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(54) HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE PROBES AND PRIMERS FOR DETECTION OF MICROORGANISMS

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(21) Appl. No.: **11/522,253**

(22) Filed: **Sep. 14, 2006**

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/236,785, filed on Sep. 27, 2005, which is a continuation of application No. 10/089,177, filed on Mar. 27, 2002, now abandoned, filed as application No. PCT/CA00/01150 on Sep. 28, 2000, Continuation-in-part of application No. 10/753,169, filed on Jan. 7, 2004, which is a continuation of application No. 09/989,643, filed on Nov. 20, 2001, now abandoned, which is a continuation of application No. 09/297,539, filed on May 3, 1999, now abandoned, filed as application No. PCT/CA97/00829 on Nov. 4, 1997.

(30) **Foreign Application Priority Data**

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May 19, 2000 (CA) 2037010

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C07H 21/00 (2006.01)

(52) **U.S. Cl.** **435/6**; 536/24.33

(57) **ABSTRACT**

Compositions and methods for the detection of vancomycin-resistant pathogens using primers and/or probes to the vanA and vanB genes.

atpD **sequences databases and main subsets.**

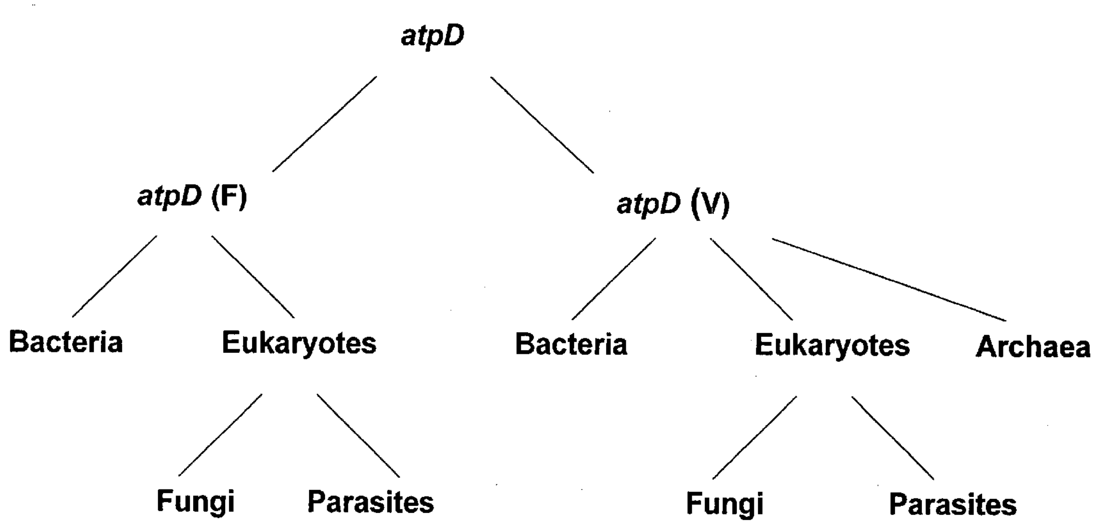


Figure 1: *atpD* sequences databases and main subsets.

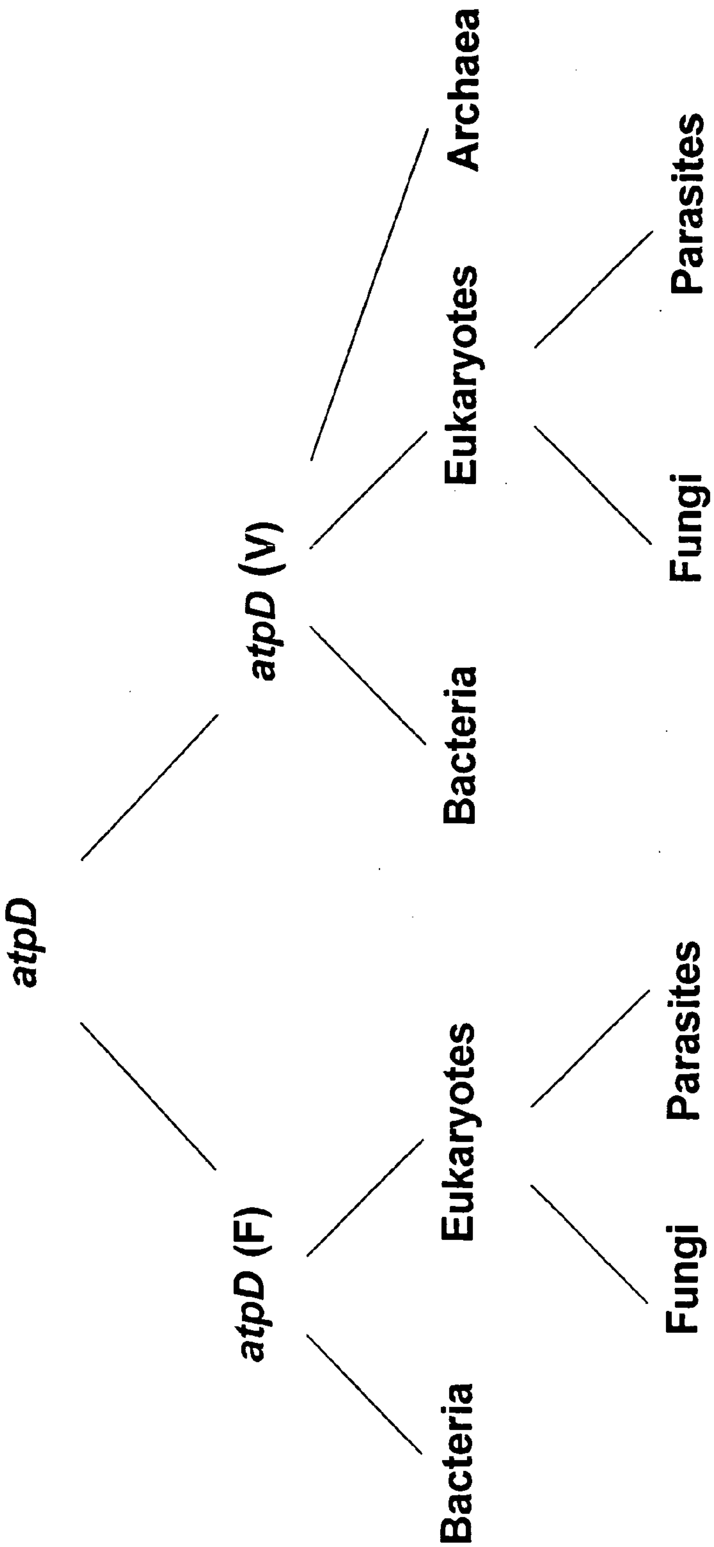


Figure 2: *tuf* sequences databases and main subsets.

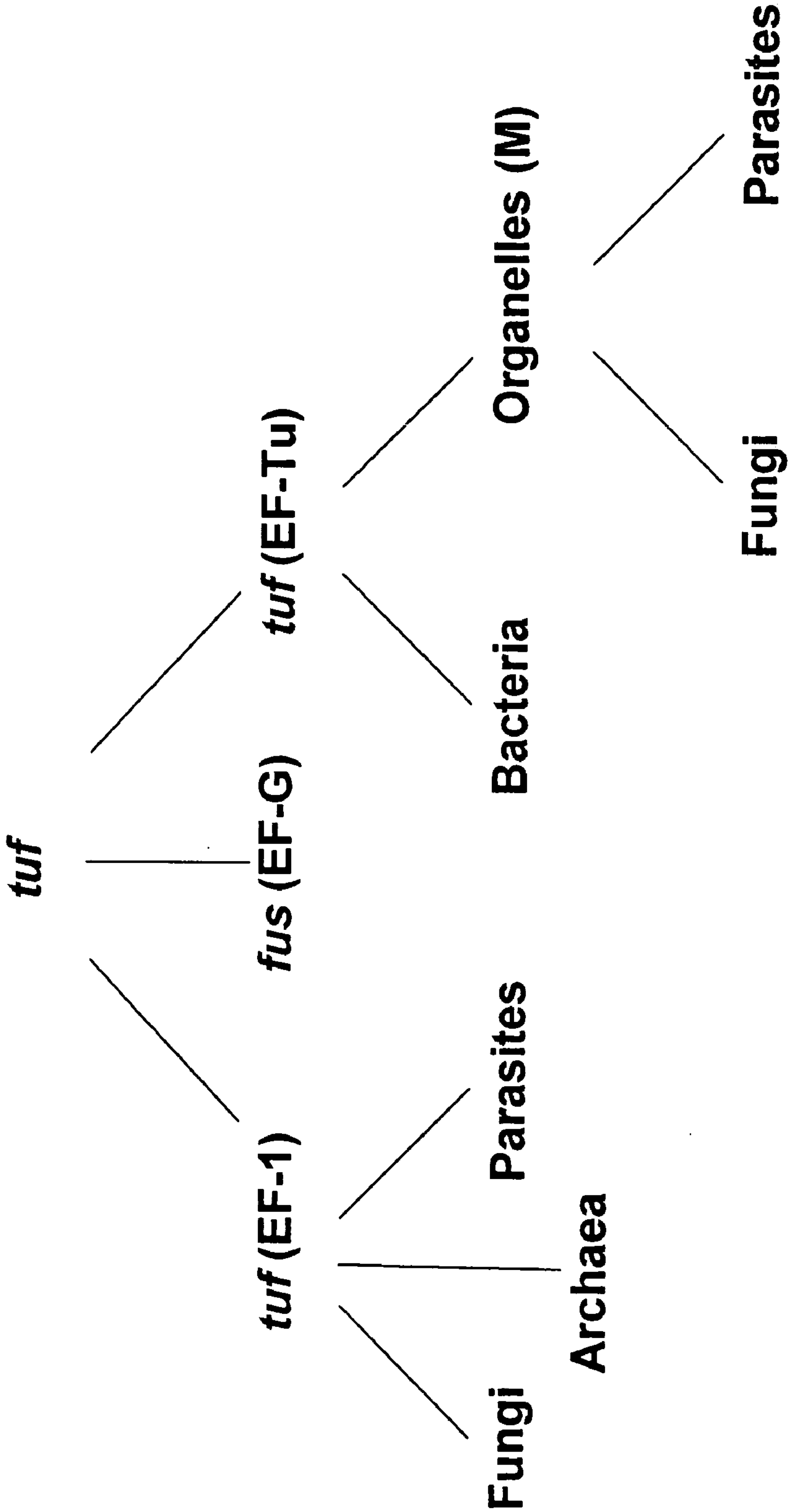
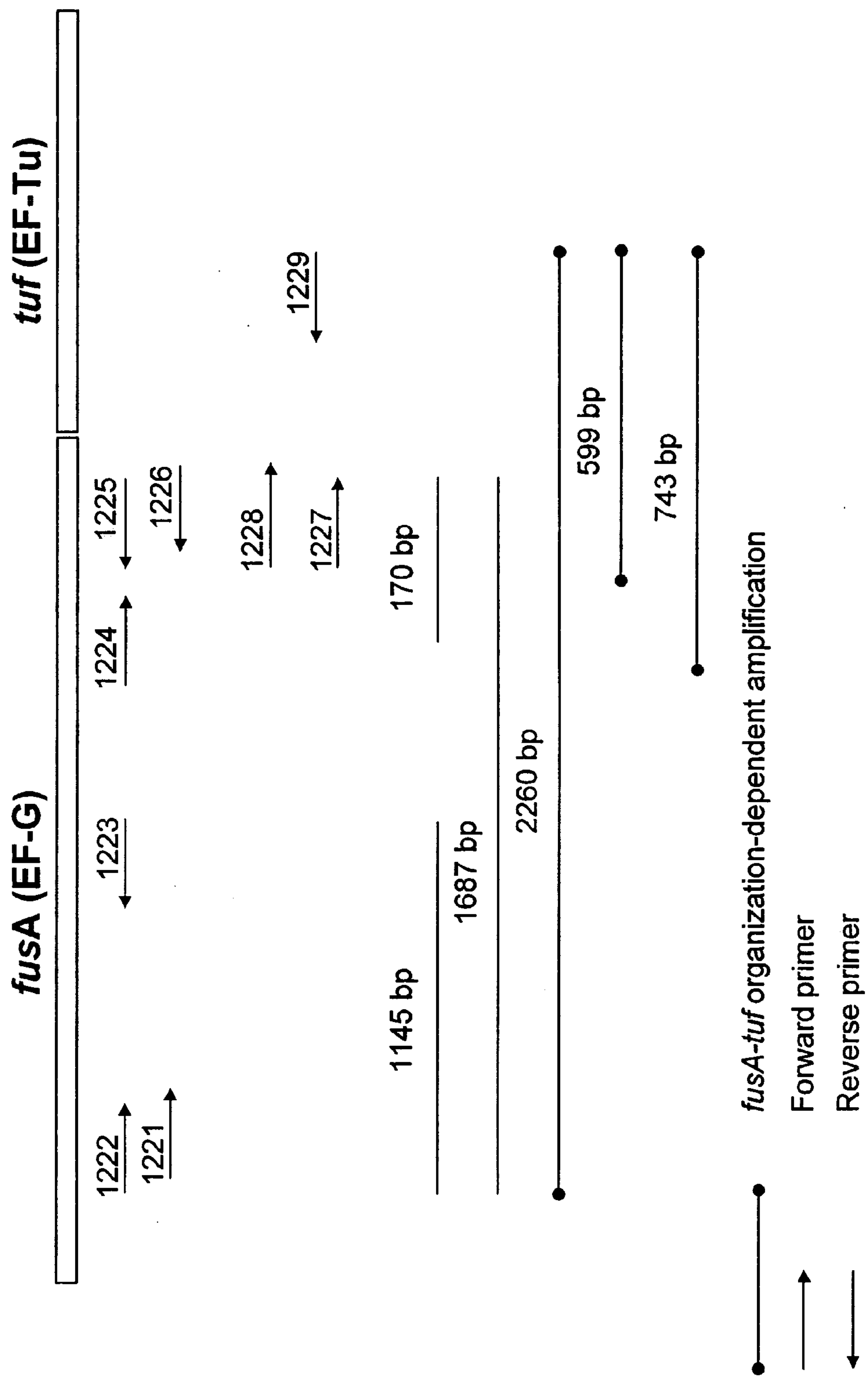


