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(54) Titre : DETECTION, IDENTIFICATION ET DIFFERENTIATION SIMULTANEEES DE TAXA D'EUBACTERIALES A
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(54) Title: SIMULTANEOUS DETECTION, IDENTIFICATION AND DIFFERENTIATION OF EUBACTERIAL TAXA USING
A HYBRIDIZATION ASSAY

(57) **Abrégé/Abstract:**

The present invention relates to a method for detection and identification of at least one microorganism, or for the simultaneous detection of several microorganisms in a sample, comprising the steps of: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, with at least one suitable primer pair, (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least one and preferably more than one of the spacer probes as mentioned in table 1a or equivalents of thereof, under the appropriate hybridization and wash conditions, and/or with a taxon-specific probe derived from any of the spacer sequences as represented in figs. 1-103 under the same hybridization and wash conditions; (iv) detecting the hybrids formed in step (iii) with each of the probes used under appropriate hybridization and wash conditions; (v) identification of the microorganism(s) present in the sample from the differential hybridization signals obtained in step (iv).





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(54) Title: SIMULTANEOUS DETECTION, IDENTIFICATION AND DIFFERENTIATION OF EUBACTERIAL TAXA USING A HYBRIDIZATION ASSAY

(57) Abstract

The present invention relates to a method for detection and identification of at least one microorganism, or for the simultaneous detection of several microorganisms in a sample, comprising the steps of: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, with at least one suitable primer pair; (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least one and preferably more than one of the spacer probes as mentioned in table 1a or equivalents of thereof, under the appropriate hybridization and wash conditions, and/or with a taxon-specific probe derived from any of the spacer sequences as represented in figs. 1-103 under the same hybridization and wash conditions; (iv) detecting the hybrids formed in step (iii) with each of the probes used under appropriate hybridization and wash conditions; (v) identification of the microorganism(s) present in the sample from the differential hybridization signals obtained in step (iv).

SIMULTANEOUS DETECTION, IDENTIFICATION AND DIFFERENTIATION OF EUBACTERIAL TAXA USING A HYBRIDIZATION ASSAY

The present invention relates to nucleic acid probes derived from the spacer region between the 16S and 23S ribosomal ribonucleic acid (rRNA) genes, to be used for the specific detection of eubacterial organisms in a biological sample by a hybridization procedure, as well as to nucleic acid primers to be used for the amplification of said spacer region of eubacterial organisms in a biological sample. The present invention also relates to new spacer region sequences from which said probes or primers may be derived.

Since the advent of the polymerase chain reaction and some other nucleic acid amplification techniques the impact of DNA-probe technology in the diagnosis of micro-organisms in biological samples of all sorts is increasing. Being often more specific and potentially more sensitive - if an adequate amplification and/or detection system is used - the DNA probe approach may eventually replace the conventional identification techniques.

The reliability of nucleic acid based tests essentially depends on the sensitivity and specificity of the probes and/or primers used. Thus the corner stone of this type of assay is the identification of nucleic acid sequences which are unique to the group of organisms of interest.

Most of the nucleic acid based tests either described in literature and/or commercially available aim at the detection of just one particular organism in a biological sample. Since most biological samples usually may contain a great variety of clinically relevant micro-organisms, a multitude of separate assays have to be performed to detect all relevant organisms possibly present. This approach would be very expensive, laborious and time-consuming. Consequently, the number of tests actually performed in most routine diagnostic labs on a particular sample is restricted to the detection of just a few of the most relevant organisms. Therefore it would be extremely convenient to have access to a system which enables the fast, easy and simultaneous detection of a multitude of different organisms. The more organisms that can be screened for in the same assay, the more cost-effective the procedure would be.

As put forward in earlier published documents, the spacer region situated between the 16S rRNA and the 23S rRNA gene, also referred to as the internal transcribed spacer (ITS), is an advantageous target region for probe development for detection of pathogens of

bacterial origin (International application WO 91/16454; Rossau et al., 1992; EP-A-0 395 292).

One of its most appreciated advantages is that sequences unique to a great variety of bacterial taxa can be found in a very limited area of the bacterial genome. This characteristic allows for an advantageous design of "probe-panels" enabling the simultaneous detection of a set of organisms possibly present in a particular type of a biological sample. Moreover, being flanked by quasi-universally conserved nucleotide sequences - more particularly located in the 3'-part of the 16S rRNA gene and the 5'-part of the 23S rRNA gene respectively - almost all spacers can be simultaneously amplified with a limited set of amplification primers. Alternatively, specific primer sets can be derived from the spacer sequences themselves, thereby allowing species- or group-specific amplifications.

The 16S-23S rRNA spacer region is a relatively short (about 200 to 1000 base pairs) stretch of DNA present in one or multiple copies in the genome of almost all eubacterial organisms. If multiple copies are present in the genome of one bacterium these copies can either be identical (as is most probably the case in some Neisseria species) or may differ from each other (as is the case for E. coli). This difference can be limited to a few nucleotides but also deletions and insertions of considerable length may be present.

Uptil now, spacer probes are only described and made publicly available for a limited number of organisms many of which were disclosed in international application WO 91/16454. As described above, it would be very advantageous to be able to detect simultaneously a panel of pathogens: e.g. a panel of pathogens possibly present in the same type of biological sample, or a panel of pathogens possibly causing the same type of disease symptoms, which are difficult to differentiate clinically and/or biochemically, or a panel of organisms belonging to the same taxon. In order to make the different panels as complete as possible, additional probes or sets of probes located in the spacer region and enabling the identification of at least the following bacterial groups or species are required :

- Mycobacterium species
- Listeria species
- Chlamydia species
- Acinetobacter species
- Mycoplasma species
- Streptococcus species

- Staphylococcus species
- Salmonella species
- Brucella species
- Yersinia species
- 5 - Pseudomonas species

These additional spacer probes need to be meticulously designed such that they can be used simultaneously with at least one other probe, under the same hybridization and wash conditions, allowing the detection of a particular panel of organisms.

10 It is thus the aim of the present invention to select probes or sets of probes, which have as target the 16S-23S rRNA spacer region, and which allow the detection and identification of at least one, and preferably more than one, of the above mentioned micro-organisms. The probes or probe sets are selected in such a way that they can be used in combination with at least one other probe, preferably also originating from the 16S-23S rRNA spacer region, under the same hybridisation and wash conditions, to allow possibly
15 the simultaneous detection of several micro-organisms in a sample.

It is also an aim of the present invention to provide for a selection method for use in the selection of said spacer probes or probe sets.

It is also an aim of the present invention to provide a rapid and reliable hybridization method for detection and identification of at least one micro-organism in a sample, or for the
20 simultaneous detection and identification of several micro-organisms in a sample.

It is more particularly an aim of the present invention to provide a hybridization method allowing simultaneous detection and identification of a set of micro-organisms, liable to be present in a particular type of sample.

It is more particularly an aim of the present invention to provide probes or sets of
25 probes for the possible simultaneous detection of micro-organisms in a sample originating from respiratory tract.

It is another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample originating from cerebrospinal fluid.

30 It is still another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample originating from urogenital tract.

2193101

4

It is still another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample taken from the gastro-intestinal tract of a patient.

5 It is still another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample originating from food or environmental samples.

10 It is moreover an aim of the present invention to provide a method for detection and identification of a particular taxon in a sample, or a set of particular taxa, said taxon being either a complete genus, or a subgroup within a genus, a species or even subtypes within a species (subspecies, serovars, sequevars, biovars...).

15 It is more particularly an aim of the present invention to provide probes or sets of probes for the detection of Mycobacterium species and subspecies, more particularly for the detection of M. tuberculosis complex strains, Mycobacterium strains from the MAIS-complex, M. avium and M. paratuberculosis, M. intracellulare and M. intracellulare-like strains, M. scrofulaceum, M. kansasii, M. chelonae, M. gordonae, M. ulcerans, M. genavense, M. xenopi, M. simiae, M. fortuitum, M. malmoeense, M. celatum and M. haemophilum.

It is also an aim of the present invention to provide probes or sets of probes for the detection of Mycoplasma strains, more particularly of M. pneumoniae and M. genitalium.

20 It is also an aim of the present invention to provide probes or sets of probes for the detection of Pseudomonas strains, more particularly P. aeruginosa.

It is also an aim of the present invention to provide probes or sets of probes for detection of Staphylococcus species, more particularly S. aureus and S. epidermidis.

25 It is also an aim of the present invention to provide probes or sets of probes for the detection of Acinetobacter strains, more particularly A. baumannii.

It is also an aim of the present invention to provide probes or sets of probes for the detection of Listeria strains, more particularly Listeria monocytogenes.

It is also an aim of the present invention to provide probes or sets of probes for the detection of Brucella strains.

30 It is also an aim of the present invention to provide probes or sets of probes for the detection of Salmonella strains.

It is also an aim of the present invention to provide probes or sets of probes for the

detection of Chlamydia strains, more particularly C. trachomatis and C. psittaci.

It is also an aim of the present invention to provide probes or sets of probes for the detection of Streptococcus strains.

5 It is also an aim of the present invention to provide probes or sets of probes for the detection of Yersinia enterocolitica strains.

10 It is also an aim of the present invention to provide primers allowing specific amplification of the 16S-23S rRNA spacer region for certain organisms. More particularly, it is an aim of the present invention to provide primers for the specific amplification of the spacer region of Mycobacterium, Chlamydia, Listeria, Brucella and Yersinia enterocolitica strains.

It is also an aim of the present invention to provide new sequences of 16S-23S rRNA spacer regions from which useful spacer probes or primers can be derived.

It is also an aim of the present invention to provide for kits for detection of at least one organism in a sample in which said probes and/or primers are used.

15 It is noted that for a few of the above-mentioned organisms spacer sequences have already been published in literature or in publicly accessible data-banks.

20 However, it should be made clear that the spacer region sequences disclosed in the current invention (figs. 1-103) are new and, in case they originate from the same species as those of which a spacer sequence was already described in the prior art, they differ to some extent from the already described sequences.

Moreover, it is the principal aim of the present invention to select, from the compilation of sequence data on spacer regions, specific probes and sets of probes enabling the detection and identification of a particular panel of organisms, be it the organisms belonging to a common taxon, or the organisms possibly present in the same type of sample.

25 The selection procedure usually consists of a theoretical and an experimental part. First of all, the different spacer sequences need to be aligned to those of the 'closest neighbours' or to the spacer sequences of other micro-organisms liable to be present in the same sample. This requires of course the sequence determination of the spacer region, as described in the examples. From the alignment, regions of divergence can be defined, from
30 which probes with desired hybridization characteristics are designed, according to guidelines known to the man skilled in the art and specified in more detail below.

Secondly, the designed probes need to be tested experimentally and evaluated for their

2193101

6

usefulness under specific hybridization conditions and/or in combination with other probes. Experimental testing can be done according to any hybridization method known in the art, but a preferred assay for the simultaneous testing of different probes under the same conditions is the reverse hybridization assay. A specific format for reverse hybridization of
5 different probes simultaneously used in the current invention is the LiPA (Line Probe Assay) as described below.

Upon experimental testing unexpected hybridization behaviour may show up when the probes are hybridized to the target nucleic acid, and specific probe adaptations may be required.

10 Moreover, specificity and sensitivity of the probes need to be tested with a large collection of strains, both belonging to the taxon to be detected and belonging to other taxa. Due to genome heterogeneity in the spacer region, or the existence of multiple spacer regions with different sequences in the same organism, it is quite often necessary to sequence spacer regions of additional strains, or to sequence additional spacer regions in the same strain, and
15 redesign the probes according to the new sequence data in order to obtain a better sensitivity and/or specificity (see e.g. example 3). In some cases it may be necessary or preferable to use several probes for the same organism (see e.g. example 2 and 7). Also, upon sequencing the spacer region, some organisms may show unexpected (un)relatedness, which may lead to a revision of strain classification contrary to classical taxonomic criteria (see e.g. examples
20 2 and 7).

In conclusion, the experimental part of the probe selection procedure is indispensable and complementary to the theoretical part. Probe design, especially under the fixed conditions of reverse hybridization (the same conditions for each probe) is not straightforward and probes have to be evaluated meticulously before they can be used in a reverse hybridization
25 format. Therefore, probes cannot always be simply derived on a theoretical basis from a known gene sequence.

For designing probes with desired characteristics the following useful guidelines may be followed.

30 Because the extent and specificity of hybridization reactions such as those described herein are affected by a number of factors, manipulation of one or more of those factors will determine the exact sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The importance and effect of various assay conditions,

explained further herein, are known to those skilled in the art.

First, the stability of the [probe : target] nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs, and by designing the probe with an appropriate T_m . The beginning and end points of the probe should be chosen so that the length and %GC result in a T_m about 2-10°C higher than the temperature at which the final assay will be performed. The base composition of the probe is significant because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs due to additional hydrogen bonding. Thus, hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures.

Conditions such as ionic strength and incubation temperature under which a probe will be used should also be taken into account in constructing a probe. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of the hybrids will increase with increasing ionic strength. On the other hand, chemical reagents, such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, will increase the stringency of hybridization. Destabilization of the hydrogen bonds by such reagents can greatly reduce the T_m . In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

It is desirable to have probes which hybridize only under conditions of high stringency. 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 is chosen to maximize the difference in stability between the hybrid formed with the target and the nontarget nucleic acid. In some examples of the current invention, e.g. when highly related organisms need to be differentiated, it may be necessary to detect single base pair changes. In those cases, conditions of very high stringency are needed.

Second, probes should be positioned so as to minimize the stability of the [probe : nontarget] nucleic acid hybrid. This may be accomplished by minimizing the length of perfect

2193101

8

complementarity to non-target organisms, avoiding GC rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible. Whether a probe sequence is useful to detect only a specific type of organism depends largely on the thermal stability difference between [probe:target] hybrids and
5 [probe:nontarget] hybrids. In designing probes, the differences in these T_m values should be as large as possible (e.g. at least 2°C and preferably 5°C).

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired
10 hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly complementary base sequence will normally primarily determine hybrid stability. While oligonucleotide probes of different lengths and base composition may be used, oligonucleotide probes
15 preferred in this invention are between about 10 to 50 bases in length and are sufficiently homologous to the target nucleic acid.

Third, regions in the target DNA or RNA which are known to form strong internal structures inhibitory to hybridization are less preferred. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of
20 two single strands of complementary nucleic acids to form a hydrogen bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in a hybrid that it will be less able to participate in formation of a new hybrid. There can be intramolecular and intermolecular hybrids formed within the molecules of one type of probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. By
25 designing a probe so that a substantial portion of the sequence of interest is single stranded, the rate and extent of hybridization may be greatly increased. Computer programs are available to search for this type of interaction. However, in certain instances, it may not be possible to avoid this type of interaction.

The probes of the present invention are designed for attaining optimal performance
30 under the same hybridization conditions so that they can be used in sets for simultaneous hybridization; this highly increases the usability of these probes and results in a significant gain in time and labour. Evidently, when other hybridization conditions should be preferred,

all probes should be adapted accordingly by adding or deleting a number of nucleotides at their extremities. It should be understood that these concomitant adaptations should give rise to essentially the same result, namely that the respective probes still hybridize specifically with the defined target. Such adaptations might also be necessary if the amplified material should be RNA in nature and not DNA as in the case for the NASBA system.

The hybridization conditions can be monitored relying upon several parameters, such as the nature and concentration of the components of the media, and the temperatures under which the hybrids are formed and washed.

The hybridization and wash temperature is limited in upper value depending on the sequence of the probe (its nucleic acid composition, kind and length). The maximum hybridization or wash temperature of the probes described in the present invention ranges from 40°C to 60°C, more preferably from 45°C to 55°C, in the specific hybridization and wash media as described in the Examples section. At higher temperatures duplexing (= formation of the hybrids) competes with the dissociation (or denaturation) of the hybrid formed between the probe and the target.

In a preferred hybridization medium of the invention, containing 3 x SSC and 20% formamide, hybridization temperatures can range from 45°C to 55°C, with a preferred hybridization temperature of 50°C. A preferred wash medium contains 3 x SSC and 20% formamide, and preferred wash temperatures are the same as the preferred hybridization temperatures, i.e. preferably between 45°C and 55°C, and most preferably 50°C.

However, when modifications are introduced, be it either in the probes or in the media, the temperatures at which the probes can be used to obtain the required specificity should be changed according to known relationships, such as those described in the following reference: Hames B and Higgins S (eds.). Nucleic acid hybridization. A practical approach, IRL Press, Oxford, U.K., 1985.

The selected nucleic acid probes derived from the 16S-23S rRNA spacer region and described by the present invention are listed in Table 1a (SEQ ID NO 1 to 64, 175 to 191, 193 to 201, and 210 to 212). As described in the examples section, some of these probes show a better sensitivity and/or specificity than others, and the better probes are therefore preferentially used in methods to detect the organism of interest in a biological sample. However, it is possible that for certain applications (e.g. epidemiology, substrain typing, ...) a set of probes including the less specific and/or less sensitive probes may be very

informative (see e.g. example 7).

The following definitions serve to illustrate the terms and expressions used in the different embodiments of the present invention as set out below.

The term "spacer" is an abbreviated term referring to the 16S-23S rRNA internal transcribed spacer region.

The term "probe" refers to single stranded sequence-specific oligonucleotides which have a sequence which is sufficiently complementary to hybridize to the target sequence to be detected.

The more specific term "spacer probe" refers to a probe as defined above having a sequence which is sufficiently complementary to hybridize to a target sequence which is located in the spacer region(s) of the organism (or group of organisms) to be detected.

Preferably said probes are 70%, 80%, 90%, or more than 95% homologous to the exact complement of the target sequence to be detected. These target sequences are either genomic DNA or precursor RNA, or amplified versions thereof.

Preferably, these probes are about 5 to 50 nucleotides long, more preferably from about 10 to 25 nucleotides. The nucleotides as used in the present invention may be ribonucleotides, deoxyribonucleotides and modified nucleotides such as inosine or nucleotides containing modified groups which do not essentially alter their hybridization characteristics. Moreover, it is obvious to the man skilled in the art that any of the below-specified probes can be used as such, or in their complementary form, or in their RNA form (wherein T is replaced by U).

The probes according to the invention can be formed by cloning of recombinant plasmids containing inserts including the corresponding nucleotide sequences, if need be by cleaving the latter out from the cloned plasmids upon using the adequate nucleases and recovering them, e.g. by fractionation according to molecular weight. The probes according to the present invention can also be synthesized chemically, for instance by the conventional phospho-triester method.

The term "complementary" nucleic acids as used herein means that the nucleic acid sequences can form a perfect base-paired double helix with each other.

The term "homologous" as used in the current application is synonymous for identical: this means that polynucleic acids which are said to be e.g. 80% homologous show 80% identical base pairs in the same position upon alignment of the sequences.

The term "polynucleic acid" corresponds to either double-stranded or single-stranded cDNA or genomic DNA or RNA, containing at least 10, 20, 30, 40 or 50 contiguous nucleotides. A polynucleic acid which is smaller than 100 nucleotides in length is often also referred to as an oligonucleotide. Single stranded polynucleic acid sequences are always represented in the current invention from the 5' end to the 3' end.

The term 'closest neighbour' means the taxon which is known or expected to be most closely related in terms of DNA homology and which has to be differentiated from the organism of interest.

The expression 'desired hybridization characteristics' means that the probe only hybridizes to the DNA or RNA from organisms for which it was designed, and not to DNA or RNA from other organisms (closest neighbours or organisms liable to be present in the same sample). In practice, this means that the intensity of the hybridization signal is at least two, three, four, five, ten or more times stronger with the target DNA or RNA from the organisms for which the probes were designed, as compared to non-target sequences.

These desired hybridization characteristics correspond to what is called later in the text "specific hybridization".

The expression "taxon-specific hybridization" or "taxon-specific probe" means that the probe only hybridizes to the DNA or RNA from the taxon for which it was designed and not to DNA or RNA from other taxa.

The term taxon can refer to a complete genus or a sub-group within a genus, a species or even subtype within a species (subspecies, serovars, sequevars, biovars...).

The term "specific amplification" or "specific primers" refers to the fact that said primers only amplify the spacer region from these organisms for which they were designed, and not from other organisms.

The term "sensitivity" refers to the number of false negatives: i.e. if 1 of the 100 strains to be detected is missed out, the test shows a sensitivity of $(100-1/100)\% = 99\%$.

The term "specificity" refers to the number of false positives: i.e. if on 100 strains detected, 2 seem to belong to organisms for which the test is not designed, the specificity of the test is $(100-2/100)\% = 98\%$.

The probes selected as being "preferential" show a sensitivity and specificity of more than 80%, preferably more than 90% and most preferably more than 95%.

The term "primer" refers to a single stranded DNA oligonucleotide sequence capable

2193101

12

of acting as a point of initiation for synthesis of a primer extension product which is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably the primer is about 5-50 nucleotides long. Specific length and sequence will depend on the complexity of the required DNA or RNA targets, as well as on the conditions of primer use such as temperature and ionic strength. The fact that amplification primers do not have to match exactly with the corresponding template sequence to warrant proper amplification is amply documented in the literature (Kwok et al., 1990).

The amplification method used can be either polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwok et al., 1989), strand displacement amplification (SDA; Duck, 1990; Walker et al., 1992) or amplification by means of Q β replicase (Lizardi et al., 1988; Lomeli et al., 1989) or any other suitable method to amplify nucleic acid molecules known in the art.

The oligonucleotides used as primers or probes may also comprise nucleotide analogues such as phosphorothioates (Matsukura et al., 1987), alkylphosphorothioates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984).

As most other variations or modifications introduced into the original DNA sequences of the invention these variations will necessitate adaptations with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. However the eventual results of hybridisation will be essentially the same as those obtained with the unmodified oligonucleotides.

The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate, a membrane (e.g. nylon or nitrocellulose) or a microsphere (bead). Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid

probe in order to facilitate fixation or improve the hybridization efficiency. Such modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH_2 groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

The term "labelled" refers to the use of labelled nucleic acids. Labelling may be carried out by the use of labelled nucleotides incorporated during the polymerase step of the amplification such as illustrated by Saiki et al. (1988) or Bej et al. (1990) or by the use of labelled primers, or by any other method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P , ^{35}S , etc.) or non-isotopic (biotin, digoxigenin, etc.).

The "sample" may be any biological material taken either directly from the infected human being (or animal), or after culturing (enrichment), or a sample taken from food or feed. Biological material may be e.g. expectorations of any kind, broncheolavages, blood, skin tissue, biopsies, lymphocyte blood culture material, colonies, etc. Said samples may be prepared or extracted according to any of the techniques known in the art.

The "target" material in these samples may be either genomic DNA or precursor RNA of the organism to be detected (= target organism), or amplified versions thereof as set out above. More specifically, the nucleic acid sequence of the target material is localized in the spacer region of the target organism(s).

Detection and identification of the target material can be performed by using one of the many electrophoresis methods, hybridization methods or sequencing methods described in literature and currently known by men skilled in the art. However, a very convenient and advantageous technique for the simultaneous detection of nucleic acids possibly present in biological samples is the Line Probe Assay technique. The Line Probe Assay (LiPA) is a reverse hybridization format (Saiki et al., 1989) using membrane strips onto which several oligonucleotide probes (including negative or positive control oligonucleotides) can be conveniently applied as parallel lines.

The LiPA technique, as described by Stuyver et al. (1993) and in international application WO 94/12670, provides a very rapid and user-friendly hybridization test. Results can be read within 4 h. after the start of the amplification. After amplification during which usually a non-isotopic label is incorporated in the amplified product, and alkaline denaturation, the amplified product is contacted with the probes on the membrane and the hybridization is carried out for about 1 to 1,5 h. Consequently, the hybrids formed are

detected by an enzymatic procedure resulting in a visual purple-brown precipitate. The LiPA format is completely compatible with commercially available scanning devices, thus rendering automatic interpretation of the results possible. All those advantages make the LiPA format liable for use in a routine setting.

The LiPA format is not only an advantageous tool for identification and detection of pathogens at the species level but also at higher or lower taxonomical levels. For instance, probe-configurations on LiPA strips can be selected in such a manner that they can detect a complete genus (e.g. Neisseria, Listeria, etc.) or can identify subgroups within a genus (e.g. subgroups in the Mycobacterium avium-intracellulare-scrofulaceum complex) or can in some cases even detect subtypes (subspecies, serovars, sequevars, biovars, etc. whatever is clinically relevant) within a species.

It should be stressed that the ability to simultaneously generate hybridization results with a number of probes is an outstanding benefit of the LiPA technology. In many cases the amount of information which can be obtained by a particular combination of probes greatly outnumbers the data obtained by using single probe assays. Therefore the selection of probes on the membrane strip is of utmost importance since an optimized set of probes will generate the maximum of information possible. This is more particularly exemplified further herein.

The fact that different probes can be combined on one strip also offers the possibility to conveniently cope with a lack of sensitivity due to sequence heterogeneity in the target region of the group of organisms to be detected. Due to this heterogeneity, two or more probes may be required to positively identify all organisms of the particular group. These probes can be applied to membrane strips at different locations and the result is interpreted as positive if at least one of these probes is positive. Alternatively these probes can be applied as a mixture at the same location, hereby reducing the number of lines on a strip. This reduction may be convenient in order to make the strip more concise or to be able to extend the total number of probes on one strip. Another alternative approach, in view of its practical benefits, is the synthesis of oligonucleotides harbouring the sequences of two (or more) different probes (=degenerate probes) which then can be further processed and applied to the strip as one oligonucleotide molecule. This approach would considerably simplify the manufacturing procedures of the LiPA-strips. For example, probes with nucleotide sequences A and B are both required to detect all strains of taxon X. In the latter alternative a probe can be synthesized having the nucleotide sequence AB. This probe will have the combined

characteristics of probes A and B.

By virtue of the above-mentioned properties the LiPA system can be considered as a preferential format for a hybridization method wherein several organisms need to be detected simultaneously in a sample. Moreover, as described in the examples section, the LiPA system is a preferred format for a selection method for the experimental evaluation and selection of theoretically designed probes.

However, it should be clear that any other hybridization assay, whereby different probes are used under the same hybridization and wash conditions can be used for the above-mentioned detection and/or selection methods. For example, it may be possible to immobilize the target nucleic acid to a solid support, and use mixtures of different probes, all differently labeled, resulting in a different detection signal for each of the probes hybridized to the target.

As an example, the procedure to be followed for the detection of one or more organisms in a sample using the LiPA format is outlined below :

- First, the nucleic acids of the organism(s) to be detected in the sample, is made available for amplification and/or hybridization.
- Secondly, the nucleic acids, if present, are amplified with one or another target amplification system, as specified below. Usually, amplification is needed to enhance the subsequent hybridization signal. However for some samples or some organisms amplification might not be necessary. This might also be the case if, for the detection of the hybrids formed, highly sensitive signal-amplification systems are used.
- Thirdly, eventually after a denaturation step, the nucleic acids present in the sample or the resulting amplified product are contacted with LiPA strips onto which one or more DNA-probes, allowing the detection of the organisms of interest, are immobilized, and hybridization is allowed to proceed.
- Finally, eventually after having performed a wash step, the hybrids are detected using a convenient and compatible detection system. From the hybridization signals or patterns observed the presence or absence of one or several organisms screened for in that particular biological sample can be deduced.

The amplification system used may be more or less universal, depending on the specific application needed.

By using universal primers located in the conserved flanking regions (16S and 23S

gene) of the rRNA spacer, the spacer region of most if not all organisms of eubacterial origin will be amplified. The same result may be obtained by using a combination of different sets of primers with reduced universality (multiplex amplification, i.e. an amplification procedure in which two or more primer sets are used simultaneously in one and the same reaction mixture).

For some applications it may be appropriate to amplify not all organisms present in the sample but more specifically, beforehand defined taxa. This may be achieved using specific primers located either in less conserved parts of the flanking genes of the spacers (e.g. MYCP1-5 for amplification of the spacer region of mycobacteria) or located in the spacers themselves (e.g. LIS-P1-P7, BRU-P1-4, CHTR-P1-2 and YEC-P1-2 for specific amplification of the spacer region(s) of Listeria species, Brucella species, Chlamydia trachomatis, and Yersinia enterocolitica respectively).

The present invention thus provides a method for detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms in a sample, comprising the steps of:

- (i) if need be releasing, isolating and/or concentrating the polynucleic acids from the micro-organism(s) to be detected in the sample;
- (ii) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, from the micro-organism(s) to be detected, with at least one suitable primer pair;
- (iii) hybridizing the polynucleic acids of step (i) or (ii) with a set of probes comprising at least two probes, under the same hybridization and wash conditions, with said probes being selected from the sequences of table 1a or equivalents thereof and/or from taxon-specific probes derived from any of the spacer sequences represented in figs. 1-103, with said taxon-specific probe being selected such that it is capable of hybridizing under the same hybridization and wash conditions as at least one of the probes of table 1a;
- (iv) detecting the hybrids formed in step (iii);
- (v) identification of the micro-organism(s) present in the sample from the differential hybridization signals obtained in step (iv).

The probes as mentioned in table 1a are all selected in such a way that they show the desired hybridization characteristics at a hybridization and wash temperature of 50°C in a preferred hybridization and wash medium of 3X SSC and 20% formamide.

The term "equivalents" of a probe, also called "variants" or "homologues" or "obvious derivatives", refers to probes differing in sequence from any of the probes specified in table 1 either by addition to or removal from any of their respective extremities of one or several nucleotides, or by changing one or more nucleotides within said sequences, or a combination of both, provided that said equivalents still hybridize with the same RNA or DNA target as the corresponding unmodified probe sequence. Said equivalents share at least 75 % homology, preferably more than 80 %, most preferably more than 85 % homology with the corresponding unmodified probe sequence. It should be noted that, when using an equivalent of a probe, it may be necessary to modify the hybridization conditions to obtain the same specificity as the corresponding unmodified probe. As a consequence, since it is the aim of this invention to use a set of probes which work under the same hybridization and wash conditions, it will also be necessary to modify accordingly the sequence of the other probes, belonging to the same set as the original unmodified probe. These modifications can be done according to principles known in the art, e.g. such as those described in Hames B and Higgins S (Eds): Nucleic acid hybridization. Practical approach. IRL Press, Oxford, UK, 1985.

The invention also provides for a method to select taxon-specific probes from the spacer region sequence(s) of said taxon, said probes being selected such that they show their desired hybridization characteristics under unified hybridization and wash conditions.

The term "unified" conditions means that these conditions are the same for the different probes enabling the detection of different taxa.

Preferentially, the present invention provides for a method as described above wherein at least 2 micro-organisms are detected simultaneously.

In a preferred embodiment, the set of probes as described in step (iii) is comprising at least two probes being selected from the sequences of table 1a, or equivalents thereof.

In another embodiment, the set of probes as described in step (iii) is comprising at least one probe being selected from the sequences of table 1a, or equivalents thereof, and at least one taxon-specific probe derived from any of the spacer sequences as represented in figs. 1-103.

In still another embodiment, the set of probes as described in step (iii) is comprising at least two taxon-specific probes derived from any of the spacer sequences as represented in figs. 1-103.

