAA ATT G – 3' GufI gene nucleotide base position from 507 to 537

SEQ ID NO 41: GufI Region 1 CanGufI-2f Forward primer: 5'- GAT GCA TCT CAA GGA
GTT CAA GC - 3' GufI gene nucleotide base position from 466 to 489

SEQ ID NO 42: GufI Region 2 CanGufI-2r Reverse primer: 5' – ATC ATG CCA AGA ATC
CAC CAA TA - 3' GufI gene nucleotide base position from 740 to 762

25 **SEQ ID NO 43:** GufI Region 3 Probe P3-CalbiGufI: 5' – CTT GTC AGC GCA CAC AAA
TAG GAC ATA CGA C – 3' GufI gene nucleotide base position from 828 to 858

SEQ ID NO 44: GufI Region 3 CanGufI-3f, Forward primer: 5'- TAT TGG TGG ATT CTT
GGC ATG AT - 3' GufI gene nucleotide base position from 740 to 762

30 **SEQ ID NO 45:** GufI Region 3 CanGufI-3r, Reverse primer: 5' – GGG AAT GCC CCA ACA
AAT ACC A- 3' GufI gene nucleotide base position from 1043 to 1064

***Aspergillus fumigatus* primers and probes:**

SEQ ID NO 46: GufI Region 1 probe P1-AfumiGufI 5' –CGC AAA CCT GCT CGA TGA
TAT ACA ATC AC- 3'

35 **SEQ ID NO 47:** GufI Region 1 AspGufI-1f, Forward primer: 5' –GCC CAT GTC GAT CAT
GGC AAA AG- 3' GufI gene nucleotide base position from 613 to 635

SEQ ID NO 48: GufI Region 1 AspGufI-1r Revere primer: 5' –ACC TCT GCA CGG AAG
TCC AC- 3' GufI gene nucleotide base position from 820 to 839

SEQ ID NO 49: GufI Region 2 probe P2-AfumiGufI 5' –CAC CAC AGA GCG TGC TCC GTG CCG GC- 3'

SEQ ID NO 50: GufI Region 2 AspGufI-2f, Forward primer: 5'- GAG GTT GGC ATC ATG TAT CC- 3' GufI gene nucleotide base position from 1339 to 1358

5 **SEQ ID NO 51:** GufI Region 2 AspGufI-2r Reverse primer: 5' –AGC TGG TTG ATA CTG TCT TC- 3' GufI gene nucleotide base position from 1573 to 1592

SEQ ID NO 52: GufI Region 3 probe P3-AfumiGufI
5' –CCACTCAAGTCAAGTGGAAAGGCTCGGAC- 3'

10 **SEQ ID NO 53:** GufI Region 3 AspGufI-3f Forward primer: 5' –GAG TAT TTC ACA CCA ACG CAG GT- 3' GufI gene nucleotide base position from 1951 to 1973

SEQ ID NO 54: GufI Region 2 AspGufI-3r Reverse primer: 5' –TTG AAT TTT GTC ACC CAT TGT C- 3' GufI gene nucleotide base position from 2183 to 2204

SEQ ID 55 to 158

GufI/LepA Sequences from public databases

15 **SEQ ID NO 55**

>Aspergillus_fumigatus Af293 GTP binding protein GufI, putative (AFUA_3G14350) mRNA, complete cds

ATGAGTATGCGTTGCACCCATCCTCCAGCGCACATCAACATACAGGATGGAGTTTA
TCGAGTATTCAAACTAAGTCTTGTCACCCGGTGTCTCCGTCCAGATCGATGGAATT
20 GACTCACGAATTCTGCCACGAGCTTCGCCGAGCCTGTCATAGATGGAATGGATGCT
TTCTCGTTTCGTGAGGTCTCTTGCTGGTCAGAAACGAGGCAGCTTTTGTTCACCTCG
ATAGTCACCTCAGCAATCCGTTGTTCCACCGCCCGGAGCTAAGCACTTTTCTCCGC
AGCAAGTATGATCCGTTGCCGCAGTTTTCCTTTCTCGGAATCGGCAGTGGAACACCTCG
25 AATCCCTCATCCCAAAGGCATTATATCCCATGCCATTGCAGTACTCCAGATGCGAG
GATGCTTGCAGTTAGCCAGATGGCTGAGCGCTGCACCGAAAGGCACAGCTGCAAGC
CTGACGAGGGCGCCGTTTCGTCCTTGCTAACGCTCCCCGGTATTTACCAGCTCCGCT
TCCTCGTGTGGATCGAGATCGACCGCTACCAAGCCGTTATCCGACCTCGAGAAGAG
AATATCTGCGATTCCAAATCGAGCGATACCGGAACCTTTGCATCGTTGCCCATGTGCA
TCATGGCAAAAGCACACTTTCGGACCGCCTGCTCGAACTCACGGGGACGAATTGAGC
30 CCGGCTCGAACAACAAGTGTTGGACAAGCTCGACGTGGAGCGTGAACGTGGCATC
ACCGTGAAGGCGCAAACCTGCTCGATGATATACAATCACAATGGTGAAGACTATTT
ATTGCACCTGGTTGATACTCCAGGCCATGTGGACTTCCGTGCAGAGGTATCCCGTAG
TTATGCTAGTTGCGGAGGAGCATTGCTCCTGGTCGATGCCAGTCAAGGAATCCAGG
CACAGACCGTTGCGAAGCTTCTACCTCGCTTTTGCGCAGGGGCTTGAATTGATTCAG
35 TCATCAACAAGGTAGATTTGCCCTCGGCTGAACCAGAGCGAGCTCTTGAGCAGATG
AAGAAATTCCTTCGAGCTCGACACCGAGAACGCAGTGATGGTTTCAGCTAAGACAGG
CTTCAATGTTGAGAAATTACTTCCGACAGTTATTGAGAAGATCCCAGCGTATGGCC
ATTTCCCGCTCGATTCTCATGAGCTGCTTCCGTTACTGACTCTTAGCAGCCCCATCG
GCGATTGCAAGAAGCCCCCTGCGAATGTTACTCGTTGACTCGTGGTACGATTCCTACA
40 AAGGCGTAATCTGTCTGGTCCGCATCTTCGACGGTGAAATTCGAGCAGGCCAGCAG
GTCTGTGTCGTTTGGGACGGGTCTTAAATACTACGTTGGCGAGGTTGGCATCATGTAT
CCAAATGAAACACCACAGAGCGTGCTCCGTGCCGGCCAAGTTGGATACATCTACTT
CAACCCAGGTATGAAACGAAGCAAGAAGCGAAGATTGGTGACACATTTACAAGG
GTCCGATTGTGAGAAAGCCGTTGAACCACTTCCTGGCTTTGAGGAGCCCCAAGCGAT
45 GGTTTTTGTGGCGGCTTACCCGGTGGATGCAGACCATTTTGAGCACTTGGAAGACA
GTATCAACCAGCTCGTGCTTAACGACAGGAGTATCACCGTGCAGAAGGAGTCCTCA
GAGGCTCTTGGCGCCGGCTTCAGATTGGGAATTTCTAGGCACACTGCACGTCTGTG
TTTGAGGATCGTTTGCCTCAGGAGCATGGTGTCTAGCATCATCATCACACCTCCATCA

GTGCCTGTCAAGATCATTGGAAGGACGGAAAAGAGGAGATCAATTACCAGCCCTGC
 CAAATTTTCCTGAAGACGAAGAACTGCGCTCCAAGGTGGCTGAGATTCAAGAGCCCT
 ACGTCTTGGCTACACTGACCTTCCCCGAGGAGTACCTTGGCAAGGTCATTGAGCTTT
 GCGAGGCCAAACAGAGGGCGAGCAGAAGAGTTAGAGTATTTACACCAAACGCAGGT
 5 CATTCTTAAAGTATGAACTGCCTCTTGCTCAACTCGTTGATGACTTCTTCGGCAAGCT
 CAAGGGGTCTACCAAAGGCTACGCCAGCCTGGACTATGAGGAGTCCGCTTGGCAA
 CAGGGAACATCGTCAAGCTGCAACTTTTGGTAAATAAGGCGCCTGTGGATGCTGTT
 GCTCGCATCGTCCACTCAAGTCAAGTGGAAAGGCTCGGACGACAATGGGTGACAAA
 ATTCAAAGGAGCATGTGATCGACAACCTGTTTCGAGGTTGTGATCCAGGCTGCTGTTG
 10 GTAAAAAGATCATCGCCCGAGAGACAGTCAAACCCTACCGAAAAGATGTCTTGGCC
 AAGCTCCATGCTAGCGATGTCAGTCGGCGCCGGAAACTGCTGGAGAAGCAGAAAG
 AAGGACGAAAGAGACTGAGAGCTGTTGGAAACGTGGTGATTGAGCACAAAGGCATT
 CCAGGCCCTTCCTCGCCAAATAA

15 **SEQ ID NO 56**

>Aspergillus_terreus NIH2624 GTP-binding protein GUF1 (ATEG_03492) mRNA, complete cds