Figure 3.


fusA (EF-G)
tuf (EF-Tu)
1222
1221
1223
1224
1225
1226
1228
1227
1229
1145 bp
1687 bp
170 bp
2260 bp
599 bp
743 bp
fusA-tuf organization-dependent amplification
Forward primer
Reverse primer

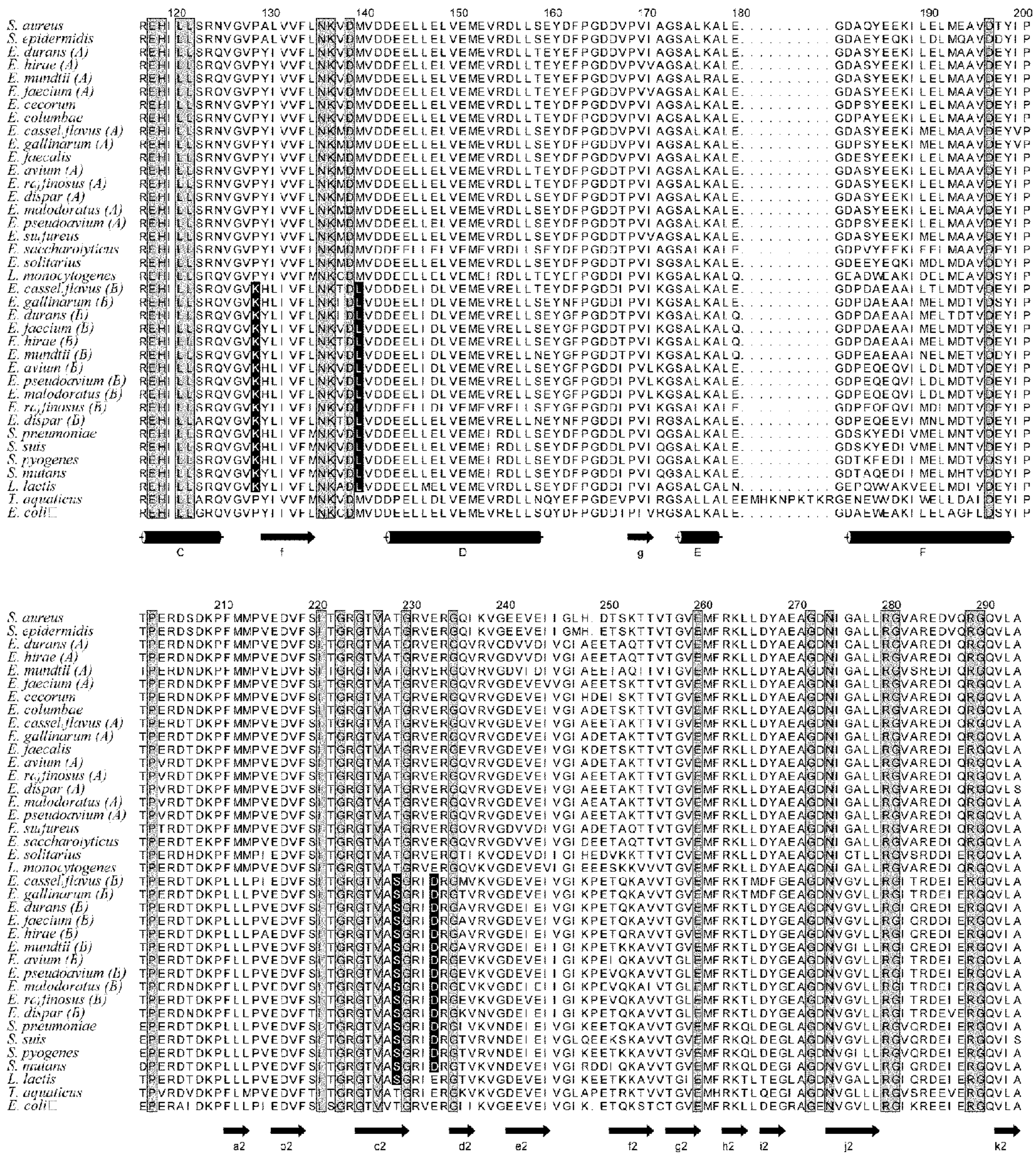


FIGURE 4.

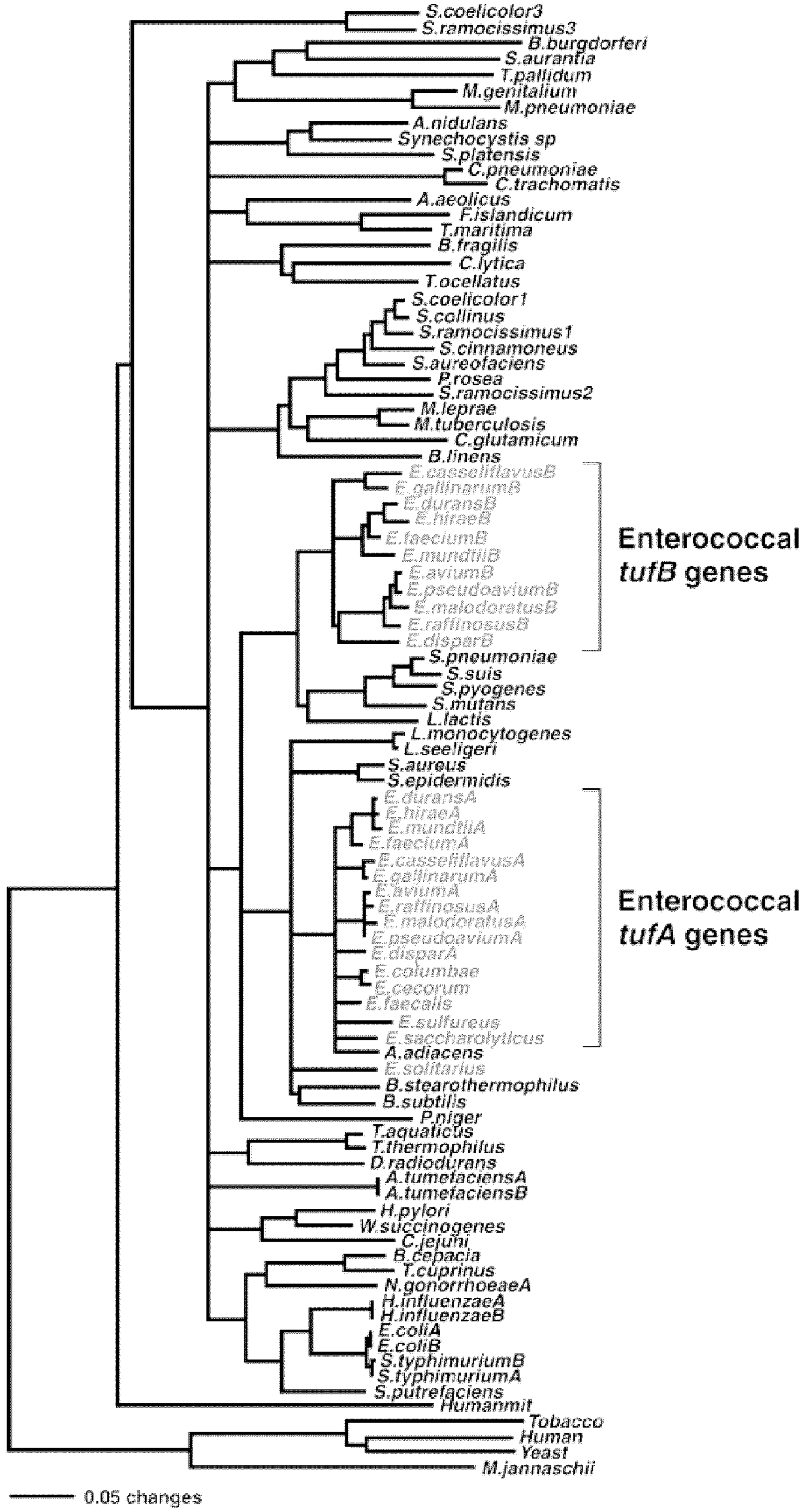

S.coelicolor3
S.ramocissimus3
B.burgdorferi
S.aurantia
T.pallidum
M.genitalium
M.pneumoniae
A.nidulans
Synechocystis sp
S.platensis
C.pneumoniae
C.trachomatis
A.aeolicus
F.islandicum
T.maritima
B.fragilis
C.lytica
T.ocellatus
S.coelicolor1
S.collinus
S.ramocissimus1
S.cinnamoneus
S.aureofaciens
P.rosea
S.ramocissimus2
M.leprae
M.tuberculosis
C.glutamicum
B.linens
E.casseliflavusB
E.gallinarumB
E.duransB
E.hiraeB
E.faeciumB
E.mundtiiB
E.aviumB
E.pseudoaviumB
E.malodoratusB
E.raffinosusB
E.disparB
Enterococcal tufB genes
S.pneumoniae
S.suis
S.pyogenes
S.mutans
L.lactis
L.monocytogenes
L.seeligeri
S.aureus
S.epidermidis
E.duransA
E.hiraeA
E.mundtiiA
E.faeciumA
E.casseliflavusA
E.gallinarumA
E.aviumA
E.raffinosusA
E.malodoratusA
E.pseudoaviumA
E.disparA
E.columbae
E.cecorum
E.faecalis
E.sulfureus
E.saccharolyticus
A.adiacens
Enterococcal tufA genes
E.solitarius
B.stearothermophilus
B.subtilis
P.niger
T.aquaticus
T.thermophilus
D.radiodurans
A.tumefaciensA
A.tumefaciensB
H.pylori
W.succinogenes
C.jejuni
B.cepacia
T.cuprinus
N.gonorrhoeaeA
H.influenzaeA
H.influenzaeB
E.coliA
E.coliB
S.typhimuriumB
S.typhimuriumA
S.putrefaciens
Humanmit
Tobacco
Human
Yeast
M.jannaschii
0.05 changes

FIGURE 5.

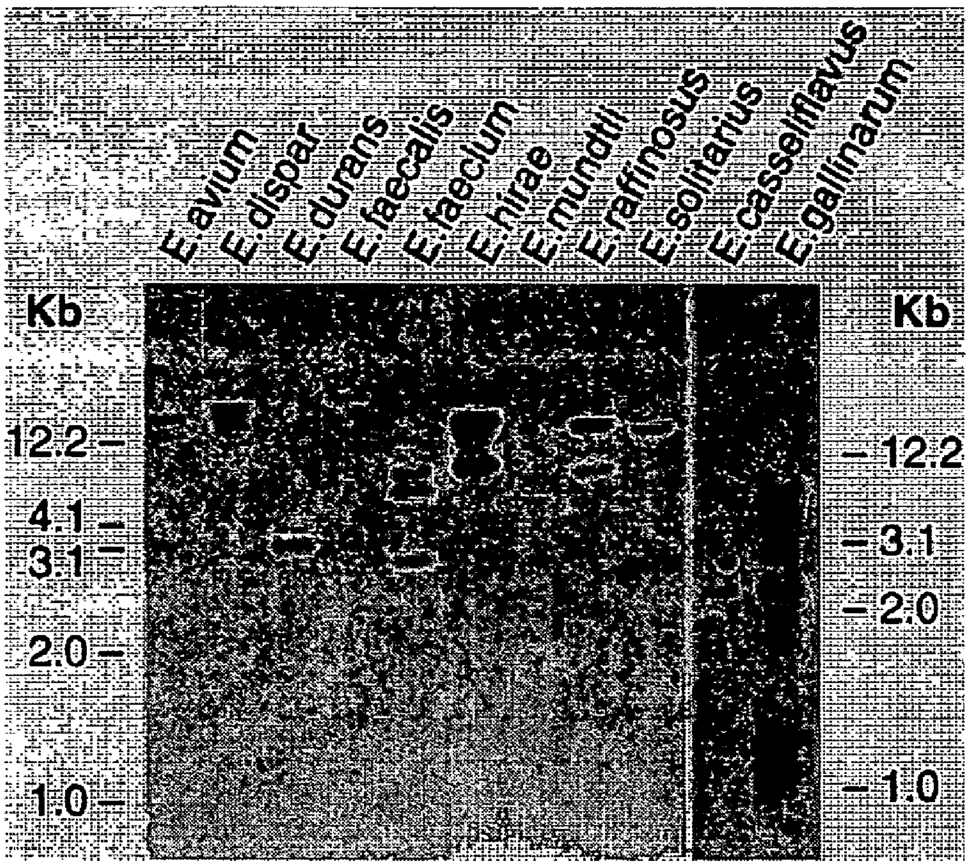

E.avium
E.dispar
E.durans
E.faecalis
E.faecium
E.hirae
E.mundtii
E.raffinosus
E.solitarius
E.casseliflavus
E.gallinarum
Kb
12.2
4.1
3.1
2.0
1.0
Kb
12.2
3.1
2.0
1.0

FIGURE 6.