The present invention also provides for a method as described above, wherein the probes as specified in step (iii) are combined with at least one other probe, preferentially also from the 16S-23S rRNA spacer region, enabling the simultaneous detection of different pathogenic bacteria liable to be present in the same sample.

The organisms of clinical relevance present in biological samples may vary considerably depending on the origin of the sample. The most common pathogenic bacteria which may be found in sputum samples, or in samples originating from the respiratory tract, are :

- Moraxella catarrhalis
- Streptococcus pneumoniae
- Haemophilus influenzae
- Pseudomonas aeruginosa
- Mycoplasma pneumoniae
- Acinetobacter species
- Mycobacterium species
- Staphylococcus aureus
- Legionella pneumophila

A LiPA-strip harbouring spacer-probes enabling the detection of most if not all of these organisms would be extremely beneficial for reasons explained above.

Evidently, this also applies for other biological samples, as there are : cerebrospinal fluid, urogenital samples, gastrointestinal samples, blood, urine, food products, soil, etc. For example, a preferred panel for cerebrospinal fluid would contain probe combinations enabling the detection and differentiation of the following organisms :

- Neisseria meningitidis
- Streptococcus pneumoniae
- Streptococcus agalactiae
- Listeria monocytogenes
- Mycobacterium tuberculosis

For some of the above mentioned organisms, spacer probes were already designed in a previous patent application (WO 91/16454). In order to be able to detect most pathogens possibly present in a sample in a single test, the probes of the present invention may be combined with at least one of the probes of WO 91/16454, or their obvious derivatives as

specified in WO 91/16454. For clarity, these probes are listed hereafter:

Neisseria gonorrhoeae: NGI1: CGATGCGTCGTTATTCTACTTCGC
 NGI2: TTCGTTTACCTACCCGTTGACTAAGTAAGCAAAC

Neisseria meningitidis: NMI1: GGTCAAGTGTGACGTCGCCCTG
 NMI2: GTTCTTGGTCAAGTGTGACGTC
 NMI3: GCGTTCGTTATAGCTATCTACTGTGC
 NMI4: TGC GTTCGATATTGCTATCTACTGTGCA
 NMI5: TTTTGTTCTTGGTCAAGTGTGACGTCGCCCTGAA
 TGGATTCTGTTCCATT
 NMI6: TTTGCCTAACATTCCGTTGACTAGAACATCAGAC

Haemophilus ducreyi: HDI1: TTATTATGCGCGAGGCATATTG

Branhamella catharralis: BCI1: TTAAACATCTTACCAAAG
 BCI2: TTGATGTTTAAACTTGCTTGGTGGA

Bordetella pertussis: BPI1: CCACACCCATCCTCTGGACAGGCTT

Haemophilus influenzae: HII1: ACGCATCAAATTGACCGCACTT
 HII2: ACTTTGAAGTGAAAACCTTAAAG

Streptococcus agalactiae: SAI1: AATCGAAAGGTTCAAATTGTT
 SAI2: GGAAACCTGCCATTTGCGTCTT
 SAI3: TCCACGATCTAGAAATAGATTGTAGAA
 SAI4: TCTAGTTTTTAAAGAACTAGGTT

Streptococcus pneumoniae: SPI1: GTGAGAGATCACCAAGTAATGCA
 SPI2: AGGAACTGCGCATTTGGTCTT
 SPI3: GAGTTTATGACTGAAAGGTCAGAA

The invention thus provides for a method as described above, wherein said sample is originating from the respiratory tract, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

MYC-ICG-1 : ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22 : CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)
 MTB-ICG-1 : GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2 : GACTTGTTCCAGGTGTTGTCCCAC (SEQ ID NO 4)
 MTB-ICG-3 : CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)
 MAI-ICG-1 : CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)

MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MIN-ICG-2 :	GCTGATGCGTTCGTCGAAATGTGTA	(SEQ ID NO 13)
MIN-ICG-22 :	CTGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 14)
MIN-ICG-222 :	TGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 15)
MIN-ICG-2222 :	GGCTGATGCGTTCGTCGAAATGTGTAA	(SEQ ID NO 16)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MHEF-ICG-1 :	TGGACGAAAACCGGGTGCACAA	(SEQ ID NO 18)
MAH-ICG-1 :	GTGTAATTTCTTTTTTA	(SEQ ID NO 19)
	ACTCTTGTGTGTAAGTAAGTG	
MCO-ICG-11 :	TGGCCGGCGTGTTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-1 :	GATGCGTTTGCTACGGGTAGCGT	(SEQ ID NO 25)
MKA-ICG-2 :	GATGCGTTGCCTACGGGTAGCGT	(SEQ ID NO 26)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)

MGO-ICG-1 :	AACACCCTCGGGTGCTGTCC	(SEQ ID NO 31)
MGO-ICG-2 :	GTATGCGTTGTCGTTTCGCGGC	(SEQ ID NO 32)
MGO-ICG-5 :	CGTGAGGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)
PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 2 :	TGAATGTTCGTGGATGAACATTGATT	(SEQ ID NO 35)
PA-ICG 3 :	CACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 36)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACCTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)
MPN-ICG 1 :	ATCGGTGGTAAATTAAACCCAAATCCCTGT	(SEQ ID NO 49)
MPN-ICG 2 :	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	(SEQ ID NO 50)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACCTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
ACI-ICG 1 :	GCTTAAGTGACAGTGCTCTAAACTGA	(SEQ ID NO 57)
ACI-ICG 2 :	CACGGTAATTAGTGTGATCTGACGAAG	(SEQ ID NO 58)

and more preferably from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MCO-ICG-11 :	TGGCCGGCGTGTTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTGCGACGTGGTGT	(SEQ ID NO 176)

2193101

23

MGV-ICG-2 :	GGTGTGTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)
PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 4 :	TGAATGTTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACCTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)
MPN-ICG 1 :	ATCGGTGGTAAATTAAACCCAAATCCCTGT	(SEQ ID NO 49)
MPN-ICG 2 :	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	(SEQ ID NO 50)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
ACI-ICG 1 :	GCTTAAGTGACAGTGCTCTAAACTGA	(SEQ ID NO 57)
ACI-ICG 2 :	CACGGTAATTAGTGTGATCTGACGAAG	(SEQ ID NO 58)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 76 to 106, 157 to 174, 124, 125, 111 to 115, 139 to 144, or 126 to 130, and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Haemophilus influenzae, Streptococcus

pneumoniae, Moraxella catarrhalis or Bordetella pertussis.

The above mentioned probes of the invention are designed for the detection of Mycobacterium species (SEQ ID NO 1 to 33 and 175 to 191), of Pseudomonas aeruginosa (SEQ ID NO 34 to 38), of Mycoplasma species (SEQ ID NO 49 to 52), of Staphylococcus aureus (SEQ ID NO 53 to 56) and of Acinetobacter baumannii (SEQ ID NO 57 and 58).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from the cerebrospinal fluid, and wherein the set of probes as described in step (iii) comprises at least one probe chosen from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTCT	(SEQ ID NO 5)
LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 1 :	AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG	(SEQ ID NO 40)
LMO-ICG 2 :	TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC	(SEQ ID NO 41)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)

and preferably from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTCT	(SEQ ID NO 5)
LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, or 213-215,

and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria meningitidis, Haemophilus influenzae or Streptococcus pneumoniae.

The above mentioned probes of the invention are designed for the detection of Mycobacterium species, and more particularly Mycobacterium tuberculosis (SEQ ID NO 1 to 5), and of Listeria species, more particularly Listeria monocytogenes (SEQ ID NO 39 to 42).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from the urogenital tract, and wherein the set of probes as described in step (iii) comprises at least one probe chosen from the following spacer probes:

CHTR-ICG 1 : GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)
 CHTR-ICG 2 : GCATTTATATGTAAGAGCAAGCATTCTATTTC (SEQ ID NO 46)
 CHTR-ICG 3 : GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)
 CHTR-ICG 4 : GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)
 CHPS-ICG 1 : GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)
 MGE-ICG 1 : CACCCATTAATTTTTTCGGTGTTAAACCC (SEQ ID NO 51)
 Mycoplasma-ICG : CAAAACCTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 122, 123, 197, 124 or 125,

with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria gonorrhoeae, Haemophilus ducreyi or Streptococcus agalactiae.

The above mentioned probes of the invention are designed for the detection of

2193101

26

Chlamydia species (SEQ ID NO 45 to 48 and 201) and of Mycoplasma species (SEQ ID NO 51 and 52).

Preferentially, at least two, three, four, five, six or seven of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from food, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

- LIS-ICG 1 : CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)
- LMO-ICG 1 : AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG (SEQ ID NO 40)
- LMO-ICG 2 : TGAGAGGTTAGTACTTCTCAGTATGTTTGTTT (SEQ ID NO 41)
- LMO-ICG 3 : AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)
- LIV-ICG 1 : GTTAGCATAAATAGGTAAGTATTTATGACACAAGTAAC (SEQ ID NO 43)
- LSE-ICG 1 : AGTTAGCATAAGTAGTGTAAGTATTTATGACACAAG (SEQ ID NO 44)
- LISP-ICG 1 : CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)
- STAU-ICG 1 : TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)
- STAU-ICG 2 : CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)
- STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)
- STAU-ICG 4 : GAACGTAAGTTCATGTAAACGTTTGACTTAT (SEQ ID NO 56)
- BRU-ICG 1 : CGTGCCGCCTTCGTTTCTCTTT (SEQ ID NO 59)
- BRU-ICG 2 : TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)
- BRU-ICG 3 : GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)
- BRU-ICG 4 : CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)
- SALM-ICG 1 : CAAAAGTACTTACGAGTCACGTTTGAG (SEQ ID NO 61)
- SALM-ICG 2 : GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)
- STY-ICG 1 : GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)
- SED-ICG 1 : GCGGTAATGTGTGAAAGCGTTGCC (SEQ ID NO 64)
- YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
- YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
- YEC-ICG 3 : GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

and preferably from the following spacer probes:

2193101

27

LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAAC TTCATGTAAACGTTTGACTTAT	(SEQ ID NO 56)
BRU-ICG 2 :	TTCGCTTCGGGGTGGATCTGTG	(SEQ ID NO 60)
BRU-ICG 3 :	GCGTAGTAGCGTTTGCGTCGG	(SEQ ID NO 193)
BRU-ICG 4 :	CGCAAGAAGCTTGCTCAAGCC	(SEQ ID NO 194)
SALM-ICG 1 :	CAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, 213-215, 139-144, 131, 132, 154, 133-138, 195 or 196, with said probes or equivalents being possibly used in combination with any probe detecting strains of Campylobacter species.

The above mentioned probes of the invention are designed for the detection of Listeria species (SEQ ID NO 39 to 44), of Staphylococcus species (SEQ ID NO 53 to 56), of Brucella species (SEQ ID NO 59, 60, 193 and 194), of Salmonella species (SEQ ID NO 61 to 64) and of Yersinia enterocolitica (SEQ ID NO 198 to 200).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from the gastrointestinal tract of a patient, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

SALM-ICG 1 :	CAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
SALM-ICG 2 :	GATGTATGCTTCGTTATTCCACGCC	(SEQ ID NO 62)

STY-ICG 1 : GGTC AACCTCCAGGGACGCC (SEQ ID NO 63)
 SED-ICG 1 : GCGGTAATGTGTGAAAGCGTTGCC (SEQ ID NO 64)
 YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3 : GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

and preferably from the following spacer probes:

SALM-ICG 1 : CAAA ACTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)
 YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3 : GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 133-138 or 195-196,

with said probes or equivalents being possibly used in combination with any probe detecting Campylobacter species.

The above mentioned probes of the invention are designed to detect Salmonella species (SEQ ID NO 61 to 64) and Yersinia enterocolitica (SEQ ID NO 198 to 200).

Preferentially, at least two, three, four, five, six or seven of said probes are used simultaneously.

The invention also relates to the use of the selected probes or their equivalents for the detection of specific bacterial taxa, said taxa being either a complete genus, or a subgroup within a genus, a species, or even a subtype within a species.

The invention thus provides for a method as described above to detect and identify one or more strains of Mycobacterium species and subspecies in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 : ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22 : CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)
 MTB-ICG-1 : GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2 : GACTTGTTCCAGGTGTTGTCCCAC (SEQ ID NO 4)
 MTB-ICG-3 : CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)

MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCTCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MIN-ICG-2 :	GCTGATGCGTTCGTCGAAATGTGTA	(SEQ ID NO 13)
MIN-ICG-22 :	CTGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 14)
MIN-ICG-222 :	TGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 15)
MIN-ICG-2222 :	GGCTGATGCGTTCGTCGAAATGTGTAA	(SEQ ID NO 16)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MHEF-ICG-1 :	TGGACGAAAACCGGGTGCACAA	(SEQ ID NO 18)
MAH-ICG-1 :	GTGTAATTTCTTTTTTAACCTCTTGTGTGTAAGTAAGTG	(SEQ ID NO 19)
MCO-ICG-11 :	TGGCCGGCGTGTTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-1 :	GATGCGTTTGCTACGGGTAGCGT	(SEQ ID NO 25)
MKA-ICG-2 :	GATGCGTTGCCTACGGGTAGCGT	(SEQ ID NO 26)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)

2193101

30

MGO-ICG-1 : AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)
 MGO-ICG-2 : GTATGCGTTGTCGTTGCGGGC (SEQ ID NO 32)
 MGO-ICG-5 : CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)
 MUL-ICG-1 : GGTTTCGGGATGTTGTCCCACC (SEQ ID NO 175)
 MG-ICG-1 : CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)
 MG-ICG-2 : GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)
 MXE-ICG-1 : GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)
 MSI-ICG-1 : CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)
 MFO-ICG-1 : TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)
 MFO-ICG-2 : ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)
 MML-ICG-1 : CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)
 MML-ICG-2 : TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)
 MCE-ICG-1 : TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)
 MHP-ICG-1 : CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

and more preferably to at least one probe of the following restricted group of spacer probes:

MYC-ICG-1 : ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22 : CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)
 MTB-ICG-1 : GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2 : GACTTGTTCCAGGTGTTGTCCCAC (SEQ ID NO 4)
 MTB-ICG-3 : CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)
 MAI-ICG-1 : CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
 MIL-ICG-11 : GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)
 MIL-ICG-22 : TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)
 MAC-ICG-1 : CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
 MAV-ICG-1 : TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
 MAV-ICG-22 : GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)
 MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
 MAL-ICG-1 : ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
 MCO-ICG-11 : TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
 MTH-ICG-11 : GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
 MTH-ICG-2 : GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
 MEF-ICG-11 : ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)

MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-5 :	CGTGAGGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGTTGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76-110, or 157-174 provided said probe hybridizes specifically to a Mycobacterium species.

The sequences represented by SEQ ID NO 76-110 and 157-174 are new.

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

As described above, the preferred restricted set of probes are those probes which

2193101

32

showed a sensitivity and specificity of more than 80%, preferably more than 90%, most preferably more than 95%, under the specific hybridization conditions as described in the examples section.

In one specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium tuberculosis complex strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MTB-ICG-1 : GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)

MTB-ICG-2 : GACTTGTTCCAGGTGTTGTCCCAC (SEQ ID NO 4)

MTB-ICG-3 : CGGCTAGCGGTGGCGTGTCT (SEQ ID NO 5)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76 provided said probe hybridizes specifically to the M. tuberculosis complex. The M. tuberculosis complex comprises M. tuberculosis, M. bovis, M. bovis BCG, M. africanum and M. microti strains.

The sequence represented in SEQ ID NO 76 is new.

Preferentially, at least two, or three of said probes are used simultaneously.

In another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium strains from the MAIS-complex, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAI-ICG-1 : CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)

MIL-ICG-11 : GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)

MIL-ICG-22 : TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)

MAC-ICG-1 : CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)

MAV-ICG-1 : TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)

MAV-ICG-22 : GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)

MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)

MIN-ICG-2 : GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)

MIN-ICG-22 : CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)

MIN-ICG-222 : TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)

MIN-ICG-2222 : GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)

MAL-ICG-1 : ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)

MHEF-ICG-1 : TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)

MAH-ICG-1 : GTGTAATTTCTTTTAACTCTTGTGTGTAAGTAAGTG

(SEQ ID NO 19)
MCO-ICG-11 : TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
MTH-ICG-11 : GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
MTH-ICG-2 : GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
MEF-ICG-11 : ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)
MSC-ICG-1 : TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77-100 or 108-110, provided said probe hybridizes specifically to strains from the MAIS complex. The MAIS complex as defined in this invention comprises all strains of M. avium, M. intracellulare and M. scrofulaceum and all strains closely related to the above mentioned species and not clearly belonging to another defined Mycobacterium species. The latter group of strains are defined in this invention as "MIC strains" (M. intracellulare complex).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more M. avium and M. paratuberculosis strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAV-ICG-1 : TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
MAV-ICG-22 : GTGGCCGGCGTTTCATCGAAA (SEQ ID NO 11)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77 and 78 provided said probe hybridizes specifically to M. avium or M. paratuberculosis.

The sequences as represented in SEQ ID NO 77 and 78 are new.

Preferentially, this embodiment uses both probes in combination.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium intracellulare strains and MIC-strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAI-ICG-1 : CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
MIL-ICG-11 : GAGGGGTTCCTCGTCTGTAGTG (SEQ ID NO 7)
MIL-ICG-22 : TGAGGGGTTCCTCGTCTGTAGTG (SEQ ID NO 8)

MAC-ICG-1 : CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
 MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
 MIN-ICG-2 : GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)
 MIN-ICG-22 : CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)
 MIN-ICG-222 : TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)
 MIN-ICG-2222 : GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)
 MAL-ICG-1 : ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
 MHEF-ICG-1 : TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)
 MAH-ICG-1 : GTGTAATTTCTTTTTTA ACTCTTGTGTGTAAGTAAGTG
 (SEQ ID NO 19)
 MCO-ICG-11 : TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
 MTH-ICG-11 : GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
 MTH-ICG-2 : GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
 MEF-ICG-11 : ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 provided said probe hybridizes specifically to M. intracellulare strains and MIC-strains.

The sequences as represented in SEQ ID NO 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 are new.

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more Mycobacterium intracellulare strains in a sample, wherein step (iii) comprises hybridizing to at least the following probes :

MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 89 provided said probe hybridizes specifically to M. intracellulare strains.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more Mycobacterium scrofulaceum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MSC-ICG-1 : TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 100 provided said probe hybridizes specifically to M. scrofulaceum.

The sequence as represented in SEQ ID NO 100 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium kansasii strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MKA-ICG-1 : GATGCGTTTGCTACGGGTAGCGT (SEQ ID NO 25)

MKA-ICG-2 : GATGCGTTGCCTACGGGTAGCGT (SEQ ID NO 26)

MKA-ICG-3 : ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)

MKA-ICG-4 : CGGGCTCTGTTCGAGAGTTGTC (SEQ ID NO 28)

MKA-ICG-5 : CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)

MKA-ICG-6 : GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)

MKA-ICG-7 : TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)

MKA-ICG-8 : GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)

MKA-ICG-9 : GATGCGTTGCCCCTACGGG (SEQ ID NO 186)

MKA-ICG-10 : CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)

and more preferably to:

MKA-ICG-3 : ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)

MKA-ICG-4 : CGGGCTCTGTTCGAGAGTTGTC (SEQ ID NO 28)

MKA-ICG-5 : CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)

MKA-ICG-6 : GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)

MKA-ICG-7 : TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)

MKA-ICG-8 : GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)

MKA-ICG-9 : GATGCGTTGCCCCTACGGG (SEQ ID NO 186)

MKA-ICG-10 : CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 101, 167, 168 or 169 provided said probe hybridizes specifically to M. kansasii.

The sequences as represented in SEQ ID NO 101, 167, 168 and 169 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium chelonae strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MCH-ICG-1 : GGTGTGGACTTTGACTTCTGAATAG (SEQ ID NO 29)

MCH-ICG-2 : CGGCAAAACGTCGGACTGTCA (SEQ ID NO 30)

MCH-ICG-3 : GGTGTGGTCCTTGACTTATGGATAG (SEQ ID NO 210)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 102, 103 or 174 provided said probe hybridizes specifically to M. chelonae. According to another preferential embodiment, these three probes are used in combination.

The sequences as represented in SEQ ID NO 102, 103 and 174 are new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium gordonae strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MGO-ICG-1 : AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)

MGO-ICG-2 : GTATGCGTTGTCGTTTCGCGGC (SEQ ID NO 32)

MGO-ICG-5 : CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)

and more preferably to:

MGO-ICG-5 : CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 104, 105 or 106 provided said probe hybridizes specifically to M. gordonae.

The sequences as represented in SEQ ID NO 104 to 106 are new.

Preferentially, at least two or three of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium ulcerans strains or Mycobacterium marinum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MUL-ICG-1 : GGTTTCGGGATGTTGTCCCACC (SEQ ID NO 175)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 157 provided said probe hybridizes specifically to M. ulcerans and M. marinum.

The sequence as represented in SEQ ID NO 157 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium genavense strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MGV-ICG-1 : CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)

MGV-ICG-2 : GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)

MGV-ICG-3 : TCGGGCCGCGTGTTTCGTCAAA (SEQ ID NO 211)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 158, 159, 160, 161 or 162 provided said probe hybridizes specifically to M. genavense.

The sequences as represented in SEQ ID NO 158 to 162 are new.

As described in the examples, M. genavense includes M. genavense strains sensu strictu and a group of closely related strains called M. simiae-like. The former group of strains can be detected specifically with probe MGV-ICG-1 while the latter group hybridizes specifically with probe MGV-ICG-3. Probe MGV-ICG-2 detects both groups.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium xenopi strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MXE-ICG-1 : GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 163 provided said probe hybridizes specifically to M. xenopi.

The sequence as represented in SEQ ID NO 163 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium simiae strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MSI-ICG-1 : CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 164 or 165 provided said probe hybridizes specifically to M. simiae.

The sequence as represented in SEQ ID NO 164 or 165 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium fortuitum strains in a sample,

wherein step (iii) comprises hybridizing to at least one of the the following probes:

MFO-ICG-1 : TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)

MFO-ICG-2 : ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)

or to equivalents of said probes or to any probe derived from SEQ ID NO 166 provided said probe hybridizes specifically to M. fortuitum.

The sequence as represented in SEQ ID NO 166 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium celatum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MCE-ICG-1 : TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 170 provided said probe hybridizes specifically to M. celatum.

The sequence as represented in SEQ ID NO 170 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium haemophilum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MHP-ICG-1 : CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 171, 172 or 173 provided said probe hybridizes specifically to M. haemophilum.

The sequences as represented in SEQ ID NO 171 to 173 are new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium malmoeense strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MML-ICG-1 : CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)

MML-ICG-2 : TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 107 provided said probe hybridizes specifically to M. malmoeense.

The sequence as represented in SEQ ID NO 107 is new.

In still another specific embodiment, the invention provides for a method as described

above to detect and identify one or more Mycobacterium strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 : ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)

MYC-ICG-22 : CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)

or to equivalents of said probes.

According to a preferred embodiment, both probes are used in combination.

The invention also provides for a method as described above to detect and identify one or more Mycoplasma strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 : ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2 : CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

MGE-ICG 1 : CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

Mycoplasma-ICG : CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 124 or 125 provided said probe hybridizes specifically with Mycoplasma species.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more Mycoplasma pneumoniae strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 : ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2 : CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 125 provided said probe hybridizes specifically to Mycoplasma pneumoniae. According to a preferred embodiment, both these probes are used in combination.

The sequence as represented in SEQ ID NO 125 is new.

In another particular embodiment, the invention provides for a method as described above to detect and identify one or more Mycoplasma genitalium strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MGE-ICG 1 : CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

or to equivalents of said probes,

2193101

40

and/or to any probe derived from SEQ ID NO 124 provided said probe hybridizes specifically to Mycoplasma genitalium.

The sequence as represented in SEQ ID NO 124 is new.

The invention also provides for a method as described above to detect and identify one or more Pseudomonas strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

PA-ICG 1 : TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 2 : TGAATGTTCGTGGATGAACATTGATT (SEQ ID NO 35)

PA-ICG 3 : CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)

PA-ICG 4 : TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC
(SEQ ID NO 37)

PA-ICG 5 : CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111, 112, 113, 114 or 115 provided said probe hybridizes specifically to Pseudomonas strains.

The sequences as represented in SEQ ID NO 111 to 115 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more Pseudomonas aeruginosa strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

PA-ICG 1 : TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 2 : TGAATGTTCGTGGATGAACATTGATT (SEQ ID NO 35)

PA-ICG 3 : CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)

PA-ICG 4 : TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC
(SEQ ID NO 37)

PA-ICG 5 : CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)

and most preferably to at least one of the following probes:

PA-ICG 1 : TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 4 : TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC
(SEQ ID NO 37)

PA-ICG 5 : CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111 provided said probe hybridizes specifically to Pseudomonas aeruginosa.

The sequence as represented in SEQ ID NO 111 is new.

Preferentially, at least two, three, four or five of said probes are used simultaneously.

The invention also provides for a method as described above to detect and identify one or more Staphylococcus species in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

STAU-ICG 1 : TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)

STAU-ICG 2 : CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)

STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)

STAU-ICG 4 : GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142, 143 or 144 provided said probe hybridizes specifically to Staphylococcus species.

The sequences as represented in SEQ ID NO 139 to 144 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more Staphylococcus aureus strains in a sample, wherein step (iii) comprises hybridizing to at least one, and preferably both of the following probes:

STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)

STAU-ICG 4 : GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142 or 143 provided said probe hybridizes specifically to Staphylococcus aureus. According to a preferred embodiment, both these probes are used in combination.

In another specific embodiment the invention provides for a method as described above to detect and identify one or more Staphylococcus epidermidis strains in a sample, wherein step (iii) comprises hybridizing to any probe derived from SEQ ID NO 144 as long as this probe can be caused to hybridize specifically to Staphylococcus epidermidis.

The invention also provides for a method as described above to detect and identify one or more Acinetobacter strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

2193101

42

ACI-ICG 1 : GCTTAAGTGCACAGTGCTCTAAACTGA (SEQ ID NO 57)

ACI-ICG 2 : CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126, 127, 128, 129 or 130 provided said probe hybridizes specifically to Acinetobacter sp.. According to a preferred embodiment, both these probes are used in combination.

The sequences as represented in SEQ ID NO 126 to 130 are new.

More particularly, the invention provides for a method as described above to detect and identify one or more Acinetobacter baumanii strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

ACI-ICG 1 : GCTTAAGTGCACAGTGCTCTAAACTGA (SEQ ID NO 57)

ACI-ICG 2 : CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126 provided said probe hybridizes specifically to Acinetobacter baumanii. According to a preferred embodiment, both these probes are used in combination.

The invention also provides for a method as described above, to detect and identify one or more Listeria strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LIS-ICG 1 : CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)

LMO-ICG 1 : AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
(SEQ ID NO 40)

LMO-ICG 2 : TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC (SEQ ID NO 41)

LMO-ICG 3 : AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

LIV-ICG 1 : GTTAGCATAAATAGGTAAGTATTTATGACACAAGTAAC
(SEQ ID NO 43)

LSE-ICG 1 : AGTTAGCATAAGTAGTGTAAGTATTTATGACACAAG

LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)

and most preferably to at least one of the following probes:

LIS-ICG 1 : CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)

LMO-ICG 3 : AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 116, 118, 119, 120, 121, 213, 214 or 215 provided said probe hybridizes specifically to Listeria species.

As described in the examples section, Listeria species encompass Listeria species sensu strictu, and a group of closely related organisms referred to as "Listeria-like organisms". The latter group can be specifically recognized by probe LISP-ICG 1.

The sequences as represented in SEQ ID NO 116, 118 to 121 and 213 to 215 are new.

Preferentially, at least two, three, four, five or six of said probes are used simultaneously.

More particularly, the invention provides for a method as described above, to detect and identify one or more Listeria monocytogenes strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LMO-ICG 1 : AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG (SEQ ID NO 40)

LMO-ICG 2 : TGAGAGGTTAGTACTTCTCAGTATGTTTGTTT (SEQ ID NO 41)

LMO-ICG 3 : AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

and most preferably to the following probe:

LMO-ICG 3 : AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 120 provided said probe hybridizes specifically to Listeria monocytogenes.

Preferentially, at least two, or three of said probes are used simultaneously.

The invention also provides for a method as described above to detect and identify one or more Brucella strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

BRU-ICG 1 : CGTGCCGCCTTCGTTTCTCTTT (SEQ ID NO 59)

BRU-ICG 2 : TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)

BRU-ICG 3 : GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)

BRU-ICG 4 : CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)

and most preferably to at least one of the following probes:

BRU-ICG 2 : TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)

BRU-ICG 3 : GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)

2193101

44

BRU-ICG 4 : CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 131, 132 or 154 provided said probe hybridizes specifically to Brucella strains.

The sequences as represented in SEQ ID NO 131, 132 and 154 are new.

The invention also provides for a method as described above to detect and identify one or more Salmonella strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

SALM-ICG 1 : CAAAACTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)

SALM-ICG 2 : GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)

STY-ICG 1 : GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)

SED-ICG 1 : GCGGTAATGTGTGAAAGCGTTGCC (SEQ ID NO 64)

and most preferably to the following probe:

SALM-ICG 1 : CAAAACTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 133, 134, 135, 136, 137 or 138 provided said probe hybridizes specifically to Salmonella strains.

The sequences as represented in SEQ ID NO 133 to 138 are new.

Preferentially, at least two, three, or four of said probes are used simultaneously.

The invention also relates to a method as described above to detect and identify one or more Chlamydia strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 : GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)

CHTR-ICG 2 : GCATTTATATGTAAGAGCAAGCATTCTATTTCA (SEQ ID NO 46)

CHTR-ICG 3 : GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)

CHTR-ICG 4 : GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)

CHPS-ICG 1 : GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 122, 123 or 197 provided that said probe hybridizes specifically to Chlamydia strains.

Preferentially, at least two, three, four or five of said probes are used simultaneously.

More particularly, the invention relates to a method as described above to detect and

identify one or more Chlamydia trachomatis strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 : GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)

CHTR-ICG 2 : GCATTTATATGTAAGAGCAAGCATTCTATTTC (SEQ ID NO 46)

CHTR-ICG 3 : GAGTAGCGTG GTGAGGACGAGA (SEQ ID NO 47)

CHTR-ICG 4 : GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 123 or 197 provided said probe hybridizes specifically to Chlamydia trachomatis.

The sequences as represented in SEQ ID NO 123 and 197 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

In another particular embodiment, the invention provides for a method as described above to detect and identify one or more Chlamydia psittaci strains in a sample, wherein step (iii) comprises hybridizing to at least the following probe:

CHPS-ICG 1 : GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 122 provided said probe hybridizes specifically to Chlamydia psittaci.

The sequence of SEQ ID NO 122 is new.