ATGCGAGGATGCCTGCAGTTAGCCAGATGGCTGAGCGCAGCGCCAACCCGCCCTTGC
 TGTAGCCATTGGCCTGGCCTTTGCGCCGCTCCGCGGTTTTTCTCCCATTCAGCGAT
 20 CTTCCGAGCCTCCGCAAGGACAGCCCGCGCGGCGGCGAGCAAACCTCCCTCCGACC
 TTGAATCGCGAATCGCGGCGATCCCAATCGAGCGGTACCGGAATTCTGCATCGTTC
 GCGCACGTGACCATGGCAAAGCACCCCTCTCTGACCGACTGCTGGAACGTACGG
 GGACGATTGAGCCGGGGACAAACAAGCAGGTTCTGGACAAACTCGATGTGGAGCG
 TGAACGGGGTATCACAGTCAAGGCGCAGACATGTACGATGATCTACAATCACAACG
 25 GCGAGGACTATCTGCTGCATCTGGTCGATACCCCGGGACATGTGGATTTCCTGTGG
 GAGGTCTCGCGCAGTTACGCCAGCTGCGGCGGTGCGTTGCTTCTTGTTGATGCGAGC
 CAGGGTGTCCAGGCGCAGACCGTGGCGAACTTCTACCTCGCCTTTCGCCAGGGTCT
 GGAGCTTATCCCTGTCAATTAACAAAGTAGATTTCGCGTCGCGCGGAGCCCGAGCGCG
 CCCTCGAGCAGATGAAACAGTCTTTCGAACTCGACACGGAAAATGCTGTGATGGTT
 30 TCGGCGAAGTCGGGACTGAATGTGGAGAACTTCTTCTACGGTGGTGGATAAGAT
 TCCAGCACCGATTGGCGACTGCAAAAAGCCCTGCGAATGCTCCTTGTTCGACTCGT
 GGTACGACTCCTACAAAGGCGTGATATGCTTGGTCCGCGTATTCGACGGCGAATC
 CGCGCGGGGGACCAATTGGTGTGCTTCGCCACGGGCATCAAGTACTACGTGGGCGA
 GGTTCGGCAICATGTATCCCAACGAGACTCCTCAGACGGTCATTCTGTGCGGGACAAG
 35 TCGGATACATCTTCTTCAACCCGGGTATGAAGCGCAGTAAGGAAGCCAAGATTGGG
 GACACCTACACGAAAGTCGGCTCCGAGAAGGCTGTTGAGCCGCTTTCGGGGCTTCGA
 AGAACCAAGGGCGATGGTCTTTGTGGCGGCATACCCCGTGGACGCCGACCAATTTCG
 AGCATCTCGAAGACAGCATCAACCAAGCTTATGCTGAACGACCGGAGCATCACGGTG
 CAGAAAGGAGTCTTCCGAGGCTCTGGGCGCGGGCTTCGGGCTCGGAATTTCTGGGAAC
 40 ACTGCATTGCTCCGTATTCGAGGACCGTCTGCGCCAGGAGCACGGCGCCAGCATCA
 TTATCACCCCGCCTTCAGTGCCCGTGAAGGTTCTGTGGAAAGACGGGCGGGAGGAG
 ATCGTACCCAGCCCTGCAAAGTTCCAGATGAAGATGAACTGCGGTCTAAGGTCCG
 GGAGATCAAGGAACCCCTACGTGCTGGCTACCTTGACGTTCCAGATGAATACCTCG
 GAAAAGTCAATGAAGTGTGCGAATCCAACCGTGGTGTCCAGCAGAGCCTCGAGTAC
 45 TTACGTTCAACGCAGGTATCTTCAAATACGAACTGCCAATGGCTCAGCTTGTGGA
 CGATTTCCTTCGGAAAGCTGAAGGGATCGACGAAGGGCTACGCGTCTTAGACTACG
 AGGAATCCGCTTGGCAGACGAGCAACATTTGTGAAGCTGCAGCTCCTTCGTCAAATAA
 GCTTCCTGTGGATGCTGTGGCGAGAATTGTGCACTATAGTCAAATTGAGAGACTTGG
 CAGGAATTGGGTGACCAAGTTCAAGGAACATGTTGATCGGCAGCTCTTCGAGGTCG
 50 TGAATCCAAGCCGCTGTCCGACGGAAGGTCATTGCGCGTGAAACGGTCAAACCGTAC
 CGCAAGACGTCTTCGCCAAACTCCATGCCAGCGATGTCAGTCGACGGAGAAAACCT
 GTTGGAGAAGCAGAAGGAGGGACGGAAGAGACTGAGAGCAGTCGGCAATGTGCTG
 ATAGAACATAAGGCGTTCCAGGCTTCTTGTCCAAGTGA

SEQ ID No 57

>Aspergillus clavatus NRRL 1 GTP binding protein Guf1, putative (ACLA_041830) mRNA, complete cds

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5  ATGCGAGGATGCCTGCAGTTAGCCAGATGGCTGAGCGCAGCACCGAAAGGCACCG
   CTGCAAGCCGTGACCAGGGCGCCAATTTGGCCTTGCTAACGCAACTCGGTTTTTCACCA
   ATTCCGCTGCGCGCGCCGGCTCAAGAGCCACTGCCAGCAAACCGGTAAACCGATCTC
   GAGAAACAGAAATATCCGCGAATCCGATCGAGCGCTACCGCAATTICTGCATCGTCCG
   CCACGTTGATCATGGCAAAAGCACCCCTTTCCGACCGGCTGCTCGAAGTGACGGGCA
10  CCATCCAGCCCGGTTTCAACAAGCAAGTCTTGACAAACTCGATGTGGAACGAGAA
   CGCGGCATCACTGTGAAGGCACAGACGTGTACTATGATATATAACCACAAACGGCGA
   AGACTACTTGTTCATCTGGTTGATACCCCGGGACATGTGGACTTCCGCGCGGAGG
   TTTCCGCGCAGTTATGCCAGCTGCCGAGGAGCACTGCTCTTGGTCGACGCCAGTCAAG
   GGAGTCCAGGCACAGACGGTTGCGAACTTTTATCTTGCTTTTGACAGGGATTAGA
   ATIGATCCCCGTCAACAAGGTGGATCTACCGTCGGCTGAGCCACAGCGGGCTT
15  TAGACCAGATGAAGCATACCTTTGAGCTGATACAGAAAACGCAGTCATGGTTTCG
   GCCAAGACAGGACTCAATGTGAGCAGTTGCTTCCGACAGTTGTTGACAAGATTCC
   TCGCGCGATCGGCGATTGCAAGAAACCCCTCCGAATGTTACTCGTTGATTCCTGGTA
   TGATTTCTTACAAGGGTGTTATCTGTTTGGTTTCGTATCTTCGACGGCGAACTGCGG
   GCTGGCCAGCAAGTTGTGTCATTGCCACGGGTCTCAAATACTATGTGGGAGAAGT
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SEQ ID No 58

>Aspergillus niger CBS 513.88 hypothetical protein (An09g04110) mRNA, complete cds

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SEQ ID NO 59

>Candida albicans SC5314 EF-type GTP-binding protein (CaO19.5483), mRNA

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10

SEQ ID NO 60

>C. glabrata CBS138 hypothetical protein (CAGL0100660g) partial mRNA

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50 LepA sequences from public databases.

SEQ ID NO 61

>Bacillus_anthraxis_0581_lepA

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35 SEQ ID NO 62

>Bacillus_cereus_10987_lepA

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20 SEQ ID NO 63

>Bacillus halodurans_lepA

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SEQ ID NO 64>*Bacillus subtilis_lepA*

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SEQ ID NO 65>*Bacillus licheniformis* DSM 13, complete genome

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20 **SEQ ID NO 66**
 >*Bacillus cereus_10987_lepA*
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 40 GAAACATCTCAAGCGCTAGGATTGGTTTCCGTTGTGGATTCTTAGGACTTCTTCAT
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SEQ ID NO 67>*Bacillus cereus*_14579_lepA

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SEQ ID NO 68>*Escherichia coli*_K12_lepA

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 10 AGTGGTGCCTCGACTTCTTCGATCGCCTGAAATCTACCTCGCGTGGTTATGCGTCTCT
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20 **SEQ ID NO 69**
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SEQ ID NO 70

>Escherichia coli_O157_H7_EDL933_lepA

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SEQ ID NO 71

>Enterobacter faecalis_V583_lepA

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20 SEQ ID NO 72

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SEQ ID NO 73

>Salmonella_Typhi_Ty2_lepA

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SEQ ID NO 74

>Salmonella_typhimurium_LT2_lepA

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20 **SEQ ID NO 75**

>Shigella flexneri

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SEQ ID NO 76

>Yersinia_pestis_KIM_lepA

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 35 AATTACCACAAGAGGCCTTCTGGCTATTCTGCATGTTGGAAAAGACAGTAAA

SEQ ID NO 77

>Streptococcus_agalactiae_NEM316

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20 **SEQ ID NO 78**

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SEQ ID NO 79

>Streptococcus_agalactiae_H36B

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SEQ ID NO 80

>Streptococcus_agalactiae_COH1

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20 SEQ ID NO 81

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SEQ ID NO 82

>Streptococcus_agalactiae_2603V/R

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SEQ ID NO 83

>Streptococcus_agalactiae_A909

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20 **SEQ ID NO 84**

>Streptococcus_agalactiae_515

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SEQ ID NO 85

>Streptococcus_pyogenes_MGAS10270

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SEQ ID NO 86

>Streptococcus_pyogenes_MGAS10750

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20 SEQ ID NO 87

>Streptococcus_pneumoniae_TIGR4

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SEQ ID NO 88

>Streptococcus_mutans_UA159

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SEQ ID NO 89

>Streptococcus_thermophilus_LMG18311

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20 **SEQ ID NO 90**

>Enterococcus_faecalis_V583

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 25 AGGCCTTAATATCTGAACGAGCCACAATTTTGTGACCAATCGCCGCTTGAAGTGGGA
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SEQ ID NO 91