FIGURE 7

```
                                                                      SEQ ID
                301        311        321                   331        NO :
E. coli         GAGATCGGTG AAGAAGAGCG TTGGG..... .......... CGATTCACCG (2620)
E. agglomerans  GACATCGGTG AAGAAGAGCG TTGGG..... .......... CGATCCACCG (2621)
P. agglomerans  GAGCTGAAAG AAGAAGATGG CAGCGCAGTA GAGATCGCCT CTATTCACCG (2622)
P. dispersa     GACCTGAAAG AAGAAGACGG CAGCGCTGTA GAGGTTTCCT CTATTCATCG (2623)
T. ptyseos      GACCTGAAGA ACGAAGATGG TAGCAATGTT GAGGTGAACT CTATTCACCG (2624)

E. coli         ~I~~G~~E~~ E~~E~~R~~W ~~A~~..... .......... I~~H~~R~~  (2625)
E. agglomerans  ~I~~G~~E~~ E~~E~~R~~W ~~A~~..... .......... I~~H~~R~~  (2626)
P. agglomerans  ~L~~K~~E~~ E~~D~~G~~S ~~A~~V~~E~ ~I~~A~~S~~ I~~H~~R~~  (2627)
P. dispersa     ~L~~K~~E~~ E~~D~~G~~S ~~A~~V~~E~ ~V~~S~~S~~ I~~H~~R~~  (2628)
T. ptyseos      ~L~~K~~N~~ E~~D~~G~~S ~~N~~V~~E~ ~V~~N~~S~~ I~~H~~R~~  (2629)
```

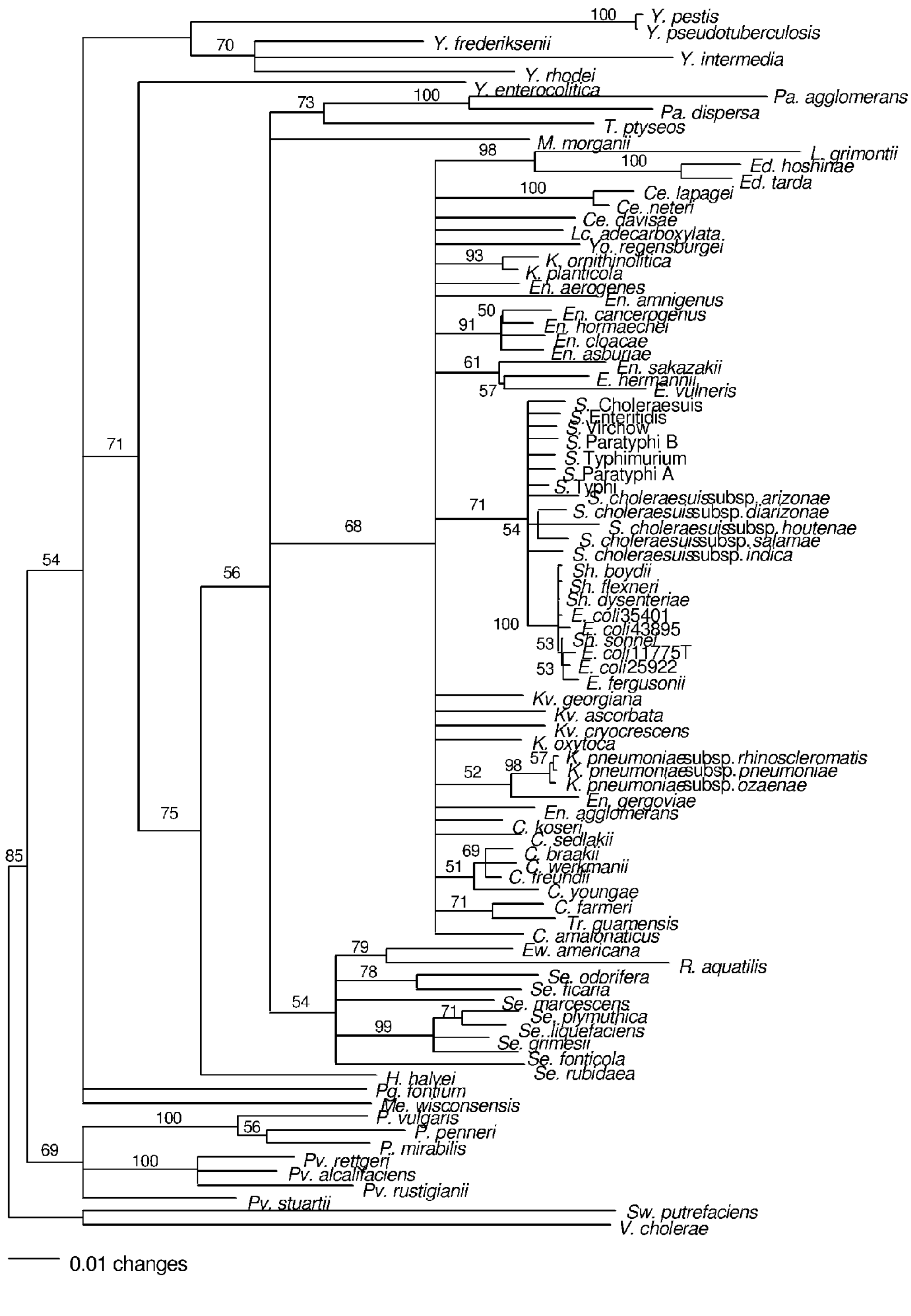
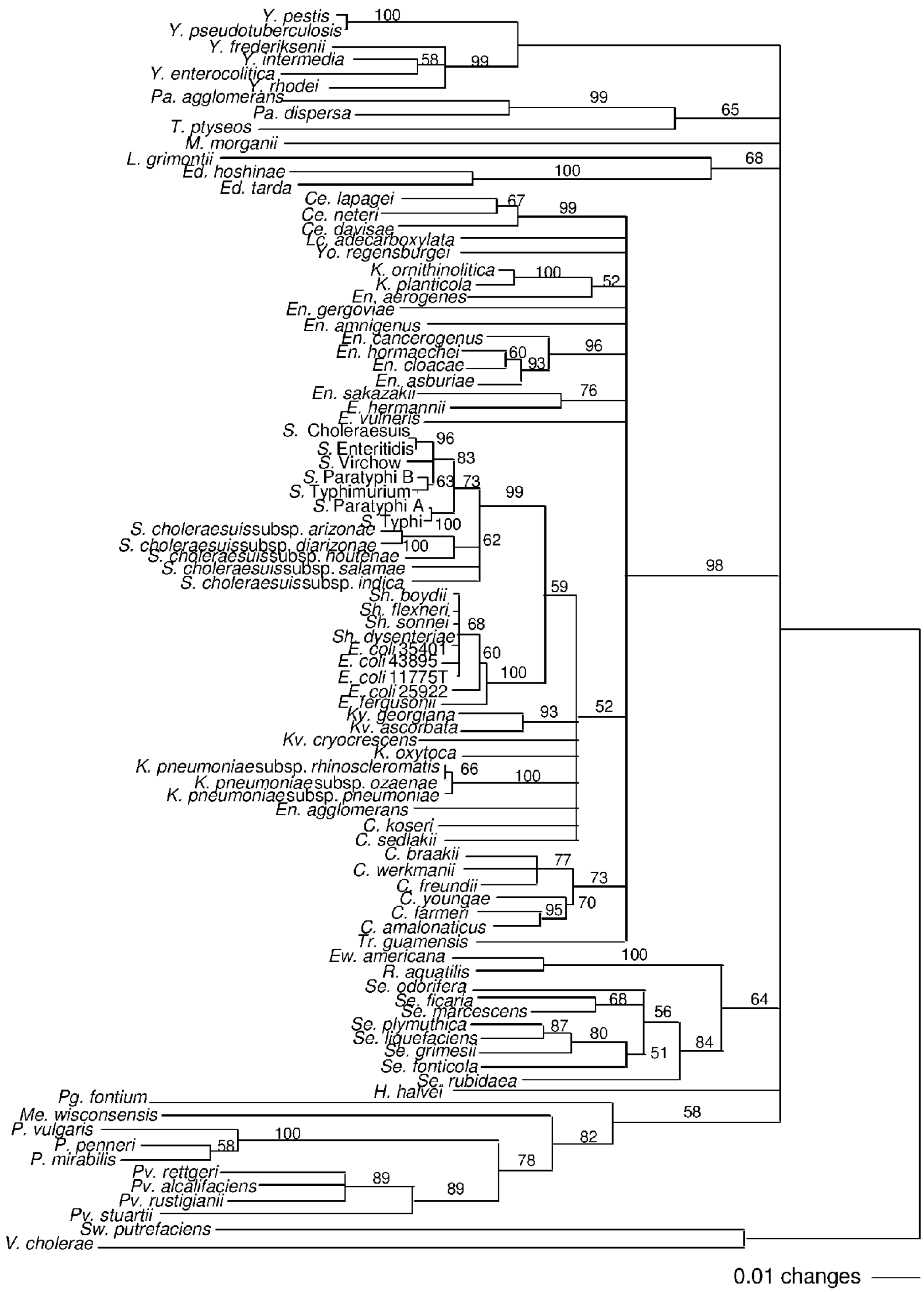

Y. pestis
Y. pseudotuberculosis
Y. frederiksenii
Y. intermedia
Y. rhodei
Y. enterocolitica
Pa. agglomerans
Pa. dispersa
T. ptyseos
M. morganii
L. grimontii
Ed. hoshinae
Ed. tarda
Ce. lapagei
Ce. neteri
Ce. davisae
Lc. adecarboxylata
Yo. regensburgei
K. ornithinolitica
K. planticola
En. aerogenes
En. amnigenus
En. cancerogenus
En. hormaechei
En. cloacae
En. asburiae
En. sakazakii
E. hermannii
E. vulneris
S. Choleraesuis
S. Enteritidis
S. Virchow
S. Paratyphi B
S. Typhimurium
S. Paratyphi A
S. Typhi
S. choleraesuis subsp. arizonae
S. choleraesuis subsp. diarizonae
S. choleraesuis subsp. houtenae
S. choleraesuis subsp. salamae
S. choleraesuis subsp. indica
Sh. boydii
Sh. flexneri
Sh. dysenteriae
E. coli 35401
E. coli 43895
Sh. sonnei
E. coli 11775T
E. coli 25922
E. fergusonii
Kv. georgiana
Kv. ascorbata
Kv. cryocrescens
K. oxytoca
K. pneumoniae subsp. rhinoscleromatis
K. pneumoniae subsp. pneumoniae
K. pneumoniae subsp. ozaenae
En. gergoviae
En. agglomerans
C. koseri
C. sedlakii
C. braakii
C. werkmanii
C. freundii
C. youngae
C. farmeri
Tr. guamensis
C. amalonaticus
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. liquefaciens
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
Me. wisconsensis
P. vulgaris
P. penneri
P. mirabilis
Pv. rettgeri
Pv. alcalifaciens
Pv. rustigianii
Pv. stuartii
Sw. putrefaciens
V. cholerae
0.01 changes