The invention also provides for a method as described above, to detect one or more Streptococcus strains in a sample, wherein step (iii) comprises hybridizing to any probe derived from SEQ ID NO 145, 146, 147, 148, 149, 150, 151, 152 or 153 provided said probe hybridizes specifically to Streptococcus strains, or equivalents of these probes.

The sequences as represented in SEQ ID NO 145, 146, 147, 148, 149, 150, 151, 152 or 153 are new.

The invention also provides for a method as described above, to detect one or more Yersinia enterocolitica strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes :

YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)

YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)

YEC-ICG 3 : GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 195 or 196, provided said probe hybridizes specifically to Yersinia enterocolitica.

The sequences as represented in SEQ ID NO 195 and 196 are new.

In some cases it may be advantageous to amplify not all organisms present in a sample, but only more specific taxa, which are considered to be relevant. In these cases the invention provides for primers allowing the specific amplification of the spacer region for only those beforehand defined taxa.

The invention thus provides for a method as described above to detect and identify specifically Chlamydia trachomatis in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

CHTR-P1 : AAGGTTTCTGACTAGGTTGGGC (SEQ ID NO 69)

CHTR-P2 : GGTGAAGTGCTTGCATGGATCT (SEQ ID NO 70)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from Chlamydia trachomatis.

Preferably both primers are used.

The invention also provides for a method as described above to detect and identify specifically Listeria species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

LIS-P1 : ACCTGTGAGTTTTCGTTCTTCTC (SEQ ID NO 71)

LIS-P2 : CTATTGTTTCAGTTTTGAGAGGTT (SEQ ID NO 72)

LIS-P3 : ATTTCCGTATCAGCGATGATAC (SEQ ID NO 73)

LIS-P4 : ACGAAGTAAAGGTTGTTTTTCT (SEQ ID NO 74)

LIS-P5 : GAGAGGTTACTCTTTTATGTCAG (SEQ ID NO 75)

LIS-P6 : CTTTATGTCAGATAAAGTATGCAA (SEQ ID NO 202)

LIS-P7 : CGTAAAAGGGTATGATTATTG (SEQ ID NO 203)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from Listeria species.

The invention also relates to a method as described above to detect and identify specifically Mycobacterium species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

MYC-P1:	TCCCTTGTGGCCTGTGTG	(SEQ ID NO 65)
MYC-P2:	TCCTTCATCGGCTCTCGA	(SEQ ID NO 66)
MYC-P3:	GATGCCAAGGCATCCACC	(SEQ ID NO 67)
MYC-P4:	CCTCCCACGTCCTTCATCG	(SEQ ID NO 68)
MYC-P5:	CCTGGGTTTGACATGCACAG	(SEQ ID NO 192)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from Mycobacterium species.

The invention also provides for a method as described above to detect and identify specifically Brucella species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or part of it, using at least one of the following primers :

BRU-P1 :	TCGAGAATTGGAAAGAGGTC	(SEQ ID NO 204)
BRU-P2 :	AAGAGGTCGGATTTATCCG	(SEQ ID NO 205)
BRU-P3 :	TTCGACTGCAAATGCTCG	(SEQ ID NO 206)
BRU-P4 :	TCTTAAAGCCGCATTATGC	(SEQ ID NO 207)

or equivalents of these primers, said equivalents differing in sequence from the above-mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region of part of it from Brucella species.

The invention also provides for a method as described above to detect and identify specifically Yersinia enterocolitica species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or part of it, using at least one of the following primers :

YEC-P1 :	CCTAATGATATTGATTCGCG	(SEQ ID NO 208)
YEC-P2 :	ATGACAGGTTAATCCTTACCCC	(SEQ ID NO 209)

or equivalents of these primers, said equivalents differing in sequence from the above-mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region of part of it from Yersinia enterocolitica species.

The invention also provides for a composition comprising at least one of the probes and/or primers as defined above.

Said composition may comprise any carrier, support, label or diluent known in the art for probes or primers, more particularly any of the labels or supports detailed in the definitions section.

The invention relates more particularly to isolated probes and primers as defined above, more particularly any of the probes as specified in Table 1a or any of the primers as specified in Table 1b.

According to another embodiment, the present invention relates also to new spacer region sequences as defined above and as set out in figures 1-103 (SEQ ID NO 76 to 154, SEQ ID NO 157 to 174, SEQ ID NO 195 to 197 and SEQ ID NO 213 to 215).

In another embodiment the invention provides for a reverse hybridization method comprising any of the probes as defined above, wherein said probes are immobilized on a known location on a solid support, more preferably on a membrane strip.

In yet another embodiment the invention provides for a kit for the detection and identification of at least one micro-organism, or the simultaneous detection and identification of several micro-organisms in a sample, comprising the following components:

- (i) when appropriate, at least one suitable primer pair to allow amplification of the intercistronic 16S-23S rRNA spacer region, or a part of it;
- (ii) at least one of the probes as defined above;
- (iii) a buffer, or components necessary to produce the buffer, enabling a hybridization reaction between said probes and the polynucleic acids present in the sample, or the amplified products thereof;
- (iv) a solution, or components necessary to produce the solution, enabling washing of the hybrids formed under the appropriate wash conditions;
- (v) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

FIGURE LEGENDS

- Fig 1 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium tuberculosis strain H37RV ATCC 27294 (SEQ ID NO 76)
- 5 Fig 2 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium avium ATCC 151.769 (ITG 4991) (SEQ ID NO 77)
- Fig 3 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium paratuberculosis strains 316F and 2E (SEQ ID NO 78)
- Fig 4 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 5513 (SEQ ID NO 79)
- 10 Fig 5 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8695 (SEQ ID NO 80)
- Fig 6 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8708 (SEQ ID NO 81)
- 15 Fig 7 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8715 (SEQ ID NO 82)
- Fig 8 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8054 (SEQ ID NO 83)
- Fig 9 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8737 (SEQ ID NO 84)
- 20 Fig 10 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8743 (SEQ ID NO 85)

2193101

50

- Fig 11 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8745 (SEQ ID NO 86)
- Fig 12 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8748 (SEQ ID NO 87)
- 5 Fig 13 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 8752 (SEQ ID NO 88)
- Fig 14 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium intracellulare serovar 12 ITG 5915 (SEQ ID NO 89)
- Fig 15 : represents the DNA sequence of the 16S-23S rRNA spacer region from
10 Mycobacterium lufu ITG 4755 (SEQ ID NO 90)
- Fig 16 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 5922 (SEQ ID NO 91)
- Fig 17 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 1329 (SEQ ID NO 92)
- 15 Fig 18 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 1812 (SEQ ID NO 93)
- Fig 19 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 5280 (SEQ ID NO 94)
- Fig 20 : represents the DNA sequence of the 16S-23S rRNA spacer region from
20 Mycobacterium strain ITG 5620 (SEQ ID NO 95)
- Fig 21 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium strain ITG 5765 (SEQ ID NO 96)

- Fig 22 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 7395 (SEQ ID NO 97)
- Fig 23 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 8738 (SEQ ID NO 98)
- 5 Fig 24 : represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 926 (SEQ ID NO 99)
- Fig 25 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium scrofulaceum ITG 4988 (SEQ ID NO 100)
- 10 Fig 26 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium kansasii ATCC 22478 (=ITG 4987) (SEQ ID NO 101)
- Fig 27 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium chelonae abcessus ITG 4975 (SEQ ID NO 102)
- Fig 28 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium chelonae chelonae ITG 9855 (SEQ ID NO 103)
- 15 Fig 29 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 7703 (SEQ ID NO 104)
- Fig 30 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 7836 (SEQ ID NO 105)
- 20 Fig 31 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 8059 (SEQ ID NO 106)
- Fig 32 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium malmoeense ITG 4842 and ITG 4832 (SEQ ID NO 107)

2193101

52

- Fig 33 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium strain 8757 (SEQ ID NO 108)
- Fig 34 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium ITG 8723 (SEQ ID NO 109)
- 5 Fig 35 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium ITG 8724 (SEQ ID NO 110)
- Fig 36 : represents the DNA sequence of the 16S-23S spacer region from Pseudomonas aeruginosa UZG 5669 (SEQ ID NO 111)
- Fig 37 : represents the DNA sequence of the 16S-23S spacer region from Pseudomonas pseudoalcaligenes LMG 1225 (SEQ ID NO 112)
- 10 Fig 38 : represents the DNA sequence of the 16S-23S spacer region from Pseudomonas stutzeri LMG 2333 (SEQ ID NO 113)
- Fig 39 : represents the DNA sequence of the 16S-23S spacer region from Pseudomonas alcaligenes LMG 1224 (SEQ ID NO 114)
- 15 Fig 40 : represents the DNA sequence of the 16S-23S spacer region from Pseudomonas putida LMG 2232 (SEQ ID NO 115)
- Fig 41 : represents the DNA sequence of the small 16S-23S spacer region from Listeria ivanovii CIP 7842 (SEQ ID NO 116)
- Fig 42 : represents the DNA sequence of the small 16S-23S spacer region from Listeria monocytogenes (SEQ ID NO 117)
- 20 Fig 43 : represents the DNA sequence of the small 16S-23S spacer region from Listeria seeligeri serovar 4A nr. 4268 (SEQ ID NO 118)

- Fig 44 : represents the partial DNA sequence of the large 16S-23S spacer region from partial sequence of the long spacer region of Listeria ivanovii CIP 7842 (SEQ ID NO 119)
- 5 Fig 45 : represents the DNA sequence of the large 16S-23S spacer region from Listeria monocytogenes IHE serovar 4B (SEQ ID NO 120)
- Fig 46 : represents the DNA sequence of the large 16S-23S spacer region from Listeria seeligeri serovar 4A nr. 4268 (SEQ ID NO 121)
- Fig 47 : represents the DNA sequence of the 16S-23S spacer region from Chlamydia psittaci 6BC (SEQ ID NO 122)
- 10 Fig 48 : represents the DNA sequence of the 16S-23S spacer region from Chlamydia trachomatis (SEQ ID NO 123)
- Fig 49 : represents the DNA sequence of the 16S-23S spacer region from Mycoplasma genitalium (U. Gobel) (SEQ ID NO 124)
- 15 Fig 50 : represents the DNA sequence of the 16S-23S spacer region from Mycoplasma pneumoniae ATCC 29432 (SEQ ID NO 125)
- Fig 51 : represents the DNA sequence of the 16S-23S spacer region from Acinetobacter baumannii LMG 1041 (SEQ ID NO 126)
- Fig 52 : represents the DNA sequence of the 16S-23S spacer region from Acinetobacter calcoaceticus LMG 1046 (SEQ ID NO 127)
- 20 Fig 53 : represents the DNA sequence of the 16S-23S spacer region from Acinetobacter haemolyticus LMG 996 (SEQ ID NO 128)
- Fig 54 : represents the DNA sequence of the 16S-23S spacer region from

Acinetobacter johnsonii LMG 999 (SEQ ID NO 129)

- Fig 55 : represents the DNA sequence of the 16S-23S spacer region from Acinetobacter junii LMG 998 (SEQ ID NO 130)
- 5 Fig 56 : represents the DNA sequence of the 16S-23S spacer region from Brucella melitensis NIDO Biovar 1 (SEQ ID NO 131)
- Fig 57 : represents the DNA sequence of the 16S-23S spacer region from Brucella suis NIDO Biovar 1 (SEQ ID NO 132)
- Fig 58 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella dublin (SEQ ID NO 133)
- 10 Fig 59 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella dublin (SEQ ID NO 134)
- Fig 60 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella enteritidis (SEQ ID NO 135)
- 15 Fig 61 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella enteritidis (SEQ ID NO 136)
- Fig 62 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella typhimurium (SEQ ID NO 137)
- Fig 63 : represents the DNA sequence of one of the 16S-23S spacer region from Salmonella typhimurium (SEQ ID NO 138)
- 20 Fig 64 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus aureus strain UZG 5728 (SEQ ID NO 139)

2193101

55

- Fig 65 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus aureus strain UZG 6289 (SEQ ID NO 140)
- Fig 66 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus aureus strain UZG 6289 (SEQ ID NO 141)
- 5 Fig 67 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus aureus strain UZG 6289 (SEQ ID NO 142)
- Fig 68 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus aureus strain UZG 6289 (SEQ ID NO 143)
- 10 Fig 69 : represents the DNA sequence of one of the 16S-23S spacer region from Staphylococcus epidermidis strain UZG CNS41 (SEQ ID NO 144)
- Fig 70 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus mitis UZG 2465 (SEQ ID NO 145)
- Fig 71 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus pyogenes UZG 3671 (SEQ ID NO 146)
- 15 Fig 72 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus sanguis UZG 1042 (SEQ ID NO 147)
- Fig 73 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus saprophyticus UZG CNS46 (SEQ ID NO 148)
- 20 Fig 74 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus species UZG 536 (84) (SEQ ID NO 149)
- Fig 75 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus species UZG 4341 (SEQ ID NO 150)

2193101

56

- Fig 76 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus species UZG 457 (44B) (SEQ ID NO 151)
- Fig 77 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus species UZG 97A (SEQ ID NO 152)
- 5 Fig 78 : represents the DNA sequence of the 16S-23S spacer region from Streptococcus species UZG 483 (76) (SEQ ID NO 153)
- Fig 79 : represents the DNA sequence of the 16S-23S spacer region from Brucella abortus NIDO Tulya biovar 3 (SEQ ID NO 154)
- 10 Fig 80 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium ulcerans ITG 1837 and Mycobacterium marinum ITG 7732 (SEQ ID NO 157)
- Fig 81 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium genavense ITG 8777 (SEQ ID NO 158)
- 15 Fig 82 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium genavense ITG 92-742 (SEQ ID NO 159)
- Fig 83 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium genavense ITG 9500 (SEQ ID NO 160)
- Fig 84 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium simiae-like ITG 7379 (SEQ ID NO 161)
- 20 Fig 85 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium simiae-like ITG 9745 (SEQ ID NO 162)
- Fig 86 : represents the DNA sequence of the 16S-23S spacer region from

Mycobacterium xenopi ITG 4986 (SEQ ID NO 163)

Fig 87 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium simiae A ITG 4485 (SEQ ID NO 164)

5 Fig 88 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium simiae B ITG 4484 (SEQ ID NO 165)

Fig 89 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium fortuitum ITG 4304 (SEQ ID NO 166)

Fig 90 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium kansasii ITG 6328 (SEQ ID NO 167)

10 Fig 91 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium kansasii ITG 8698 (SEQ ID NO 168)

Fig 92 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium kansasii ITG 8973 (SEQ ID NO 169)

15 Fig 93 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium celatum ITG 94-123 (SEQ ID NO 170)

Fig 94 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium haemophilum ITG 776 (SEQ ID NO 171)

Fig 95 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium haemophilum ITG 778 (SEQ ID NO 172)

20 Fig 96 : represents the DNA sequence of the 16S-23S spacer region from
Mycobacterium haemophilum ITG 3071 (SEQ ID NO 173)

- Fig 97 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium chelonae ITG 94-330 and ITG 94-379 (SEQ ID NO 174)
- Fig 98 : represents the DNA sequence of a 16S-23S spacer region from Yersinia enterocolitica strain P95 (SEQ ID NO 195)
- 5 Fig 99 : represents the DNA sequence of a 16S-23S spacer region from Yersinia enterocolitica strain P95 (SEQ ID NO 196)
- Fig 100 : represents the DNA sequence of the 16S-23S spacer region from Chlamydia trachomatis strain SSDZ 94 M 1961 (SEQ ID NO 197)
- 10 Fig 101 : represents the DNA sequence of a 16S-23S spacer region from Listeria -like isolate MB 405 (SEQ ID NO 213)
- Fig 102 : represents the DNA sequence of a 16S-23S spacer region from Listeria -like isolate MB 405 (SEQ ID NO 214)
- Fig 103 : represents the DNA sequence of a 16S-23S spacer region from Listeria -like isolate MB 405 (SEQ ID NO 215)

TABLE LEGENDS

- Table 1a: List of all new probes originating from the 16S-23S rRNA spacer region
- Table 1b: List of possible primers to be used for taxon-specific amplification of the spacer region or part of it.
- 5 Table 2: Hybridization results for Pseudomonas
- Table 3: Different probe patterns obtained for mycobacterial strain-types
- Table 4: Mycobacteria strains tested in LiPA
- Table 5: Hybridization results for Listeria (Probes LMO1, 2, LSE1, LIV1, LIS1)
- Table 6: Hybridization results for Listeria (Probes LMO3, LIS1)
- 10 Table 7: Hybridization results for Chlamydia
- Table 8: New mycobacterial probes and hybridization results
- Table 9: Hybridization results for Brucella
- Table 10: Hybridization results for Staphylococcus

2193101

60

Table 1a

	<u>PROBE</u>	<u>SEQUENCE</u>	<u>SEQ ID NO</u>
	MYC-ICG-1	: ACTGGATAGTGGTTGCGAGCATCTA	1
	MYC-ICG-22	: CTTCTGAATAGTGGTTGCGAGCATCT	2
5	MTB-ICG-1	: GGGTGCATGACAACAAAGTTGGCCA	3
	MTB-ICG-2	: GACTTGTTCCAGGTGTTGTCCCAC	4
	MTB-ICG-3	: CGGCTAGCGGTGGCGTGTTCT	5
	MAI-ICG-1	: CAACAGCAAATGATTGCCAGACACAC	6
	MIL-ICG-11	: GAGGGGTTCCCGTCTGTAGTG	7
10	MIL-ICG-22	: TGAGGGGTTCTCGTCTGTAGTG	8
	MAC-ICG-1	: CACTCGGTTCGATCCGTGTGGA	9
	MAV-ICG-1	: TCGGTCCGTCCGTGTGGAGTC	10
	MAV-ICG-22	: GTGGCCGGCGTTCATCGAAA	11
	MIN-ICG-1	: GCATAGTCCTTAGGGCTGATGCGTT	12
15	MIN-ICG-2	: GCTGATGCGTTCGTCGAAATGTGTA	13
	MIN-ICG-22	: CTGATGCGTTCGTCGAAATGTGT	14
	MIN-ICG-222	: TGATGCGTTCGTCGAAATGTGT	15
	MIN-ICG-2222	: GGCTGATGCGTTCGTCGAAATGTGTAA	16
	MAL-ICG-1	: ACTAGATGAACGCGTAGTCCTTGT	17
20	MHEF-ICG-1	: TGGACGAAAACCGGGTGCACAA	18
	MAH-ICG-1	: GTGTAATTTCTTTTTTA ACTCTTGTGTGTAAGTAAGTG	19
	MCO-ICG-11	: TGGCCGGCGTGTTTCATCGAAA	20
	MTH-ICG-11	: GCACTTCAATTGGTGAAGTGCGAGCC	21
	MTH-ICG-2	: GCGTGGTCTTCATGGCCGG	22
25	MEF-ICG-11	: ACGCGTGGTCCTTCGTGG	23
	MSC-ICG-1	: TCGGCTCGTTCTGAGTGGTGTC	24
	MKA-ICG-1	: GATGCGTTTGCTACGGGTAGCGT	25
	MKA-ICG-2	: GATGCGTTGCCTACGGGTAGCGT	26
	MKA-ICG-3	: ATGCGTTGCCCTACGGGTAGCGT	27
30	MKA-ICG-4	: CGGGCTCTGTTTCGAGAGTTGTC	28
	MCH-ICG-1	: GGTGTGGACTTTGACTTCTGAATAG	29
	MCH-ICG-2	: CGGCAAAACGTCGGACTGTCA	30

	MCH-ICG-3	: GGTGTGGTCCTTGACTTATGGATAG	210
	MGO-ICG-1	: AACACCCTCGGGTGCTGTCC	31
	MGO-ICG-2	: GTATGCGTTGTCGTTTCGCGGC	32
	MGO-ICG-5	: CGTGAGGGGTCATCGTCTGTAG	33
5	MUL-ICG-1	: GGTTTCGGGATGTTGTCCCACC	175
	MGV-ICG-1	: CGACTGAGGTCGACGTGGTGT	176
	MGV-ICG-2	: GGTGTTTGAGCATTGAATAGTGGTTGC	177
	MGV-ICG-3	: TCGGGCCGCGTGTTTCGTCAA	211
	MXE-ICG-1	: GTTGGGCAGCAGGCAGTAACC	178
10	MSI-ICG-1	: CCGGCAACGGTTACGTGTTC	179
	MFO-ICG-1	: TCGTTGGATGGCCTCGCACCT	180
	MFO-ICG-2	: ACTTGGCGTGGGATGCGGGAA	181
	MKA-ICG-5	: CCCTCAGGGATTTTCTGGGTGTTG	182
	MKA-ICG-6	: GGACTCGTCCAAGAGTGTTGTCC	183
15	MKA-ICG-7	: TCGGGCTTGGCCAGAGCTGTT	184
	MKA-ICG-8	: GGGTGCGCAACAGCAAGCGA	185
	MKA-ICG-9	: GATGCGTTGCCCCTACGGG	186
	MKA-ICG-10	: CCCTACGGGTAGCGTGTTCTTTTG	187
	MML-ICG-1	: CGGATCGATTGAGTGCTTGTCCC	188
20	MML-ICG-2	: TCTAAATGAACGCACTGCCGATGG	189
	MCE-ICG-1	: TGAGGGAGCCCGTGCCTGTA	190
	MHP-ICG-1	: CATGTTGGGCTTGATCGGGTGC	191
	PA-ICG 1	: TGGTGTGCTGCGTGATCCGAT	34
	PA-ICG 2	: TGAATGTTTCGTGGATGAACATTGATT	35
25	PA-ICG 3	: CACTGGTGATCATTCAAGTCAAG	36
	PA-ICG 4	: TGAATGTTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	37
	PA-ICG 5	: CTCTTTCACTGGTGATCATTCAAGTCAAG	38
	LIS-ICG 1	: CAAGTAACCGAGAATCATCTGAAAGTGAATC	39
	LMO-ICG 1	: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG	40
30	LMO-ICG 2	: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC	41
	LMO-ICG 3	: AGGCACTATGCTTGAAGCATCGC	42
	LIV-ICG 1	: GTTAGCATAAATAGGTAACATTTATGACACAAGTAAC	43

2193101

62

	LSE-ICG 1	: AGTTAGCATAAGTAGTGTAAGTATTTATGACACAAG	44
	LISP-ICG 1	: CGTTTTTCATAAGCGATCGCACGTT	212
	CHTR-ICG 1	: GGAAGAAGCCTGAGAAGGTTTCTGAC	45
	CHTR-ICG 2	: GCATTTATATGTAAGAGCAAGCATTCTATTTCA	46
5	CHTR-ICG 3	: GAGTAGCGTGGTGAGGACGAGA	47
	CHPS-ICG 1	: GGATAACTGTCTTAGGACGGTTTGAC	48
	MPN-ICG 1	: ATCGGTGGTAAATTAAACCCAAATCCCTGT	49
	MPN-ICG 2	: CAGTTCTGAAAGAACATTTCCGCTTCTTTC	50
	MGE-ICG 1	: CACCCATTAATTTTTTCGGTGTTAAAACCC	51
10	Mycoplasma-ICG	: CAAAACCTGAAAACGACAATCTTTCTAGTTCC	52
	STAU-ICG 1	: TACCAAGCAAAACCGAGTGAATAAAGAGTT	53
	STAU-ICG 2	: CAGAAGATGCGGAATAACGTGAC	54
	STAU-ICG 3	: AACGAAGCCGTATGTGAGCATTGAC	55
	STAU-ICG 4	: GAACGTAACCTTCATGTTAACGTTTGACTTAT	56
15	ACI-ICG 1	: GCTTAAGTGCACAGTGCTCTAACTGA	57
	ACI-ICG 2	: CACGGTAATTAGTGTGATCTGACGAAG	58
	BRU-ICG 1	: CGTGCCGCCTTCGTTTCTCTTT	59
	BRU-ICG 2	: TTCGCTTCGGGGTGGATCTGTG	60
	BRU-ICG 3	: GCGTAGTAGCGTTTGCGTCGG	193
20	BRU-ICG 4	: CGCAAGAAGCTTGCTCAAGCC	194
	SALM-ICG 1	: CAAAACCTGACTTACGAGTCACGTTTGAG	61
	SALM-ICG 2	: GATGTATGCTTCGTTATTCCACGCC	62
	STY-ICG 1	: GGTCAAACCTCCAGGGACGCC	63
	SED-ICG 1	: GCGGTAATGTGTGAAAGCGTTGCC	64
25	YEC-ICG 1	: GGAAAAGGTACTGCACGTGACTG	198
	YEC-ICG 2	: GACAGCTGAAACTTATCCCTCCG	199
	YEC-ICG 3	: GCTACCTGTTGATGTAATGAGTCAC	200
	CHTR-ICG 4	: GAGTAGCGCGGTGAGGACGAGA	201

63

Table 1b

	<u>PRIMERS</u>	<u>SEQUENCE</u>	<u>SEQ ID NO</u>
	MYC-P1	: TCCCTTGTGGCCTGTGTG	65
	MYC-P2	: TCCTTCATCGGCTCTCGA	66
5	MYC-P3	: GATGCCAAGGCATCCACC	67
	MYC-P4	: CCTCCCACGTCCTTCATCG	68
	MYC-P5	: CCTGGGTTTGACATGCACAG	192
	CHTR-P1	: AAGGTTTCTGACTAGGTTGGGC	69
	CHTR-P2	: GGTGAAGTGCTTGCATGGATCT	70
10	LIS-P1	: ACCTGTGAGTTTTCGTTCTTCTC	71
	LIS-P2	: CTATTTGTTTCAGTTTTGAGAGGTT	72
	LIS-P3	: ATTTTCCGTATCAGCGATGATAC	73
	LIS-P4	: ACGAAGTAAAGGTTGTTTTTCT	74
	LIS-P5	: GAGAGGTTACTCTCTTTTATGTCAG	75
15	LIS-P6	: CTTTTATGTCAGATAAAGTATGCAA	202
	LIS-P7	: CGTAAAAGGGTATGATTATTTG	203
	BRU-P1	: TCGAGAATTGGAAAGAGGTC	204
	BRU-P2	: AAGAGGTCGGATTTATCCG	205
	BRU-P3	: TTCGACTGCAAATGCTCG	206
20	BRU-P4	: TCTTAAAGCCGCATTATGC	207
	YEC-P1	: CCTAATGATATTGATTCGCG	208
	YEC-P2	: ATGACAGGTTAATCCTTACCCC	209

EXAMPLE 1 : Pseudomonas aeruginosa

Pseudomonas aeruginosa is a significant human pathogen, usually in the context of serious underlying disease. It is also a major cause of nosocomial infections, which are characteristically prone to resistance to antimicrobial agents. This gram-negative, non-fermentative rod can be responsible for different clinical manifestations, like wound infections, bacteremia, respiratory and urinary tract infections, and is also a major cause of morbidity and mortality in patients with cystic fibrosis.

Pseudomonas species are currently differentiated based on growth characteristics and several biochemical features implying a time schedule of 24h to 72h to get a correct identification of the pathogen.

Already the development of monoclonal or polyclonal antibodies significantly improved the identification of Pseudomonas species. Recently however it has been shown that it is possible to detect organisms directly in clinical samples on a very sensitive and specific way using DNA probes with or without a prior amplification of the target DNA.

DNA probes to study Pseudomonas aeruginosa are already described and are mainly used for epidemiological typing (Ogle et al., 1987; Samadpour et al., 1988; McIntosh et al., 1992). However, none of these probes have been derived from the 16S-23S spacer.

The 16S-23S rRNA gene spacer region and a part of the 23S rRNA gene was amplified with conserved primers (upper primer: TGGGGTGAAGTCGTAACAAGGTA . SEQ ID NO 155; lower primer: CCTTCCCTCACGGTACTGGT, SEQ ID NO 156) using the polymerase chain reaction for the following species :

- Pseudomonas aeruginosa 5669
- Pseudomonas alcaligenes LMG 1224^T
- Pseudomonas fluorescens LMG 5167
- Pseudomonas putida LMG 2232
- Pseudomonas stutzeri LMG 2333^T
- Pseudomonas pseudoalcaligenes LMG 1225^T

To facilitate cloning of the obtained amplicons a *NotI* recognition site was added to the lower primer. After purification and digestion of the fragment with *NotI*, the amplicon was cloned in a *EcoRV/NotI* digested pBluescript SK⁺ plasmid vector.

Sequencing of the 16S-23S rRNA gene spacer region was performed according the

dideoxy-chain terminating chemistry either using double stranded plasmid DNA combined with primers located in the plasmid vector or directly on the PCR products after purification combined with internal PCR primers.

Fig. 36 to 40 represent the nucleotide sequence of the 16S-23S rRNA gene spacer regions from the different Pseudomonas species described above. For P. fluorescens only partial sequence information was obtained.

From the nucleic acid sequence of the spacer from P. aeruginosa strain 5669 five oligonucleotide-probes were chosen and chemically synthesized. The sequences of the oligonucleotides are the following :

10 PA1 = PA-ICG 1 : TGGTGTGCTGCGTGATCCGATA
PA2 = PA-ICG 2 : TGAATGTTTCGTGGATGAACATTGATT
PA3 = PA-ICG 3 : CACTGGTGATCATTCAAGTCAAG

15 Specificity and sensitivity testing of the oligonucleotide-probes was carried out using a reverse hybridization assay. Genomic DNA of the different bacteria tested was amplified using biotinylated primers (idem primers as for cloning procedure, see above). The obtained amplicon, spanning the 16S-23S rRNA gene spacer region, was denatured and hybridized to a membrane-strip onto which the different oligonucleotide probes were immobilized in a line-wise fashion (LiPA). Hybridization was carried out in a mixture of 3xSSC (1xSSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 20% formamide (FA) at a temperature of 50°
20 C for one hour. Washing was done in the same mixture at the same temperature for 15 min.

Hybrids were detected using a streptavidine conjugate coupled to alkaline phosphatase and the probes were visualized through a precipitation reaction using NBT (nitrobluetetrazolium) and BCIP (bromo-chloro-indolylphosphate).

25 The hybridization results obtained with probes PA1, PA2 and PA3 are given in table 4 and show that probes PA1 and PA3 were 100% specific for Pseudomonas aeruginosa and hybridized to all the strains tested. The hybridization signal with probe PA3 at 50° C was not optimal, so the oligonucleotide-probe was improved by adding some additional nucleotides to the specific probe. This newly designed probe is PA5.

PA5 = PA-ICG 5 : CTCTTTCAGTGGTGATCATTCAAGTCAAG

30 Hybridization experiments with probe PA5 proved that this probe also shows a 100% specificity and 100% sensitivity for P. aeruginosa.

Oligonucleotide-probe PA2 hybridized only to 5 out of 17 P. aeruginosa strains tested.

2193101

66

Direct sequencing of the 16S-23S rRNA gene spacer region of the strains which did not hybridize to these probes, showed some heterogeneity between different strains. Two mismatches were seen in comparison to the first developed PA2 probe. To overcome this heterogeneity between different strains in the region of probe PA2 a new probe PA4 was designed. This probe is degenerated at the position of the mismatches and some additional nucleotides were added to improve the hybridization signal at 50° C.