>Enterococcus_faecium_DOctg621

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SEQ ID NO 92

>Streptococcus_sanguinis

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20 SEQ ID NO 93

>Streptococcus_gordonii_str_Challis

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SEQ ID NO 94

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SEQ ID NO 95

>Listeria_monocytogenes_4b_F2365_lepA

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20 **SEQ ID NO 96**

>Listeria_welshimeri

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SEQ ID NO 97>*Listeria innocua*

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SEQ ID NO 98>*Mycobacterium bovis* AF2122/97

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SEQ ID NO 99

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SEQ ID NO 100

>Mycobacterium_tuberculosis_str_Haarlem

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>Mycobacterium_tuberculosis_C

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SEQ ID NO 102

>Mycobacterium_tuberculosis_F11

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20 SEQ ID NO 103

>Mycobacterium_tuberculosis_H37Rv

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SEQ ID NO 104

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SEQ ID NO 106

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SEQ ID NO 107

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SEQ ID NO 115

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SEQ ID NO 116

20 >Staphylococcus aureus_MRSA252
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SEQ ID NO 117

>Staphylococcus_aureus_COL

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35 SEQ ID NO 118

>Staphylococcus_aureus_MW2

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 15

SEQ ID NO 119

20 >Staphylococcus aureus_Mu3
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SEQ ID NO 120

>Staphylococcus_aureus_RF122

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35 SEQ ID NO 121

>Staphylococcus_aureus_JH9

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SEQ ID NO 122

>Staphylococcus_aureus_JH1

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SEQ ID NO 123

>Staphylococcus_aureus_Newman

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 25 TCTACTACTAAAAATCGCGCCCTCACAAGCTGCCAAAGAACGTGACACTTCATAIGT
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SEQ ID NO 124

>Staphylococcus_aureus_MSSA476

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 40 CCACTTAACTAAATTACTTTCTTTATTTTCGATGAATTCATAATCAAAATGATGCATA
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 45 TTTGTGCTGGGTATCAACCGTCACTTCTGAACCGTCCCTTAAAATACATTTGATAAA
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SEQ ID NO 125

>Staphylococcus_aureus_NCTC 8325

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 30 TGTCTCTCTTAAATCATTATAAATTTTGTATCTATTGGGAACAGTCCGCAATATACC
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 35 TAACAACACCGTCCACAATTCTTATCGATGAAATTAACCCCTCTATATGGATCATACT
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 40 TTATCTAATGCTAAATAACAATTTGCTAATGTTTGTGCTTCGATACCTTGAGCCGCA
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SEQ ID NO 126

>Staphylococcus_epidermidis_RP62A

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 TGTTCGTGCAACAATTTTIGACCAATAGCCGCTGAACTGGCACTTCAAATTTGTTG
 TCGTGGAAITTAATGTTTTTAATTTTTCGACAAGCGCTTCCCTCTTTTCATATGCAAAA

TCTCTATGGACAATAAACTTAGAGCATCAACTTTATCTCCATTTCAGTAATATGTCC
 ATTTTAAACAAGATTACTTTCTTTATTTCTCAATAAATTCATAATCAAAAGAAGCATAC
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 5 CCTCTTTTACGTTGACATAATTCCATCACTGCACCTACATAATCATTCCGGCACCATC
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 10 GTGAAGACTCTGGTTCAAACCTCTAAGGATGCGTCATTAAGTTGTAATTTTCTAAAG
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 15 TTCTGTAACTTCAAATTCCTTACC GG TAGCCATCATTTTAATCCTATCTCCAGCTTTA
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 25 AAAATCCATAGAGTCAAGTAATTGATCTTGCATTTCTCGAGTTTCAACTGATTTGTAT
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SEQ ID NO 127

30 >Staphylococcus_epidermidis_ATCC_12228
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10 **SEQ ID NO 128**

>Staphylococcus_haemolyticus_JCSC1435

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45 **SEQ ID NO 129**

>Staphylococcus_saprophyticus_ATCC_15305

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25

SEQ ID NO 130

>*Lactobacillus sakei* subsp. *sakei*

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 30 ACAATTTCCGTTTCCGGGTGATGTCACCACCGTAACACTTGGCAAGCACGTTTTC
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 CTCCGTTCAAAGGCAAAGTCCCGATGCACGATGAAACTCAAGGCATCAACCGCTTC
 ACCATTCALGAGAATATCAATCTTAACCAAGGTCACTGGCACGATAGCCITGTCACIT
 35 CGTAATCAAACGATGCGTAACCCCTTGGTGTGATTTCAAATCATCGAAGAAATCA
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 40 GTCAAGGCAACGTTGTAATCAACCGATGGTGTCTGTCATGATCAAGTCTAAATTA
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 5 TGAFTTACCATGATCAATATGCGCGATGATCGAAAAGTTACGAATGTGTTTTCGCC
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SEQ ID NO 131

>Lactobacillus_delbrueckii_subsp.Bulgaricus_ATCC11842

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 15 GGCCCCGTCCCCGGGCTTCTTCGCGGTGGGCGATGAAGCTCAAGGCATCGAATGGTT
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 30 TCATGATCATGATTTCTGCGCTGGCTTAACCGTGCCCTTCTTCAATTCTGACTGACAT
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 40 GATTTTTCAGCTGCCGCTTGGAAACCGTGTCCGTCAGTTCCAGAATCCGGTCGGCAA
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SEQ ID NO 132

>Lactobacillus_acidophilus

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 CGATATGGCTTAATCGTAGCACGTGAAATAATCTTAGCACCGATTCACCTTGAAT
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TAAATCCATTGTAACAAATTCGCCGCGTTACGTTGGCAAAGTTCCATTACAGGACCA
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 TATTCACCAGCATCTGGTAAATCAGATGGATTATCAATCACCTTAGTCGTACCATCA
 TTCATTAATTGCATGATAGTCAACACTTGGTGCGGTATGATTAAATCAAGGTCAAAC
 5 TCTTGGTCCAATCTTTCTTGAACACTACGTCCATATGAAGTAAGCCAAGGAACCACAA
 CGGAAACCAAATCCTAAAGCAGTAGAAGTTTCTGGTTCAAATTCCAAAGCAGCATC
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 GGATACATACCAGAGTATACCATTTGGTGGAAATTTGACGGTAACCTGGAAGTGGTTC
 AGCAGTTGGGTGCCAGCATCAGTGAATTGTATCACCTACACGAGTTTCACGAACTG
 10 ATTTAAATGTTGGCAGTAATATACTCTACATCACCGAGGATTAATAATCTTCTTTA
 CTGGATGAGGACTTGAAACACCAACTTCAGTAACTTCATATTCCTTGCCAGTATTC
 TAAATTTGAATCTTATCACCGGGCTTAACTGTACCATCTTCAATTCTAACTGATAAAA
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 15 TTCCCTTGACCTGTTTTCCAGATACTTCAACAGCGTCTGAAGCGTCAAGACCTAAC
 TTTCCTTCGATTTCTTTCTTAGCATTTTCTGGATCAGCAGAAGGTAAATCAAATTTAT
 GATTACTGGTACAATTTCCAAATCGTCATCAATGGCTAAGTAAGTATTAAGCTAAAGT
 TTGFGCTTGAACACCTTGTGAAGCATCAACCACCAATAATGCACCTTCACATGCAGC
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 20 GGAATAATATAATCTTCGCCATTTTGGCATGATACTTAACTTCAACCGAGTTCATCT
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 ATTGTCTTTTCAGATACTGTATCTGTAACTCTAAGATTCCGGTCAGCAATTGTGTGACTT
 ACCATGGTCAATATGGGCAACAATTGAAAAGTTACGAATGTGATTITGATAATCTT
 TTAATTTTTTATATCCAT

25

SEQ ID NO 133

>Rickettsia prowazekii_Madrid E

TTATTCACCTCCAAATTTGCAATGCTGCAATAAATGCAGATTGTGGGAITTCIATAIT
 ACCGTACIGTCTCATTTTCTTTTTACCCGCTTTTGTCTTCTCAAGTAGITACGTTTAC
 30 GGCTTAATATCACCGCCATAACATTTTGATAAAAACATCTTTACGTAAGGCITTAATAG
 TTTACAGAGCAATAATACGACTACCAACACTTGTCTGAATTGCTATATCGATCTGT
 GTCTTGGTATTAATCCTTTAATCTTATGCAATAAGCCCTACCACGCTGTTCGGCAC
 GTGATCGATGTACTATAGTTGATAACGCATCAACAACCTCCCCATTAACATAAAATCC
 TAAAGATTAACAAGATCAGAAAGTTCAATAACATCCATTTGCCATTCAAAACTTGCA
 35 TAACTTTAGAACAGCTTTTCAAACGATCATAAAAATCATAACTATCTCATTTAGC
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 ATAGTTGCCITTAATCCATGGCTCTTCCATCGATGCGATTTTTTGCAAATCAGGTAA
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 40 ACATTTGGAGCAGTAGTGATTAAATCTAAATCGAATTCCTACTTAAACGTTCTTGT
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 TGAGGAGCTTTCCATTTCTACTCAAACTGGCATCATTAAGACGTAATTTAGCTAA
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 45 CAGTAATAGTATCACCAACTTTACAACTCTGATACCCGTTTTATAGAAGCTGTAAAA
 AACCTATTTCCCTGCAATAAAACATCCGAATATGTTTTTTGGAGTAAAAAAC
 CAACATGCTCGACGGTATAAACAGAAITTTGTCTCTATCATTTTAACACGCATATTT
 TACGTAAAGCACCATCAATAATACGTACCAAAATAACTACACCAAGATACGGATCG
 TACCAACTATCAACAAGTAACGCTTTTAAGATATCTGTGCTACTTCTTTTGGGGCA
 50 GGTAATTTAATTTGATTAATTGCTTCTAGAACTGAATCAATGCCTATACCGTTTTAGCA
 GATATTAACACAGCTTCACITGCATCAATGCCAATTATATCCTCTATTTGCGTCTTA
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 ATGATCAATTCGCAATTGCTTGATAAACATTTGCAAGAGTTTGAGCCTCTACTCCITG
 AGTACTATCCACTACTAATAACGAACCTTCACATGCAGCCAAGGACCTACTAACCT