FIGURE 8 (1/2)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Y. pseudotuberculosis
Y. frederiksenii
Y. intermedia
Y. enterocolitica
Y. rhodei
Pa. agglomerans
Pa. dispersa
T. ptyseos
M. morganii
L. grimontii
Ed. hoshinae
Ed. tarda
Ce. lapagei
Ce. neteri
Ce. davisae
Lc. adecarboxylata
Yo. regensburgei
K. ornithinolitica
K. planticola
En. aerogenes
En. gergoviae
En. amnigenus
En. cancerogenus
En. hormaechei
En. cloacae
En. asburiae
En. sakazakii
E. hermannii
E. vulneris
S. Choleraesuis
S. Enteritidis
S. Virchow
S. Paratyphi B
S. Typhimurium
S. Paratyphi A
S. Typhi
S. choleraesuis subsp. arizonae
S. choleraesuis subsp. diarizonae
S. choleraesuis subsp. houtenae
S. choleraesuis subsp. salamae
S. choleraesuis subsp. indica
Sh. boydii
Sh. flexneri
Sh. sonnei
Sh. dysenteriae
E. coli 35401
E. coli 43895
E. coli 11775T
E. coli 25922
E. fergusonii
Kv. georgiana
Kv. ascorbata
Kv. cryocrescens
K. oxytoca
K. pneumoniae subsp. rhinoscleromatis
K. pneumoniae subsp. ozaenae
K. pneumoniae subsp. pneumoniae
En. agglomerans
C. koseri
C. sedlakii
C. braakii
C. werkmanii
C. freundii
C. youngae
C. farmeri
C. amalonaticus
Tr. guamensis
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. liquefaciens
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
Me. wisconsensis
P. vulgaris
P. penneri
P. mirabilis
Pv. rettgeri
Pv. alcalifaciens
Pv. rustigianii
Pv. stuartii
Sw. putrefaciens
V. cholerae
100
58
99
99
65
100
68
67
99
100
52
60
93
96
76
96
83
63
73
99
100
100
62
59
98
68
60
100
93
52
66
100
77
73
95
70
100
68
56
87
80
51
84
64
100
58
89
89
78
82
58
0.01 changes

FIGURE 8 (2/2)

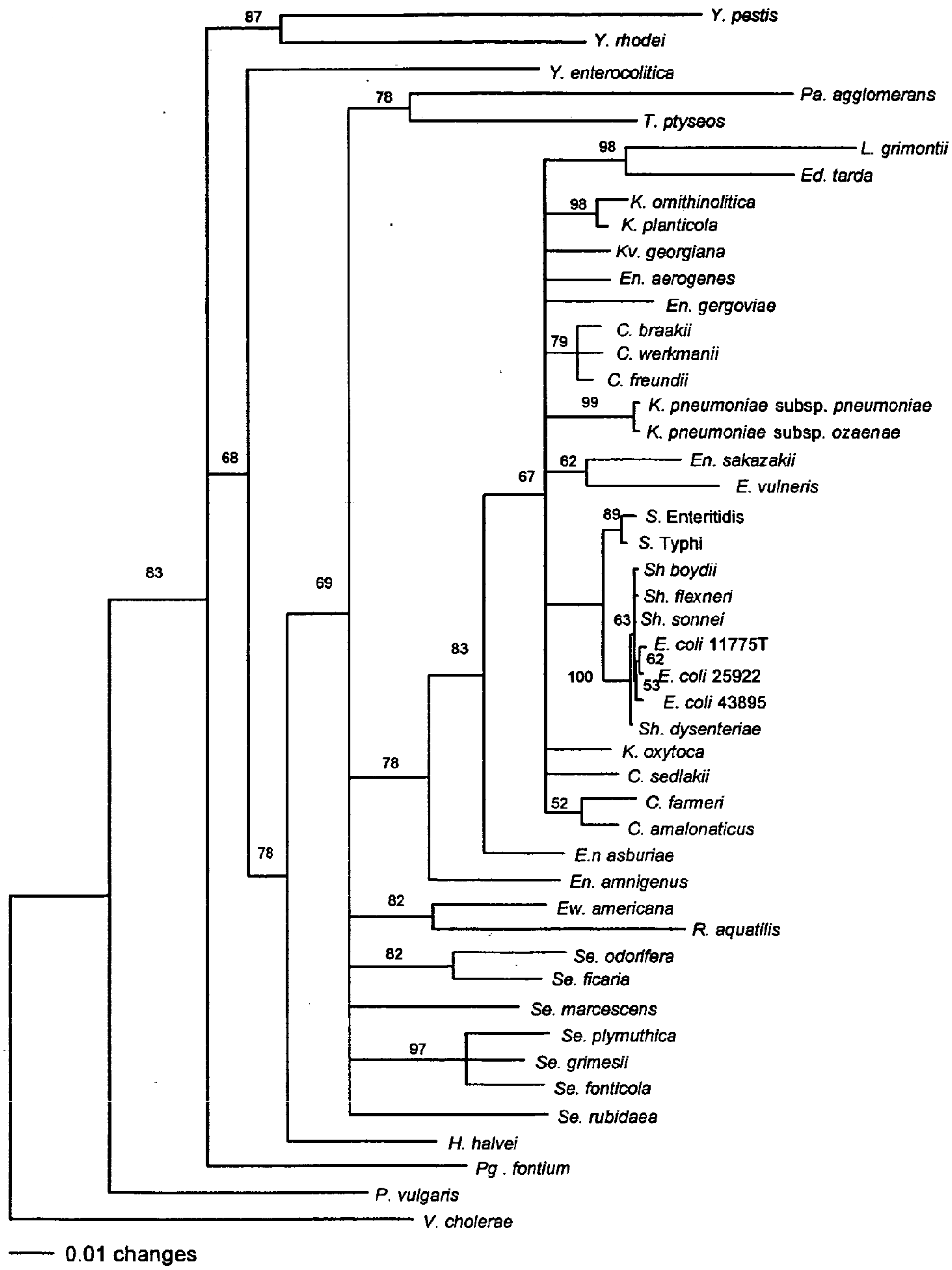
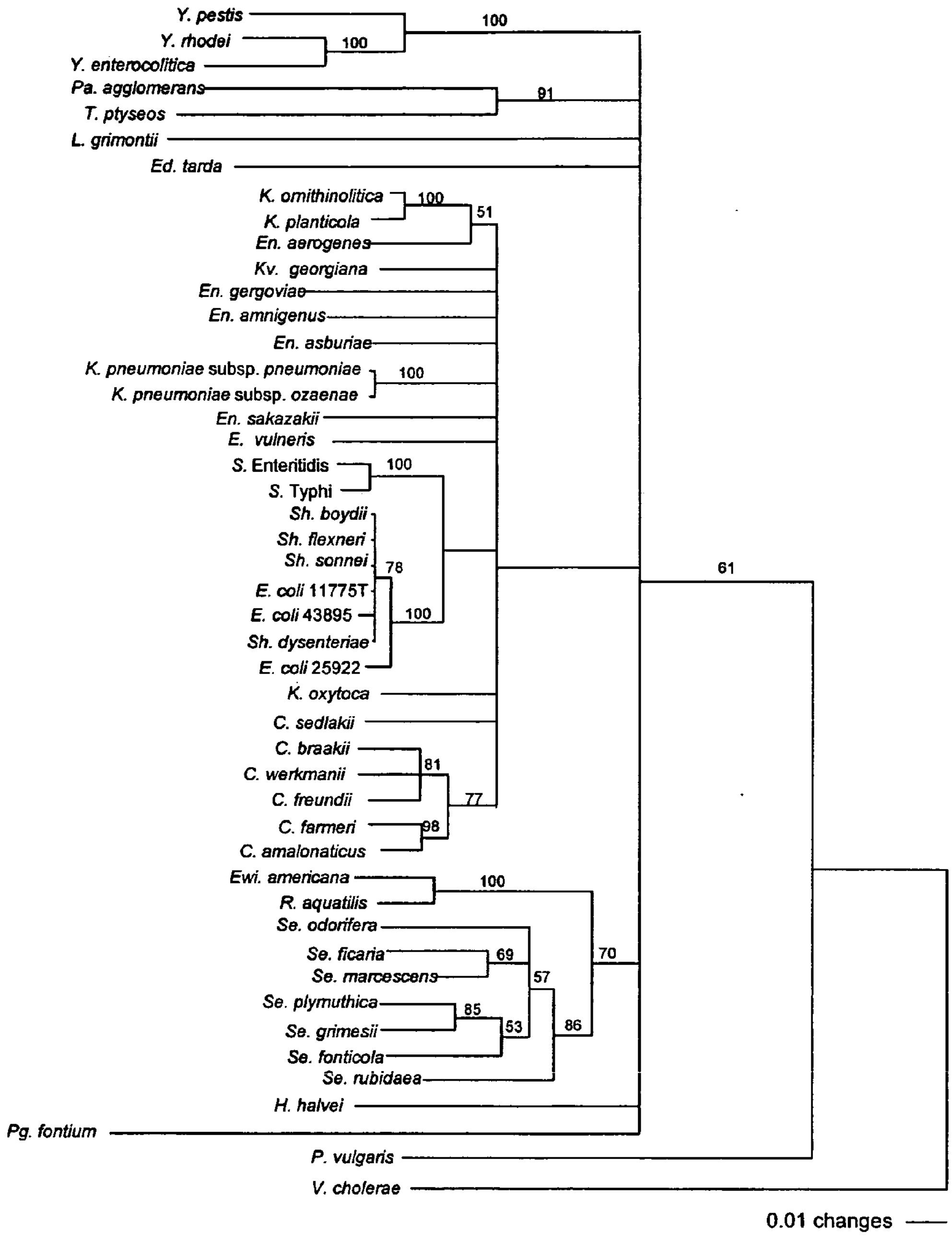

Y. pestis
Y. rhodei
Y. enterocolitica
Pa. agglomerans
T. ptyseos
L. grimontii
Ed. tarda
K. ornithinolitica
K. planticola
Kv. georgiana
En. aerogenes
En. gergoviae
C. braakii
C. werkmanii
C. freundii
K. pneumoniae subsp. pneumoniae
K. pneumoniae subsp. ozaenae
En. sakazakii
E. vulneris
S. Enteritidis
S. Typhi
Sh boydii
Sh. flexneri
Sh. sonnei
E. coli 11775T
E. coli 25922
E. coli 43895
Sh. dysenteriae
K. oxytoca
C. sedlakii
C. farmeri
C. amalonaticus
E.n asburiae
En. amnigenus
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg . fontium
P. vulgaris
V. cholerae
87
78
98
98
79
99
62
67
89
63
62
53
100
52
83
68
69
78
83
78
82
82
97
0.01 changes

FIGURE 9 a)



Y. pestis
Y. rhodei
Y. enterocolitica
100
100
Pa. agglomerans
T. ptyseos
91
L. grimontii
Ed. tarda
K. ornithinolitica
K. planticola
100
51
En. aerogenes
Kv. georgiana
En. gergoviae
En. amnigenus
En. asburiae
K. pneumoniae subsp. pneumoniae
K. pneumoniae subsp. ozaenae
100
En. sakazakii
E. vulneris
S. Enteritidis
S. Typhi
100
Sh. boydii
Sh. flexneri
Sh. sonnei
78
E. coli 11775T
E. coli 43895
100
Sh. dysenteriae
E. coli 25922
61
K. oxytoca
C. sedlakii
C. braakii
C. werkmanii
C. freundii
81
77
C. farmeri
C. amalonaticus
98
Ewi. americana
R. aquatilis
100
Se. odorifera
Se. ficaria
Se. marcescens
69
57
70
Se. plymuthica
Se. grimesii
85
53
86
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
P. vulgaris
V. cholerae
0.01 changes

FIGURE 9 b)