PA4 = PA-ICG 4 : TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

A 100% specificity and 100% sensitivity was obtained with this degenerated probe as is shown by the hybridization results.

taxa tested	PA1	PA2	PA3	PA4	PA5
<u>Pseudomonas aeruginosa</u>	17/17	5/17	17/17	17/17	17/17
<u>Pseudomonas alcaligenes</u>	0/1	0/1	0/1	0/1	0/1
<u>Pseudomonas fluorescens</u>	0/1	0/1	0/1	0/1	0/1
<u>Pseudomonas putida</u>	0/1	0/1	0/1	0/1	0/1
<u>Pseudomonas pseudoalcaligenes</u>	0/1	0/1	0/1	0/1	0/1
<u>Pseudomonas stutzeri</u>	0/1	0/1	0/1	0/1	0/1
<u>Pseudomonas cepacia</u>	0/1	0/1	0/1	ND	ND
<u>Neisseria gonorrhoeae</u>	0/1	0/1	0/1	ND	ND
<u>Escherichia coli</u>	0/1	0/1	0/1	ND	ND
<u>Bordetella pertussis</u>	0/1	0/1	0/1	ND	ND
<u>Bordetella parapertussis</u>	0/1	0/1	0/1	ND	ND
<u>Bordetella bronchiseptica</u>	0/1	0/1	0/1	ND	ND
<u>Mycobacterium tuberculosis</u>	0/1	0/1	0/1	ND	ND
<u>Mycobacterium avium</u>	0/1	0/1	0/1	ND	ND
<u>Moraxella catarrhalis</u>	0/4	0/4	0/4	ND	ND
<u>Haemophilus influenzae</u>	0/2	0/2	0/2	ND	ND
<u>Streptococcus pneumoniae</u>	0/3	0/3	0/3	ND	ND
<u>Acinetobacter calcoaceticus</u>	0/1	0/1	0/1	ND	ND
<u>Staphylococcus aureus</u>	0/2	0/2	0/2	ND	ND

Table 2 : Hybridization results for Pseudomonas

(n/m: number of strains positive/number of strains tested)

(ND: not done)

EXAMPLE 2: Mycobacterium

A variety of mycobacterial species may be involved in serious human infectious disease. Notorious examples are *Mycobacterium tuberculosis* and *Mycobacterium leprae*.
5 Recently other species such as *M. avium*, *M. intracellulare* and *M. kansasii* have been more frequently encountered as human pathogens especially in immunocompromised hosts.

Consequently, laboratory diagnosis of mycobacterial infections should not be restricted to the *M. tuberculosis* complex but should ideally include most other clinically relevant mycobacterial species.

10 The identification and differentiation of pathogenic mycobacteria at the species level by conventional laboratory techniques is, in general, difficult and time-consuming.

To overcome these problems DNA-techniques were implemented. The techniques described extended from straightforward DNA-probing to automated sequence analysis. Several approaches have been recently reported (Jonas et al., 1993; Frothingham and Wilson,
15 1993; Tomioka et al., 1993; Saito et al., 1989; Vaneechoutte et al., 1993; Telenti et al., 1993; Böttlinghaus et al., 1990).

However, these methods all have their particular disadvantages, and most of them still rely on culture. Moreover, and most importantly, none of these techniques allows for a simultaneous detection of the different clinically relevant mycobacterial species in a single
20 test run. Besides, the differentiation of particular groups within the *Mycobacterium avium-intracellulare* complex is problematic and often even impossible.

To overcome the above-mentioned disadvantages, a LiPA-test was developed which allows for the simultaneous and reliable detection and differentiation of a number of *Mycobacterium* species and groups. The sets of probes used to achieve these goals were all
25 derived from the 16S-23S rRNA spacer region. The methods used are analogous to those mentioned in example 1.

The 16S-23S rRNA spacer region, and part of the 16S and 23S rRNA flanking genes, was amplified by PCR with primers conserved for the genus *Mycobacterium*. At least one of the following primers located in the 16S gene were used as upper primers:

30 MYC-P1: TCCCTTGTGGCCTGTGTG (SEQ ID NO 65)
MYC-P5: CCTGGGTTTGACATGCACAG (SEQ ID NO 192)

At least one of the following primers, located in the 23S gene, were used as lower primers

for the amplification:

MYC-P2: TCCTTCATCGGCTCTCGA (SEQ ID NO 66)

MYC-P3: GATGCCAAGGCATCCACC (SEQ ID NO 67)

MYC-P4: CCTCCCACGTCCTTCATCG (SEQ ID NO 68)

5 All the above mentioned primers amplified the spacer region of all *Mycobacterium* strains tested, except primer MYC-P2 which was not functional for *M. chelonae*. In order to enhance the sensitivity of the detection, a nested PCR was sometimes carried out, using P5 and P4 as outer primers and P1 and P3 as inner primers.

10 In order to be able to design and select the probes and probe combinations which fit our purpose, the 16S-23S rRNA spacer region of a number of mycobacterial strains was sequenced. The obtained sequences were compared to each other and to those already known from literature (e.g. Frothingham et al., 1993, 1994; Kempell et al., 1992; Suzuki et al., 1988; EP-A-0395292; Van der Giessen et al., 1994;) or from publicly accessible data banks. The corresponding sequences are represented in fig.1 to 35 (SEQ ID NO 76 to SEQ
15 ID NO 110).

The probes derived from these data were all adjusted in such a way that the desired hybridization-behaviour was obtained using unified hybridization and wash conditions (i.e. 3xSSC, 20% deionized formamide, 50°C). The set of adjusted probes used for hybridization to different mycobacterial strains is represented in table 1a, SEQ ID NO 1-33. Please note
20 that the probe nomenclature used in this example is an abbreviated version of the one used in table 1a: i.e. the letters "ICG" have always been omitted. According to the specific hybridization pattern obtained, the strains tested could be assigned to one of the following species or species groups: *M. tuberculosis* complex, *M. avium*, *M. intracellulare* or *M. intracellulare* complex, *M. kansasii*, *M. chelonae* and *M. gordonae*. The strains tested which
25 belong to each group are summarized in Table 4. All strains were obtained from the Institute of Tropical Medicine, Antwerp, Belgium. The different probe-patterns obtained for each group are illustrated in Table 3, and are discussed in more detail hereafter.

M. tuberculosis complex

30 The *M. tuberculosis* complex harbours all strains belonging to *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*. The probes Mtb1, Mtb2 and Mtb3 hybridize with DNA originating from all *M. tuberculosis* complex strains tested. None of the other strains tested hybridized with these probes at the conditions used.

2193101

70

In addition, *M. tuberculosis* complex strains, as is the case with all other mycobacterial strains tested, hybridize with either the **myc1** or the **myc22** probe or both. The latter two probes are designed as general *Mycobacterium* probes, either alone or in combination with each other.

5 *M. avium/M. paratuberculosis*

All *M. avium* and *M. paratuberculosis* strains studied reveal an identical hybridization pattern with the set of probes. For this type of organisms positive hybridization signals are obtained with the probes **myc1/myc22**, **mail**, **mil11**, **mav1**, **mahl** and **mav22**. The latter two probes hybridize exclusively with *M. avium* and *M. paratuberculosis* strains, and can thus be used as species-specific probes. Since the 16S-23S spacer sequences of *M. avium* isolates and *M. paratuberculosis* isolates are identical or nearly identical these two taxa cannot be discriminated from each other. This finding supports 16S rRNA sequencing data which indicate that *M. avium* and *M. paratuberculosis* should in fact be considered as belonging to one geno-species (Rogal et al., 1990), *M. avium* ssp. *avium* and *M. avium* ssp. *paratuberculosis*.

15 *M. intracellulare* and *M. intracellulare* complex (MIC)

MIC strains are genotypically highly related organisms, which, according to sequence data of the 16S-23S rRNA spacer region, belong to a distinct cluster which is separate from other *Mycobacterium* species. *M. avium* and *M. scrofulaceum* are their closest relatives. Almost all strains tested which are generally referred to as *M. avium* complex (MAC) strains (the former MAIS-complex) can be found in the MIC group. Thus, the MIC group defined in the current invention encompasses the MAC-type strains described by Frothingham and Wilson (1993) with the exception of MAC-G which appears to be *M. scrofulaceum*. Also *M. intracellulare* strains *sensu stricto* (*M. intracellulare* s.s.) are part of this cluster.

Because this MIC group contains a quite large group of strains with, among them, subgroups showing different hybridization characteristics to the set of probes, a further subdivision into MIC-types was envisaged.

Type MIC 1 harbours *M. intracellulare* s.s., together with some other MAC-strains. All MIC 1 type isolates, without exception, hybridize to the following probes: **myc1/myc22**, **mail** and **mac1**. The following probes can be used to make further subdivisions within the MIC 1 group : **mil11**, **min1**, **min2** to **2222**, **mil22** and

mhef1.

M. intracellulare sensu stricto strains (type MIC 1.1.a) can be distinguished from other subtypes in this group by virtue of probe **min1** which is positive only for this group of strains. All strains of type MIC 1.1.a strains are positive when tested with the *M. intracellulare* probe of the Gen-Probe Rapid Diagnostic system for MAC.

Type MIC 1.1.b and MIC 1.2 harbour strains which are highly related to *M. intracellulare*. They can be differentiated by using probes **mil11** and **mil22** (see Table 3). Further subdivision within these groups was not attempted although this could be achieved by using the probes : **min2**, **min22**, **min222** and **min2222**. Further subdivision might be of value for epidemiological reasons.

Only two of our collection of strains tested group as MIC 2 strains. One of these strains is a "*Mycobacterium lufu*" strain (ITG 4755). The specific probe pattern generated by these strains is characterized by a positive hybridization signal with the following probes : **myc1/myc22**, **mail**, **mil22**, **mah1** and **mal1**. Variable hybridization results are obtained with probes **min2222**, **mac1** and **mhef1**. The other probes are negative. It is not unlikely that MIC 2 would eventually prove to be a heterogeneous group when more strains of this type are being identified. The variable probes may help in a further differentiation, if this would become relevant.

Type MIC 3 groups a fairly high number of MAC-strains which are rather remotely related to *M. intracellulare s.s.* strains and most other MAC-strains. This cluster should be regarded as distinct from *M. avium* and *M. intracellulare* on genotypical grounds. All MIC 3 subtypes hybridize to probes **myc1/myc22**, **mail**, **mil22** and **mco1**. A positive signal with the latter probe (**mco1**) is characteristic for MIC 3 strains. Variable hybridization results are obtained with the following probes : **mac1**, **mhef1** and **mah1**. MIC 3 can be further subdivided into four subtypes by using three probes : **mth11**, **mth2** and **mef11**. Probe **mth2** is specific for type MIC 3.1 which encompasses a group of highly related MAC-strains isolated from immunocompromised human beings. Most MIC 3 strains are located in the MIC 3.1 subtype. Eventually species status may be assigned to this group of strains, as might also be the case for other groups of MAC strains, yet unnamed. In subtypes MIC 3.4, MIC 3.3 and MIC 3.2 only two, one and one strain are found respectively in our collection of strains tested.

2193101

72

Type MIC 4 is a collection of "MAIS" strains (including *M. malmoeense*) which are remotely related to *M. intracellulare*. The only probe of the above-described set which hybridizes to MIC 4, apart from the general myc1/myc22 probes, is the mail probe. This probe shows a broad specificity, hybridizing also with *M. avium*, *M. intracellulare* and other MIC strains and *M. scrofulaceum*.

M. scrofulaceum

All *M. scrofulaceum* strains tested reveal an identical hybridization pattern with the set of probes. A positive signal with probe msc1 is unique to *M. scrofulaceum* strains. The only other probes with a positive signal for this species are evidently myc1/myc22 and also mail.

M. kansasii

Probes mka3 and mka4 are specific for *M. kansasii*: i.e. a distinct positive signal is obtained on the LiPA strip when amplified DNA from the *M. kansasii* strains is used in the hybridization whilst with all other organisms tested the signal is absent. Although the sequences of probes mka1 and mka2 are not absolutely complementary to the target sequence (3 and 1 mismatches, respectively), these probes also proved to be useful since they hybridized exclusively to *M. kansasii* DNA and not to any other mycobacterial DNA tested under the conditions used (50°C, 3xSSC, 20% formamide). This illustrates that probes not necessarily have to match perfectly to the target to be useful, and that modifications in sequence and length may be allowed up to a certain degree.

M. chelonae

The species *M. chelonae* encompasses *M. chelonae* ssp. *chelonae* and *M. chelonae* ssp. *abscessus* strains. The spacer region was sequenced for one strain of each subspecies and small differences were noticed (SEQ ID NO 103 and SEQ ID NO 102). Probes mch1 and mch2 hybridize to both strains. All other probes are negative for these 2 strains except for myc1/myc22.

Upon testing of probes mch1 and mch2 with 2 additional *M. chelonae* strains not mentioned in table 4, i.e. *M. chelonae* 94-379 and *M. chelonae* 94-330, both obtained from the Institute of Tropical Medicine in Antwerp, Belgium, it appeared that they did not hybridize to probe mch1. This was confirmed by sequencing the spacer region of these two strains (SEQ ID NO 184). Cluster analysis of the spacer region with

other mycobacteria revealed that *M. chelonae* strains can be subdivided in two groups. A third probe *mch3* was designed to specifically detect this second group of strains, to which 94-379 and 94-330 belong.

This illustrates that the use of DNA probes derived from the 16S-23S rRNA spacer region can be helpful in differentiating different groups of strains, which belong to the same species according to the classical identification methods, and possibly can be used to detect and describe new species within the mycobacteria. In this case *mch2* detects all *M. chelonae* strains, whereas *mch1* and *mch3* differentiate between different subgroups.

10 *M. gordonae*

The five *M. gordonae* strains tested all hybridize to probe *mgo5*. Positive hybridization signals are also obtained with probes *myc1/myc22*, and some *M. gordonae* strains also hybridize to probes *mgo1* and *mgo2*.

other mycobacterial species

15 Strains belonging to other mycobacterial species than those mentioned above only hybridize to the general probes *myc1/myc22*. This indicates that these strains most probably belong to the genus *Mycobacterium*, but do not belong to one of the species or groups which can be specifically identified by using one or more of the other probes described.

20

In conclusion we can state that, according to the particular combinations of probes of the invention used, DNA probe tests at different levels can be provided.

When all probes are used in one and the same LiPA-test, differentiation at the species level as well as subtyping of certain groups of mycobacteria can be achieved. However, the probe-assembly on one strip could be restricted to those probes which are species-specific; in that case identification is performed at the species level. A further reduction of the number of probes on the strip might lead to the specific detection of only one or just a few species. Obviously, LiPA strips can be designed which solely attempt to subtype strains, e.g. those belonging to the *M. intracellulare* complex (MIC). Depending on the particular needs of the laboratoria performing diagnosis and/or typing of mycobacteria, all these different applications might be of value. However, it is clear that by using a combination of probes in a LiPA-format the amount of information obtained as to the identity of the organisms

present in the clinical sample, is considerably increased as compared to DNA probe tests using only a single probe. For some groups, or at least for further subdivision of some groups, a single probe uniquely hybridizing to this (sub)group could not be designed. In that case only probe-patterns are able to provide the information needed. For these applications
5 the LiPA is an advantageous format.

Table 3 : Different probe patterns obtained for mycobacterial (sub)species

[illegible]

2193101

76

Table 3: continued

Mycobacterium	mco1	mth11	mth2	mef11	mhef1	mah1	mal1	msc1	mka1,2,3,4	meh 1,2,3	ngo1,2	ngo5
M. tuberculosis	-	-	-	-	-	-	-	-	-	-	-	-
M. bovis	-	-	-	-	-	+	-	-	-	-	-	-
M. avium	-	-	-	-	-	-	-	-	-	-	-	-
M. paratuberculosis	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.1.a	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.1.b	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.2	-	-	-	-	±	-	-	-	-	-	-	-
MIC 2	-	-	-	-	±	+	+	-	-	-	-	-
MIC 3.4	+	-	-	+	+	±	-	-	-	-	-	-
MIC 3.3	+	+	-	+	+	+	-	-	-	-	-	-
MIC 3.1	+	+	+	-	±	+	-	-	-	-	-	-
MIC 3.2	+	-	-	-	+	+	w	-	-	-	-	-
MIC 4	-	-	-	-	-	-	-	-	-	-	-	-
M. scrofulaceum	-	-	-	-	-	-	-	+	-	-	-	-
M. kansasii	-	-	-	-	-	-	-	-	+	-	-	-
M. chelonae	-	-	-	-	-	-	-	-	-	±	-	-
M. goodii	-	-	-	-	-	-	-	-	-	-	+	-
Mycobacterium sp.	-	-	-	-	-	-	-	-	-	-	-	-

w : weak / v : very weak / ± : + or -, variable according to the strain tested

Table 4 Mycobacteria strains tested in LiPA

species/group	strain numbers from Institute of Tropical Medicine Antwerp (except those between parentheses)
M. tuberculosis complex	7602, 8004, 8017, 8647, 8872, 9081, 9129, 9173, 9517, (ATCC 27294), 8324, 8428
M. avium/ M. paratuberculosis	1101, 1983, 2070, 2074, 4176, 4189, 4191, 4193, 4197, 4204, 4386, 4991, 5872, 5874, 5884, 5887, 5893, 5894, 5897, 5903, 5904, 5905, 5927, 5983, 8180, 8750, (ATCC 25291), <u>M. paratub</u> : (316F), (2E)
M. intracellulare (MIC 1.1.a)	4199, 4208, 5701, 5880, 5906, 5908, 5909, 5913, 5915, 5917, 5918, 5920, 5921, 5924, 5925, 5929, 8713, 8717, 8718, 8720, 8721, 8722, 8732, 8740, 8741, 8742, 8744, 8747, 8749
MIC 1.1.b	8694, 8745, 8754 8708 5513, 8743 8054, 8190
MIC 1.2	8710, 8711, 8712, 8714, 8715, 8716, 8725, 8729, 8733, 8737, 8746, 8751, 8752 5919 8695 8748
MIC 2	5922 4755 (M. lufu)
MIC 3.4	1815 8707
MIC 3.3	5620
MIC 3.1	925, 926, 1329, 1788, 1794, 1812, 1818, 2069, 2073, 2076, 4541, 4543, 5074, 5280, 5789, 7395, 8739, 8753 8738
MIC 3.2	5765
M. scrofulaceum	4979, 4988, 5907, 8706, 8726, 8727, 8735, (MB022), (MB023), (MB024)
M. kansasii	4987, (ATCC 22478)
M. chelonae	4975, 9855
M. gordonae	7703, 7704, 7836, 7838, 8059
MIC 4	8723, 8724 8757 4842 (M. malmoeense)
other mycobacterial species	7732 (M. marinum), 94-123 (M. celatum), 778 (M. haemophilum), 8777 (M. genavense), 4484 (M. siniae), 4986 (M. xenopi), 4304 (M. fortuitum), 1837 (M. ulcerans)

EXAMPLE 3: Listeria

Listeria species are a group of Gram-positive rods widely spread in nature. Within this group it seems that only L. monocytogenes is pathogenic to humans and animals. L. monocytogenes is the causative agent of listeriosis, giving rise to meningitis, abortions, encephalitis and septicemia. Immunocompromised individuals, newborn infants and pregnant women are high risk groups for this foodborn disease. Most cases have been caused by the consumption of food of animal origin, particularly soft cheeses. Therefore, the presence of L. monocytogenes should be excluded from food. For safety measurements, in some countries, the absence of all Listeria species is required in food products.

The classical identification method for L. monocytogenes in dairy products involves an enrichment culture for 48 h and subsequently colony forming on selective agar medium for 48 h followed by a whole set of biochemical and morphological assays (Farber and Peterkin, 1991). This procedure could be very much simplified by the use of gene probes.

Several DNA probes are already described for the identification of L. monocytogenes. Some probes are derived from genes responsible for the pathogenicity of the organism, for instance the listeriolysin O gene (Datta et al., 1993) or the invasion-associated-protein (iap) (Bubert et al., 1992).

A commercially available identification system, based on a specific 16S rRNA probe, was introduced by GenProbe (Herman and De Ridder, 1993; Ninet et al., 1992).

These specific probes are used as confirmation assays on colonies obtained after enrichment and plating on selective agar medium.

Recently several publications reported on the use of the polymerase chain reaction to amplify the target region for the DNA probes, which can shorten the time of the assay without interfering with the specificity and the sensitivity of the assay. Different primer sets are described that can specifically amplify L. monocytogenes DNA. These primer sets were derived from the listeriolysin O gene (Golstein Thomas et al., 1991), and the iap gene (Jaton et al., 1992).

We used the 16S-23S rRNA gene spacer region as the target for the development of a genus-specific probe for Listeria and a probe specific for Listeria monocytogenes.

Using conserved primers derived from the 3' end of the 16S rRNA and the 5' end of the 23S rRNA (sequences are given in example 1) the spacer region was amplified using the

polymerase chain reaction and subsequently cloned in a suitable plasmid vector following the same procedures as in example 3.

Two amplicons differing in length (800 bp and 1100 bp) were obtained. Both PCR fragments were cloned for the following Listeria species :

- 5 - Listeria monocytogenes, serovar 4b, IHE (Instituut voor Hygiëne en Epidemiologie, Belgium)
- Listeria ivanovii CIP 78.42 (Collection Nationale de Cultures de Microorganismes de l'Institut Pasteur, France)
- Listeria seeligeri serovar 4a, nr. 42.68 (Bacteriologisches Institut, Südd,
10 Versuchs- und Forschungsanstalt für Milchwirtschaft Weihenstephan, Germany)

The sequence of the spacer region between the 16S and 23S rRNA gene was determined using the cloned material originating from the 800 bp PCR fragment and this was done for the three described Listeria species. Fig. 41 to 43 show the sequences of the different short spacer regions obtained. The sequence of this short spacer region of L.
15 monocytogenes was also retrieved from the EMBL databank (LMRGSPCR).

Based on this sequence information, following oligonucleotides for species-specific detection were chosen and chemically synthesized :

LMO-ICG-1 : AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

LMO-ICG-2 : TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC

20 LSE-ICG-1 : AGTTAGCATAAGTAGTGTA ACTATTTATGACACAAG

LIV-ICG-1 : GTTAGCATAAATAGGTA ACTATTTATGACACAAGTAAC

Also, a genus specific probe for Listeria was designed:

LIS-ICG-1 : CAAGTAACCGAGAATCATCTGAAAGTGAATC

The oligonucleotide-probes were immobilized on a membrane strip and following reverse
25 hybridization with biotinylated PCR fragments, the hybrids were visualized using a precipitation reaction. The hybridization results of different Listeria species are summarized in table 5.

Table 5

Species	n	LIS1	LMO1	LMO2	LSE1	LIV1
<u>L. monocytogenes</u>	1	+	+	+	—	—
<u>L. seeligeri</u>	2	+	+	±	+	±
<u>L. ivanovii</u>	3	+	±	—	±	+
<u>L. welshimeri</u>	3	+	+	±	—	—
<u>L. innocua</u>	2	+	+	+	—	—

These hybridization results show that probe LIS1 can detect all described Listeria species, but also that the species-specific probes cross-hybridize to each other. Hence, from this short spacer region probes with sufficient specificity could not be found.

For Listeria monocytogenes the 16S-23S rRNA gene spacer was also determined originating from the 1100 bp fragment. Fig. 45 shows the sequence obtained for this species. This sequence information was also obtained for L. seeligeri (see fig. 46) and partial sequence information of the large spacer region was obtained for L. ivanovii (see fig. 44).

Based on sequence alignment with L. seeligeri following oligonucleotide-probe was chosen to specifically detect L. monocytogenes.

LMO-ICG-3.: AGGCACTATGCTTGAAGCATCGC

Initial hybridization results (not shown) indicated that no cross-hybridization with other Listeria species was seen with this L. monocytogenes probe LMO3, and that all Listeria strains used hybridized to the general probe LIS1.

The oligonucleotide-probes, LIS1 for detection of all Listeria species and LMO3 for specific detection of L. monocytogenes, were immobilized on a membrane strip and hybridized to labeled amplicons, containing the 16S-23S rRNA spacer region, derived from different organisms. The hybridization results are shown in the following table.

An excellent specificity and sensitivity were obtained for probes LMO3 and LIS1 respectively at the species and genus level.

Table 6

	Taxa tested	n	LIS1	LMO3
5	<u>Listeria monocytogenes</u>	44	+	+
	<u>Listeria ivanovii</u>	10	+	-
	<u>Listeria seeligeri</u>	11	+	-
	<u>Listeria welshimeri</u>	16	+	-
	<u>Listeria innocua</u>	23	+	-
10	<u>Listeria murrayi</u>	3	+	-
	<u>Listeria grayi</u>	2	+	-
	<u>Brochotrix thermosphacta</u>	1	-	-
	<u>Brochotrix campestris</u>	1	-	-
	<u>Bacillus cereus</u>	3	-	-
	<u>Bacillus brevis</u>	2	-	-
15	<u>Bacillus coagulans</u>	1	-	-
	<u>Bacillus pumilis</u>	1	-	-
	<u>Bacillus macerans</u>	1	-	-
	<u>Bacillus lentus</u>	1	-	-
	<u>Bacillus firmus</u>	2	-	-
20	<u>Bacillus subtilis</u>	2	-	-
	<u>Bacillus megantum</u>	1	-	-
	<u>Enterococcus faecalis</u>	1	-	-
	<u>Enterococcus faecium</u>	1	-	-
	<u>Enterococcus durans</u>	1	-	-
25	<u>Lactococcus lactis</u>	3	-	-
	<u>Lactococcus casei</u>	1	-	-
	<u>Escherichia coli</u>	1	-	-
	<u>Hafnia halvei</u>	1	-	-
30	<u>Agrobacterium tumefaciens</u>	2	-	-
	<u>Mycoplasma dimorpha</u>	1	-	-
	<u>Clostridium tyrobutyricum</u>	1	-	-
	<u>Clostridium perfringens</u>	1	-	-
	<u>Clostridium sporogenes</u>	1	-	-
35	<u>Clostridium acetobutyricum</u>	1	-	-
	<u>Brucella abortus</u>	1	-	-
	<u>Brucella suis</u>	1	-	-
	<u>Brucella melitensis</u>	1	-	-
	<u>Staphylococcus aureus</u>	1	-	-
40	<u>Salmonella typhimurium</u>	1	-	-
	<u>Salmonella enteritidis</u>	1	-	-
	<u>Yersinia enterocolitica</u>	1	-	-

n: number of strains tested

2193101

82

These two probes can be used for the detection of Listeria species and Listeria monocytogenes directly on food samples or after enrichment of the samples in liquid broth. In both cases amplification problems can occur with the conserved primerset due to the enormous background flora in these samples.

5 To circumvent this problem, we designed several sets of primers derived from the 16S-23S rRNA spacer regions of Listeria species.

Primers LIS-P1 and LIS-P2 are upper primers, whereas LIS-P3 and LIS-P4 are lower primers. These primersets amplify the smaller 16S-23S rRNA spacer region as well as the larger spacer of Listeria species (except L. grayi and L. murrayi). If needed these primers
10 can be used in a nested PCR assay where LIS-P1/LIS-P4 are the outer primers and LIS-P2/LIS-P3 are the inner primers.

For the specific detection of Listeria monocytogenes probe LMO-ICG-3 was designed and derived from the large 16S-23S rRNA spacer region. In order to specifically amplify only this large spacer region for an improved detection of this pathogen directly in samples
15 a set of primers was derived from the part of sequence information from the large 16S-23S rRNA spacer region that is not present in the smaller rRNA spacer. For this aim, primers LIS-P5 and LIS-P6 are used as the upper primers and LIS-P7 is used as the lower primer.

	LIS-P1	: ACCTGTGAGTTTTTCGTTCTTCTC	71
	LIS-P2	: CTATTTGTTTCAGTTTTGAGAGGTT	72
20	LIS-P3	: ATTTTCCGTATCAGCGATGATAC	73
	LIS-P4	: ACGAAGTAAAGGTTGTTTTTCT	74
	LIS-P5	: GAGAGGTTACTCTCTTTTATGTCAG	75
	LIS-P6	: CTTTATGTCAGATAAAGTATGCAA	202
	LIS-P7	: CGTAAAAGGGTATGATTATTG	203

25 During the evaluation of the probes for Listeria spp. an organism was isolated from cheese that resembled Listeria according to the classical determination methods. This isolate (MB 405) showed the following characteristics (similar to Listeria spp.) : Gram positive, growth on Oxford and Tryptic Soy Agar, catalase positive. The only difference with the Listeria spp. was the motility, which was negative.

30 Using the conserved primers as described in example 1 in order to amplify the 16S-23S rRNA spacer region of this isolate MB 405, the same amplicon pattern was obtained with this strain as with Listeria spp. Hybridization of the amplicon showed that there was no

signal obtained with any of the probes for Listeria spp.

Sequencing of the 16S rRNA of isolate MB 405 and subsequent comparison with Listeria spp. and relatives showed that the organism was more closely related to Listeria spp. than to any other species described in the literature until now. Taxonomical studies will show if this isolate does or does not belong to the genus Listeria. This isolate, and subsequently isolated organisms from the same type, are referred to in this application as Listeria like organisms.

Isolate MB 405 seemed to contain at least 3 different 16S-23S rRNA spacer regions which were cloned and sequenced. Following alignment with Listeria spp. an oligonucleotide-probe was chosen to specifically detect Listeria-like strains:

LISP-ICG-1 : CGTTTTTCATAAGCGATCGCACGTT

Reverse hybridization reactions of this probe with the 16S-23S rRNA spacer regions of Listeria spp. showed that there was no cross-hybridization.

2193101

84

EXAMPLE 4: Chlamydia trachomatis

Chlamydia trachomatis is a small obligate intracellular gram-negative bacterium, which has 15 serovars (A-K, Ba, L1, L2, and L3) distinguished by the major outer membrane protein (MOMP) and contains a cryptic plasmid required for intracellular growth. The A-K and Ba serovars constitute the trachoma biovar, while the L1, L2, and L3 serovars constitute the LGV biovar.

Serovars A, B, Ba, and C are commonly associated with trachoma, the leading cause of preventable blindness worldwide. The D-K serovars are found mainly in sexually transmitted infections and are the major cause of cervicitis and pelvic inflammatory disease in women, and urethritis and epididymitis in men. Serovars L1, L2 and L3 are involved in lymphogranuloma venereum, a rare sexually transmitted disease.

Cell culture is regarded as the benchmark method for laboratory diagnosis, although specimen viability is difficult to maintain during transport and laboratory techniques are time-consuming and technically demanding. Therefore, a number of more rapid test kits were developed, such as an enzyme-linked immunosorbent assay, and direct fluorescent-antibody staining. However, none of these immunoassays have been shown to have high levels of sensitivity or specificity.