CATAAGCAAAATCAACATGACCCGGAGTATCCATGAGATTCAAATAGTAAGTAITTA
CCGTTTTTAGCTTTATATACAAGCCGTACAGTTTGGGCTTTAATAGTAATACCTCGT
TCTCTCTATATCCATCGAATCTAATACTTGCTGACTCATTTCCCTAGCATTCLAAC
CTCCACAATACTCGATTAGCCTATCAGCTAACGTAGATTTACCATGATCTATATGAG
5 CGATTATTGAGAAATTCCTTATATATTTTIGATTATTCAT

SEQ ID NO 134

>gi|33591275:2575127-2576920 Bordetella pertussis Tohama I, complete genome

CTATTTGTCTTCGACTTGCGAGGATGGCGAGGAACGCCTCTTGCGGGATTTCACGCT
10 GCCGACCTGCTTCATGCGCTTCTTGCCGGCCCTCTGCTTTTCCAGCAACTCTCTCTTG
CGCGAGATAICGCCGCCATAGCACTTGCCAGCACGTTCTTGCGCAGCGCCTTGAC
GTCTCGCGCGCGATGACCTCCGCGCCGATTGCCGCCTGGATGGCCACGTCGAACA
TCTGGCGCGGGATCAGGCCGCGCATGCGCGAAACCACCTCGCGCGCCCGGTAGCGC
GCATTGTTGCGGTGCGCGATCATGGCCAGCGCATCGACGCGGTGCGCGTTGATCAG
15 CAGATCCACCTTGACCACGTGCGCCGAGCGGTATTCCACGAACTCGTAGTCCATCG
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CATGAACAGCGTCACCTTGACGATGGGTTCGCGGATATCGGCGATCTTGCGGATTT
20 CCGGCATGCGCGACGGGCTTTCGATGGTACGACCGTGCCGTGCGCGCTGTCGACC
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AGCCAGCGCCTGCGACACTTCGGGCTCGAACATCAGCGCGCGGTTCGTGAGCTTC
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25 GCGCGCGAACACCTGCGGCTTGACTTCCTTGAAGCCCGGCAGCGGCTCGGCCGCCG
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30 CCACGTAGTTGTGGAACACGAATCGATGATCAGCGCCTGCAGCGGCGCGGACGGA
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CATGCCGGTCTTGGCGCTGGCCAGCACCGCCTCGGACGCGTGCATGCCGATCACGT
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GCACCGGCAGCACTTCCATGCCAGTTCGATGGCCGTGTAGCAGTTGGCCACGGTC
35 TGGGCCCTCGACGCCCTGCGAAGCGTCGACCACCAGCAAGGCGCCTTCGCAAGGCCGA
CAGCGAACGACTGACTTCGTACGAGAAATCGACGTGTCCGGGGGTATCGATCAGGT
TGAGGTGTAGACCGTGCCGTCTTGCAGCTTGTACTGCAGGGACGCGGTCTGCGCC
TTGATCGTGATACCCCGTTCCGCGCTCGATATCCATGGAATCAAGCACITTCGCGCGAC
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40 TTACCGTGATCAATGTGGGCAATGATGGAAAAGTTGCGGATATGCTGCAT

SEQ ID NO 135

>gi|5932441:340-2133 Bordetella pertussis lep operon, (also Tomaha I) complete sequence

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45 GCCGACCTGCTTCATGCGCTTCTTGCCGGCCCTCTGCTTTTCCAGCAACTCTCTCTTG
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GTCTCGCGCGCGATGACCTCCGCGCCGATTGCCGCCTGGATGGCCACGTCGAACA
TCTGGCGCGGGATCAGGCCGCGCATGCGCGAAACCACCTCGCGCGCCCGGTAGCGC
GCATTGTTGCGGTGCGCGATCATGGCCAGCGCATCGACGCGGTGCGCGTTGATCAG
50 CAGATCCACCTTGACCACGTGCGCCGAGCGGTATTCCACGAACTCGTAGTCCATCG
ACGCATAGCCGCGCGACACCGATTTCAGTTTGTGGAAGAAATCGAGCACGATCTCG
GCCAGCGGAATCTCGTAGGTACGGTGCACCTGGCGCCCGTGGTAGCTCATGTTGAG
CTGGACGCCGCGCTTGTGTTGCACAGTGTATCACCGGCCCCACGTATTCCTGGGG
CATGAACAGCGTCACCTTGACGATGGGTTCGCGGATATCGGCGATCTTGCGGATTT

CCGGCATGCGCGACGGGCTTTCGATGGTCACGACCGTGCCGTCGCGCTGTTCGACC
 TCATAGACCACCGATGGCGCGGTGGTGATGATGTCCATGTGGAACCTCGCGCTCCAG
 GCGCTCCIGCACGATTTCCATGTGCAGCAGCCCCAGGAAGCCGCAGCGAAAGCCGA
 AGCCCAGCGCCTGCGACACTTCGGGCTCGAACATCAGCGCGGCGTGGTTGAGCTTC
 5 AGCTTTTCCAGCGAATCGCGCAGCTGGTCTGATTTCGGAGCTTTCCACCGGATACAG
 GCCGGCGAACACCTGCGGCTTGACTTCCTTGAAGCCCGGCAGCGGCTCGGCCGCCG
 GCTTGCCCCGCCAGGGTGATGGTGTGCCCCACCTTGGCGTGTTCCAGTTCCTTGATGC
 CGGCGATGACAAAGCCCACTTCGCCGGCCGACAACCTCCGGCCGCGGCTGCGATTG
 GCGTGAAACACGCCGATCTGCTCGCACAGATGCGTGCGGTGGGACGCCATCAGCAG
 10 GATCTTGTCTTGGGCTTGAGCACGCCGTGACGATGCGCACCAGCATGACCACGC
 CCACGTAGTTGTGCAACCACGAATCGATGATCAGCGCCTGCAGCGGCGCGGACGGA
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 CTTCGACCTTCCTGGCGCGCGGCTTCGGGTGCGGCTGCGGCAGGTCCATCTTGTGTA
 15 GCACCGGCAGCACTTCCATGCCCCAGTTTCGATGGCCGTGTAGCAGTTGGCCACGGTC
 TGGGCAICGACGCCCTGCGAAGCGTCGACCACCAGCAAGGCGCCTTCGCAGGCCGA
 CAGCGAACGACTGACTTCGTACGAGAAATCGACGTGTCCGGGGGTATCGATCAGGT
 TGAGGTGTAGACCGTGCCGTCTGCGACTTGTACTGCAGGGACGCGGTCTGCGCC
 TTGATCGTGATACCCCGTTGCGGCTCGATATCCATGGAATCAAGCACTTGCGCGGAC
 20 ATTCGCGCTCGGCCAGCCCCCGCAACGGTGGATCAGGCGATCGGCGAGCGTCGA
 TTTACCGTGATCAATGTGGGCAATGATGGAAAAGTTGCGGATATGCTGCAI