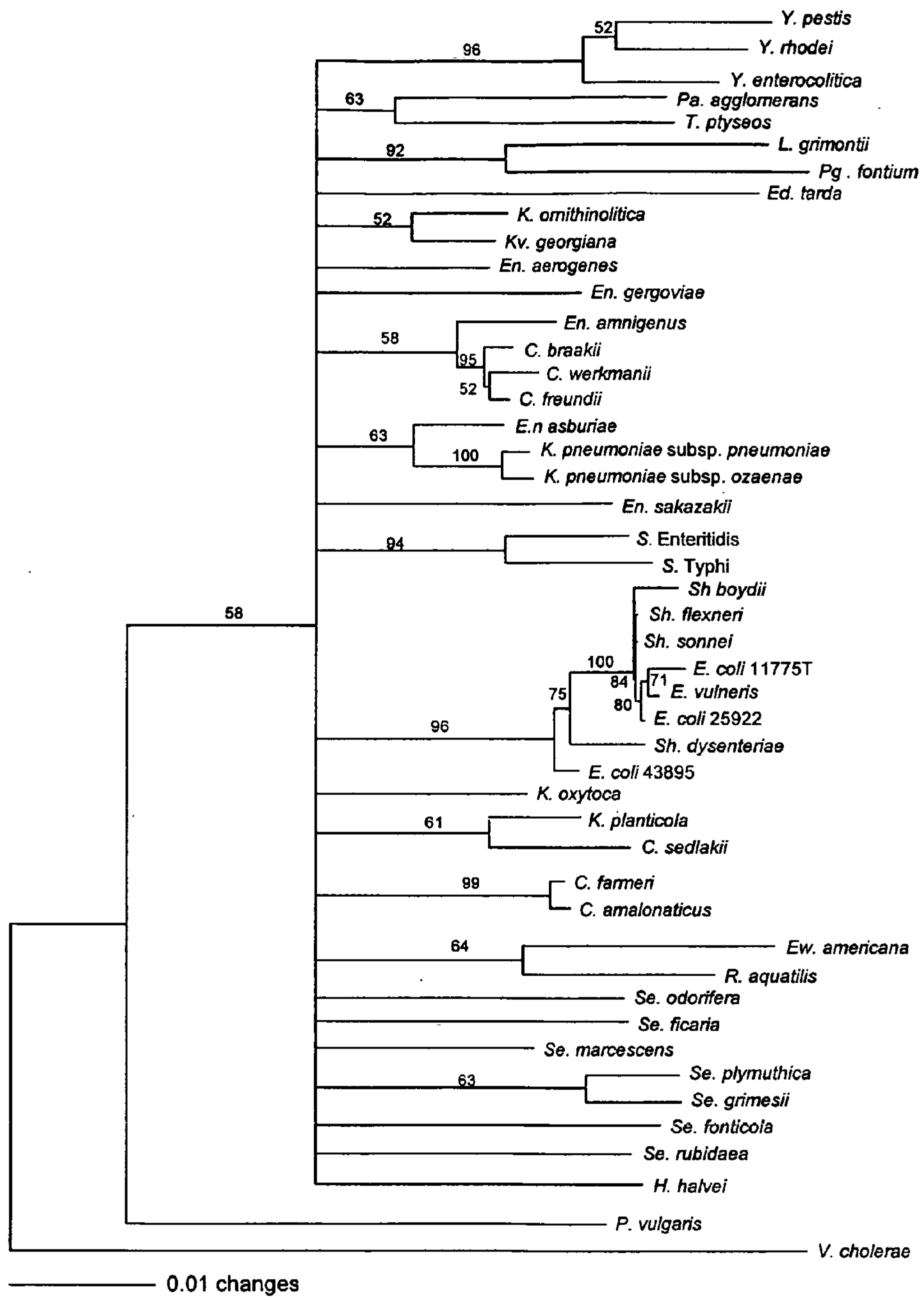

Y. pestis
Y. rhodei
Y. enterocolitica
Pa. agglomerans
T. ptyseos
L. grimontii
Pg . fontium
Ed. tarda
K. ornithinolitica
Kv. georgiana
En. aerogenes
En. gergoviae
En. amnigenus
C. braakii
C. werkmanii
C. freundii
E.n asburiae
K. pneumoniae subsp. pneumoniae
K. pneumoniae subsp. ozaenae
En. sakazakii
S. Enteritidis
S. Typhi
Sh boydii
Sh. flexneri
Sh. sonnei
E. coli 11775T
E. vulneris
E. coli 25922
Sh. dysenteriae
E. coli 43895
K. oxytoca
K. planticola
C. sedlakii
C. farmeri
C. amalonaticus
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
P. vulgaris
V. cholerae
52
96
63
92
52
58
95
52
63
100
94
58
100
84
71
75
80
96
61
99
64
63
0.01 changes

Figure 9 c)

FIGURE 10 a)

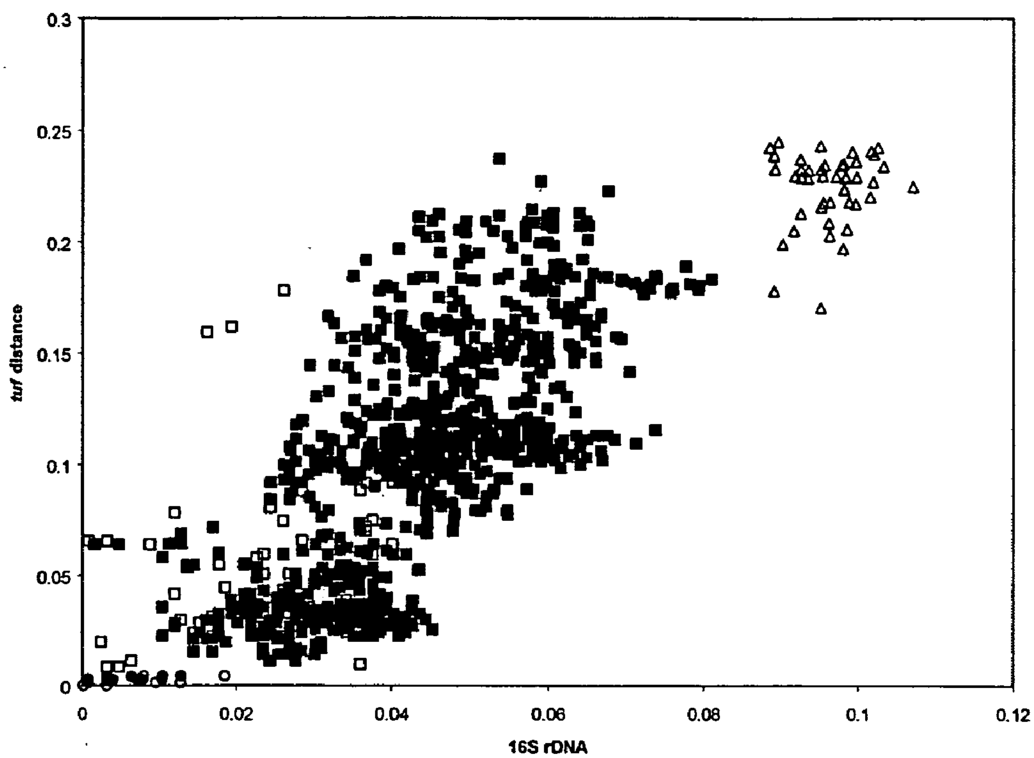

0.3
0.25
0.2
0.15
0.1
0.05
0
tuf distance
0
0.02
0.04
0.06
0.08
0.1
0.12
16S rDNA

FIGURE 10 b)

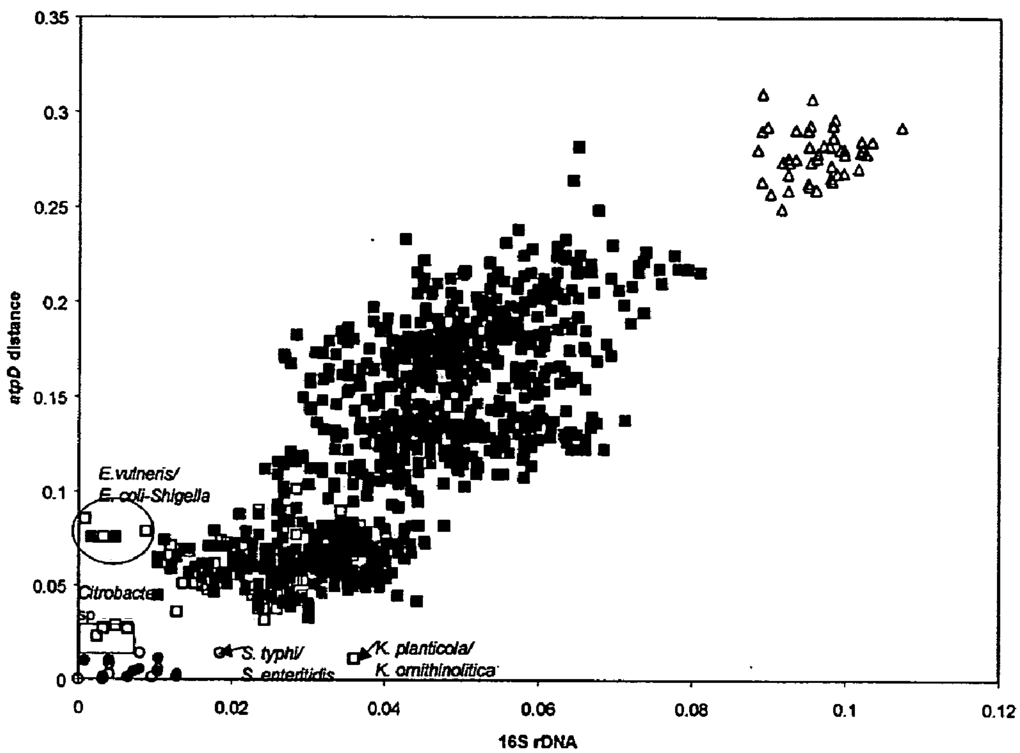

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
atpD distance
E.vulneris/
E.coli-Shigella
Citrobacter
sp
S. typhi/
S. enteritidis
K. planticola/
K. ornithinolitica
0
0.02
0.04
0.06
0.08
0.1
0.12
16S rDNA

FIGURE 10 c)

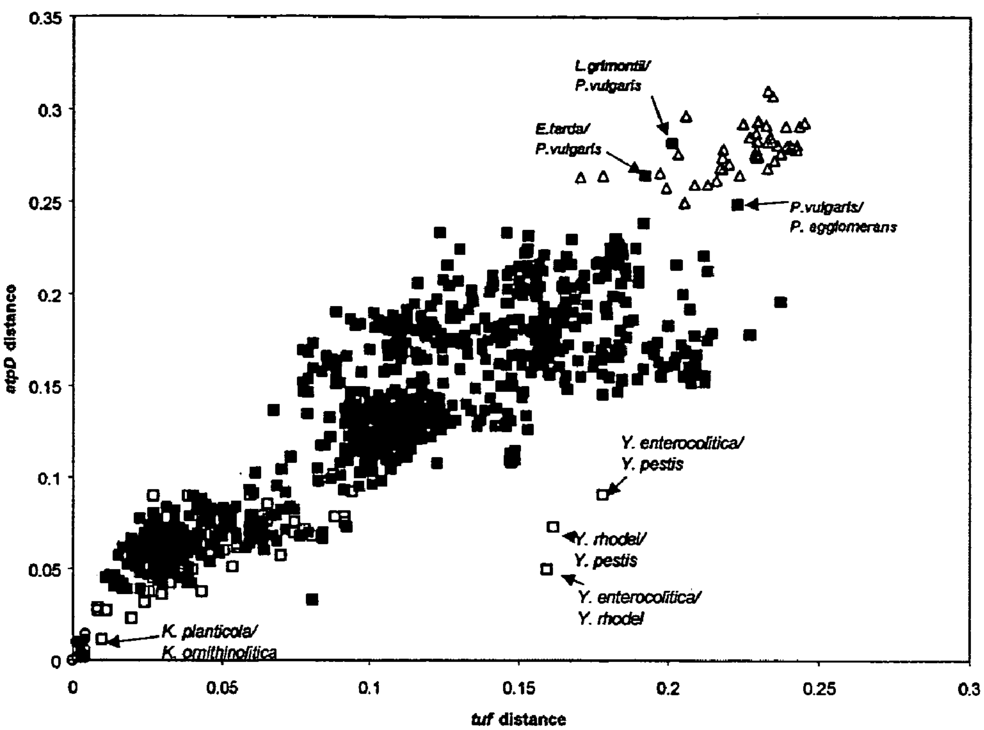
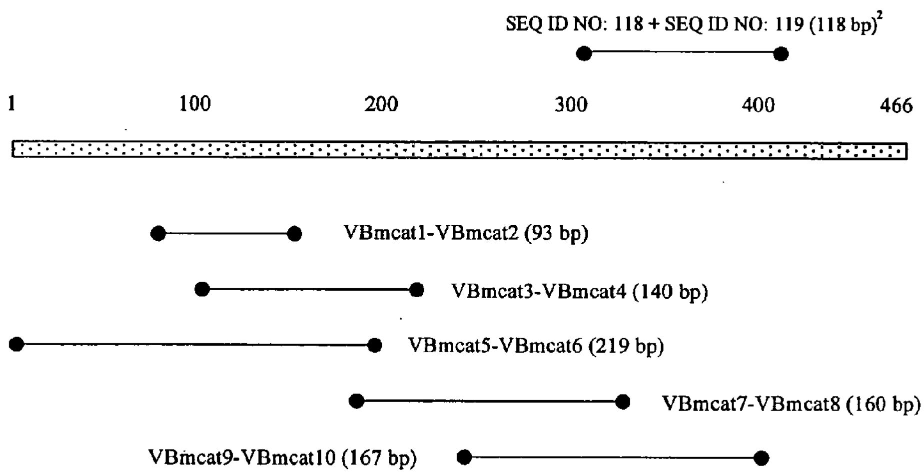
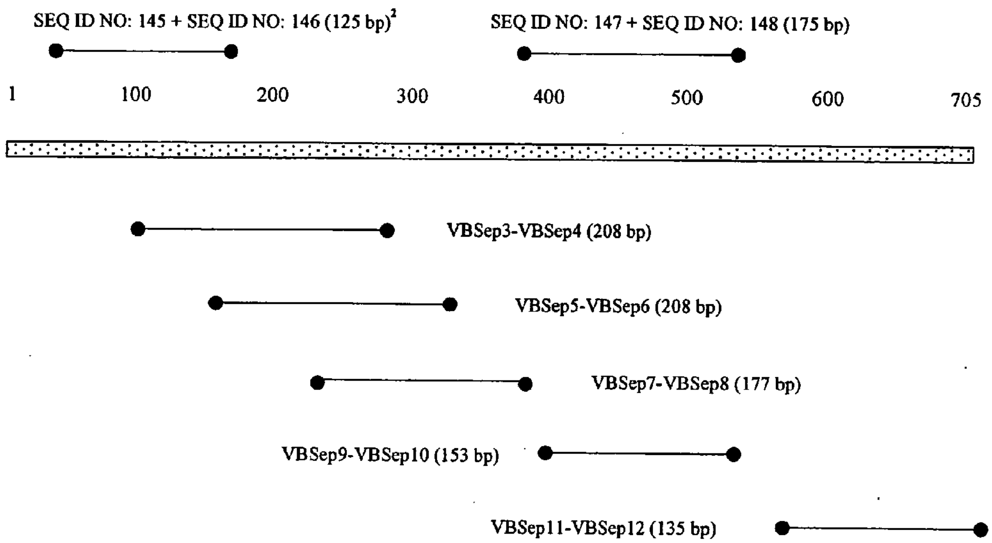

L.grimontii/
P.vulgaris
E.tarda/
P.vulgaris
P.vulgaris/
P. agglomerans
Y. enterocolitica/
Y. pestis
Y. rhodei/
Y. pestis
Y. enterocolitica/
Y. rhodei
K. planticola/
K. ornithinolitica
atpD distance
tuf distance
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.05
0.1
0.15
0.2
0.25
0.3

FIGURE 11: Position of the 5 new primer pairs selected from the *M. catarrhalis*-specific 466-bp DNA fragment (SEQ ID NO: 29)[1].