A nonisotopic DNA probe assay (Gen-Probe PACE; Woods et al., 1990) that detects chlamydial rRNA is commercially available. Recently, the polymerase chain reaction (PCR) method has been used for detection of Chlamydia infections. Detection was targeted at either the cryptic plasmid (Loeffelholz et al., 1992), or the *omp1* gene, which encodes for the major outer membrane protein (Taylor-Robinson et al., 1992). Compared with other techniques, PCR has higher sensitivity and specificity (Ossewaarde et al., 1992). None of these assays make use of DNA probes derived from the 16S-23S rRNA gene spacer region.

For a Chlamydia trachomatis L2 and a Chlamydia psittaci 6BC strain, a part of the ribosomal RNA cistron, containing the 16S-23S rRNA spacer region was amplified using conserved primers (see example 1) and subsequently cloned in a plasmid vector. The 16S-23S rRNA spacer region was sequenced using the dideoxychain terminating chemistry.

The sequence of the spacer region of both Chlamydia species is shown in fig. 47 to 48.

Based on this sequence information, following oligonucleotide-probes were chemically synthesized :

CHTR-ICG-1 : GGAAGAAGCCTGAGAAGGTTTCTGAC

CHTR-ICG-2 : GCATTATATGTAAGAGCAAGCATTCTATTTCA

5 CHTR-ICG-3 : GAGTAGCGTGGTGAGGACGAGA

CHPS-ICG-1 : GGATAACTGTCTTAGGACGGTTTGAC

The oligonucleotide-probes were immobilized in a line-wise fashion on a membrane strip and subsequently used in a reverse hybridization assay with biotinylated PCR products, containing the 16S-23S rRNA spacer region, as target.

10 Hybridizations were done in a solution of 3xSSC and 20% formamide (FA) at a temperature of 50°C.

The hybridization results with the different probes are shown in the following table.

Table 7

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Strains tested	CHTR1	CHTR2	CHTR3	CHPS1
<u>Chlamydia trachomatis</u> L2	+	+	+	-
<u>Chlamydia psittaci</u> 6BC	-	-	-	+
<u>Chlamydia psittaci</u> CP	-	-	-	+
<u>Chlamydia psittaci</u> TT	-	-	-	+
<u>Haemophilus ducreyi</u> CIP 542	-	-	-	-
<u>Haemophilus influenzae</u> NCTC 8143	-	-	-	-
<u>Neisseria gonorrhoeae</u> NCTC 8375	-	-	-	-
<u>Moraxella catarrhalis</u> LMG 5128	-	-	-	-
<u>Escherichia coli</u> B	-	-	-	-
<u>Streptococcus pneumoniae</u> S92-2102	-	-	-	-

30

As shown in the table at a hybridization temperature of 50°C the probes CHTR1, CHTR2 and CHTR3 are specific for Chlamydia trachomatis and probe CHPS1 is specific for Chlamydia psittaci.

35

Several clinical isolates, obtained from the SSDZ, Delft, Netherlands, identified as Chlamydia trachomatis using conventional methods were tested in a reverse hybridization assay with the different oligonucleotide-probes. All Chlamydia trachomatis specific probes gave a positive hybridization signal and none of the isolates reacted with the Chlamydia psittaci probe. For some clinical isolates the CHTR2 probe reacted significantly weaker than

CHTR1 or CHTR3. The spacer region of one of these isolates (94 M 1961) was sequenced (SEQ ID NO 197) and the sequence revealed one mismatch with the spacer sequence of strain L2. An additional probe (CHTR4) was derived from this new spacer sequence :

CHTR-ICG-4 : GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)

- 5 This probe gives a stronger hybridization signal than CHTR2 with some clinical isolates from Chlamydia trachomatis. It can be used alone, or in combination with the CHTR2 probe (e.g. both probes applied in one LiPA-line).

- 10 In order to develop very sensitive assays for the detection of Chlamydia trachomatis directly in clinical specimens a specific primerset was derived from the 16S-23S rRNA spacer region, CHTR-P1 (upper primer) and CHTR-P2 (lower primer), amplifying specifically the spacer region of Chlamydia species.

CHTR-P1 : AAGGTTTCTGACTAGGTTGGGC 69

CHTR-P2 : GGTGAAGTGCTTGCATGGATCT 70

EXAMPLE 6: Mycoplasma pneumoniae and Mycoplasma genitalium

Mycoplasmas are a group of the smallest prokaryotes known that are able to grow in cell-free media, lack a cell wall, and have very small genomes with a low G+C content. More than 100 different species have been isolated from humans, animals, plants, and insects.

In humans, mycoplasmas have been recognized either as pathogenic organisms or as commensals. The best known pathogen is Mycoplasma pneumoniae, the causative agent of primary atypical pneumonia, especially in children and young adults. The diagnosis of M. pneumoniae has been based on the direct isolation by the culture method or on the detection of specific antibodies against M. pneumoniae in the patient's serum.

Another pathogen, first isolated from urethral specimens from patients with nongonococcal urethritis, has been described as Mycoplasma genitalium. This mycoplasma has several properties in common with M. pneumoniae. Both species are pathogenic, and both possess the capability to adhere to erythrocytes, various tissue cells, glass, and plastic surfaces. Furthermore, M. genitalium and M. pneumoniae share antigens, giving rise to extensive cross-reactions in serological tests. The observation that M. genitalium could also be found in respiratory tract specimens from patients with pneumonia and isolated from a mixture with M. pneumoniae has raised questions to the possible pathogenicity of M. genitalium.

Since cultivation of both species is time-consuming and serology lacks specificity, more rapid and more specific assays were developed to identify these mycoplasmas. The use of hybridization assays with DNA probes was described for these species, but despite good specificities these tests do not allow the detection of low levels of M. pneumoniae or M. genitalium. So more recently, DNA hybridization techniques were developed using the polymerase chain reaction. M. pneumoniae-specific PCR assays have been reported using the P1 adhesin gene (Buck et al., 1992) and the 16S rRNA gene (Kuppeveld et al., 1992). Specific PCR assays for M. genitalium were described using sequences from the adhesin gene and the 16S rRNA gene.

The spacer sequences of clinical isolates of M. pneumoniae and M. genitalium (obtained from U. Göbel, University of Freiburg, Germany) were determined. They are shown in fig. 49 to 50. The sequences show some differences to those from other strains of

the same species deposited in the EMBL databank (MPMAC and MGG37 respectively). Based on this information four probes were derived: one general *Mycoplasma* probe, two *M. pneumoniae* specific, and one *M. genitalium* specific probe :

Mycoplasma-ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC

5 MPN-ICG-1: ATCGGTGGTAAATTAAACCCAAATCCCTGT

MPN-ICG-2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC

MGE-ICG-1: CACCCATTAATTTTTTCGGTGTTAAAACCC

10 The probes were applied to LiPA strips and hybridized under standard conditions (3X SSC, 20% formamide at 50°C) to amplified spacer material from four *M. pneumoniae* strains, one *M. genitalium* strain and twenty-two non-*Mycoplasma* species strains. The general probe hybridized only to the five *Mycoplasma* strains tested, while the specific probes hybridized only to strains of the species for which they were designed.

EXAMPLE 7: Other mycobacterial species

With the steady improvement of laboratory techniques the information on the systematics and clinical significance of the so called "potentially pathogenic environmental mycobacteria" increased rapidly. With the emergence of newly recognized diseases, additional syndromes associated with different mycobacterial species have emerged and have assumed major importance.

In order to extend the LiPA test for the simultaneous detection of different mycobacterial species as described in example 2, a new set of DNA probes was designed to specifically identify the following species : Mycobacterium ulcerans, Mycobacterium genavense, Mycobacterium xenopi, Mycobacterium simiae, Mycobacterium fortuitum, Mycobacterium malmoeense, Mycobacterium celatum and Mycobacterium haemophilum.

These probes were derived from the 16S-23S rRNA spacer region sequence. For the above mentioned species this information was obtained through direct sequencing of PCR products or after cloning of the PCR-amplified spacer region. The sequences obtained are represented in fig. 80 to 97, and in fig. 38 for M. malmoeense.

The sequences of the spacer region of the above-mentioned mycobacterial species were compared and aligned to those already described in example 2 or in publicly available sources. From the regions of divergence, species-specific DNA probes were designed. The probes were selected and designed in such a way that the desired hybridization behaviour (i.e. species-specific hybridization) was obtained under the same conditions as those specified for the other mycobacterial probes mentioned in example 2, i.e. 3X SSC, 20% deionized formamide, 50°C. This allows simultaneous detection of at least two, and possibly all, of the mycobacterial species described in the current invention.

The following oligonucleotide probes were designed from the spacer region sequence of respectively M. ulcerans, M. genavense, M. xenopi, M. simiae, M. fortuitum, M. malmoeense, M. celatum and M. haemophilum:

MUL-ICG-1 : GGTTCGGGATGTTGTCCCACC

MGV-ICG-1 : CGACTGAGGTCGACGTGGTGT

MGV-ICG-2 : GGTGTTTGAGCATTGAATAGTGGTTGC

MXE-ICG-1 : GTTGGGCAGCAGGCAGTAACC

MSI-ICG-1 : GCCGGCAACGGTTACGTGTTC

MFO-ICG-1 : TCGTTGGATGGCCTCGCACCT

MFO-ICG-2 : ACTTGGCGTGGGATGCGGGAA

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC

MML-ICG-2: TCTAAATGAACGCACTGCCGATGG

5 MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA

MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC

10 The probes were immobilized on a LiPA strip and hybridized with amplified biotinylated material derived from a set of representative mycobacterial species as described in example 2. Amplification of the spacer region was carried out by PCR using a primer set as described in example 2. The different strains used for specificity testing are shown in table 8 together with the hybridization results obtained. The strains were obtained from the collection of the Institute for Tropical Medicine, Antwerp, Belgium.

15 The probes tested (MSI-ICG1, MXE-ICG-1, MFO-ICG-1, MFO-ICG-2, MML-ICG-1, MML-ICG-2, MCE-ICG-1 and MHP-ICG-1) specifically detected M. simiae, M. xenopi, M. fortuitum, M. malmoeense, M. celatum and M. haemophilum respectively and showed no cross-hybridization with the other mycobacterial species tested. Thus, these probes allow a specific detection of mycobacterial species which were not further identifiable using the set of DNA probes described in example 2. M. malmoeense was classified in example 2 as a "MIC 4"-type, while the other species mentioned above were only hybridizing to the general probes MYC1/MYC22 for the genus Mycobacterium, and were thus classified in example 20 2 as "other mycobacterial species".

25 All tested M. genavense isolates reacted with MGIV-ICG1 and MGIV-ICG2, and not with MSI-ICG1 designed for M. simiae, closely related to M. genavense. A group of "intermediate" organisms, situated in between M. simiae and M. genavense, were received from the Tropical Institute of Medicine, Antwerp, where they were classified as "M. simiae - like" (strains 4358, 4824, 4833, 4844, 4849, 4857, 4859, 7375, 7379, 7730, 9745, 94-1228). These strains reacted only with probe MGIV-ICG2 and not with probe MSI-ICG1 which specifically detects M. simiae strains sensu stricto. Sequencing of the 16S-23S rRNA spacer region of two of these "M. simiae-like" isolates (strains 7379 and 9745) (see SEQ ID NO 161 and 162) confirmed that they were more closely related to M. genavense than to M. simiae. A new probe MGIV-ICG3 was designed to specifically detect this group of organisms, 30 which possibly belong to a new species.

MGV-ICG 3 : TCGGGCCGCGTGTTCGTCAAA

This illustrates again that the use of DNA probes derived from the 16S-23S spacer region can be helpful in differentiating different groups of strains, which are also found indeterminate by classical taxonomic criteria. The use of these DNA probes may possibly lead to the description of new (sub)species within mycobacteria. In this case, the MGV-1 probe would react only with M. genavense strains sensu stricto, MGV-3 probe would react only with the intermediate "M. simiae-like" strains, and MGV-2 probe would detect both types of strains.

The probe MUL-ICG-1 reacted with all M. ulcerans strains tested, but also showed cross-hybridization with M. marinum strain ITG 7732. Sequencing of the spacer region of this M. marinum strain indeed revealed an identical sequence to that of M. ulcerans strain 1837 (see fig. 80). Further differentiation between M. marinum and M. ulcerans can be done using a probe from the 16S-rRNA gene of M. ulcerans, part of which is co-amplified with the spacer region when primers MYC P1-P5 are used for amplification. A species-specific 16S rRNA probe for M. ulcerans, which can work under the same hybridization conditions as the spacer probes for mycobacterium species differentiation, is for example:

TGGCCGGTGCAAAGGGCTG (SEQ ID NO 216)

The above paragraph shows that, although it is preferable to use probes derived from the spacer region, it is also possible, and sometimes necessary, to combine the spacer probes with probes derived from other gene sequences, e.g. the 16S rRNA gene. Here again, these additional probes are selected such that they show the desired hybridization characteristics under the same hybridization and wash conditions as the spacer probes.

For M. kansasii, additional strains to the ones mentioned in example 2 have been tested with probes MKA-ICG-1, 2, 3 and 4 described in example 2. Since none of these probes was entirely satisfactory, additional probes were designed for M. kansasii detection. Therefore, the spacer region of some of the additional M. kansasii strains ITG 6328, 8698 and 8973 was sequenced (see fig.90 to 92). These strains were also obtained from the Institute of Tropical Medicine in Antwerp, Belgium. Apparently, M. kansasii strains constitute a quite heterogeneous group, with remarkable differences in the spacer sequence between different strains. Additional probes MKA-ICG-5, 6, 7, 8, 9 and 10 were designed, all hybridizing again under the same conditions as those earlier described, i.e. 3X SSC, 20% deionized formamide, 50°C. The probes were tested with a collection of test strains obtained from the

2193101

92

Institute of Tropical Medicine, Antwerp, Belgium, and results are shown in table 8.

None of the M. kansasii probes hybridizes with a species other than M. kansasii, as far as tested. However, due to the heterogeneous character of this species, none of the M. kansasii probes hybridizes with all M. kansasii strains. The different M. kansasii probes
5 recognize different strains of M. kansasii. This differential hybridization may be of clinical significance. On the other hand, if detection of all M. kansasii strains is desirable, a combination of different M. kansasii probes can be envisaged.

Table 8: additional mycobacterial probes

species/type	strain	MUL ICG-1	MGV ICG- 1 2 3	MXE ICG-1	MFO ICG-1 ICG-2	MSI ICG-1	MML ICG-1 ICG-2	MCE ICG-1	MHP ICG-1
M. tuberculosis	8004	-	-	-	-	-	-	-	-
M. avium	5887	-	-	-	-	-	-	-	-
M. intracellulare	5915 5913	-	-	-	-	-	-	-	-
MIC 3.1 strain	1812	-	-	-	-	-	-	-	-
MIC-4 strain	8724	-	-	-	-	-	-	-	-
M. scrofulaceum	4979	-	-	-	-	-	-	-	-
M. kansasii	4987 2795 6238 6362 8698 8973 8974 8971	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -
M. ulcerans	1837 3129 5114 5115	+ + + +	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
M. marinum	7732	+	-	-	-	-	-	-	-
M. malmoense	4832 4842	- -	- -	- -	- -	- -	+ +	- -	- -
M. goodii	7703	-	-	-	-	-	-	-	-

2193101

Table 8 continued

[illegible]

— = negative reaction, + = positive reaction, w = weak reaction, ± = variable reaction, blanc = non tested

Table 8 continued

species/type	strain	MKA ICG-3	MKA ICG-4	MKA ICG-5	MKA ICG-6	MKA ICG-7	MKA ICG-8	MKA ICG-9	MKA- ICG-10
<i>M. tuberculosis</i>	8004	-	-	-	-	-	-	-	-
<i>M. avium</i>	5887	-	-	-	-	-	-	-	-
<i>M. intracellulare</i>	5915 5913	-	-	-	-	-	-	-	-
MIC 3.1 strain	1812	-	-	-	-	-	-	-	-
MIC-4 strain	8724	-	-	-	-	-	-	-	-
<i>M. scrofulaceum</i>	4979	-	-	-	-	-	-	-	-
<i>M. kansasii</i>	4987 2795 6238 6362 8698 8973 8974 8971	+ + + + - - - -	+ + - - - - - -	- - + + - - - -	- - - - - + + +	- - - - + - - -	- - + + - + + +	- - + + + - - -	+ + + + w - - -
<i>M. ulcerans</i>	1837 3129 5114 5115	-	-	-	-	-	-	-	-
<i>M. marinum</i>	7732	-	-	-	-	-	-	-	-
<i>M. malmoense</i>	4832 4842	-	-	-	-	-	-	-	-
<i>M. goodii</i>	7703	-	-	-	-	-	-	-	-

2193101

Table 8 continued

[illegible]

EXAMPLE 8: Brucella

Brucellosis is a very widespread and economically important zoonosis which also affects humans.

For the identification of Brucella spp., mainly bacteriological and immunological detection techniques are being used. These tests are time-consuming and often give false-positive results. Quick and reliable identification methods are being developed, mainly based on DNA amplification and hybridization.

Specific detection of Brucella spp. based on the amplification of a 43 kDa outer membrane protein (Fekete A. et al., 1990) or of a part of the 16S rRNA gene (Herman and De Ridder, 1992) were already described.

In order to develop specific DNA probes and primers for the detection of Brucella spp. we analyzed the 16S-23S rRNA gene spacer region. Using conserved primers (sequences are given in example 1) the spacer region was amplified and subsequently cloned into the Bluescript SK+ vector following the same procedures as in example 1.

The obtained amplicon of about 1400 bp in length was cloned for the following Brucella species :

- Brucella abortus NIDO Tulya biovar 3 (SEQ ID NO 154)
- Brucella melitensis NIDO biovar 1 (SEQ ID NO 131)
- Brucella suis NIDO biovar 1 (SEQ ID NO 132)

*Hind*III digestion of the constructs, followed by subcloning of the obtained fragments (n=3) facilitated the sequencing of the spacer region for the three described Brucella spp..

Fig. 56, 57 and 79 represent the sequences of the spacer regions obtained for the above-mentioned strains of respectively Brucella melitensis, Brucella suis and Brucella abortus.

Due to the high homology of these spacer region sequences between different Brucella species, no species-specific DNA probes were deduced from this sequence information, and only genus-specific probes were designed.

For this purpose, the following probes were chemically synthesized:

- BRU-ICG 1 : CGTGCCGCCTTCGTTTCTCTTT
- BRU-ICG 2 : TTCGCTTCGGGGTGGATCTGTG
- BRU-ICG 3 : GCGTAGTAGCGTTTGCGTCGG
- BRU-ICG 4 : CGCAAGAAGCTTGCTCAAGCC

The oligonucleotides were immobilized on a membrane strip and following reverse

2193101

98

hybridization with biotinylated PCR fragments, the hybrids were visualized using a precipitation reaction. The hybridization results of the immobilized probes with different Brucella spp. and related organisms are represented in the table 9.

These hybridization results show that probes BRU-ICG 2, BRU-ICG 3 and BRU-ICG 4 are specific for Brucella spp. and can be used in a reverse hybridization assay for detection of these pathogens. Probe BRU-ICG 1 cross-hybridizes with Ochrobactrum antropi and Rhizobium loti strains, which are two taxonomically highly related organisms, but which are not expected to be present in the same sample material as used for Brucella detection.

As described in previous examples (e.g. 3 and 4) also for Brucella specific primers were chosen from the 16S-23S rRNA spacer region, in order to specifically amplify the spacer region from Brucella strains.

BRU-P1 and BRU-P2 are used as upper primers, while BRU-P3 and BRU-P4 are used as lower primers. When used in a nested PCR assay the combination BRU-P1/BRU-4 is the outer primerset whereas the combination BRU-P2/BRU-P3 is the inner primerset.

BRU-P1	: TCGAGAATTGGAAAGAGGTC	204
BRU-P2	: AAGAGGTCGGATTTATCCG	205
BRU-P3	: TTCGACTGCAAATGCTCG	206
BRU-P4	: TCTTAAAGCCGCATTATGC	207

TABLE 2

TAXA TESTED	n	BRU-ICG 1	BRU-ICG 2	BRU-ICG 3	BRU-ICG 4
<u>Brucella abortus</u>	6	+	+	+	+
<u>Brucella suis</u>	3	+	+	+	+
<u>Brucella melitensis</u>	4	+	+	+	+
<u>Brucella ovis</u>	2	+	+	+	+
<u>Brucella canis</u>	2	+	+	+	+
<u>Brucella neotomae</u>	1	+	+	+	+
<u>Phyllobacterium rubiacearum</u>	1	-	-	NT	NT
<u>Ochrobactrum anthropi</u>	8	+	-	-	-
<u>Agrobacterium tumefaciens</u>	2	-	-	NT	NT
<u>Agrobacterium rhizogenes</u>	1	-	-	NT	NT
<u>Mycoplana dimorpha</u>	1	-	-	NT	NT
<u>Rhizobium loti</u>	1	+	-	-	-
<u>Rhizobium meliloti</u>	1	-	-	NT	NT
<u>Rhizobium leguminosarum</u>	1	-	-	NT	NT
<u>Bradyrhizobium japonicum</u>	1	-	-	NT	NT
<u>Brochothrix thermosphacta</u>	1	-	-	NT	NT
<u>Brochothrix campestris</u>	1	-	-	NT	NT
<u>Bacillus cereus</u>	3	-	-	NT	NT
<u>Bacillus brevis</u>	2	-	-	NT	NT
<u>Bacillus coagulans</u>	1	-	-	NT	NT
<u>Bacillus pumilis</u>	1	-	-	NT	NT
<u>Bacillus macerans</u>	1	-	-	NT	NT
<u>Bacillus lentus</u>	1	-	-	NT	NT
<u>Bacillus firmus</u>	2	-	-	NT	NT
<u>Bacillus subtilis</u>	2	-	-	NT	NT
<u>Bacillus megaterium</u>	1	-	-	NT	NT
<u>Enterococcus faecalis</u>	1	-	-	NT	NT
<u>Enterococcus faecium</u>	1	-	-	NT	NT
<u>Enterococcus durans</u>	1	-	-	NT	NT
<u>Lactobacillus lactis</u>	3	-	-	NT	NT
<u>Lactobacillus casei</u>	1	-	-	NT	NT
<u>Leuconostoc lactis</u>	1	-	-	NT	NT
<u>Escherichia coli</u>	1	-	-	NT	NT
<u>Hafnia halvei</u>	1	-	-	NT	NT
<u>Clostridium tyrobutyricum</u>	1	-	-	NT	NT
<u>Clostridium perfringens</u>	1	-	-	NT	NT
<u>Clostridium sporogenes</u>	1	-	-	NT	NT
<u>Clostridium acetobutylicum</u>	1	-	-	NT	NT
<u>Staphylococcus aureus</u>	1	-	-	NT	NT
<u>Salmonella enteritidis</u>	1	-	-	NT	NT
<u>Yersinia enterocolitica</u>	1	-	-	NT	NT
<u>Listeria monocytogenes</u>	1	-	-	NT	NT
<u>Listeria ivanovii</u>	1	-	-	NT	NT
<u>Listeria seeligeri</u>	1	-	-	NT	NT
<u>Listeria welshimeri</u>	1	-	-	NT	NT
<u>Listeria innocua</u>	1	-	-	NT	NT
<u>Listeria murrayi</u>	1	-	-	NT	NT
<u>Listeria grayi</u>	1	-	-	NT	NT

NT = Not tested

n = number of strains tested

2193101

100

EXAMPLE 9: *Staphylococcus aureus*

Staphylococcus aureus is the staphylococcal species most commonly associated with human and animal infections. *Staphylococcus aureus* strains have been identified as important etiologic agents in both community-acquired and nosocomial infections. Recently nosocomial infection with methicillin-resistant *S. aureus* (MRSA) appear to be increasingly prevalent in many countries. The strains belonging to this species are also causative agents of food spoilage and poisoning.

In order to discriminate in a fast and specific way *S. aureus* strains from other staphylococci, the use of molecular techniques based on DNA probes and/or PCR were already described in the literature. Examples of target genes used for the development of these DNA based assays are the 16S rRNA gene (De Buyser et al., 1992; Geha et al., 1994), the *mecA* gene (Ubukata et al., 1992; Shimaoka et al., 1994) and the *nuc* gene (Brakstad et al., 1992; Chesneau et al., 1993).

As a target for the development of specific DNA probes we chose the 16S-23S rRNA gene spacer region. Amplification using conserved primers derived from the 16S and the 23S rRNA genes (sequences, see example 1) showed that the pattern obtained was not similar in all *S. aureus* strains tested. A lot of variation was seen in either the number of fragments obtained and in the size of these different fragments.

One spacer region from strain UZG 5728 and four spacer regions (differing in length) from strain UZG 6289 were cloned into Bluescript SK+ vector and subsequently sequenced. The sequences are represented in fig. 64 to fig. 68 (SEQ ID NO 139 to SEQ ID NO 143). For the development of specific DNA probes these different spacer regions were compared to each other and to the spacer region derived from *Staphylococcus epidermidis* strain UZG CNS41 (SEQ ID NO 144).

The following probes were chemically synthesized :

STAU-ICG 1 : TACCAAGCAAAACCGAGTGAATAAAGAGTT

STAU-ICG 2 : CAGAAGATGCGGAATAACGTGAC

STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC

STAU-ICG 4 : GAACGTAACCTTCATGTTAACGTTTGACTTAT

The oligonucleotides were immobilized on a membrane strip and following reverse hybridization with biotinylated PCR fragments, the hybrids were visualized using a

colorimetric precipitation reaction.

The hybridization results of the immobilized probes with different *Staphylococcus* spp. and non-staphylococcal organisms are represented in Table 10.

These hybridization results show that only probes STAU-ICG 3 and STAU-ICG 4 are specific for *Staphylococcus aureus* strains. Probe STAU-ICG 1 reacts with all *Staphylococcus* spp. tested and probe STAU-ICG 2 cross-hybridizes with the *S. lugdinensis* strain.

Neither probe STAU-ICG 3 nor probe STAU-ICG 4 detects all *S. aureus* strains tested, but when both probes are used simultaneously in a LiPA assay, all *S. aureus* strains tested hybridize with one of these probes or with both.

2193101

102

Table 10

Strains tested	n	STAU-ICG 1	STAU-ICG 2	STAU-ICG 3	STAU-ICG 4
<i>Staphylococcus aureus</i>	13	+	+	+	+
<i>Staphylococcus aureus</i>	10	+	+	-	+
<i>Staphylococcus aureus</i>	3	+	+	w	+
<i>staphylococcus aureus</i>	1	+	+	+	-
<i>Staphylococcus epidermidis</i>	11	+	-	-	-
<i>Staphylococcus saprophyticus</i>	1	+	-	-	-
<i>Staphylococcus haemolyticus</i>	1	+	-	-	-
<i>Staphylococcus capitis</i>	1	+	-	-	-
<i>Staphylococcus lugdinensis</i>	1	+	+	-	-
<i>Staphylococcus hominis</i>	1	+	-	-	-
<i>Bordetella pertussis</i>	1	+	-	-	-
<i>Bordetella parapertussis</i>	1	-	-	-	-
<i>Bordetella bronchiseptica</i>	1	-	-	-	-
<i>Mycobacterium tuberculosis</i>	1	-	-	-	-
<i>Mycobacterium avium</i>	1	-	-	-	-
<i>Moraxella catarrhalis</i>	4	-	-	-	-
<i>Haemophilus influenzae</i>	2	-	-	-	-
<i>Streptococcus pneumoniae</i>	3	-	-	-	-
<i>Pseudomonas cepacia</i>	1	-	-	-	-
<i>Pseudomonas aeruginosa</i>	3	-	-	-	-
<i>Acinetobacter calcoaceticus</i>	1	-	-	-	-

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2193101

104

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2193101

108

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2193101

110

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CLAIMS

1. A method for the detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms, in a sample, comprising the steps of:

(i) releasing or isolating or concentrating polynucleic acids from micro-organisms in the sample;

(ii) amplifying a 16S-23S rRNA spacer region, or a part thereof, in the polynucleic acids, with at least one primer pair;

(iii) simultaneously hybridizing the polynucleic acids with a set of probes comprising at least two probes at a hybridization and wash temperature of 50°C in a hybridization and wash medium of 3xSSC and 20% formamide, wherein said probes are selected from a panel of probes, wherein said panel of probes comprises two or more probes having a probe sequence selected from the group consisting of:

MYC-ICG-1: ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)

MYC-ICG-22: CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)

MTB-ICG-1: GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)

MTB-ICG-2: GACTTGTTCCAGGTGTTGTCCAC (SEQ ID NO 4)

MTB-ICG-3: CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)

MAI-ICG-1: CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)

MIL-ICG-11: GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)

MIL-ICG-22: TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)

MAC-ICG-1: CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)

MAV-ICG-1: TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)

MAV-ICG-22: GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)

MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)

MIN-ICG-2: GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)

MIN-ICG-22: CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)

MIN-ICG-222: TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)

MIN-ICG-2222: GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)

MAL-ICG-1: ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)

MHEF-ICG-1: TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)

MAH-ICG-1: GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG (SEQ ID NO 19)

MCO-ICG-11: TGGCCGGCGGTGTTTCATCGAAA (SEQ ID NO 20)

MTH-ICG-11: GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)

MTH-ICG-2: GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)

MEF-ICG-11: ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)

MSC-ICG-1: TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)

MKA-ICG-1: GATGCGTTTGCTACGGGTAGCGT (SEQ ID NO 25)

MKA-ICG-2: GATGCGTTGCCTACGGGTAGCGT (SEQ ID NO 26)

MKA-ICG-3: ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)

MKA-ICG-4: CGGGCTCTGTTTCGAGAGTTGTC (SEQ ID NO 28)

MCH-ICG-1: GGTGTGGACTTTGACTTCTGAATAG (SEQ ID NO 29)

MCH-ICG-2: CGGCAAAACGTCGGACTGTCA (SEQ ID NO 30)

MCH-ICG-3: GGTGTGGTCCTTGACTTATGGATAG (SEQ ID NO 210)

MGO-ICG-1: AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)

MGO-ICG-2: GTATGCGTTGTCGTTTCGCGGC (SEQ ID NO 32)

MGO-ICG-5: CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)

MUL-ICG-1: GGTTCGCGGATGTTGTCCCACC (SEQ ID NO 175)
 MGV-ICG-1: CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)
 MGV-ICG-2: GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)
 MGV-ICG-3: TCGGGCCGCGTGTTTCGTCAAA (SEQ ID NO 211)
 MXE-ICG-1: GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)
 MSI-ICG-1: CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)
 MFO-ICG-1: TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)
 MFO-ICG-2: ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)
 MKA-ICG-5: CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)
 MKA-ICG-6: GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)
 MKA-ICG-7: TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)
 MKA-ICG-8: GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)
 MKA-ICG-9: GATGCGTTGCCCCCTACGGG (SEQ ID NO 186)
 MKA-ICG-10: CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)
 MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)
 MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)
 MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)
 MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)
 PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)
 PA-ICG 2: TGAATGTTTCGTGGATGAACATTGATT (SEQ ID NO 35)
 PA-ICG 3: CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)
 PA-ICG 4: TGAATGTTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC
 (SEQ ID NO 37)
 PA-ICG 5: CTCTTTCCTGTTGATCATTCAAGTCAAG (SEQ ID NO 38)
 LIS-ICG 1: CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)
 LMO-ICG 1: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
 (SEQ ID NO 40)
 LMO-ICG 2: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC (SEQ ID NO 41)
 LMO-ICG 3: AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)
 LIV-ICG 1: GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC
 (SEQ ID NO 43)
 LSE-ICG 1: AGTTAGCATAAGTAGTGTAACCTATTTATGACACAAG (SEQ ID NO 44)
 LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)
 CHTR-ICG 1: GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)
 CHTR-ICG 2: GCATTTATATGTAAGAGCAAGCATTCTATTCA (SEQ ID NO 46)
 CHTR-ICG 3: GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)
 CHPS-ICG 1: GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)
 MPN-ICG 1: ATCGGTGGTAAATTAACCCAAATCCCTGT (SEQ ID NO 49)
 MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)
 MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAACCC (SEQ ID NO 51)
 Mycoplasma-ICG: CAAAACCTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)
 STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)
 STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)
 STAU-ICG 3: AACGAAGCCGTATGTGAGCATTGAC (SEQ ID NO 55)
 STAU-ICG 4: GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)
 ACI-ICG 1: GCTTAAGTGACAGTGCTCTAAACTGA (SEQ ID NO 57)
 ACI-ICG 2: CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)
 BRU-ICG 1: CGTGCCGCCTTCGTTTCTCTTT (SEQ ID NO 59)
 BRU-ICG 2: TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)
 BRU-ICG 3: GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)
 BRU-ICG 4: CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)

SALM-ICG 1: CAAAACCTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)
 SALM-ICG 2: GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)
 STY-ICG 1: GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)
 SED-ICG 1: GCGGTAATGTGTGMAGCGTTGCC (SEQ ID NO 64)
 YEC-ICG 1: GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2: GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3: GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)
 CHTR-ICG 4: GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence;

(iv) detecting hybrids formed in step (iii); and,
 (v) identifying a micro-organism present in the sample based on hybrids detected in step (iv).