SEQ ID NO 136

>gi33572656:143317-145110 *Bordetella pertussis* strain Tohama I

25 CTATTTGTCTTCGACTTCAGGATGGCGAGGAACGCCTCTTGCGGGATTTCACGCCT
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 GTTCTCGCGCGCGATGACCTCCGCGCCGATTCGCCGCTGGATGGCCACGTGGAACA
 TCTGGCGCGGGATCAGGCCGCGCATGCGCGAAACCACCTCGCGCGCCCGGTAGCGC
 30 GCATGTGTGCGGTGCGCGATCATGGCCAGCGCATCGACGCGGTGCGCGTTGATCAG
 CAGATCCACCITGACCACGTGCGCCGAGCGGTATTCACGAACCTCGTAGTCCATCG
 ACGCATAGCCGCGCGACACCGATTTAGTTTGTGCAAGAAATCGAGCACGATCTCG
 GCCAGCGGAATCTCGTAGGTGACCTGGCGCCCGTGGTAGCTCATGTGAG
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 35 CATGAACAGCGTCACCTTGACGATGGGTTGCGGGATATCGGCGATCTTGGCGATT
 CCGGCAITGCGCGACGGGCTTTCGATGGTCACGACCGTGCCGTGCGGCTGTTCGACC
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 GCGCTCCIGCACGATTTCCATGTGCAGCAGCCCCAGGAAGCCGCAGCGAAAGCCGA
 AGCCCAGCGCCTGCGACACTTCGGGCTCGAACATCAGCGCGGCGTGGTTGAGCTTC
 40 AGCTTTTCCAGCGAATCGCGCAGCTGGTCTGATTTCGGAGCTTTCCACCGGATACAG
 GCCGGCGAACACCTGCGGCTTGACTTCCTTGAAGCCCGGCAGCGGCTCGGCCGCCG
 GCTTGCCCCGCCAGGGTGATGGTGTGCCCCACCTTGGCGTGTTCCAGTTCCTTGATGC
 CGGCGATGACAAAGCCCACTTCGCCGGCCGACAACCTCCGGCCGCGGCTGCGATTG
 GCGTGAAACACGCCGATCTGCTCGCACAGATGCGTGCGGTGGGACGCCATCAGCAG
 45 GATCTTGTCTTGGGCTTGAGCACGCCGTGACGATGCGCACCAGCATGACCACGC
 CCACGTAGTTGTGCAACCACGAATCGATGATCAGCGCCTGCAGCGGCGCGGACGGA
 TCGCCCCTTGGGCGGCGGCACCCGCGCCACGATGGATTTCGAGGATCTCGTCGATGCC
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 CTTCGACCTTCCTGGCGCGCGGCTTCGGGTGCGGCTGCGGCAGGTCCATCTTGTGTA
 50 GCACCGGCAGCACTTCCATGCCCCAGTTTCGATGGCCGTGTAGCAGTTGGCCACGGTC
 TGGGCCICGACGCCCTGCGAAGCGTCGACCACCAGCAAGGCGCCTTCGCAGGCCGA
 CAGCGAACGACTGACTTCGTACGAGAAATCGACGTGTCCGGGGGTATCGATCAGGT
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 TTGATCGTGATACCCCGTTGCGGCTCGATATCCATGGAATCAAGCACTTGCGCGGAC

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TTTACCGTGATCAATGTGGGCAATGATGGAAAAGTTGCGGATATGCTGCAT

SEQ ID NO 137

5 >gi|33598993:3971204-3972997 *Bordetella bronchiseptica* RB50, complete genome
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10 TCTGGCGCGGGATCAGGCCGCGCATGCGCGAAACCACCTCGCGCGCCCGGTAGCGC
GCATTGTTCGCGTGCGCGATCATGGCCAGCGCATCGACGCGGTGCGCGTTGATCAG
CAGATCCACCTTGACCACGTGCGCCGAGCGGTATTCCACGAACTCGTAGTCCATCG
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GCCAGCGGAATCTCGTAGGTGAGGTGCACCTGACGCCCCGTGATAGCTCATGTTGAG
15 CTGGACGCGCGCTTGTGTTGTCACAGCGTCATCACCGGCCCCACGTAATCCTGGGG
CATGAACAGCGTCACCTTGACGATGGGTTCGCGGATATCGGCGATCTTGGCGATTTC
CCGGCATGCGCGACGGGCTTTCGATGGTCAACGACCGTGCCGTGCGGCTGTTCGACC
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GCGCTTCCTGCACGATTTCCATGTGCAGCAGCCCCAGGAAGCCGCGAGCGAAAGCCGA
20 AGCCCAGCGCCTGCGACACTTCGGGTTCGAACATCAGCGCGGCGTCTGAGCTTC
AGCTTTTCAGCGAATCGCGCAGCTGGTCTGATTCGGAGCTTTCCACCGGATACAG
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25 GCGGTGAACACGCCGATCTGCTCGCACAGATGCGTGCGGTGGGACGCCATCAGCAG
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CATGCCGGTCTTGGCGCTGGCCAGCACCGCCTCGGACGCGTCGATGCCGATCACGT
30 CTTCGACCTCTTGGCGCGCGGCTTCCGGATCGGCCTGCGGCAGGTTCATCTTGTGA
GCACCGGCAGCACTTCCACGCCAGCTCGATGGCCGTGTAGCAGTTGGCCACGGTC
TGGGCTTCGACGCCCTGCGAGGCGTCGACCACCAGCAAGGCGCCTTCGCAGGCCGA
CAGCGAACGACTGACTTCGTACGAGAAATCGACGTGTCCGGGGGTATCGATCAGGT
TGAGGTGTGAGACCGTGCCGTCTTGCAGCTTGTACTGCAGGGACGCGGTCTGCGCC
35 TTGATCGTGATACCCCGTTTCGCGCTCGATATCCATGGAATCAAGCACTTGCGCGGAC
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TTTACCGTGATCGATGTGGGCAATGATGGAAAAGTTGCGGATATGCTGCAT

SEQ ID NO 138

40 >gi|33577019:140181-141974 *Bordetella bronchiseptica* strain RB50, complete genome;
segment 12/16
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45 GTTCCTCGCGCGCGATGACCTCCGCGCCGATTGCCGCCTGGATGGCCACGTTCGAACA
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GCATTGTTCGCGTGCGCGATCATGGCCAGCGCATCGACGCGGTGCGCGTTGATCAG
CAGATCCACCTTGACCACGTGCGCCGAGCGGTATTCCACGAACTCGTAGTCCATCG
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50 GCCAGCGGAATCTCGTAGGTGAGGTGCACCTGACGCCCCGTGATAGCTCATGTTGAG
CTGGACGCGCGCTTGTGTTGTCACAGCGTCATCACCGGCCCCACGTAATCCTGGGG
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GCGCTCC'IGCAGGATTTCCATGTGCAGCAGCCCCAGGAAGCCGCAGCGAAGGCCGA
 AGCCCAGCGCCTGCGACACTTCGGGTTCGAACATCAGCGCGGCGTCCGTTGAGCTTC
 AGCTTTTCCAGCGAATCGCGCAGCTGGTTCGTATTCGGAGCTTTCCACCGGATACAG
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 5 GCTTGCCCCGCCAGGGTGATGGTGTGCGCCACCTTGCGGTGTTCCAGTTCCTTGATGC
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 GGCGTGAACACGCCGATCTGCTCGCACAGATGCGTGCGGTGGGACGCCATCAGCAG
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 CCACGTAGTTGTGCAACCACGAATCGATGATCAGCGCCTGCAGCGGCGCGGACGGA
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 TGGGCCCTCGACGCCCTGCGAGGCGTGCACACCAGCAAGGCGCCTTCGCAGGCCGA
 15 CAGCGAACGACTGACTTCGTACGAGAAATCGACGTGTCCGGGGGTATCGATCAGGT
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 TTGATCGTGATACCCCGTTTCGCGCTCGATATCCATGGAATCAAGCACTTGCGCGGAC
 ATTTTCGCGCTCGGCCAGCCCCCGCAACGGTGATCAGGCGATCGGCGAGCGTCTGA
 TTTACCGTGATCGATGTGGGCAATGATGGAAAAGTTGCGGATATGCTGCAT

20

SEQ ID NO 139

>gi|33574176:76674-78467 Bordetella parapertussis strain 12822, complete genome; segment
 11/14

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 25 GCGGACCTGCTTCATGCGCTTCTTGCCGGCCTTCTGCTTTTCCAGCAACTTCTTCTTG
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 30 CAGATCCACCTTGACCACGTGCGCGGAGCGGTATTCACGAACCTCGTAGTCCATCG
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 35 CCGGCATGCGCGACGGGCTTTCGATGGTCAAGACCGTGCCGTGCGGCTGTTCGACC
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 40 GCGGCGGAACACCTGCGGCTTGACTTCCTTGAAGCCCGGCAGCGGCTCGGCCGCCG
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 45 CCACGTAGTTGTGCAACCACGAATCGATGATCAGCGCCTGCAGCGGCGCGGACGGA
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 50 TGGGCCCTCGACGCCCTGCGAGGCGTGCACACCAGCAAGGCGCCTTCGCAGGCCGA
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 TTGATCGTGATACCCCGTTTCGCGCTCGATATCCATGGAATCAAGCACTTGCGCGGAC

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SEQ ID NO 140

5 >gil163258032:1817720-1819513 *Bordetella petrii* strain DSM 12804, complete genome
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GTTCTCGCGCGCGATGACCTCGGCGCCGATAGCCGCCTGAATGGCCACGTTCGAACA
10 TCTGGCGCGGGATCAGCCCGCGCATGCGCGTGACACGTTCGCGCGCGCGATAGCGG
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CAGGTTCGACCCGCACACGTCGGCCGAACGGTATTCCAGGAATTTCGTAATCCATCG
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GCCAGCGGAATCTCGTAGGTGAGGTGCACCTGGCGGCCGTGGTAGCTCATGTTGAT
15 CTGCACGCGCGCTTGTGTTGACACAACGTTCATGACCGGACCCACGTATTCCTGGGG
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20 GCCCCAGCGCTGCGAGACCTCGGGTTCGAACATCAGCGCGGCATCGTTTGAGCTTGA
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CGGCGAACACCTGCGGCTTGACTTCTTGAAGCCCCGGCAGCGGCGCGTTGGCCGGC
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GCAATGATGAACCCGACCTCGCCCGCGGACAGCTCGGTGCGCGGCTGCGACTTTGGG
25 CGTGAACACGCCGACCTGCTCGCACAGGTGGGTGGCGCCCGAGGCCATCAGCAGG
ATCTTGTCTTTTGGGACGCAGCACGCCGTTCGATGATGCGCACCAAGCATACCAACGCC
CACGTAGTTGTCTGAACCAAGGAGTCGATGATCAGGGCCTGCAGTGCGCCGTTCGGGT
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ATGCCCGTCTTGCGGCTGGCCAGCACCGCGTTTCGAGGCGTCGATGCCGATGACGTC
30 TTCGACCTCTTGCGCGCGCGGCTCGGGATCGGCCTGCGGCAGGTCCAATCTGTTGAG
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AGCGAACGGCTGACTTCGTACGAGAAGTCGACGTGCCCCGGGTGTTCGATGAGGTT
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SEQ ID NO 141