[1] All SEQ ID NOs. in this Figure are from US patent 6,001,564.
[2] Amplicon size is given in parenthesis.

FIGURE 12: Position of the 5 new primer pairs selected from the *S. epidermidis*-specific 705-bp DNA fragment (SEQ ID NO: 36)[1].



[1] All SEQ ID NOs. in this Figure are from US patent 6,001,564.
[2] Amplicon size is given in parenthesis.

Figure 13A

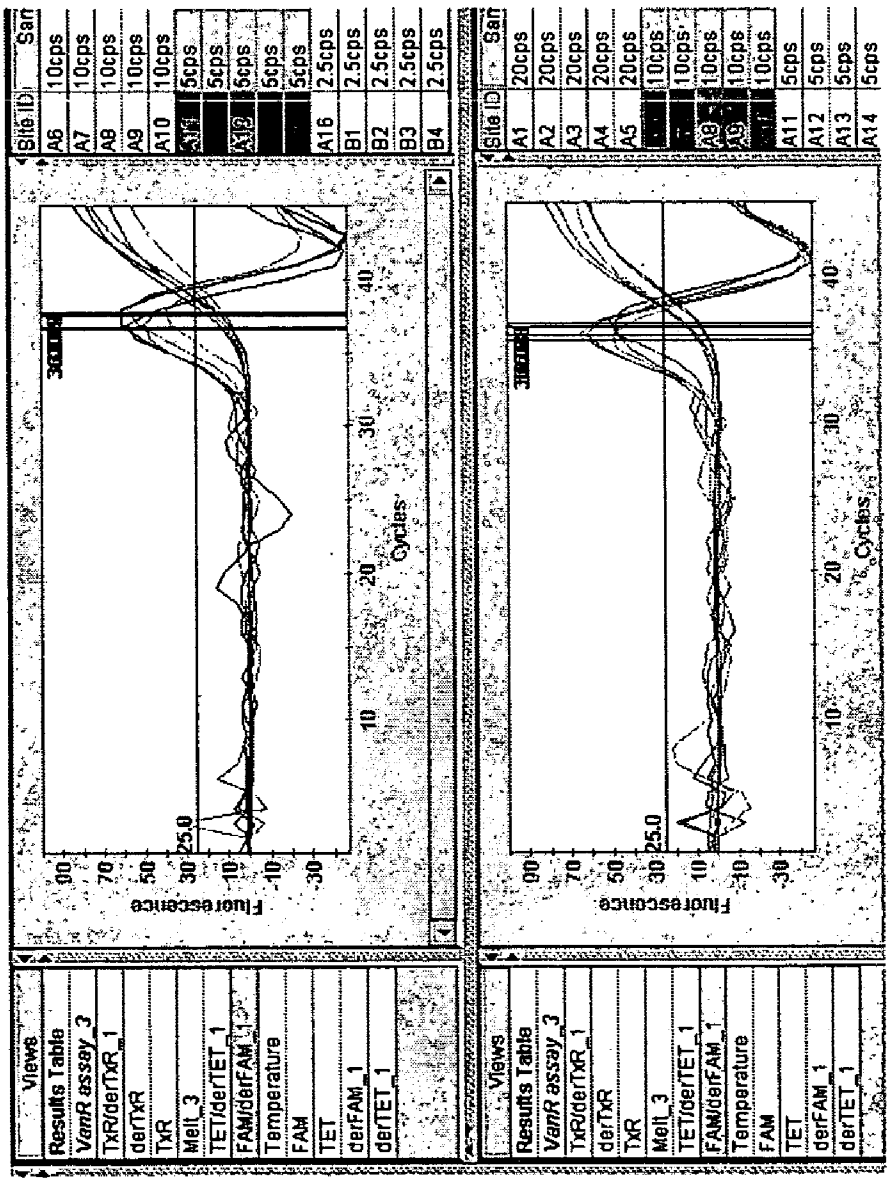

Views
Results Table
VanR assay_3
TxR/derTxR_1
derTxR
TxR
Melt_3
TET/derTET_1
FAM/derFAM_1
Temperature
FAM
TET
derFAM_1
derTET_1
Fluorescence
Cycles
25.0
Site ID
A6 10cps
A7 10cps
A8 10cps
A9 10cps
A10 10cps
5cps
A16 2.5cps
B1 2.5cps
B2 2.5cps
B3 2.5cps
B4 2.5cps
A1 20cps
A2 20cps
A3 20cps
A4 20cps
A5 20cps
10cps
A11 5cps
A12 5cps
A13 5cps
A14 5cps

Figure 13B

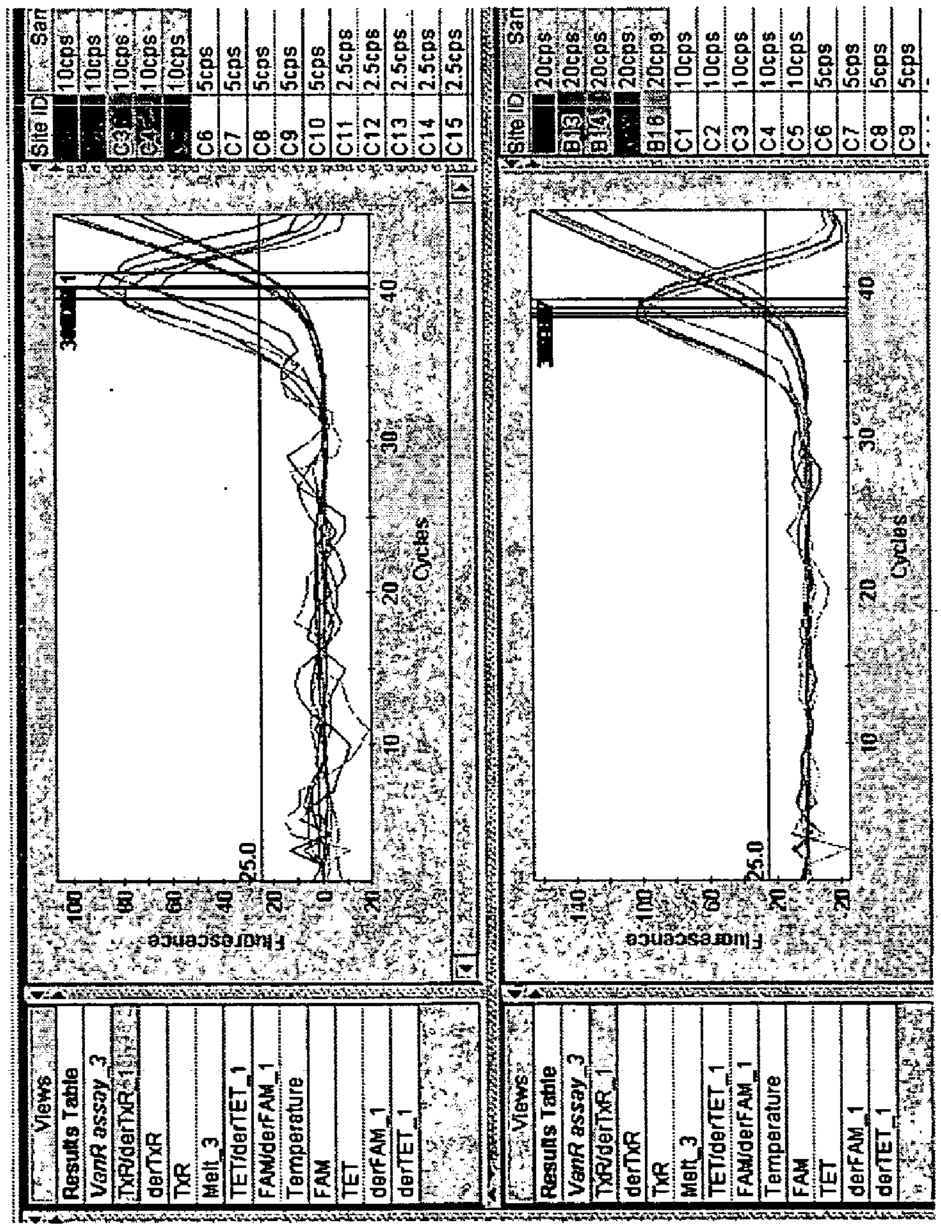
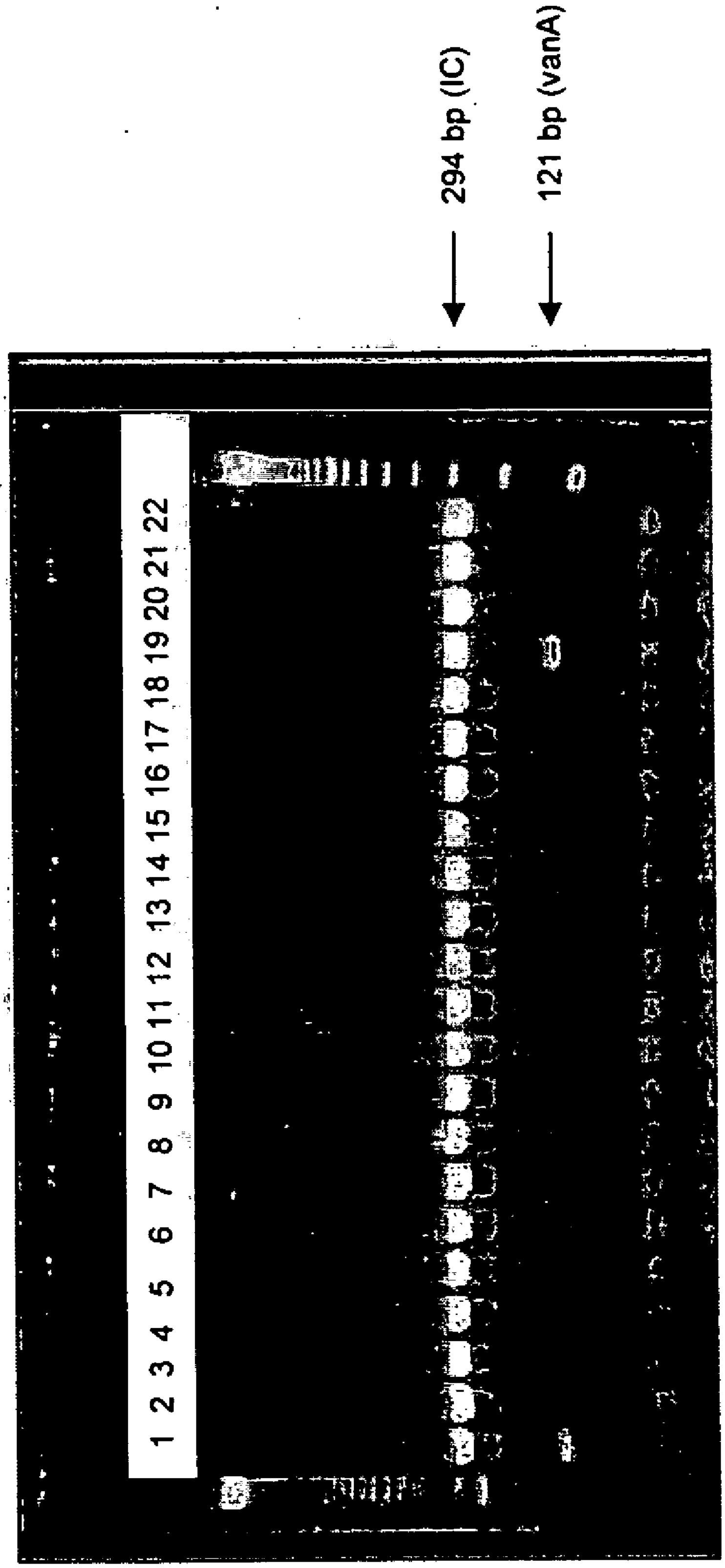