2. The method according to claim 1, wherein said sample originates from a respiratory tract and wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MYC-ICG-1: ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22: CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)
 MTB-ICG-1: GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2: GACTTGTTCCAGGTGTTGTCCAC (SEQ ID NO 4)
 MTB-ICG-3: CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)
 MAI-ICG-1: CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
 MIL-ICG-11: GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)
 MIL-ICG-22: TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)
 MAC-ICG-1: CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
 MAV-ICG-1: TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
 MAV-ICG-22: GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)
 MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
 MIN-ICG-2: GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)
 MIN-ICG-22: CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)
 MIN-ICG-222: TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)
 MIN-ICG-2222: GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)
 MAL-ICG-1: ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
 MHEF-ICG-1: TGGACGAAAACCGGGTGACAA (SEQ ID NO 18)
 MAH-ICG-1: GTGTAATTTCTTTTTTA ACTCTTGTGTGTAAGTAAGTG (SEQ ID NO 19)
 MCO-ICG-11: TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
 MTH-ICG-11: GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
 MTH-ICG-2: GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
 MEF-ICG-11: ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)
 MSC-ICG-1: TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)
 MKA-ICG-1: GATGCGTTTGCTACGGGTAGCGT (SEQ ID NO 25)
 MKA-ICG-2: GATGCGTTGCCTACGGGTAGCGT (SEQ ID NO 26)
 MKA-ICG-3: ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)
 MKA-ICG-4: CGGGCTCTGTTTCGAGAGTTGTC (SEQ ID NO 28)
 MKA-ICG-5: CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)

MKA-ICG-6: GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)
 MKA-ICG-7: TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)
 MKA-ICG-8: GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)
 MKA-ICG-9: GATGCGTTGCCCCTACGGG (SEQ ID NO 186)
 MKA-ICG-10: CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)
 MCH-ICG-1: GGTGTGGACTTTGACTTCTGAATAG (SEQ ID NO 29)
 MCH-ICG-2: CGGCAAAACGTCGGACTGTCA (SEQ ID NO 30)
 MCH-ICG-3: GGTGTGGTCCTTGACTTATGGATAG (SEQ ID NO 210)
 MGO-ICG-1: AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)
 MGO-ICG-2: GTATGCGTTGTCGTTTCGCGGC (SEQ ID NO 32)
 MGO-ICG-5: CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)
 MUL-ICG-1: GGTTTCGGGATGTTGTCCCACC (SEQ ID NO 175)
 MGv-ICG-1: CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)
 MGv-ICG-2: GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)
 MGv-ICG-3: TCGGGCCGCGTGTTTCGTCAAA (SEQ ID NO 211)
 MXE-ICG-1: GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)
 MSI-ICG-1: CCGGCAACGGTTACGTGTTT (SEQ ID NO 179)
 MFO-ICG-1: TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)
 MFO-ICG-2: ACTTGGCGTGCGGATGCGGGAA (SEQ ID NO 181)
 MML-ICG-1: CGGATCGATTGAGTGCTTGTCCT (SEQ ID NO 188)
 MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)
 MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)
 MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)
 PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)
 PA-ICG 2: TGAATGTTTCGTGGATGAACATTGATT (SEQ ID NO 35)
 PA-ICG 3: CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)
 PA-ICG 4: TGAATGTTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC
 (SEQ ID NO 37)
 PA-ICG 5: CTCTTTCCTGCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)
 MPN-ICG 1: ATCGGTGGTAAATTAACCCAAATCCCTGT (SEQ ID NO 49)
 MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)
 MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAACCC (SEQ ID NO 51)
 Mycoplasma-ICG: CAAAACCTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)
 STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)
 STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)
 STAU-ICG 3: AACGAAGCCGTATGTGAGCATTGAC (SEQ ID NO 55)
 STAU-ICG 4: GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)
 ACI-ICG 1: GCTTAAGTGACAGTGCTCTAAACTGA (SEQ ID NO 57)
 ACI-ICG 2: CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

3. The method according to claim 1, wherein said sample is a sample taken from cerebrospinal fluid, and wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MYC-ICG-1: ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22: CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)

MTB-ICG-1: GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2: GACTTGTTCCAGGTGTTGTCCAC (SEQ ID NO 4)
 MTB-ICG-3: CGGCTAGCGGTGGCGTGTCT (SEQ ID NO 5)
 LIS-ICG 1: CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)
 LMO-ICG 1: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
 (SEQ ID NO 40)
 LMO-ICG 2: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTTC (SEQ ID NO 41)
 LMO-ICG 3: AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)
 LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

4. The method according to claim 1, wherein said sample is from a urogenital tract, and wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

CHTR-ICG 1: GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)
 CHTR-ICG 2: GCATTTATATGTAAGAGCAAGCATTCTATTTCA (SEQ ID NO 46)
 CHTR-ICG 3: GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)
 CHTR-ICG 4: GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)
 CHPS-ICG 1: GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)
 MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)
 MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)
 Mycoplasma-ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

5. The method according to claim 1, wherein said sample is from a food, and wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

LIS-ICG 1: CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)
 LMO-ICG 1: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
 (SEQ ID NO 40)
 LMO-ICG 2: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTTC (SEQ ID NO 41)
 LMO-ICG 3: AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)
 LIV-ICG 1: GTTAGCATAAATAGGTAACATTTATGACACAAGTAAC
 (SEQ ID NO 43)
 LSE-ICG 1: AGTTAGCATAAGTAGTGTAACATTTATGACACAAG (SEQ ID NO 44)
 LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)
 STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)
 STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)
 STAU-ICG 3: AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)
 STAU-ICG 4: GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)
 BRU-ICG 1: CGTGCCGCCTTCGTTTCTCTTT (SEQ ID NO 59)
 BRU-ICG 2: TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)
 BRU-ICG 3: GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)

BRU-ICG 4: CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)
 SALM-ICG 1: CAAAAGTACTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)
 SALM-ICG 2: GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)
 STY-ICG 1: GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)
 SED-ICG 1: GCGGTAATGTGTGMAGCGTTGCC (SEQ ID NO 64)
 YEC-ICG 1: GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2: GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3: GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

6. The method according to claim 1, wherein said sample is from a gastrointestinal tract of a patient, and wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

SALM-ICG 1: CAAAAGTACTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)
 SALM-ICG 2: GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)
 STY-ICG 1: GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)
 SED-ICG 1: GCGGTAATGTGTGMAGCGTTGCC (SEQ ID NO 64)
 YEC-ICG 1: GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2: GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3: GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

7. The method according to claim 1, for detecting and identifying one or more strains of *Mycobacterium* species or subspecies, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MYC-ICG-1: ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)
 MYC-ICG-22: CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)
 MTB-ICG-1: GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2: GACTTGTTCCAGGTGTTGTCCAC (SEQ ID NO 4)
 MTB-ICG-3: CGGCTAGCGGTGGCGTGTCT (SEQ ID NO 5)
 MAI-ICG-1: CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
 MIL-ICG-11: GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)
 MIL-ICG-22: TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)
 MAC-ICG-I: CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
 MAV-ICG-1: TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
 MAV-ICG-22: GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)
 MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
 MIN-ICG-2: GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)
 MIN-ICG-22: CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)
 MIN-ICG-222: TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)
 MIN-ICG-2222: GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)

MAL-ICG-1: ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
 MHEF-ICG-1: TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)
 MAH-ICG-1: GTGTAATTTCTTTTTTA ACTCTTGTGTGTAAGTAAGTG
 (SEQ ID NO 19)
 MCO-ICG-11: TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
 MTH-ICG-11: GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
 MTH-ICG-2: GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
 MEF-ICG-11: ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)
 MSC-ICG-1: TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)
 MKA-ICG-1: GATGCGTTTGCTACGGGTAGCGT (SEQ ID NO 25)
 MKA-ICG-2: GATGCGTTGCCTACGGGTAGCGT (SEQ ID NO 26)
 MKA-ICG-3: ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)
 MKA-ICG-4: CGGGCTCTGTTTCGAGAGTTGTC (SEQ ID NO 28)
 MKA-ICG-5: CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)
 MKA-ICG-6: GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)
 MKA-ICG-7: TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)
 MKA-ICG-8: GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)
 MKA-ICG-9: GATGCGTTGCCCCCTACGGG (SEQ ID NO 186)
 MKA-ICG-10: CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)
 MCH-ICG-1: GGTGTGGACTTTGACTTCTGAATAG (SEQ ID NO 29)
 MCH-ICG-2: CGGCAAAACGTCGGACTGTCA (SEQ ID NO 30)
 MGO-ICG-1: AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)
 MGO-ICG-2: GTATGCGTTGTCGTTTCGCGGC (SEQ ID NO 32)
 MGO-ICG-5: CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)
 MUL-ICG-1: GGTTTCGGGATGTTGTCCCACC (SEQ ID NO 175)
 MGV-ICG-1: CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)
 MGV-ICG-2: GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)
 MGV-ICG-3: TCGGGCCGCGTGTTTCGTCAAA (SEQ ID NO 211)
 MXE-ICG-1: GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)
 MSI-ICG-1: CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)
 MFO-ICG-1: TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)
 MFO-ICG-2: ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)
 MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC (SEQ ID NO 188)
 MML-ICG-2: TCTAAATGAACGCACTGCCGATGG (SEQ ID NO 189)
 MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)
 MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

8. The method according to claim 7, for detecting and identifying one or more *Mycobacterium* tuberculosis complex strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MTB-ICG-1: GGGTGCATGACAACAAAGTTGGCCA (SEQ ID NO 3)
 MTB-ICG-2: GACTTGTTCCAGGTGTTGTCCAC (SEQ ID NO 4)
 MTB-ICG-3: CGGCTAGCGGTGGCGTGTTCT (SEQ ID NO 5)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

9. The method according to claim 7, for detecting and identifying one or more *Mycobacterium* strains from the MAIS-complex, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MAI-ICG-1: CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
 MIL-ICG-11: GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)
 MIL-ICG-22: TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)
 MAC-ICG-1: CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
 MAV-ICG-1: TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
 MAV-ICG-22: GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)
 MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
 MIN-ICG-2: GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)
 MIN-ICG-22: CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)
 MIN-ICG-222: TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)
 MIN-ICG-2222: GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)
 MAL-ICG-1: ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
 MHEF-ICG-1: TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)
 MAH-ICG-1: GTGTAATTTCTTTTAACTCTTGTGTGTAAGTAAGTG
 (SEQ ID NO 19)
 MCO-ICG-11: TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
 MTH-ICG-11: GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
 MTH-ICG-2: GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
 MEF-ICG-11: ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)
 MSC-ICG-1: TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

10. The method according to claim 9, for detecting and identifying one or more *M. avium* or *M. paratuberculosis* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MAV-ICG-1: TCGGTCCGTCCGTGTGGAGTC (SEQ ID NO 10)
 MAV-ICG-22: GTGGCCGGCGTTCATCGAAA (SEQ ID NO 11)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

11. The method according to claim 9, for detecting and identifying one or more *Mycobacterium intracellulare* strains and MIC strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MAI-ICG-1: CAACAGCAAATGATTGCCAGACACAC (SEQ ID NO 6)
MIL-ICG-11: GAGGGGTTCCCGTCTGTAGTG (SEQ ID NO 7)
MIL-ICG-22: TGAGGGGTTCTCGTCTGTAGTG (SEQ ID NO 8)
MAC-ICG-I: CACTCGGTCGATCCGTGTGGA (SEQ ID NO 9)
MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)
MIN-ICG-2: GCTGATGCGTTCGTCGAAATGTGTA (SEQ ID NO 13)
MIN-ICG-22: CTGATGCGTTCGTCGAAATGTGT (SEQ ID NO 14)
MIN-ICG-222: TGATGCGTTCGTCGAAATGTGT (SEQ ID NO 15)
MIN-ICG-2222: GGCTGATGCGTTCGTCGAAATGTGTAA (SEQ ID NO 16)
MAL-ICG-1: ACTAGATGAACGCGTAGTCCTTGT (SEQ ID NO 17)
MHEF-ICG-1: TGGACGAAAACCGGGTGCACAA (SEQ ID NO 18)
MAH-ICG-1: GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG
(SEQ ID NO 19)
MCO-ICG-11: TGGCCGGCGTGTTTCATCGAAA (SEQ ID NO 20)
MTH-ICG-11: GCACTTCAATTGGTGAAGTGCGAGCC (SEQ ID NO 21)
MTH-ICG-2: GCGTGGTCTTCATGGCCGG (SEQ ID NO 22)
MEF-ICG-11: ACGCGTGGTCCTTCGTGG (SEQ ID NO 23)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

12. The method according to claim 9, for detecting and identifying one or more *Mycobacterium intracellulare* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MIN-ICG-1: GCATAGTCCTTAGGGCTGATGCGTT (SEQ ID NO 12)

or a probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

13. The method according to claim 9, for detecting and identifying one or more *Mycobacterium scrofulaceum* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MSC-ICG-1: TCGGCTCGTTCTGAGTGGTGTC (SEQ ID NO 24)

or a probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

14. The method according to claim 7, for detecting and identify one or more *Mycobacterium kansasii* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MKA-ICG-1: GATGCGTTTGCTACGGGTAGCGT (SEQ ID NO 25)
MKA-ICG-2: GATGCGTTGCCTACGGGTAGCGT (SEQ ID NO 26)
MKA-ICG-3: ATGCGTTGCCCTACGGGTAGCGT (SEQ ID NO 27)
MKA-ICG-4: CGGGCTCTGTTCGAGAGTTGTC (SEQ ID NO 28)

120

MKA-ICG-5: CCCTCAGGGATTTTCTGGGTGTTG (SEQ ID NO 182)
 MKA-ICG-6: GGACTCGTCCAAGAGTGTTGTCC (SEQ ID NO 183)
 MKA-ICG-7: TCGGGCTTGGCCAGAGCTGTT (SEQ ID NO 184)
 MKA-ICG-8: GGGTGCGCAACAGCAAGCGA (SEQ ID NO 185)
 MKA-ICG-9: GATGCGTTGCCCCTACGGG (SEQ ID NO 186)
 MKA-ICG-10: CCCTACGGGTAGCGTGTTCTTTTG (SEQ ID NO 187)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

15. The method according to claim 7, for detecting and identifying one or more *Mycobacterium chelonae* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MCH-ICG-1: GGTGTGGACTTTGACTTCTGAATAG (SEQ ID NO 29)
 MCH-ICG-2: CGGCAAAACGTCGGACTGTCA (SEQ ID NO 30)
 MCH-ICG-3: GGTGTGGTCCTTGACTTATGGATAG (SEQ ID NO 210)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

16. The method according to claim 7, for detecting and identifying one or more *Mycobacterium gordonae* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MGO-ICG-1: AACACCCTCGGGTGCTGTCC (SEQ ID NO 31)
 MGO-ICG-2: GTATGCGTTGTCGTTTCGCGGC (SEQ ID NO 32)
 MGO-ICG-5: CGTGAGGGGTCATCGTCTGTAG (SEQ ID NO 33)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

17. The method according to claim 7, for detecting and identifying one or more *Mycobacterium ulcerans* strains or *M. marinum* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MUL-ICG-1: GGTTTCGGGATGTTGTCCCACC (SEQ ID NO 175)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

18. The method according to claim 7, for detecting and identifying one or more *Mycobacterium genavense* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MGV-ICG-1: CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)
MGV-ICG-2: GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)
MGV-ICG-3: TCGGGCCGCGTGTTCGTCAAA (SEQ ID NO 211)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

19. The method according to claim 7, for detecting and identifying one or more *Mycobacterium xenopi* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MXE-ICG-1: GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

20. The method according to claim 7, for detecting and identifying one or more *Mycobacterium simiae* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MSI-ICG-1: CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

21. The method according to claim 7, for detecting and identifying one or more *Mycobacterium fortuitum* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MFO-ICG-1: TCGTTGGATGGCCTCGCACCT (SEQ ID NO 180)
MFO-ICG-2: ACTTGGCGTGGGATGCGGGAA (SEQ ID NO 181)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

22. The method according to claim 7, for detecting and identifying one or more *Mycobacterium celatum* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA (SEQ ID NO 190)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

23. The method according to claim 7, for detecting and identifying one or more *Mycobacterium haemophilum* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC (SEQ ID NO 191)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

24. The method according to claim 7, for detecting and identifying one or more *Mycobacterium* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MYC-ICG-1: ACTGGATAGTGGTTGCGAGCATCTA (SEQ ID NO 1)

MYC-ICG-22: CTTCTGAATAGTGGTTGCGAGCATCT (SEQ ID NO 2)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

25. The method according to claim 1, for detecting and identifying one or more *Mycoplasma* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MPN-ICG 1: ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

MGE-ICG 1: CACCCATTAATTTTTTCGGTGTTAAAACCC (SEQ ID NO 51)

Mycoplasma-ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

26. The method according to claim 25, for detecting and identifying one or more *Mycoplasma pneumoniae* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

MPN-ICG 1: ATCGGTGGTAAATTAAACCCAAATCCCTGT (SEQ ID NO 49)

MPN-ICG 2: CAGTTCTGAAAGAACATTTCCGCTTCTTTC (SEQ ID NO 50)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

27. The method according to claim 25, for detecting and identifying one or more *Mycoplasma genitalium* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises a probe having the probe sequence:

MGE-ICG 1: CACCCATTAATTTTTTCGGTGTAAAACCC (SEQ ID NO 51)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

28. The method according to claim 1, for detecting and identifying one or more *Pseudomonas* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 2: TGAATGTTCGTGGATGAACATTGATT (SEQ ID NO 35)

PA-ICG 3: CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)

PA-ICG 4: TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC (SEQ ID NO 37)

PA-ICG 5: CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

29. The method according to claim 28, for detecting and identifying one or more *Pseudomonas aeruginosa* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

PA-ICG 1: TGGTGTGCTGCGTGATCCGAT (SEQ ID NO 34)

PA-ICG 2: TGAATGTTCGTGGATGAACATTGATT (SEQ ID NO 35)

PA-ICG 3: CACTGGTGATCATTCAAGTCAAG (SEQ ID NO 36)

PA-ICG 4: TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC (SEQ ID NO 37)

PA-ICG 5: CTCTTTCACCTGGTGATCATTCAAGTCAAG (SEQ ID NO 38)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

30. The method according to claim 1, for detecting and identifying one or more *Staphylococcus* species in a sample, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

STAU-ICG 1: TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)

STAU-ICG 2: CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)

STAU-ICG 3: AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)

STAU-ICG 4: GAACGTAACCTCATGTAAACGTTTGACTTAT (SEQ ID NO 56)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

31. The method according to claim 30, for detecting and identifying one or more *Staphylococcus aureus* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

STAU-ICG 3: AACGAAGCCGTATGTGAGCATTGAC (SEQ ID NO 55)

STAU-ICG 4: GAACGTAACCTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

32. The method according to claim 1, for detecting and identifying one or more *Acinetobacter* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

ACI-ICG 1: GCTTAAGTGACAGTGCTCTAAACTGA (SEQ ID NO 57)

ACI-ICG 2: CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

33. The method according to claim 32, for detecting and identifying one or more *Acinetobacter baumannii* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

ACI-ICG 1: GCTTAAGTGACAGTGCTCTAAACTGA (SEQ ID NO 57)

ACI-ICG 2: CACGGTAATTAGTGTGATCTGACGAAG (SEQ ID NO 58)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

34. The method according to claim 1, for detecting and identifying one or more *Listeria* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

LIS-ICG 1: CAAGTAACCGAGAATCATCTGAAAGTGAATC (SEQ ID NO 39)

LMO-ICG 1: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
(SEQ ID NO 40)

LMO-ICG 2: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTTC (SEQ ID NO 41)

LMO-ICG 3: AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

LIV-ICG 1: GTTAGCATAAATAGGTAACCTATTATGACACAAGTAAC
(SEQ ID NO 43)

LSE-ICG 1: AGTTAGCATAAGTAGTGTAACCTATTATGACACAAG (SEQ ID NO 44)

LISP-ICG 1: CGTTTTTCATAAGCGATCGCACGTT (SEQ ID NO 212)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

35. The method according to claim 34, for detecting and identifying one or more *Listeria monocytogenes* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

LMO-ICG 1: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG
(SEQ ID NO 40)

LMO-ICG 2: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTTC (SEQ ID NO 41)

LMO-ICG 3: AGGCACTATGCTTGAAGCATCGC (SEQ ID NO 42)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

36. The method according to claim 1, for detecting and identifying one or more *Brucella* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

BRU-ICG 1: CGTGCCGCCTTCGTTTCTCTTT (SEQ ID NO 59)

BRU-ICG 2: TTCGCTTCGGGGTGGATCTGTG (SEQ ID NO 60)

BRU-ICG 3: GCGTAGTAGCGTTTGCGTCGG (SEQ ID NO 193)

BRU-ICG 4: CGCAAGAAGCTTGCTCAAGCC (SEQ ID NO 194)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

37. The method according to claim 1, for detecting and identifying one or more *Salmonella* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

SALM-ICG 1: CAAAACCTGACTTACGAGTCACGTTTGAG (SEQ ID NO 61)

SALM-ICG 2: GATGTATGCTTCGTTATTCCACGCC (SEQ ID NO 62)

STY-ICG 1: GGTCAAACCTCCAGGGACGCC (SEQ ID NO 63)

SED-ICG 1: GCGGTAATGTGTGMAGCGTTGCC (SEQ ID NO 64)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

38. The method according to claim 1, for detecting and identifying one or more *Chlamydia* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

CHTR-ICG 1: GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)
 CHTR-ICG 2: GCATTTATATGTAAGAGCAAGCATTCTATTTCA (SEQ ID NO 46)
 CHTR-ICG 3: GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)
 CHTR-ICG 4: GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)
 CHPS-ICG 1: GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

39. The method according to claim 38, for detecting and identifying one or more *Chlamydia trachomatis* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

CHTR-ICG 1: GGAAGAAGCCTGAGAAGGTTTCTGAC (SEQ ID NO 45)
 CHTR-ICG 2: GCATTTATATGTAAGAGCAAGCATTCTATTTCA (SEQ ID NO 46)
 CHTR-ICG 3: GAGTAGCGTGGTGAGGACGAGA (SEQ ID NO 47)
 CHTR-ICG 4: GAGTAGCGCGGTGAGGACGAGA (SEQ ID NO 201)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

40. The method according to claim 38, for detecting and identifying one or more *Chlamydia psittaci* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

CHPS-ICG 1: GGATAACTGTCTTAGGACGGTTTGAC (SEQ ID NO 48)

or a probe sequence differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

41. The method according to claim 1, for detecting and identifying *Chlamydia trachomatis*, wherein the primer pair specifically amplifies the 16S-23S rRNA spacer region, or a part thereof, of *Chlamydia trachomatis*, and the primer pair comprises at least one primer having the primer sequence:

CHTR-P1: AAGGTTTCTGACTAGGTTGGGC (SEQ ID NO 69)
 CHTR-P2: GGTGAAGTGCTTGCATGGATCT (SEQ ID NO 70)

or a primer sequence differing therefrom by a single nucleotide deletion, substitution or addition.

42. The method according to claim 1, for detecting and identifying *Listeria* species, wherein the primer pair specifically amplifies the 16S-23S rRNA spacer region, or a part thereof, of *Listeria*, and the primer pair comprises at least one primer having the primer sequence:

LIS-P1: ACCTGTGAGTTTTTCGTTCTTCTC (SEQ ID NO 71)
 LIS-P2: CTATTTGTTTCAGTTTTTGAGAGGTT (SEQ ID NO 72)
 LIS-P3: ATTTTCCGTATCAGCGATGATAC (SEQ ID NO 73)
 LIS-P4: ACGAAGTAAAGGTTGTTTTTCT (SEQ ID NO 74)
 LIS-P5: GAGAGGTTACTCTCTTTTATGTCAG (SEQ ID NO 75)
 LIS-P6: CTTTATGTCAGATAAAGTATGCAA (SEQ ID NO 202)
 LIS-P7: CGTAAAAGGGTATGATTATTTG (SEQ ID NO 203)

or a primer sequence differing therefrom by a single nucleotide deletion, substitution or addition.

43. The method according to claim 1, for detecting and identifying specifically *Mycobacterium* species, wherein the primer pair specifically amplifies the 16S-23S rRNA spacer region, or a part thereof, of *Mycobacterium*, and the primer pair comprises at least one primer having the primer sequence:

MYC-P1: TCCCTTGTGGCCTGTGTG (SEQ ID NO 65)
 MYC-P2: TCCTTCATCGGCTCTCGA (SEQ ID NO 66)
 MYC-P3: GATGCCAAGGCATCCACC (SEQ ID NO 67)
 MYC-P4: CCTCCCACGTCCTTCATCG (SEQ ID NO 68)
 MYC-P5: CCTGGGTTTGACATGCACAG (SEQ ID NO 192)

or a primer sequence differing therefrom by a single nucleotide deletion, substitution or addition.

44. The method according to claim 1, for detecting and identifying one or more *Yersinia enterocolitica* strains, wherein said panel of probes is selected from the group defined in claim 1 and comprises at least one probe having a probe sequence selected from the group consisting of:

YEC-ICG 1: GGAAAAGGTACTGCACGTGACTG (SEQ ID NO 198)
 YEC-ICG 2: GACAGCTGAAACTTATCCCTCCG (SEQ ID NO 199)
 YEC-ICG 3: GCTACCTGTTGATGTAATGAGTCAC (SEQ ID NO 200)

and probe sequences differing therefrom only by addition or removal of one nucleotide from either end of the probe sequence.

45. The method according to claim 1, for detecting and identifying *Brucella* species, wherein the primer pair specifically amplifies the 16S-23S rRNA spacer region, or a part thereof, of *Brucella*, and the primer pair comprises at least one primer having the primer sequence:

BRU-P1: TCGAGAATTGGAAAGAGGTC (SEQ ID NO 204)
 BRU-P2: AAGAGGTCGGATTTATCCG (SEQ ID NO 205)
 BRU-P3: TTCGACTGCAAATGCTCG (SEQ ID NO 206)
 BRU-P4: TCTTAAAGCCGCATTATGC (SEQ ID NO 207)

or a primer sequence differing therefrom by a single nucleotide deletion, substitution or addition.

46. The method according to claim 1, for detecting and identifying specifically *Yersinia enterocolitica* species in a sample, wherein the primer pair specifically amplifies the 16S-23S rRNA spacer region, or a part thereof, of *Yersinia enterocolitica*, and the primer pair comprises at least one primer having the primer sequence:

YEC-P1: CCTAATGATATTGATTCGCG (SEQ ID NO 208)

YEC-P2: ATGACAGGTTAATCCTTACCCC (SEQ ID NO 209)

or a primer sequence differing therefrom by a single nucleotide deletion, substitution or addition.

47. A composition comprising at least two of the probes as defined in any one of claims 1 to 40 and 44.

48. The method according to claim 1, wherein the probes of step (iii) are immobilized on a known location on a solid support.

49. A kit for the detection and identification of at least one micro-organism, or the simultaneous detection and identification of several micro-organisms in a sample, comprising the following components:

- (i) at least one suitable primer pair for amplification of a 16S-23S rRNA spacer region, or a part thereof;
- (ii) at least two of the probes as defined in any one of claims 1 to 40 and 46;
- (iii) a hybridization buffer, or components necessary to produce the hybridization buffer, for buffering the hybridization reaction between the probes and the polynucleic acids;
- (iv) a wash solution, or components necessary for producing the wash solution, for washing the hybrids; and,
- (v) means for detecting the hybrids.