40 >gil163854304:1817720-1819513 *Bordetella petrii* DSM 12804, complete genome
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SEQ ID NO 142

>DSM4927-B.pertussis

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SEQ ID NO 143

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SEQ ID NO 145

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SEQ ID NO 146

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SEQ ID NO 147

25 >DSM-10303-B.bronchiseptica
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SEQ ID NO 148

>DSM4922-B.parapertussis

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20 SEQ ID NO 149

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SEQ ID NO 151

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SEQ ID NO 154

>B.holmesiiDSM-13416-

50 GCGATCACTTCCGCACCGATAGCGGCTTGAATGGCCACGTCATACAATTCACGGGG
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 15 GCGCGCGGCACACGCGCGACGACCATTTCAGGATCTCGTCGATCCCCATCCCTGT
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SEQ ID NO 155

>B.holmesiiCCUG-53681

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 30 ATCTCATAGGTCAAATGCACCTGGCGTCCGTGGTAGGTCATGTTGATCTGCGTGCCG
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 35 CACGATTTCCATGTGCAGCAGGCCCCAGAAAGCCGCAGCGAAAGCCAAACCCCAAG
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 40 CAAAACCCACTTCCGCCGCGGACAGATGGGGGCGTTGCTGCGATTGGGGCGTGAAC
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SEQ ID NO 156

>B.hinziiCCUG-32379-

45 ACSTGCGGCGCGCGATAGCCGCTGGATGGCCACGTCATACATCTGGCGCGGGATG
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 5 GGTGATGGTGTGCGCCACCTTGGCATGCGCCAGCTCCTTGATGCCCGGCGATGACGA
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SEQ ID NO 157

10 >B.hinziiDSM-11333-
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 25 GTCGCCCACCTTGGCATGCGCCAGCTCCTTGATGCCGGCGATGACGAAGCCCACT
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SEQ ID NO 158

>B.trematumCCUG-24272
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40 SEQ ID NO 159

>M.africanumtype1
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 50 CGTTCGCCGTTCGATGTCCATCCGATCCAAGTACTGGGCCCGCATAGAGCGTTCGTG
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SEQ ID NO 160

>M.africanum2

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5 GCGGTAAACGGTCCGGGTCCGCGCGGGCAGGTCGATCTTGTTGAGCACCGGGATGA
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10 CGTTCCCGCTCGATGTCCATCCGATCCAAGTACTGGGCCCGCATAGAGCGTTCTGTG
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SEQ ID NO 161

15 >M.africanum3

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SEQ ID NO 162

>M.africanum11

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SEQ ID NO 163

>M.bovis

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SEQ ID NO 164

>M.bovisBCG

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15

SEQ ID NO 165

>M.canetti

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SEQ ID NO 166

>M.canetti

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SEQ ID NO 167

>M.caprae

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SEQ ID NO 168

5 >M.caprae2
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SEQ ID NO 169

>M.microtti
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SEQ ID NO 170

>M.pinipedii
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40 CCTTCGCGGGCATCGACCAACAGCACCGCACCTTCGCAAGCCTCCAGCGCACGCGA
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SEQ ID NO 171

>M.tuberculosis
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5

SEQ ID NO 172>*M.smegmatis*

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20 **SEQ ID NO 173**>*M.celatum*

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25 CGGCAGGTTCGATCTTGTTGAGCACCGGGATGATCGCCAGGTTCGCGGTCCAGCGCCA
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30 CCAGGTACTTCGCCCCGATGGACCGCTCGTCGACGACTCCGGTCAGCTGCAGCATC
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SEQ ID NO 17435 >*M.fortuitum*

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45 CGACGACGCCGGTGAGCTGCAGCATCCGGTCGGCCAGCGTCGACTTGCCATGGTTCG
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SEQ ID NO 175>*M.intracellulare*

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CTCGCAGGCCTCCAGCGCGCGGACACCTCGTAGGTGAAGTCGACGTGGCCCCGGG
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 5 CAGCGTCGACTTGCCGTGATCGATGTGGGCGATGATGCAGAAGTTCCTAATCTGCG
 CCG

SEQ ID NO 176

>M.malmoense

10 CAGAGTCGAAGATCATCGCGCGGGTGGGCGCGTCGGCGTCGCCCTGCGGCGGCGGC
 ACCTGACGGACCACTCGTCGAGAAGGTGCGCGACGCCCTCGCCGGTCTTGCCGGA
 CACCCGACGACCGTCGGTCGGCTCGCAGCCCATGATGTGGGCGATCTCGGCCCGGT
 AGCGGTCCCGATCGGCGGCGCGGAGATCGATCTTGTGAGCACCGGGATGATGGTC
 AGGTCACGGTCCAGCGCCAGGTAGAGGTTGGCCAGGGTCTGGGCCTCGATGCCCTG
 15 GGCCGCGTCGACCAGCAGCACCGCGCCCTCACAGGCCTCCAGCGCGCGGAGACCT
 CGTAGGTGAAGTCGACGTGACCAGGGGTGTGATCAGGTGCAGGACGTACTGGGTTC
 CCATCGACTTGCCAGGGCAGCCGGACGTTCTGGGCCTTGATGGTGATCCCGCGCTC
 CCGCTCGATGTCCATCCGGTCCAGGTATTGGGCGCGCATCGATCGCTCGTCGACGA
 CGCCGGTGAGCTGGAGCATCCGGTCCGCGAGCGTCGACTTGCCATGGTCGATGTGA
 20 GCGATGATGCAGAAGTTCCTAATCTGCGCCG

SEQ ID NO 177

>M.paratuberculosis

25 CAGAGTCGAAGATCATCGCGCGCAGCGGCGCATCGGCCCTGCCCCTGCGGCGGCGGC
 ACCTGGCGCACCACTCGTCGAGCAGCGCGCCACGCCCTCCCCGGTTTGCCGGA
 CACCCACAGCACGTGTCGGGTTTCGACCCGATGATGTGGGCGAGCTCGCCGGCGT
 AGCGATCCGGGTGCGGCGGCGGAGGTGATCTTGTGAGGACCGGGATGATGGTC
 AGATCGCGGTCCAGCGCCAGGTAGAGGTTGGCCAGCGTCTGGGCCTCGATGCCCTG
 CGCGGCGTCGACCAGCAGCACGGCACCTTCGACAGGCCTCCAGTGCAGCGGACACCT
 30 CGTAGGTGAAGTCGACGTGGCCCGGGGTGTGATCAGGTGCAGGACAAAATCTTTG
 CCGGCGTCTCGCCGCCGAGACCTGCCAGGGCAGCCGCACGTTCTGCGCCCTTGAT
 GGTGATGCCGCGCTCCCGCTCGATGTCCATCCGGTCCAGGTACTGGGCGCGCATCG
 ACCGCTCGTCGACGACGCCGGTGAGCTGCAGCATCCGGTCGGCCAGCGTCGACTTG
 CCGTGATCGATGTGGGCGATGATGCAGAAGTTCCTAATCTGCGCCG
 35

SEQ ID NO 178

>M.scrofulaceum

40 GTAAACGGAGTCGAAAATCATTGCGCGGGTAGGTGCCTCGGCGTCGCCCTGAGGGG
 GCGGCACCTGTCCGACCACTCGTCGAGCAGGTCCGACACGCCCTTCGCCGGTTTG
 CCGGACACCCGCAACACCTCGGCCGGCTCGCAGCCGATGATGTGTGCCATCTCGGC
 GCGGTAAACGGTCCGGGTGCGCCGCGGGCAGGTGATCTTGTGAGCACCGGGATGA
 TGTGCAGGTTCGCGGTCCAACGCCAGGTAGAGGTTGCCAGCGTCTGCGCCTCGATG
 CCTTGCGCGGCATCGACCAACAGCACCGCACCTTCGCAAGCCTCCAGCGCACGCGA
 GACTTCGTAGGTGAAGTCGACATGGCCCGGGGTGTGATCAGATGCAGCACGTAGT
 45 CGGTCTTGTCGACCCGCCAGGGTAGCCGCACATTCTGGGCCTTGATGGTGATGCCG
 CGTTCCCGCTCGATGTCCATCCGATCCAAGTACTGGGCCCCGATAGAGCGTTTCGTCG
 ACCACTCCGGTGAGCTGCAGCATCCGGTTCGGCCAACGTTGACTTGCCGTGGTTCGAT
 GTGGGCGATGATGCAAAAGTTCCTAATCTGCGCCG