Views
Results Table
VanR assay_3
TxR/derTxR_1
derTxR
TxR
Melt_3
TET/derTET_1
FAM/derFAM_1
Temperature
FAM
TET
derFAM_1
derTET_1
Fluorescence
Cycles
Site ID

Figure 14A


294 bp (IC)
121 bp (vanA)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

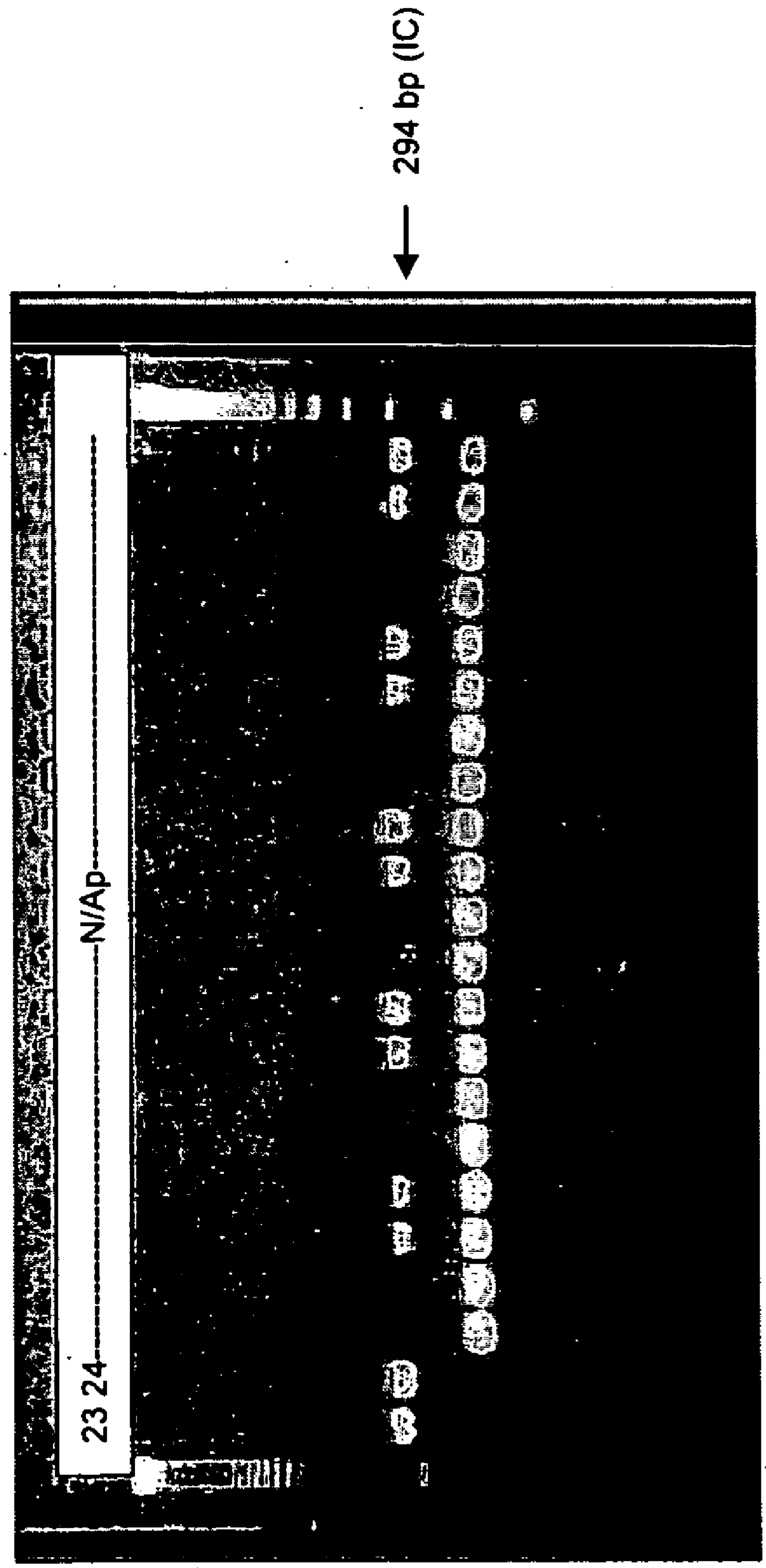


Figure 14B

Figure 15A

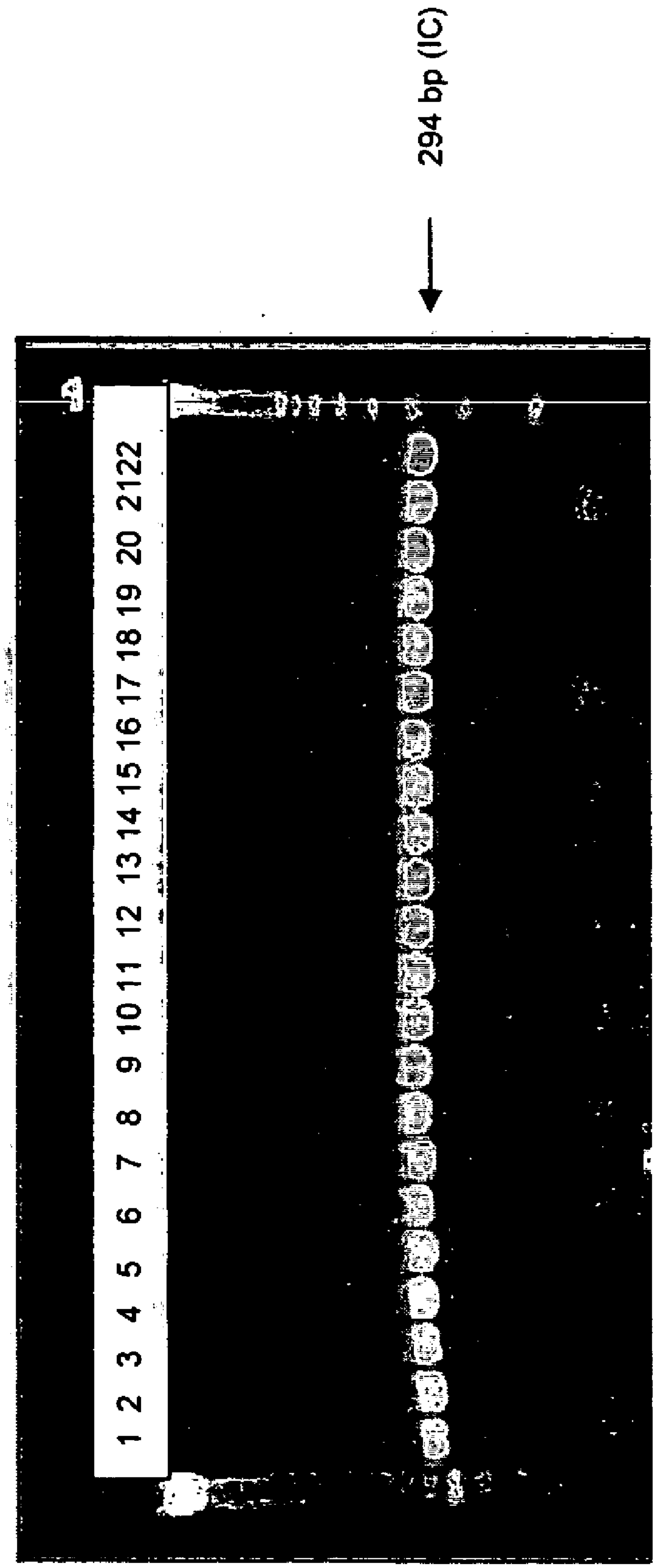


Figure 15B

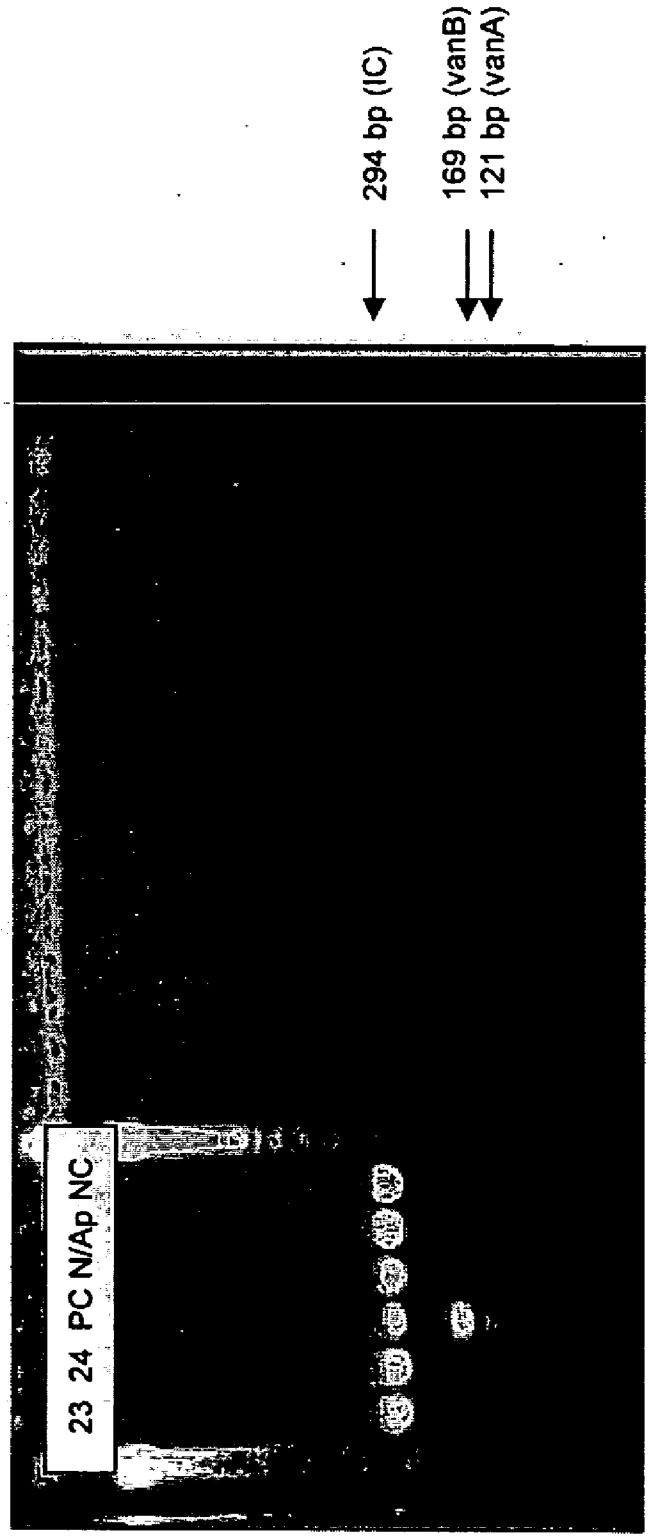


Figure 16A

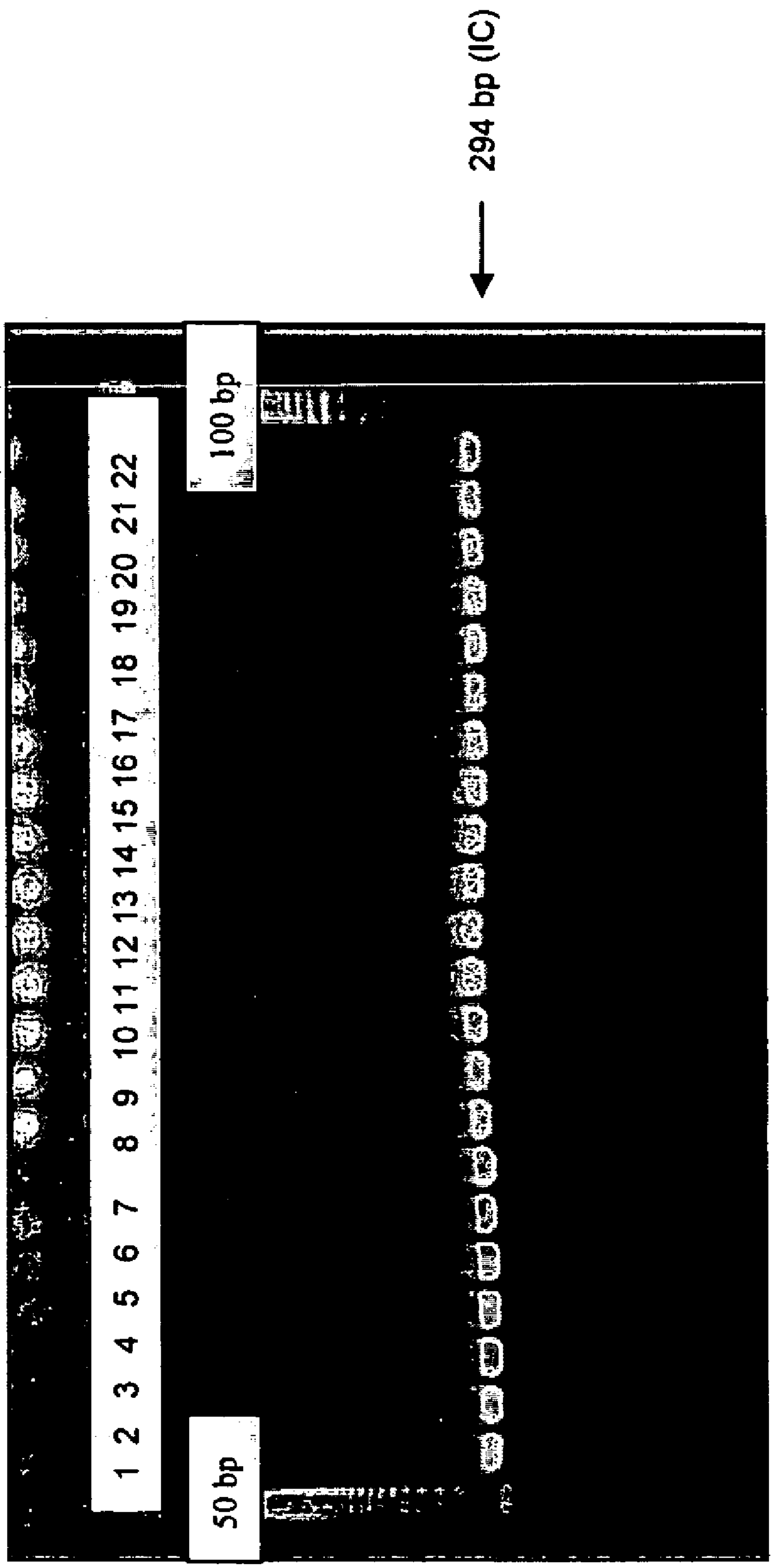

294 bp (IC)
100 bp