Figure 1

AAGGAGCACC ACGAAACGC CCAACTGGT GGGCGTAGG CCGTGAGGGG TTCTTGTCTG TAGTGGGCGA
GAGCCGGGTG CATGACAACA AAGTTGGCCA CCAACACACT GTTGGGTCCT GAGGCAACAC TCGGACTTGT
TCCAGGTGTT GTCCACCGC CTTGGTGGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCAGCATC
AATGGATACG CTGCCGGCTA GCGGTGGCGT GTTCTTTGTG CAATATTCTT TGGTTTTTGT TGTGT

(SEQ ID NO 76)

2193101

2/103

Figure 2

AAGGAGCACC ACGAAAAGCA CCCCAACTGG TGGGGTGCGA GCCGTGAGGG GTTCCCCTCT GTAGTGGACG
GGGGCCGGNT GCGCAACAGC AAATGATGCG CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCCGTC
CGTGTGGAGT CCTCCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TGGTCTTCGT GGCCGGCGGT CATCGAAATG TGTAATTCT TCCTTAATC TTGTGTGT

(SEQ ID NO 77)

3/103

Figure 3

AAGGAGCACC ACGAAAAGCA CCCCAACTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCCGTC
CGTGTGGAGT CCTCCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TGGTCTTCGT GGCCGGCGCTT CATCGAAATG TGTAATTCT TTTTAACTC TTGTGTGT

(SEQ ID NO 78)

Figure 4

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGTGCCGA GCCGTGAGGG GTTCCCCTCT GTAGTGGACG
GGGGCCGGNT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTGT GGCTGATGCG CTCGTCGAAA TGTGTAATTT CTTCTTTGGT GTNTGTGTGT

(SEQ ID NO 79)

2193101

5/103

Figure 5

AAGGAGCACCC ACGAAAAGCA TCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTTG TGGCTGATGC GTTCATCAAA ATGTGTAATT TCTTTTGTGG TTTNTGTGTG

T

(SEQ ID NO 80)

Figure 6

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCCTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGCG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGCCCTTGC GGCTGATGCG TTCGNCGAAA TGTGTAATTT CTTCTCTGGT TTCTGTGTGT

(SEQ ID NO 81)

7/103

Figure 7

AAGGAGCACC ACGAAAGCA CTCCAATTGG TGGGTGCCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GNAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTCG TGGCTGATGC GTTCATCGAA ATGTGTAATT TCTTCTTTGG TTTTGGGTGT

GT

(SEQ ID NO 82)

2193101

8/103

Figure 8

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCCTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATCGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTTG GGGCTGATGT GTTTCATCAA AATGTGTAAT TTCTTTTNG GTTTNGTGT

GT

(SEQ ID NO 83)

2193101

9/103

Figure 9

AAGGAGCACCC ACGAAAGCA CTCCAATTGG TGGGTGCCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGT TGAATAATTG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTCG TGGCTGATGC GTTCATTGAA ATGTGTAATT TCTTCTCTGG TTTTGTGTG

T

(SEQ ID NO 84)

2193101

10/103

Figure 10

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTGT GGCTGATGCG CTCGTCGAAA TGTGTAATT CTTCTTTGGT TTTTGTGTGT

(SEQ ID NO 85)

11/103

Figure 11

AAGGAGCACC ACGAAAGCA CTCCAATTGG TGGGTGCGA GCCGTGAGGG GTTCCCCTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTTGGTGT TTGAGTATTG GATAGTGTT GCGAGCATCT
AGATGAGCGC GTAGTCCTTG TGGCTGATGC GTTCGTCGAA ATGTGTAATT TCTTCTTTGG GTTTTGTGT

GT

(SEQ ID NO 86)

12/103

Figure 12

AAGGAGCACC ACGAAAAGCA CCCCAATTGG TGGGTGCCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GNAGCCGGNT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGNCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTNGTGTG TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGGGCGCG TAGTCCTTTG TGA CTGATGC GTTCATCAAA ATGTGTAAT TCTTTTTTGN NTTTNGTGTG

T

(SEQ ID NO 87)

2193101

13/103

Figure 13

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTTG TGGCTGACGC GTTCATCGAA ATGTGTAATT TCTTCTTTGG TTTTGTGTG

T

(SEQ ID NO 88)

14/103

Figure 14

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGC GA GCCGTGANGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTAG GGCTGATGCG TTCGTCGNAA TGTGTAATTT CTTCTTTTGGT TTTTGTGTGT

(SEQ ID NO 89)

2193101

15/103

Figure 15

AAGGAGCAC CACGAAAGCA TCCCAATTGG TGGGGTGC GA GCCGTGAGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATAATTGC CAGACACACT ATTGGGCCCT GAGACAAACAC TCGGTCGATC
CGTGTGGTGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG TGGCTGACGT GTTCATCGAA ATGTGTAATT TCTTNTNTTA ACTCTTGTGT

GT

(SEQ ID NO 90)

16/103

Figure 16

AAGGAGCACC ACGAAAAGCA CCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCAGTC
CGTGTGGTGT CCTCCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTGT GACTGACGTG TTCAATCGAA TGTGTAATTT CTTTCTTAAC TCTTGTGTGT

(SEQ ID NO 91)

2193101

17/103

Figure 17

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCTCCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATAT CTTCTCTGGT TTTCGGTGTG

T

(SEQ ID NO 92)

2193101

18/103

Figure 18

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGNT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTTTNNAC TCTTGTGTGT

(SEQ ID NO 93)

2193101

19/103

Figure 19

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACAAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGT TGAATAATGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTCTTTGGT TTTNGTGTGT

(SEQ ID NO 94)

2193101

20/103

Figure 20

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGTG TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG NGGNCNGCGT GTTCATCGAA ATGTGTAATT TCTNTTNTAA CTCNGTGTG

T

(SEQ ID NO 95)

SUBSTITUTE SHEET (RULE 26)

2193101

21/103

Figure 21

AAGGAGCACC ACGAAAAGCA TCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG GGGCCGGCGT GTTCATCGAA ATGTGTAATT TCTTTTTTAA CTC TTGTGTG

T

(SEQ ID NO 96)

22/103

Figure 22

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGT TGAATAATGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCAATCGAAA TGTGTAATTT CTTCTTTAAC TCTTGTGTGT

(SEQ ID NO 97)

23/103

Figure 23

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGN AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCNGCGTG TTCATCGAAA TGTGTAATTT CTTTTTTTAAAC TCTTGTGTGT

(SEQ ID NO 98)

2193101

24/103

Figure 24

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTTTTTAAC TCTTGTGTGT

(SEQ ID NO 99)

25/103

Figure 25

AAGGAGCACC ACGAAAAGCA CCCCAACCTGG TGGGGTGCGA GCCGTGAGGG GTCCTCGCCT GTAGTGGGCG
 GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGGCAACAC TCGGCTCGTT
 CTGAGTGGTG TCCCTCCATC TTGGTGGTGG GGTGTGGTGT TTGAGTATTG GATAGTGGTT GCGAGCATCT
 AACGGATGC GTGGCCGGCA ACGGTGGCGT GTTCGTTGAA ATGTGTAATT TCTTTTGG TTTTGTGTG

T

(SEQ ID NO 100)

26/103

Figure 26

AAGGAGCACC ACGAAAAGCA TCCCAACAAG TGGGTGCAA NCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACGACAAC AAGCAAAGCC AGACACACTA TTGGGTCCTG AGGCAACACT CGGGCTCTGT
TCGAGAGTTG TCCCACCATC TTGGTGGTGG GGTGTGGTGT TTGAGAAATTG GATAGTGGTT GCGAGCATCA
AATGGATGCG TTGCCCTACG GGTAGCGTGT TCTTTTGTGC AATTTTATTC TTTGGTTTTT GTGT

(SEQ ID NO 101)

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27/103

Figure 27

AAGGAGCACC ATTTCCCAAGT CGATGAACTA GGGAACATAA AGTAGGCATC TGTAGTGGAT ATCTACTTGG
TGAATATGTT TTGTAAATCC TGTCACACCC GTGGATGGGT AGTCGGCAA ACGTCGGACT GTCATAAGAA
TTGAAACGCT GGCACACTGT TGGGTCCTGA GGCAACACGT TGTGTTGTCA CCCTGCTTGG TGGTGGGGTG
TGGACTTTGA CTTCTGAATA GTGGTTGCCA GCATCTAAAC ATAGCCTCGC TCGTTTTCGA GTGGGGCTGG
TTTTGCAATT TTA

(SEQ ID NO 102)

SUBSTITUTE SHEET (RULE 26)

28/103

Figure 28

AAGGAGCACC ATTTCCCACT CGGATGAACT AGGGAACATA AAGTAGGCAT CTGTAGTGGG TATCTACTTG
GTGAATATGT TTTGTAAATC CTGTCCACCC CCGTGGATGG GTAGTCGGCA AAACGTCGGA CTGTCATAAG
AATTGAAACG CTGGCACACT GTTGGGTCCT GAGGCAACAC GTTGTGTTGT CACCCTGCTT GGTGGTGGGG
TGTGGACTTT GACTTCTGAA TAGTGGTTGC GAGCATCTAA ACATAGCCTC GCTCGTTTC GAGTGAGGCT
GGTTTTTGCA ATTTA

(SEQ ID NO 103)

29/103

Figure 29

AAGGAGCACC ACGAAGAGCA CTCCAATTGG TGGGTGCCGA GCCGTGAGGG GTCATCGTCT GTAGTGGACG
AAGACCGGGT GCACGACAAC AAGCTAAGCC AGACACACTA TTGGGTCCCTG AGGCAACACC CTCGGGTGCT
GTCCCCCAT CTGGTGGTG GGTGTGGTG TTGAGAATT GGATAGTGGT TGCAGCATC AAAATGTATG
CGTGTGCGT CTCGGCAACG TGTTCTTTT GTGCAATTA TTCTTTGGT TTTGTAGTGT TTGT

(SEQ ID NO 104)

30/103

Figure 30

AAGGAGCACC ACGAAGAGCA CTCCAATTGG TGGGGTGCGA GCCGNGAGGG GTCATCGTCT GTAGTGGACG
AAGACTGGGT GCACGACAAC AAAGCAAGCC AGACACACTA TTGGGTCCCTG AGGCAACACC CTCGGGTGCT
GCCCCCTCCAT CTTGGTGGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCAGCATC AAAAATGTAT
GCGTTGTCGT TCGCGACAAC GTGTTCTTTT TGTGCAATT TAATCTTTT GGTTTGGTA GTGTTTGT

(SEQ ID NO 105)

31/103

Figure 31

AAGGAGCACC ACGAGAAGCA CTCCAATTGG TGGGGTGCAA GCCGTGAGGG GTCATCGTCT GTAGTGGACG
AAGACCGGGT GCACGACAAC AAGCAAAGCC AGACACACTA TTGGGTCCCTG AGGCAACACC CTCGGGTGCT
GTCCCCCAT CTTGGTGGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCAGCATC AAAATGTATG
CGTTGTCGTT CGCGGCAACG TGTTCTTTTT GTGCAATTTT TATCTTTGG TTTTGTAGT GTTTGT

(SEQ ID NO 106)

32/103

Figure 32

AAGGAGCACC ACGAAAAGCA CCCCAATTGG TGGGGTGCAA GCCGTGAGGG GTTCCCGCCT GTAGTGGGCG
GGGCCGGGTG CGCAACAGCA AATGATTGCC AGACACACTA TTGGGCCCTG AGGCAACACT CGGATCGATT
GAGTGCTTGT CCCCCCATCT TGGTGGTGGG GTGTGGTGTT TGAGAACTGG ATAGTGGTTG CGAGCATCTA
AATGAACGCA CTGCCGATGG TGGTGTGTTC GTTTGTGTA ATTTATTCT TTGGTTTGTG TGTTTGT

(SEQ ID NO 107)

Figure 33

AAGGAGCACC ACGAAAGCA CTCCAATTGG TGGGTGCCGA GCCGTNAGGG GTTCTCGTCT GTAGTGGATG
GCAGCCGGGT GCACANCAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCAGTC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGNGTT TGAGTATTGG ATAGTGGTTG CGANCACTA
GATGAACGCG TAGTCCTCNG TGGCTGACGT GTTCATCAAA ATGTGTAATT TCTTTTANGG GTTTNGGTGT

CT

33/103

(SEQ ID NO 108)

34/103

Figure 34

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGNGAGGG GTTCTCGCCT GTAGTGGNCG
AGGGCCGGAT GCACAACAAC ACATGATTGC CAGACACACT ATTGGGCCCT GANACAACAC TCGGCCAGTC
CGTGTGGTGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATNGG ATAGTNGTTG NGANCATCTA
AACGGCTGCG TNGNCNNGAA CCGTGGCGTG TTCGNTAAAA TGTGTAATT CTTTNNGGT TTGGGTGTNT

(SEQ ID NO 109)

35/103

Figure 35

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGTGCCGA GCCGTGAGGG GTTCTCGCCT GTAGTGGGCG
ANGGCCGGGT GCACAACAAC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGCCAGTC
CGTGTGGTGT CCCNCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
AANGGNTGCG TTGCCGNNAN CNGTGGCGTN TTCGNTAAAA TGTGTAANTT CTTTNNGGT TTGTGTGTGT

(SEQ ID NO 110)

36/103

Figure 36

ATCGAAGATC CCGGCTTCTT CATAAGCTCC CACACGAATT GCTTGATTCA CTGGTTAGAC GATTGGGTCT
GTAGCTCAGT TGGTTAGAGC GCACCCCTGA TAAGGGTGAG GTCGGCAGTT CGAATCTGCC CAGACCCACC
AATTGTTGGT GTGCTGCGTG ATCCGATACG GGGCCATAGC TCAGCTGGGA GAGCGCCTGC TTTGCACGCA
GGAGGTCAGG AGTTCGATCC TCCTTGGCTC CACCATCTAA AACAAATCGTC GAAAGCTCAG AAATGAATGT
TCGTGGATGA ACATTGATTT CTGGTCTTTG CACCAGAACT GTTCTTTAAA AATTCGGGTA TGTGATAGAA
GTAAGACTGA ATGATCTCTT TCACTGGTGA TCATTCAAGT CAAGGTAAAA TTTGCGAGTT CAAGCGCGAA
TTTTTCGGCGA ATGTCGTCTT CACAGTATAA CCAGATTGCT TGGGGTTATA T

(SEQ ID NO 111)

Figure 37

ATCGAAGACA TCAGCTTCTT CATAAGTATC CACACGAATT GCTTGATTCA TAGTCGAACG AATGCTGTAA
CGCGACCCGT GTTATAGGTC TGAGCTCAG TTGGTTAGAG CGCACCCCTG ATAAGGGTGA GGTCGGCAGT
TCAAAATCTGC CCAGACCCTAC CAATTGCTTG GTCGAGAAGA ATACGGGGCC ATAGCTCAGC TGGAGAGCGG
CCTGCCCTGC ACGCAGGAGG TCAGCGGTC GATCCCGCTT GGCTCCACCA CTCCTCTCGT TTGCGGTGAG
TGTTAAAGAG TTCAGAAATG ATGCCGCTC AGGTTGTCC TGTGAGTGC TGATTTCTGG TCTTTTGACC
GGTACGAAA TCGTCTTTA AAAATTGGA TATGTGATAG AAGTGACTGA TTAATTGCTT TCACTGGCAA
TTGATCTGGT CAAGGTAAA TTTGTAGTTC TCAAGACGCA AATTTCGGC GAATGTCGTC TTCACGATTG
AGACAGTAAC CAGATTGCTT GGGTTATAT

37/103

(SEQ ID NO 112)

Figure 38

ATCGAAGACA CCGGCTTCGT CATAAGCTCC CACACGAATT GCTTGATTCA CTTGCGAAAG GCGATTGGGT
TTAGACCCGA GAGTAACGAT TGGGTCTGTA GCTCAGTTGG TTAGAGCGCA CCCCTGATAA GGGTGAGGTC
GGCAGTTCGA ATCTGCCCCAG ACCCACCAAT CGAAGGGGCC ATAGCTCAGC TGGGAGAGCG CCTGCTTTGC
ACGCAGGAGG TCAGCGGTTC GATCCCGCTT GGCTCCACCA TTAAGTCTAG TCGCCGAAAG CTCAGAAATG
AGTGTTTACC AGGATGAGGT TGATTGCCCTG GGTGAACAT TGATTTCTGG ACTTTGCGCC AGAACTGTTT
TTAAAAAAT TGGGTATGTG ATAGAAGTAG ACCGATGTGT TGCTTTCACT GGCAGCATGT CGCGTCAAGG
TAAAAATTGC GTGTTCTCTA TGCAAAATTT CGGCGAATGT CGTCTTCACG TTATAGACAG TAACCAGATT
GCTTGGGGT ATAT

38/103

(SEQ ID NO 113)

Figure 39

ATCGAAGACT TCAGCTTCTT CATAAGTTCC CACACGAATT GCTTGATTCA CTTGCGAAAA GCGATTGGGT
TGAGACCCGA GAGTGACGAT TGGGTCTGTA GCTCAGTTGG TTAGAGCGCA CCCCTGATAA GGGTGAGGTC
GGCAGTTCGA ATCTGCCCAG ACCACCAAT TGTCGGGATG GCCAGTGTC AATGGGCCA TAGCTCAGCT
GGGAGAGCGC CTGCTTTGCA CGCAGGAGGT CAGGAGTTCG ATCCTCCTTG GCTCCACCAT CAACTCACGA
TCGCTGAAAG CTCAGAAATG AACATTGGTA GTTCAATGTT GATTCTGGT CTTTGCGCCA GAACTGTTCT
TTAAAAATT GGGTATGTGA TAGAAGTGAC TAACAGCGTG TTCACTGCA CGTTGTTAAT CAAGGCCAAA
TTTGCGAGTT CAAGCGCGAA TTTTCGGCGA ATGTCGTCTT CACGTTACGA ATCTATAACC AGATTGCTTG
GGTTATAT

39/103

(SEQ ID NO 114)

Figure 40

ATCGACGACA TCAGCTGTCT CATAAGCTCC CACACGAATT GCTTGATTCA TTGAAGAAGA CGATTAGGTT
AGCAACCTTC GATTGGGTCT GTAGCTCAGT TGGTTAGAGC GCACCCCTGA TAAGGGTGAG GTCGGCAGTT
CGAATCTGCC CAGACCCACC AATTGCTGG GGCCATAGCT CAGCTGGGAG AGCGCCTGCC TTGCACGCAG
GAGGTCAGCG GTTCGATCCC GCTTGGCTCC ACCACCCCGC TTGCCAGTTT GTCAAAGCTT AGAAATGAAT
ATTGCGGTCG AATATTGATT TCTGAACTTT ATCAGAATCG TTCTTTAAAA ATTTGGGTAT GTGATAGAAA
GATAGACTGG ACAGCACTTT CACTGGTGTG TGTTCAGGCT AAGGTAAAT TTGTGAGTAA TTACAAGTTT
TCGGCGAATG TTGTCTTCAC AGTATAACCA GATTGCTTGG GGTATAT

40/103

(SEQ ID NO 115)

Figure 41

TAAGGAAAAG GAAACCTGTG AGTTTTCGTT CTTCTCTGTT TGAGAGGTTA ATTCTTCTCT
ATACTGTTTG TTCTTTTGAAA ACTAGATAAG AAAGTTAGTA AAGTTAGCAT AAATAGGTAA CTATTTATGA
CACAAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGAAAA
ATCAGAAAAA CAACCTTTAC TTCATCGAAG TAAATT

(SEQ ID NO 116)

42/103

Figure 42

CTAAGGAAAA GGAACCTGT GAGTTTTCGT TCTTCTCTAT TTGTTTCAGTT TTGAGAGGTT AGTACTTCTC
AGTATGTTTG TTCTTTGAAA ACTAGATAAG AAAGTTAGTA AAGTTAGCAT AGATAATTFA TTATTTATGA
CACAAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGGAAA
ATCAGAAAAA CAACCTTTAC TTCGTAGAAG TAAATT

(SEQ ID NO 117)

43/103

Figure 43

TAAGGAAAAG GAAACCTGTG AGTTTCGTT CTTCTCTGTT TGAGAGGTTA TTA CTCTCT
GTATGTTTGT TCTTTGAAAA CTAGATAAGA AAGTTAGTAA AGTAGGTAA CTATTATGA
CACAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TAATTCGACG TATCATCGCT GATACAGACA
ATTAGAAAA CAACCTTTAC TTCGACGAAG TAAATT

(SEQ ID NO 118)

44/103

Figure 44

GGCCTATAGC TCAGCTGGTT AGAGCGCACG CCTGATAAGC GTGAGGTCGA TGGTTCGAGT CCATTTAGGC
CCACTTTTTC TTCTGACAG AAGAAACACT GTATAACCTA TTTAAGGGGC CTTAGCTCAG CTGGGAGAGC
GCCTGCTTTG CACGCAGGAG GTCAGCGGTT CGATCCCCT AGGCTCCACC AAAATTGTTC TTTGAAAACT
AGATAAGAAA GTTAGTAAAG TTAGCATAAA TAGGTAACTA TTTATGACAC AAGTAACCGA GAATCATCTG
AAAGTGAATC TTTCATCTGA TTGGAAGTAT CATCGCTGAT ACGAAAAATC AGAAAAACAA CCTTACTTC
ATCGAAGTAA ATT

(SEQ ID NO 119)

2193101

45/103

Figure 45

TAAGGAAAAG GAAACCTGTG AGTTTTCGTT CTTCCTATT TGTTCAAGTT TGAGAGGTTA CTCTCTTTTA
TGTCAGATAA AGTATGCAAG GCACTATGCT TGAAGCATCG CGCCACTACA TTTTGTGACGG GCCTATAGCT
CAGCTGGTTA GAGCGCACGC CTGATAAGCG TGAGGTCGAT GGTTCGAGTC CATTAGGCC CACTTTTCT
TTCTGACATA AGAAATACAA ATAATCATAC CCTTTACGG GGCCTTAGCT CAGCTGGGAG AGCGCCTGCT
TTGCACGCAG GAGGTCAGCG GTTCGATCCC GCTAGGCTCC ACCAAAATTG TTCTTTGAAA ACTAGATAAG
AAAGTTAGTA AAGTTAGCAT AGATAATTA TTATTATGA CACAAGTAAC CGAGAATCAT CTGAAAGTGA
ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGGAAA ATCAGAAAAA CAACCTTTAC TTCGTAGAAG
TAAATT

(SEQ ID NO 120)

2193101

Figure 46

TAAGGAAAAG GAAACCTGTN AGTTTNCGTN CTTCTCTGTT TGTNCAGTTT TNAGAGGTTA CTCCTTTNA
TGTCAGATAA AGTACGCACG GCACGTTGCC TTGGGCAAG AGCCACTACA TTATTGACGG GCCTATAGCT
CAGCTGGTTA GAGCGCACGC CTGATAAGCG TGAGGTCGAT GGTTCGAGTC CATTTAGGCC CACTTTTCT
TTCTGACAGA AGAAATCATT TGCACATCCT ATTAATAAGG GNCCTTAGCT CAGCTGGGAG AGCGCCTGCT
TTGCACGCAG GAGGTCAGCG GTTCGATCCC GCTAGGCTCC ACCCAAATT GTTCTTTGAA AACTAGATAA
GAAAGTTAGT AAAGTTAGCA TAAAGTAGTAT AACTATTTAT GACACAAGTA ACCGAGAATC ATCTGAAAGT
GAATCTTTCA TCTAATTCCA CGTATCATCG CTGATACAGA CAATTNGAAA AACAACTTT ACTTCGACGA
AGTAAATT

(SEQ ID NO 121)

47/103

Figure 47

TAAGGATAAG GATAACTGTC TTAGGACGGT TTGACTAGGT TGGGCAAGCG TTTTTTTAAAT CTTGTATTCT
ATTCCTTTTG CATTGTTAAG CGTTGTTTCC AAAACATTTA GTTACGATC AAGTATGTTA TGTAATAAAT
ATGGTAACAA GTAAATTTCAC ATATAATAAT AGACGTTTAA GAATATATGT CTTAGGTGA TGTTAACTTG
CATGGATCAA TAATTTACA

(SEQ ID NO 122)

2193101

48/103

Figure 48

TAAGGATAAG GAAGAAGCCT GAGAAGGTTT CTGACTAGGT TGGGCAAGCA TTTATATGTA AGAGCAAGCA
TTCTATTCA TTTGTGTTGT TAAGAGTAGC GTGGTGAGGA CGAGACATAT AGTTTGTGAT CAAGTATGTT
ATTGTAAAGA AATAATCATG GTAACAAGTA TATTCACGC ATAATAATAG ACGTTTAAGA GTATTTGTCT
TTTAGGTGAA GTGCTTGCAAT GGATCTATAG AAATTACA

(SEQ ID NO 123)

2193101

49/103

Figure 49

CAAATGGAGT TTTTATTTT TATTATCTT AACACCCCAT TAAATTTTTC GGTGTTAAAA CCCAAATCAA
TGTTTGGTCT CACAACTAAC ACATTTGGTC AGTTTGTATC CAGTTCTGAA AGAATGTTT TGAACAGTTC
TTTCAAAAC T GAAAACGACA ATCTTCTAG TTCCAAAAAT AAATACCAAA GGATCAATAC AATAAGTTAC
TAAGGGCTTA TGGT

(SEQ ID NO 124)

2193101

50/103

Figure 50

CTAATGAAGT TTTTACTTT TTCTTTTCAT CTTAATAAA GATAAATACT AAACAAAACA TCAAAATCCA
TTTATTATC GTGGTAAAT TAAACCCAAA TCCCTGTTG GTCTCACAAC TAACATATTT GGTGAGATTG
TATCCAGTTC TGAAGAACA TTTCGGCTTC TTCAAAACT GAAAACGACA ATCTTCTAG TTCCAAATAA
ATACCAAAGG ATCAATACAA TAAGTTACTA AGGGCTTATG GT

(SEQ ID NO 125)

2193101

Figure 51

AACGAAAGAT TGACGATTGG TAAGAATCCA CAACAAGTTG TTCTTCATAG ATGTATCTGA GGGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTGAGA CCCACCATGA
CTTGACTGG TTGAAGTTAT AGATAAAAGA TACATGATTG ATGATGTAAG CTGGGGACTT AGCTTAGTTG
GTAGAGCGCC TGCTTTGCAC GCAGGAGGTC AGGAGTTCGA CTCCTCTAGT CTCCACCAGA ACTTAAGATA^{51/103}
AGTTCGGATT ACAGAAATTA GTAAATAAAG ATTGAGATCT TGGTTTATTA ACTTCTGTGA TTTCATTATC
ACGGTAATTA GTGTGATCTG ACGAAGACAC ATTAATCTAG TAACAGATTG GCAAAATTGA GTCGAAATA
AATTGTTTCA TCAAGAGTTT AGGTTAAGCA ATTAATCTAG ATGAATTGAG AACTAGCAAA TTAACCTGAAT
CAAGCGTTT GGTATGTGAA TTAGATTGA AGCTGTACAG TGCTTAAGTG CACAGTGCTC TAAACTGAAA
TGTTGAAGTT ACTAACTTGT AGGTAACATC GACTGTTTGG GGTGTAT

(SEQ ID NO 126)

52/103

Figure 52

AACGAAAGAT TGACGATTGG TAAGAATCCA CGACAAGTTG TTCTTCATAG ATGTATCTGA GGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTGAGA CCCACCATGA
CTTTGACTGG TTGAAGTTAT AGAAAAGAAG ATACATAACT GATGATGTAA GCTGGGGACT TAGCTTAGTT
GGTAGAGCGC CTGCTTTGCA CGCAGGAGGT CAGGAGTTCTG ACTCTCCTAG TCTCCACCA

(SEQ ID NO 127)

53/103

Figure 53

AACGAAAGAT TGATGGCCGG TAAGAATCCA CAACAAGTTG TTCTTCGAAG ATGTATCTGA GGGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTGAGA CCCACCAAAAT
CTGAAAGATA TGTCGTTTCA TATGATTAAA GCTGGGGACT TAGCTTAGTT GGTAGAGCGC CTGCTTTGCA
CGCAGGAGGT CAGGAGTTCG ACTCTCCTAG TCTCCACCA

(SEQ ID NO 128)

54/103

Figure 54

AACGAAAGAT TGACGATTGG TAAGAAATCCA CAACAAGTTG TTCTTCATGA CGATGTATCT GAGGGTCTGT
AGCTCAGTTG GTTAGAGCAC ACGCTTGATA AGCGTGGGGT CACAAGTTCA AGTCTTGTC A GACCCACCAA
ATCTGACTAA CAAGCATTAT TAAATGCTGA ATACAGAAAA ACAGAGACAT TGACTTATTG ATAAGCTGGG
GACTTAGCTT AGTTGGTAGA GCGCCTGCTT TGCACGCAGG AGGTCAGGAG TTCGACTCTC CTAGTCTCCA

CCA

(SEQ ID NO 129)

SUBSTITUTE SHEET (RULE 26)

Figure 55

AACGAAAGAT TGGTGACCGG TAAGAATCCA CAACAAGTTG TTCTTCGAAG ATGTATCTGA GGGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTGAGA CCCACCACTA
CTGACGAAGT GATGAATAAT CACAAGCTGC TAGATGAAA GATATGTCGT TCATTATGAT TAAAGCTGGG
GACTTAGCTT AGTTGGTAGA GCGCCTGCTT TGCACGCAGG AGGTCAGGAG TTCGACTCTC CTAGTCTCCA

CCA

55/103

(SEQ ID NO 130)

2193101

56/103

Figure 56

TAAGGAAGAT	CGAGAATTGG	AAAGAGGTCG	GATTATATCCG	GATGATCCTT	CTCCATCTTA	TTAGAACATA
GATCGCAGGC	CAGTCAGCCT	GACGATCGCT	TGCAGGCGTG	CCGCCCTTCGT	TTCTCTTTCT	TCATTGTTGA
TTGCTCACGG	GCCGTACCGC	AGCTGACGCT	GCTGGCCCTG	CGCAGGCGCG	GCCCATCAGG	GCCGACGGCC
GGTCGGCCTT	GCNAAGCTTC	GCTTCGGGGT	GGATCTGTGG	ATCGCGTAGT	AGCGTTTGCG	TCGGTATCTG
GGCTTGTAGC	TCAGTTGGTT	AGAGCACACG	CTTGATAAGC	GTGGGGTTCG	AGGTTCAAGT	CCTCCCAGGC
CCACCAAGTT	ACTTGATGAG	GGGCCGTAGC	TCAGCTGGGA	GAGCACCTGC	TTTGCAAGCA	GGGGGTCGTC
GGTTCGATCC	CGTCCGGCTC	CACCATCATG	TTGGTGTTGA	GACGGATATT	GGCAATCAAC	AAAAGAAAGA
AACAAGTTTG	CGGACTNNTA	CGAAAGTCTG	CCTGTTCTGT	ATGAAATCGT	GAAGAGAAGA	TGTAATCGGA
TCAACTGAAG	AGTTGATGTC	GCAAGAAGCT	TGCTCAAGCC	TTGCATAATG	ATTGATGTGT	TTAACCGCCA
TCACCCGATTG	TATCTCGAGA	AGCTGGTCTT	TCTGCTGATA	CTGTTGAAAC	GAGCATTTGC	AGTCGAAATGG
CAACATTTCGG	CGTCGCATAA	TGCGGCTTTA	AGAGCTGAGT	TTTGATGGAT	ATTGGCAATG	AGAGTGATCA
AGTGTCCTAA	GGGCATTGGT	GGATGCCCTTG	GCATGCAC			

(SEQ ID NO 131)

2193101

57/103

Figure 57

TAAGGAGGAT CGAGAAATTGG AAAGAGGCCG GATTATATCCG GATGATCCCTT CTCCATCTTA TTAGAACATA
GATCGCAGNC CAGTCAGCCT GACGATCGCT TGCAGGCGTG CCGCCTTCGT TTCTCTTTCT TCATTGTTGA
TTGCTCACGG GCCGTACCGC AGCTGACGCT GCTGGCCCTG CGCAGGCGCG GNCCATCAGG GCCGACGGCC
GGTCGGCCTT GCGAAGCTTC GCTTCGGGT GGATCTGTGG ATCGCGTAGT AGCGTTTGCG TCGGTATCTG
GGCTTGTAGC TCAGTTGGTT AGAGCACACG CTTGATAAGC GTGGGTCGG AGGTTCAAGT CCTCCCAGGC
CCACCAAGTT ACTTGATGAG GGGCCGTAGC TCAGCTGGGA GAGCACCTGC TTTGCAAGCA GGGGTCGTC
GGTTCGATCC CGTCCGGCTC CACCATCATG TTGGTGTGA GACGGATATT GGCAATCAAC AAAAGAAAGA
AACAAGTTG CGGACTNNTA CGAAAGTCTG CCTGTCTGT ATGAAATCGT GAAGAGAAGA TGTAAATCGGA
TCAACTGAAG AGTTGATGTC GCAAGAAGCT TGCTCAAGCC TTGCATAATG ATTGATGTGT TTAACCGCCA
TCACCGATTG TATCTCGAGA AGCTGGTCTT TCTGCTGATA CTGTTGAAAC GAGCATTTGC AGTCGAATGG
CAACATTCGG CGTCGCATAA TGCGGCTTTA AGAGCTGAGT TTTGATGGAT ATTGGCAATG AGAGTGATCA
AGTGTCTTAA GGGCATTTGGT GGATGCCCTG GCATGCAC