Claims

1. A diagnostic kit for a bacterial species and/or fungal and/or yeast species comprising at least
5 one oligonucleotide probe capable of binding to at least a portion of the LepA and/or GufI genes or its corresponding mRNA
2. A kit as claimed in claim 1, wherein the portion of the LepA gene is equivalent to a portion of the region of the gene from base pair position 57 to base pair position 228, position 57 to 74, position 209 to 229, position 112 to 134, or from position 522 to 659 of *Staphylococcus aureus* LepA gene, from base pair position 66 to 215, position 66 to 81, position 200 to 215 or from position 167 to 189 in *Group B Streptococcus* LepA gene, from base pair position 618 to 772 in *Mycobacterium* species, from base pair position 1203 to 1817 in *Mycobacterium* species, or from base pair position 160 to 612, position 552 to 1081 or from position 1006 to 1638 in *Bordetella* species and/or wherein the portion of the GufI gene is
10 equivalent to a portion of the region of the gene from base pair position 190 to base pair position 2204 of the gene, from base pair position 190 to 1064, position 270 to 300, position 190 to 212, position 466 to 491, position 507 to 537, position 466 to 489, position 740 to 762, position 828 to 858, position 740 to 762 or from position 1043 to 1064 of the *C. albicans* GufI gene, or from base pair position 613 to 2204, position 613 to 635, position
15 820 to 839, position 1339 to 1358, position 1573 to 1592, position 1951 to 1973 and 2183 to 2204 of the *A. fumigatus* GufI gene.
3. A kit as claimed in claim 1 or 2 comprising a probe selected from the group comprising SEQ ID NO 11 or SEQ ID NO 12, SEQ ID NO 15, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 30 SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO
20 37, SEQ ID NO 40, SEQ ID NO 43, SEQ ID NO 46, SEQ ID NO 49, SEQ ID NO 52 or sequences substantially similar or complementary thereto which can also act as a probe, and/or a forward primer selected from the group comprising of SEQ ID NO 1, 3, 5, 7, 9, 13, 16, 18, 22, 24, 28, 31, 33, 38, 41, 44, 47, 50 or 53 or sequences substantially similar or complementary thereto which can also act as a forward amplification primer and/or a
25 reverse primer selected from the group consisting of SEQ ID NO 2, 4, 6, 8, 10, 14, 17, 19, 23, 25, 29, 32, 34, 39, 42, 45, 48, 51 or 54, sequences substantially similar or complementary thereto which can also act as a reverse amplification primer.
4. A nucleic acid molecule selected from the group consisting of: SEQ ID NO 1 through SEQ ID NO 178 and sequences substantially homologous or substantially complementary thereto
30 or to a portion thereof and having a function in diagnostics based on the LepA and or GufI gene.

5. A kit substantially as described herein with reference to the examples and/or the accompanying figures.

Figure 1:

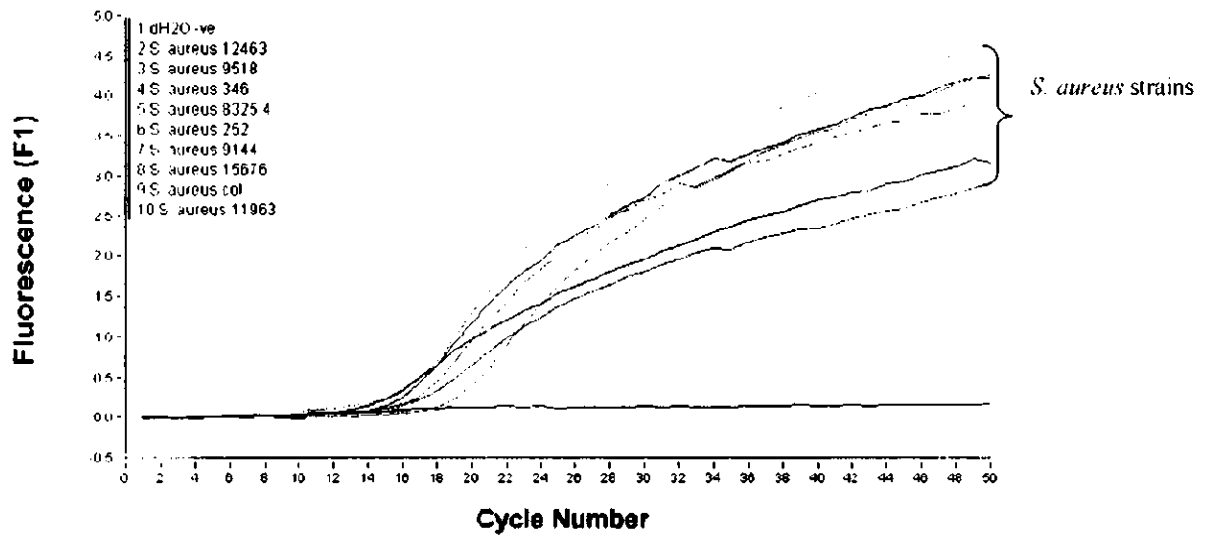


Figure 2:

5

Step 1: Baseline Step 2: Analysis

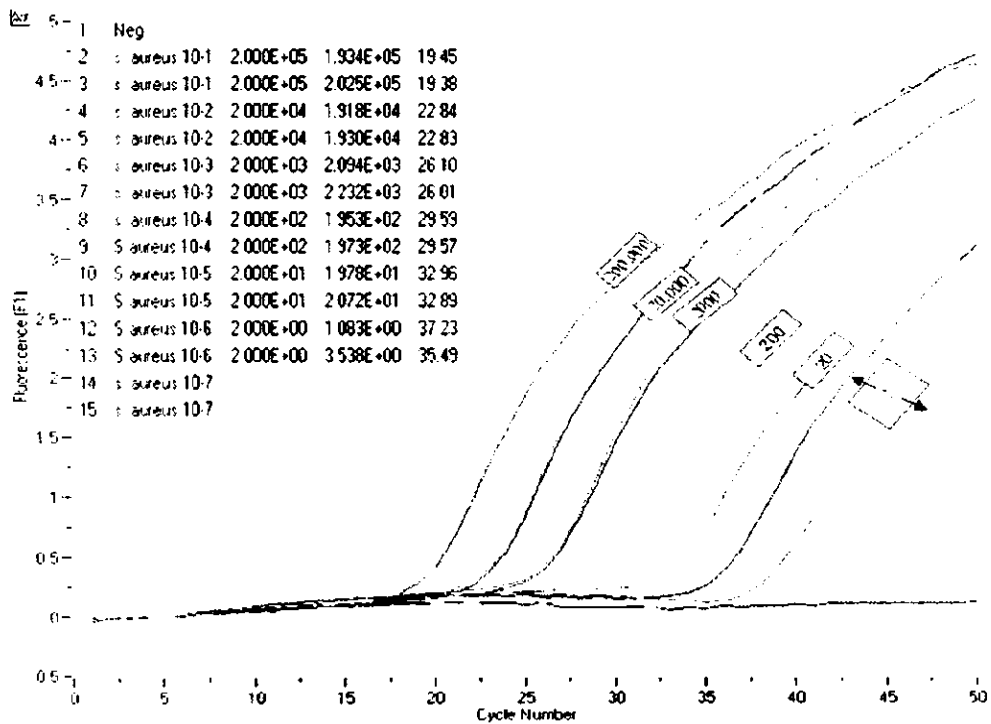
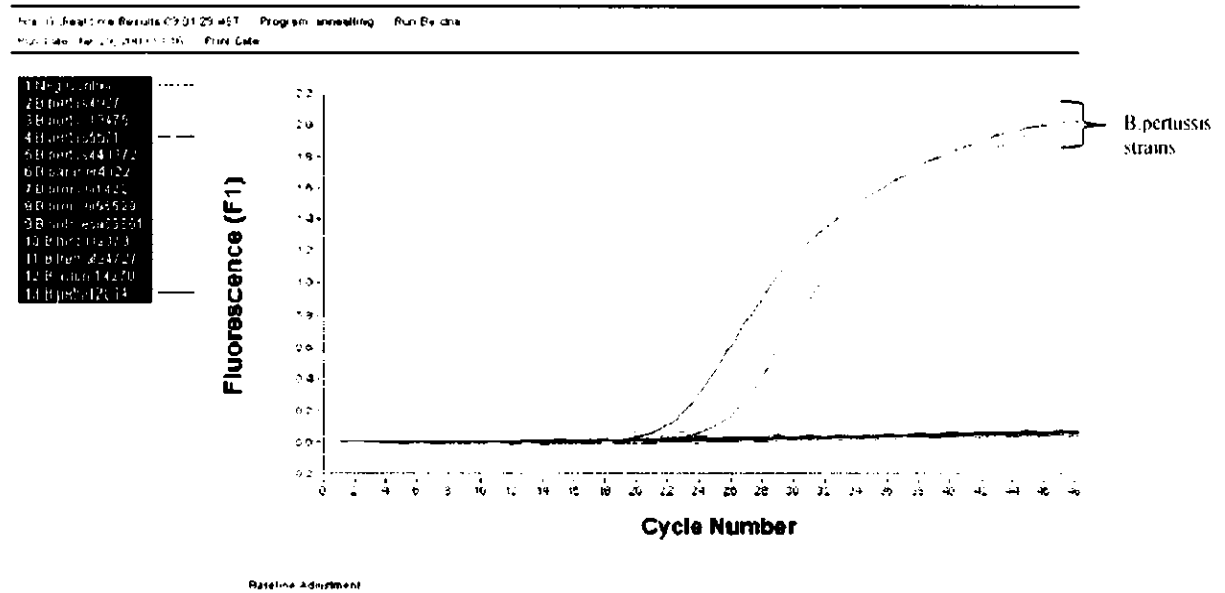


Figure 3:



5

Figure 4:

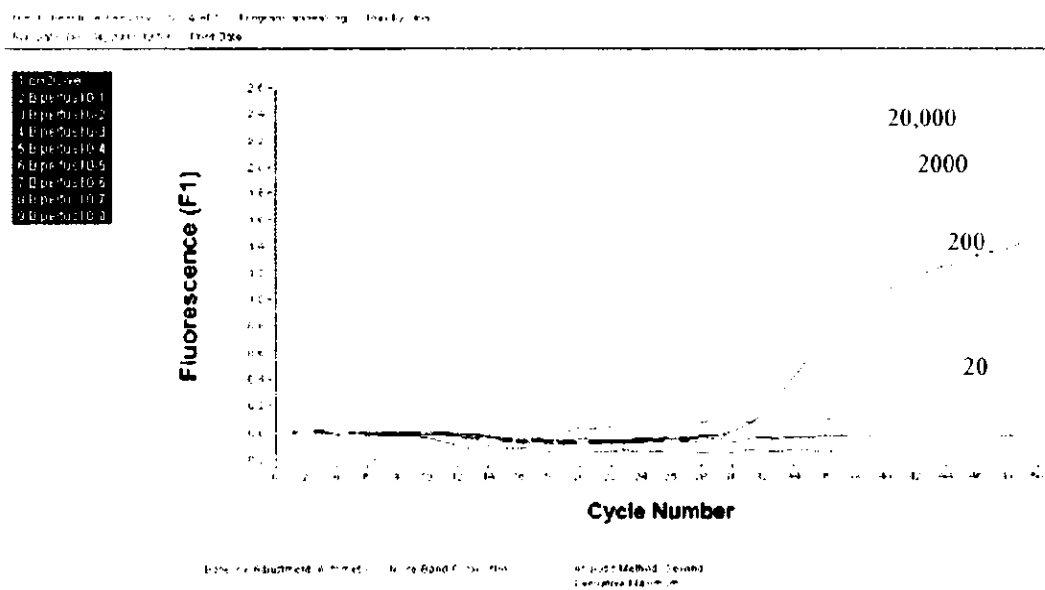


Figure 5:

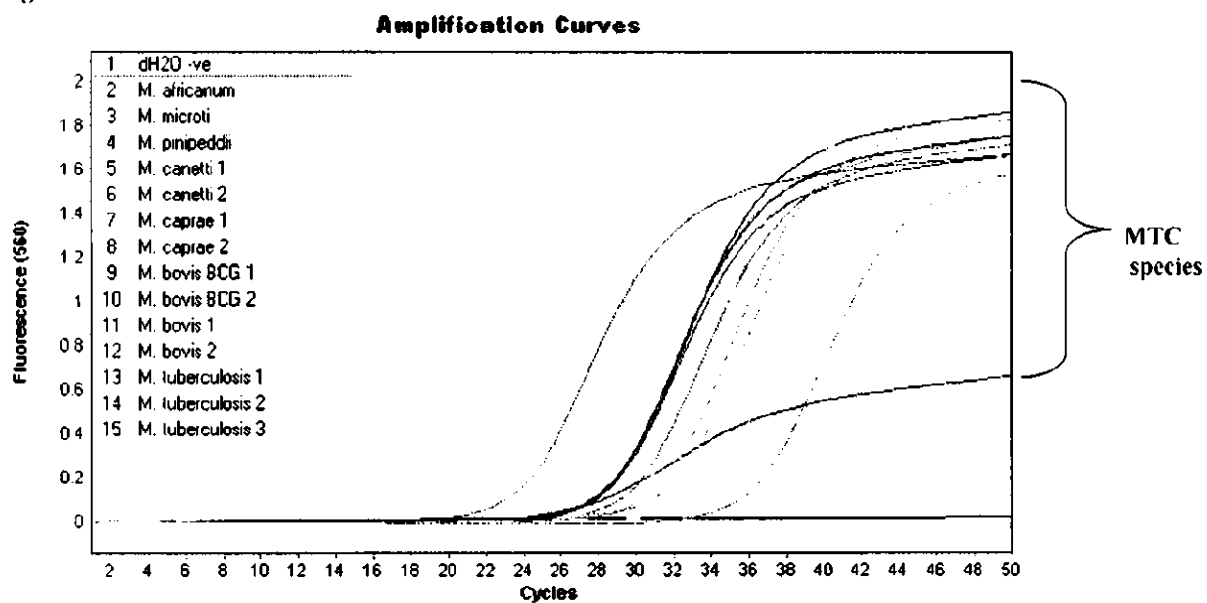
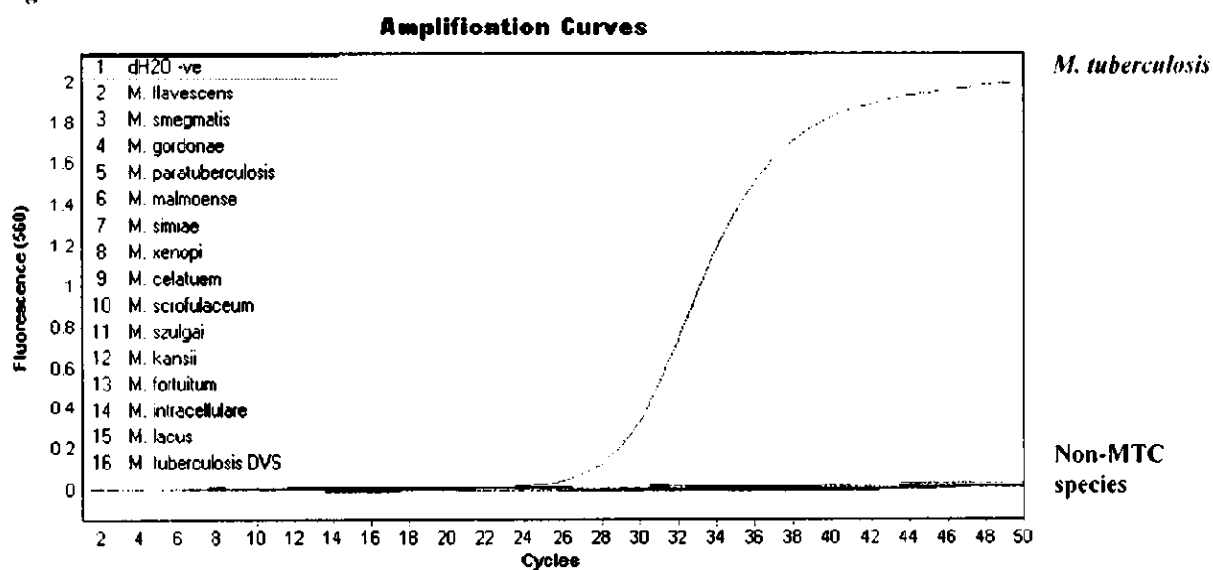
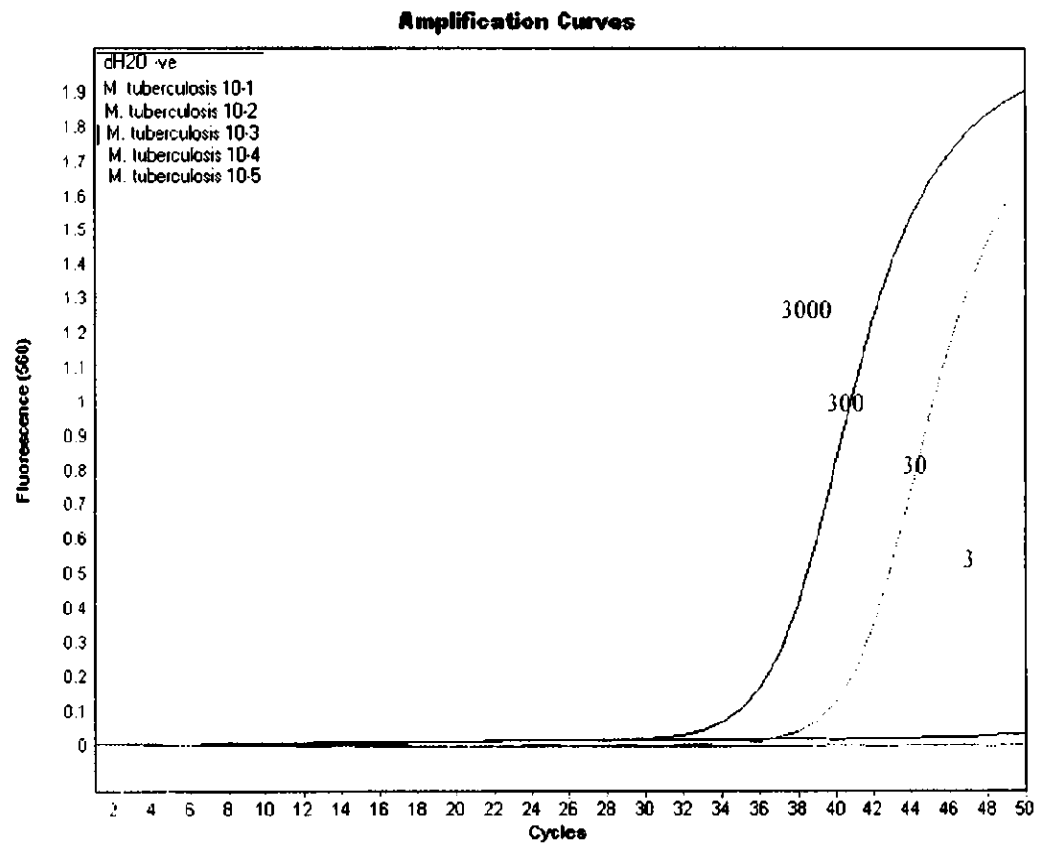


Figure 6:



5 **Figure 7**spec2678