(SEQ ID NO 132)

58/103

Figure 58

CCTTAAAGAA CTGTTCTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAAG TAAATAGCAA GCGTCTTGC
GAAGCAGACT GATACGTCCC CTTCGTCTAG AGGCCCAGGA CACCGCCCTT TCACGGCGGT AACAGGGGTT
CGAATCCCCT AGGGACGCC ACTTGCCGG TAAATGTGA AAGCGTTGCC ATCAGTATCT CAAAACTGAC
TTACGAGTCA CGTTTGAGAT ATTTGCTCTT TAAAATCTG GATCAAGCTG AAAATTGAAA CACAGAACAA
CGAAAGTTGT TCGTGAGTCT CTCAAATTTT CGCAACACCA TGATGAATCG TAAGAAACAT CTTCCGGTTG
TGA

(SEQ ID NO 133)

59/103

Figure 59

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CCTTAAAGAA CTGTTCTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTCAAGT CCACTCAGGC
CTACCAAAT TTCCCTGAAT ACTGCCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCCACG
CCTTGCTCTCA GGAAAAATTA TCGGTAAAGA GGTTCTGACT ACACGATGGG GCTATAGCTC AGCTGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCCG TTCGATCCCG CATAGCTCCA CCATATCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAGGG TTCACTGCCA AGTTTGTCTC TTTAAAAATC TGGATCAAGC
TGAAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCCTCAAAT TTCGCAACAC GATGATGAAT
CGTAAGAAAC ATCTTCGGGT TGTGA
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(SEQ ID NO 134)

60/103

Figure 60

CCTTAAAGAA GCGTACTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAG TAAATAGCAA GCGTCTTGC
GAAGCAGACT GATACGTCCC CTTGCTCTAG AGGCCCAGGA CACCGCCCTT TCACGGCGGT AACAGGGGT
CGAATCCCCT AGGGGACGCC ACTTGCGCGG TAATGTGTGA AAGCGTTGCC ATCAGTATCT CAAAAC TGAC
TTACGAGTCA CGTTTGAGAT ATTGCTCTT TAAAAATCTG GATCAAGCTG AAAATTGAAA CACAGAACAA
CGAAAGTTGT TCGTGAGTCT CTCAAATTT CGCAACACGA TGATGAATCG TAAGAAACAT CTTCCGGTTG

TGA

(SEQ ID NO 135)

61/103

Figure 61

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CCTTAAAGAA CTGTTCTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTTCAAGT CCACTCAGGC
CTACCAAATT TTCCCTGAAT ACTGCCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCACG
CCTTGCTCA GGAAAAATTA TCGGTAAAGA GGTTCTGACT ACACGATGGG GCTATAGCTC AGCTGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCCG TTCGATCCCG CATAGCTCCA CCATCTCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAGGG TTCACTGCCA AGTTTGTCTC TTTAAAAATC TGGATCAAGC
TGAAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCTCAAATT TTCGCAACAC G
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(SEQ ID NO 136)

62/103

Figure 62

CCTTAAAGAA GCGTACTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAG TGAATAGCAA GGCGTCTTGC
GATTGAGACT TCAGTGTTCC CTTCGTCTAG AGGCCCAGGA CACCGCCCTT TCACGGCGGT AACAGGGGTT
CGAATCCCCCT AGGGGACGCC AGCGTTCAAA CTGATGAGGT CAAACCTCCA GGGACGCCAC TTGCTGGTTT
GTGAGTGAAA GTCACCTGCC TTAATATCTC AAAACTGACT TACGAGTCAC GTTGAGATA TTGCTCTTT
AAAAATCTGG ATCAAGCTGA AAATTGAAAC ACAGAACAAC GAAAGTTGTT CGTGAGTCTC TCAAATTTTC
GCAACACGAT GATGAATCGT AAGAAACATC TTCGGGTTGT GA

(SEQ ID NO 137)

63/103

Figure 63

CCTTAAAGAA ACGGTCTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTTCAAGT CCACTCAGGC
CTACCAAATT TTCCCTGAAT ACTGCCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCCACG
CCTTGCTCA GGAATAAATA TCGGTAAAGA GGTCTGACT ACACGATGG GCTATAGCTC AGCTGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCCG TTCGATCCC GATAGCTCCA CCATCTCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAGGG TTCACTGCCA AGTTTGTCTC TTTAAAAAATC TGGATCAAGC
TGAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCCTCAAAT TTGCAACAC GATGATGAAT
CGTAAGAAAC ATCTTCGGGT TGTGA

(SEQ ID NO 138)

64/103

Figure 64

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CTAAGGATAT ATTCGGAACA TCTTCTTCGG AAGATGCCGA ATAACGTGAC ATATTGTATT CAGTTTGTGAA
TGTTTATTTA ACATTCAAAT ATTTTGTGGT TAAAGTGATA TTGCTTTTGA AAATAAAGCA GTATGCCGAGC
GCTTGACTAA AAAAAATTGT ACATTGAAA AAGTAAATA TAGATTTTAC CAAGCAAAAC
CGAGTGAATA AAGAGTTTAA AATAAGCTTG AATCATAAG AAATAATCGC TAGTGTTTGA AAGAACACTC
ACAAGATTAA TAACGCGGTT AAATCTTTT ATAAAAGAAC GTAACTTCAT GTTAACTGTTT GACTTATAAA
AATGGTGGAA ACATA
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(SEQ ID NO 139)

65/103

Figure 65

CTAAGGATAT ATTCGGAACA TCTTCTTCAG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGTTTGGAA
TGTTATTTA ACATTCAAAT ATTTTGTGGT TAAAGTGATA TTGCTTATGC GAGCNCTTGA CAATCTATTC
TTTTTAAAGA AAGCGGTTGT CAGACAATGC CAGACAATGC AGTTTACTTT TGTAATGAG
CATTGATT TTTGAAAATA AAGCAGTATG CGAGCGCTTG ACTAAAAGA AATTGTACAT TGAAAACTAG
ATAAGTAAGT AAAATATAGA TTTTACCAAG CAAACCGAG TGAATAAGA GTTTTAAATA AGCTTGAATT
CATAAGAAAT AATCGCTAGT GTTCGAAAGA AACTCACAA GATTAAATAC GCGTTTAAAT CTTTATTATA
AAGAAAACGT TTAGCAGACA ATGAGTTAAA TTATTTTAAA GCAGAGTTTA CTTATGTAAA TGAGCATTTA
AAATAATGAA AACGAAGCCG TATGTGAGCA TTTGACTTAT AAAAATGGTG GAAACATA

(SEQ ID NO 140)

SUBSTITUTE SHEET (RULE 26)

Figure 66

CTAAGGATAT ATTCGGAACA TCTTCTTCAG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGTTTGAA
TGTTTATTTA ACATTCAAAT ATTTTGTGTT TAAAGTGATA TTGCTTATGC GAGCGCTTGA CAATCTATTC
TTTTTAAAGA AAGCGGTTGT CAGACAATGC ATTAAGAAAA ATTAAGCCGG AGTTTACTTT TGTAATGAG
CATTGTATTT TTGAAAATA AAGCAGTATG CGAGCGCTTG ACTAAAANGA AATTGTACAT TGAAAACCTAG
ATAAGTAAGT AAAATATAGA TTTTACCAAG CAAAACCGAG TGAATAAAGA GTTTTGAATA AGCTTGAATT
CATAAGAAAT AATCGCTAGT GTTCGAAAGA ACACTCACAA GATTAATAAC GCGTTTAAAT CTTTTTATAA
AAGAACGTAA CTTCATGTTA ACGTTTGACT TATAAAAATG GTGGAAACAT A

66/103

(SEQ ID NO 141)

67/103

Figure 67

CTAAGGATAT ATTCGGAACA TCTTCTTCAG AAGATGCGGA ATACGTGAC ATATTGTATT CAGNTTTGAA
TGTTTATTTA ACATTCAAAA AATGGGCCCTA TAGCTCAGCT GGTAGAGCG CACGCCCTGAT AAGCGTGAGG
TCGGTGGTTC GAGTCCACTT AGGCCACCA TTATTGTAC ATTGAAAAC AGATAAGTAA GTAAAAATA
GATTTACCA AGCAAAACCG AGTGAATAAA GAGTTTAA TAAGCTTGAA TTCATAAGAA ATAATCGCTA
GTGTCGAAA GAACACTCAC AAGATTAATA ACGCGTTTAA ATCTTTTAT AAAAGAACGT AACTTCATGT
TAACGTTTGA CTTATAAAAA TGGTGGAAC ATA

(SEQ ID NO 142)

68/103

Figure 68

CTAAGGATAT ATTCGGAACA TCTTCYTCAG AAGATGCCGA ATAATGTGAC ATATTGTATT CAGTTTGTGAA
TGTTTATTTA ACATTCAAAT ATTTTGTGGT TAAAGTGATA TTGCTTATGC GAGCGCTTGA CTAAAAAGAA
ATTGTACATT GAAACTAGA TAAGTAAGTA AAANTATAGA TTTTACCAAG CAAAACCGAG TGAATAAAGA
GTTTAAATA AGCTTGAATT CATAAGAAAT AATCGCTAGT GTTCGAAAGA AACTCACAA GATTAATAAC
GCGTTTAAAT CTTTATATAA AAGAACGTAA CTTCATGTTA ACGTTTGACT TATAAAATG GTGGAACAT

A

(SEQ ID NO 143)

69/103

Figure 69

CTAAGGATAT ATTCGGAACA TCTTCTACGA AGATGAGGGA ATAACGTGAC ATATTGTATT CAGTTTGGAA
TGTTTATTAA CATTCAATTG TACATTGAAA ACTAGATAAG TAAGTAAGAT ,TTTACCAAGC AAAACCGAGT
GAATAGAGTT TTAAATAAGC TTGAATTTCAT AAATAATCGC TAGTGTTCCGA AAGACNTCCA CAAGATTAAAT
AACTAGTTT AGCTATTTAT TTTGAATAAC AATTCAAAAT ATGGTGGGAC ATA

(SEQ ID NO 144)

Figure 70

AAGGATAAGG AACTGCACAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTGCGACGCA GGAGGTCAGC GGTCGATCC CGTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAAGA GTTTATGACT GAAAGGTCAA AAAATAA

(SEQ ID NO 145)

2193101

71/103

Figure 71

AAGGAAATGG AACACGTTTA TCGTCTTATT TAGTTTGGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTNGCACGCA GGAGGTCAGC GGTTGATCC CGCTAGGCTC CATCAGGATA CANTCCTACT
AACTTAATA CAAGTGAAGT TGAACACGCA ACTCACTTCC TAGGAAAATA GACAACTCTC GCTTGTGTGC
AAGGCACACA TGGTCAGATT CCTAATTTC TACAGAAAGT TCGCTAAAGC GAGCGTTGCT TAGTATCCTA
TATAATAGTC CATNGAAAAT TGAATATCTA TATCAAATTC CACGATCTAG AAATAGATTG TGGAAAACGTA
ACAAGAAATT AACCCGNAAA CGCTG

(SEQ ID NO 146)

72/103

Figure 72

AAGGATAAGG AACTGCACAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTC GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTGACACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAAT AAACCGAAAC GCTGTAGTAT
TAAAGAGTT TATGACTGAA AGGTCAGAAA ATAA

(SEQ ID NO 147)

73/103

Figure 73

CTAAGGATAT ATTGGAACA TCTTCTTACG AAGATGCAGG AATAACATTG ACATATTGTA TTCAGNTGTG
AATGCTCATT GGAGNATTCA TNGCATNATT TGGTNCATTG ACANCTAGAT AAGNAAGTAA AATTATGAT
TTTACCAAGC AAAACCGAGT GAATTAGAGT TNTNNAACAA GCTTTGATTT CAAAAAGAAA TAATCGCTAG
TGTTGAAAG AACACTCACA GATTANTAAC ATCTTGGGTT TTCACCCGAC TTGTTTCGTNT CGAAAGTCAA

AAAA

(SEQ ID NO 148)

74/103

Figure 74

AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTG TG GGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTGACACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAA AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAAA AAATAA

(SEQ ID NO 149)

Figure 75

AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTC GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTGACACGCA GGAGGTCAGC GGTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAGA AAAATAA

(SEQ ID NO 150)

Figure 76

AAGGAAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTT GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCAACGA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAGA AAAATAA

(SEQ ID NO 151)

77/103

Figure 77

AAGGATAAGG AACTGCCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATGTGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAC GCTGTAGTAT
TAAAAGAGTT TATGACTGAA AGGTCAGAAA ATAA

(SEQ ID NO 152)

78/103

Figure 78

AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTGACACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAC GCTGTAGTAT
TAAAGAGTT TATGACTGAA AGTCAAAA TAA

(SEQ ID NO 153)

SUBSTITUTE SHEET (RULE 26)

Figure 79

TAAGGAAGAT CGAGAATTGG AAAGAGGTCG GATTATCCG GATGATCCTT CTCCATCTTA TTAGAACATA
GATCGCAGGC CAGTCAGCCT GACGATCGCT TGCAGGCGTG CCGCCTTCGT TTCTCTTTCT TCATTGTTGA
TTGCTCACGG GCCGTACCGC AGCTGACGCT GCTGGCCCTG CGCAGGCGCG GCCCATCAGG GCCGAACGGC
CGGTCGGCCT TGCNAAGCTT CGCTTCGGGG TGGATCTGTG GATCGCGTAG TAGCGTTTGC GTCGGTATCT
GGGCTTG TAG CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGTCG GAGGTTCAAG TCCTCCCAGG^{79/103}
CCCACCAAGT TACTTGATGA GGGCCGTAG CTCAGCTGGG AGAGCACCTG CTTTGCAAGC AGGGGTCGT¹⁰³
CGGTTGATC CCGTCCGGCT CCACCATCAT GTTGGTGTTG AGACGGATAT TGGCAATCAA CAAAAGAAAAG
AAACAAGTT GCGGACTNNT ACGAAAGTCT CGCAAGAAGC TTGCTCAAGC CTTGCATAAT GATTGATGTG TTAAACCGCC
ATCAACTGAA GAGTTGATGT CGCAAGAAGC AAGCTGGTCT TTCGTCTGAT ACTGTTGAAA CGAGCATTTG CAGTCGAATG
ATCACCGATT GTATCTCGAG AAGCTGGTCT TTCGTCTGAT ACTGTTGAAA CGAGCATTTG CAGTCGAATG
GCAACATTCG GCGTCGCATA ATGCGGCTTT AAGAGCTGAG TTTTGATGGA TATTGGCAAT GAGAGTGATC
AAGTGTCCTA AGGGCATTTG TGGATGCCCTT GGCATGCAC

(SEQ ID NO 154)

Figure 80

AAGGAGCACG ACGAGAAACA CTCCAATTGG TGGGGTGTA GCGGTGAGGG GTTCTCGTCT GTAGTGGACG
GAAGCCGGGT GCACAACAAC AAGCAAGCCA AAGCAAGCCA GACACACTAT TGGGTCCCTGA GGCAACATCT CTGTTGGTTT
CGGATGTTG TCCACCATC TTGGTGGTGG GGTGTGGTGT TCGAGAAATG GATAGTGGTT GCGAGCATCA
ATTGGATGCG CTGCCCTTTG GTGGCGTGT CTGTTGTGCA ATTTATTCT TTGGTTTTTG TGTTTAT

(SEQ ID NO 157)

81/103

Figure 81

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGCCCGGGT GCACAACAAC AGGCAATCGC CGGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGACT
GAGTCGACG TGGTGTCCTT CCATCTTGGT GGTGGGGTGT GGTGTTGAG CATTGAATAG TGGTTGCCGAG
CATCTAGCCG GATGCGTTCC CCAGTGGTGC GCGTTCGTCA AAAATGTGTA ATTTTCTTT TGGTTTGTGT
GTTCGT

(SEQ ID NO 158)

SUBSTITUTE SHEET (RULE 26)

Figure 82

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAAC AGGCAATCGC CGGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGACT
GAGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG CATTGAATAG TGGTTGCGAG
CATCTAGACG GATGCCGTTCC CCAGTGGTGC GCGTTCGTCA AAAATGTGTA ATTTTCTTT TGGTTTGTGT
GTTCGT

(SEQ ID NO 159)

83/103

Figure 83

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGNNCGGGT NNACAACAAC NGCCAATCGC CGGACACACT ATTGGGNCCT GAGACAACAC TCGGCCGACT
GAGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTGAG CATTGAATAG TGGTTGCGAG
CATCTAGCCG GATGCGTTCC CCAGTGGTGC GCGTTCGTCA AAAATGTGTA ATTTTCTNT TGGTTT'TTGT
GTTCGT

(SEQ ID NO 160)

84/103

Figure 84

AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGAGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAGC AGACAATCGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGACT
TTGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTGAG CATGAATAG TGGTGGCAG
CATCTAGACG GATGCGTTGC CCTCGGGCCG CGTGTTCGTC AAAAATGTGT AATTTTCT TTTGGTTTTT
GTGTTCTGT

(SEQ ID NO 161)

85/103

Figure 85

AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGAGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAAC AGGCAATCGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGGCT
TTGAGTCGAA GTGGTGTCCC TCCATCTTGG TGGTGGGGTG TGGTGTGA GCATTGAATA GTGGTTGCCA
GCATCTAGAC GGATGCCGTTG CCTTCGGGCC GCGTGTTCGT CAAAAATGTG TAATTTTTC TTTGGTTT
TGTGTTCGT

(SEQ ID NO 162)

86/103

Figure 86

AGGGAGCACC GNAACGCAAT CCCGCGTGGG GTGTGGGTTC GGCGTGTGT GGCCTCGGNC CGAGGTGTTG
GGCAGCAGGC AGTAACCNCC GGAACACTGT TGGGTTTGA GNNAACACCC GTGGTGGTGT TGTGCTCCCC
GTGGTGNCGG GGTGTGGTGT TTGAGTGTG GATAGTGGTT GCGAGCATCT GGCAAAGACT GTGGTAAGCG
GTTTTGTTG ANTGTTTCT GGTGTTGT

(SEQ ID NO 163)

SUBSTITUTE SHEET (RULE 26)

87/103

Figure 87

AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGNCGGGT GCACAACAAC AGNCAATCGC CAGACACACT ATTGNNCCCT GAGACAACAC TCGGCCGACT
TNGGTTGAAG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG TATTGGATAG TGGTTGCGAG
CATCTAANTG AACGCGTCGC CGNCAACGGT TACGTGTTTCG TTTTGTGTAA TTNTTTCTAT TGGTTTTTGT
GTTCGT

(SEQ ID NO 164)

88/103

Figure 88

AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAAC AGGCAATCGC CAGACACACT ATTGGNCCCT GAGACAACAC TCGGCCGACT
TTGGTCGAAG TGGTGTCCCC CCATCTTGGT GGTGGGGTGT GGTGTTTGAG TATTGGATAG TGGTTGCCGAA
CATCTAAATG AACGCGTTGC CGGCAACGGT TACGTGTTCTG TTTTAGTGTA ATTNTTCTA ATGGTTTTTG
TGTTTCGT

(SEQ ID NO 165)

SUBSTITUTE SHEET (RULE 26)

2193101

89/103

Figure 89

AAGGAGCACC ACGAGACCTG GGCCGGCCCC GCAGATCGCG GGATCAGCTG AGCTTTCAGG CGATTTCGTTG
GATGGCCTCG CACCTGTAGT GGGTGGGGGT CTGGTGCACT CAACAACCTT GGCCTGGGAT GCGGAAAGC
ATCTGCGGAA AATCATCAGA CACACTATTG GGCTTTGAGA CAACAGGCC CCAGNCCCTGN CCCGTTGGGG
GCAGNGGGTG TGTGTGTC TCACTTTGGT GGTGGGGGTG GTGTTTGATT TGTGGATAGT GGTGCGGAGC
ATCTAGCGCG CAGAATGTGT GGTCTCACTC CTTGTGGGTG GGGCCTGGTT TTGTGTGCGA TTGATGTGCA
ATTCTTTTG AAATCATTT TTTGGTTTT GTGTGT

(SEQ ID NO 166)

SUBSTITUTE SHEET (RULE 26)

90/103

Figure 90

AAGGAGCACC ACGAAAAACT CCCCAATTGG TGGGGTGTAAGCCGTGAGGG GTTCCCCGTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAGCGAAACG CCGGACACAC TATTGGGTCC TGAGGCAACA CTCGGGTTTG
TCCCCCTCAG GGATTTTCTG GGTGTTGTCC CACCATCTTG GTGGTGGGGT GTGGTGTGTTG AGAATTGGAT
AGTGGTTGCG AGCATCAAAT GGATGCCGTTG CCCCTACGGG TAGCGTGTTT TTTGTGCAA TTTTATTCNT
TGGTTTGT GTTTGT

(SEQ ID NO 167)

91/103

Figure 91

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AAGGAGCACC ACGAGAAGCA CTCCAACTGG TGGGGTGCAA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGAGCCGGGT GCGCGACAAC GAACGAGCCA GACACACTAT TGGGTCCCTGA GGCAACACTC GGGCTTGGCC
AGAGCTGTTG TCCCAACCATC TTGGTGGTGG GGTGTGGTGT TTGAGAAATG GATAGTGGTT GCGAGCATCA
AATGGATGCG TTGCCCCCTAC GGGTGGCGTG TTCCTTTGTG CAATTTTATT CTTTGGTTT TGTGTTTGT
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(SEQ ID NO 168)

Figure 92

AAGGAGCACC ACGAAAAACA CCCCAACTGG TGGGTGTAA GCCGTGAGGG GCTCCCGTCT GTAGTAGACG
GGCGCCGGGT GCGCAACAGC AAGCGAGCCA GACACACTAT TGGGTCCCTGA GGCAACACTC GGGCTTGTCT
TGGACTCGTC CAAGAGTGTT GTCCCACCAT CTTGGTGGTG GGGTGTGGTG TTGAGAAAT GGATAGTGGT
TGCGAGCATC ANCTGGATGC GTTGCCCCCA GGGTAGCGT GTTCTTTTGT GCAATTNTAT TCNNTGGTTT
TTGTGTAGT

92/103

(SEQ ID NO 169)

SUBSTITUTE SHEET (RULE 26)

93/103

Figure 93

AAGGAGCACC ACGAAAAACA CTCGCAATCC GGTGGGGTGT GAGCCGTGAG GGAGCCCGTG CCTGTAGTGG
GTGTGGGTG GTGCGCGAC AACAAATGGG AAAAATCGCT GGCACACTA TTGGGCTTTG AGGCAACACC
TGGTTTGT TGGGTGGTGT CGCTCCATCT TGGTGGTGG GTGTGGTGT TGAGTTGTGG ATAGTGGTTG
CGAGCATCTA AGCAAAAGCT GTTGTGAC GGTTTTGTG GAGTGTGTG TGTGT

(SEQ ID NO 170)

SUBSTITUTE SHEET (RULE 26)

94/103

Figure 94

AAGGAGCACC ACGAAAAACA CTCCAATTGG TGGGGTGTAAGCCGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AAATGAATCG CCAGACACAC TGTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCCATCTTG GTGGTGGGGT GTGGTGTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGATACGTT GCCAGTAATG GTGGCGTATT CATTGAAAAT GTGTAATTTT
CTTCTTTGGT TTGTGTGT

(SEQ ID NO 171)

95/103

Figure 95

AAGGAGCACC ACGAAAAACA CTCCAATTGG TGGGGTGTAAGCCGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AAATGAATCG CCAGACACAC TGTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCCATCTTG GTGGTGGGGT GTGGTGTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGATACGTT GCCAGTAATG GTGGCGTGTT CATTGAAAAT GTGTAATTTT
CTTCTTTGGT TTTGTGTGT

(SEQ ID NO 172)

96/103

Figure 96

AAGGAGCACC ACGAAAACA CTCCAATTGG TGGGGTGTA GCGGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AATGAATCG CCAGACACAC TGTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCATCTTG GTGGTGGGT GTGGTGTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGANACGTT GCCAGTAATG GTGGCGTGTT CATTGAAAAAT GTGTAATTTT
CTTCTTTGGT TTTGTGTGT

(SEQ ID NO 173)

97/103

Figure 97

AAGGAGCACC ATTCTCAGT CGAATGAACT GAGAACATAA AGCGAGTATC TGTAGTGGAT ACATGCTTGG
TGAATATGTT TTATAAATCC TGTCACCCCG GTGGATAGGT AGTCGGCAAA ACGTCGGACT GTCATAAGAA
TTGAAACGCT GGCACACTGT TGGTCCCTGA GGCAACACAT TGTGTTGTCA CCCTGCTTGG TGGTGGGGTG
TGGTCCCTGA CTATGGATA GTGGTTGCCA GCATCTAAAC ATAGCCTCGC TCGTTTTCGA GTGAGGCTGG
TTTTTGCAAT TTTATTAGCT

(SEQ ID NO 174)

98/103

Figure 98

CCTAATGATA TTGATTTCGCG TGAAGTGCTC ACACAGATTG TCTGATGAAA AAGTAACGAG CAGAAATACC
TTTATAGGCT TGTAGCTCAG GTGGTTAGAG CGACCCCTG ATAAGGTGA GGTCGGTGGT TCAAGTCCAC
TCAGGCCTAC CACTTCTCGA AGTGAAAAG GTACTGCACG TGA CTGTATG GGGCTATAGC TCAGCTGGGA
GAGCGCCTGC CTTGCACGCA GGAGGTCAGC GGTCGATCC CGCTTAGCTC CACCATATAG TCCTGTATT
CAATACTCA GAGTGTA CTG GCAACAGTAT GCTGCCAAGT ATTTGCTCT TTAACAATCT GGAACAAGCT
GAAAATTGAA ACATGACAGC TGAAACTTAT CCCTCCGTAG AAGTATTGGG GTAAGGATTA ACCTGTCATA
GAGTCTCTCA AATGTAGCAG CACGAAAGTG GAAACACCTT CGGGTTGTGA

(SEQ ID NO 195)

Figure 99

CCTAATGATA TTGATTTCGCG TGAAGTGCTC ACACAGATTG TTTGATAGAA ACGTAATGAG CAAAAGCGCT
ACCTGTTGAT GTAATGAGTC ACTGACTCAT GCTGATACGA ACCGATTAAAG ACAGTCAGTT TAATCGGATT
TTCGTGTCCC CATCGTCTAG AGGCCTAGGA CACTGCCCTT TCACGGCTGT AACAGGGGT CGAATCCCCT
TGGGGACGCC ATTCGATAAT GAGTGAAAGA CATTATCACC GGTCTTGGA ACCGAAAACA TCTTAAAGAT
GACTCTTGCG AGTCGTGTTT AAGATAATTG CTTTAAACAA TCTGGAACAA GCTGAAAATT GAAACATGAC
AGCTGAAACT TATCCCTCCG TAGAAGTATT GGGTAAGGA TTAACCTGTC ATAGAGTCTC TCAAATGTAG
CAGCACGAAA GTGGAACAC CTCGGGTTG TGA

99/103

(SEQ ID NO 196)

100/103

Figure 100

TAAGGATAAG GAAGAAGCCT GAGAAGGTTT CTGACTAGGT TGGGCAAGCA TTTATATGTA AGAGCAAGCA
TTCTATTCA TTTGTGTTGT TAAGAGTAGC GCGGTGAGGA CGAGACATAT AGTTGTGAT CAAGTATGTT
ATTGTAAGA AATAATCATG GTAACAAGTA TATTTCACGC ATAATAATAG ACGTTTAAGA GTATTGTCT
TTAGGTGAA GTGCTTGCAAT GGATCTATAG AAATTACA

(SEQ ID NO 197)

101/103

Figure 101

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTG AGAGGTTTCAT CTCTCAAAAC
GTGTTCTTTG AAAACTAGAT AAGAAAAGTT AGTGTA AAAA GACGAAGAGA AACCGTAGGT TTTTCTTCAA
CCAAAACCGA GAATCAAACC GAGAAAGAAT CTTCCGTTT TCATAAGCGA TCGCACGTTT ATGAAAACAC
AACAAACACCT TCGTAAGAAG GATGA

(SEQ ID NO 213)

102/103

Figure 102

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTTG AGAGGTCAAT GACGCTCATA
CTGAGTACCA GGTGACACGT TTTTGAGGTG TCTCTTCGTA TGAGGGGCTT ATAGCTCAGC TGGTTAGAGC
GCACGCCCTGA TAAGCGTGAG GTCGGTGGTT CGAGTCCACT TAGGCCCACT TTTTGAATA AACCTTCTT
TTTTATATGT TAATAAGGG CCTTAGCTCA GCTGGGAGAG CGCTGCTTT GCACGCAGGA GTCAGCGGT
TCGATCCCGC TAGGCTCCAC CAAAGATAGT TTGTTCTTTG AAAACTAGAT AAGAAAAGTT AGTGTAATAA
GACGAAGAGA AACCGTAGGT TTTTCTTCAA CCAAAACCGA GAATCAAACC GAGAAAGAAT CTTTCCGTTT
TCATAAGCGA TCGCACGTTT ATGAAAACAC AACAAACACCT TCGTAAGAAG GATGA

(SEQ ID NO 214)

103/103

Figure 103

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTGT AGAGGTCAAT GACGCTCATA
CTGAGTACCA GGTGACACGT TTTTGAGGTG TCTCTTCGTA TGAGGGGCCT ATAGCTCAGC TGGTTAGAGC
GCACGCCCTGA TAAGCGTGAG GTCGGTGGTT CGAGTCCACT TAGGCCCACT TTTTGAATA AACCTTTCTT
TTTTATATGT TAATAAGGGG CCTTAGCTCA GCTGGGAGAG CGCCTGCTTT GCACGCAGGA GGTACGCGGT
TCGATCCCGC TAGGCTCCAC CAAAGATAGT TTGTTCTTTG AAACTAGAT AAGAAAAGTT AGTGTAATAA
GACGAAGAGA AACCGTAGGT TTTTCTTCAA CCAAAACCGA GAAAGAATCT TTCCGTTTTC ATAAGCGATC
GCACGTTTAT GAAAACACAA CAACACCTTC GTAAGAAGGA TGA

(SEQ ID NO 215)