50 bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Figure 16B

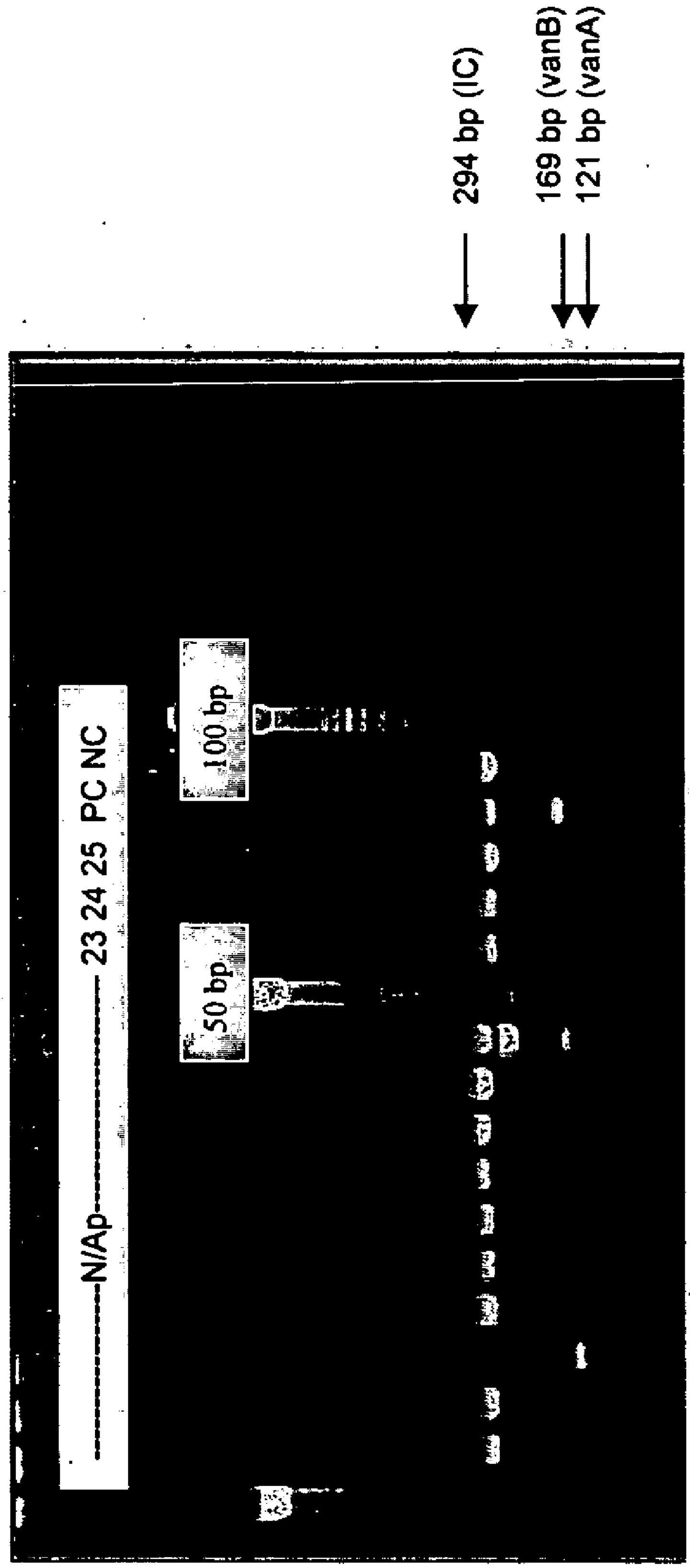


Figure 17

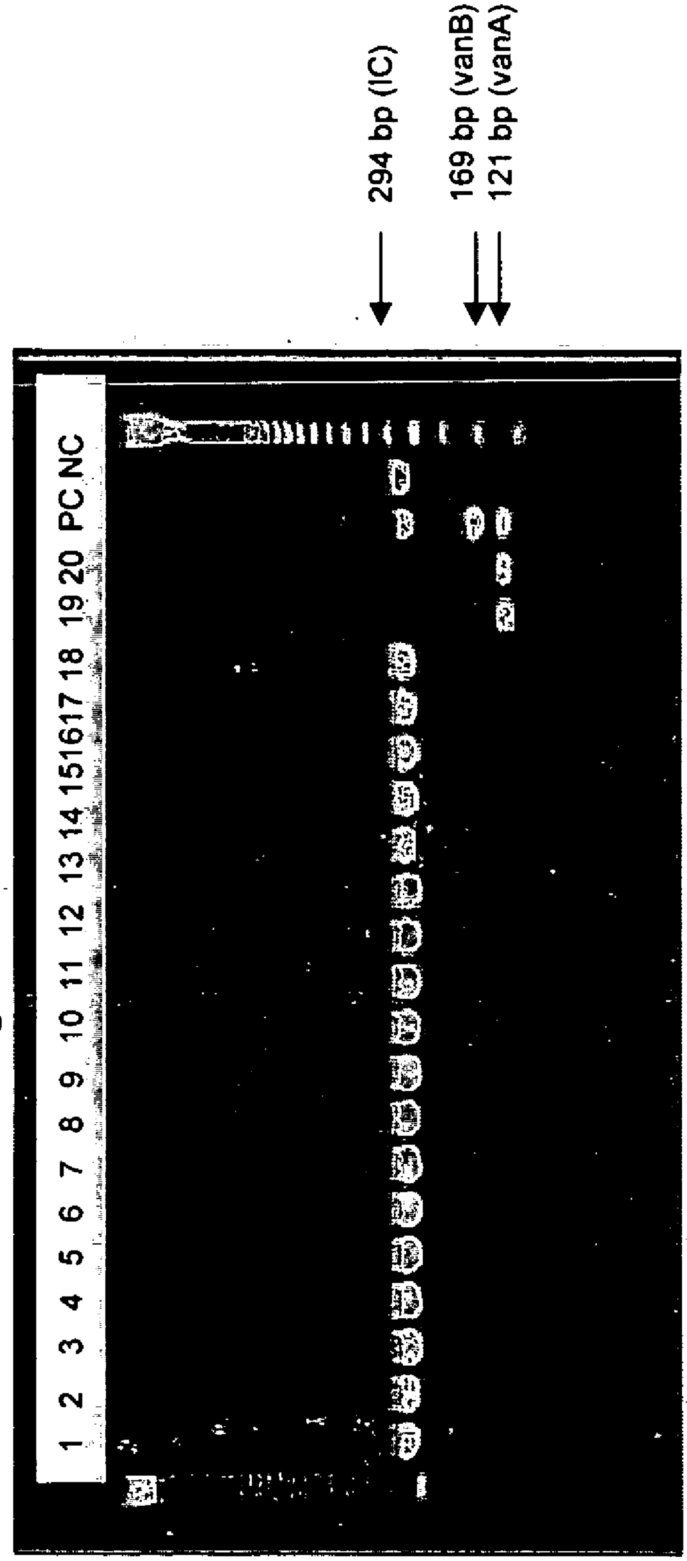

294 bp (IC)
169 bp (vanB)
121 bp (vanA)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PC NC

Figure 18A

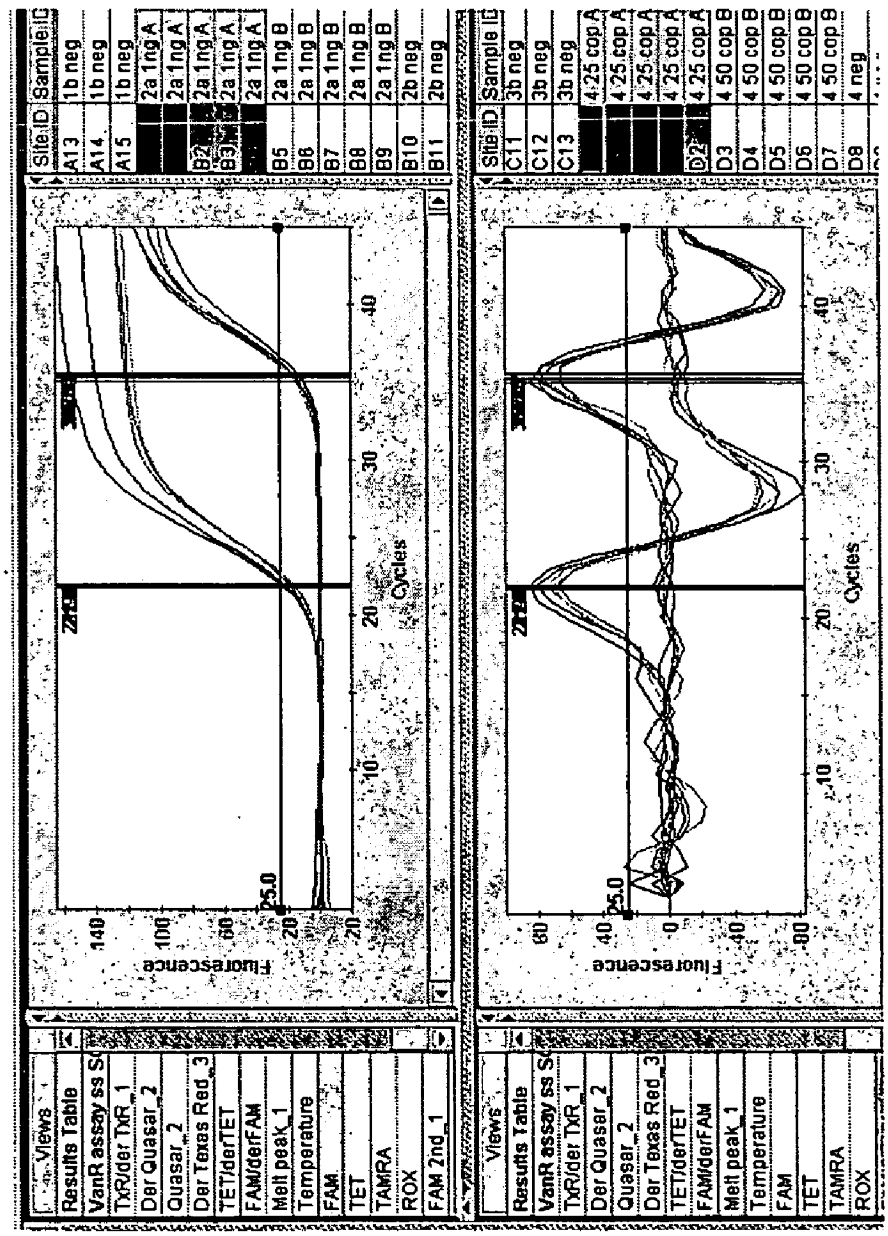

Views
Results Table
VanR assay ss
TxR/der TxR_1
Der Quasar_2
Quasar_2
Der Texas Red_3
TET/derTET
FAM/derFAM
Melt peak_1
Temperature
FAM
TET
TAMRA
ROX
FAM 2nd_1
Fluorescence
Cycles
Site ID
Sample ID

Figure 18B

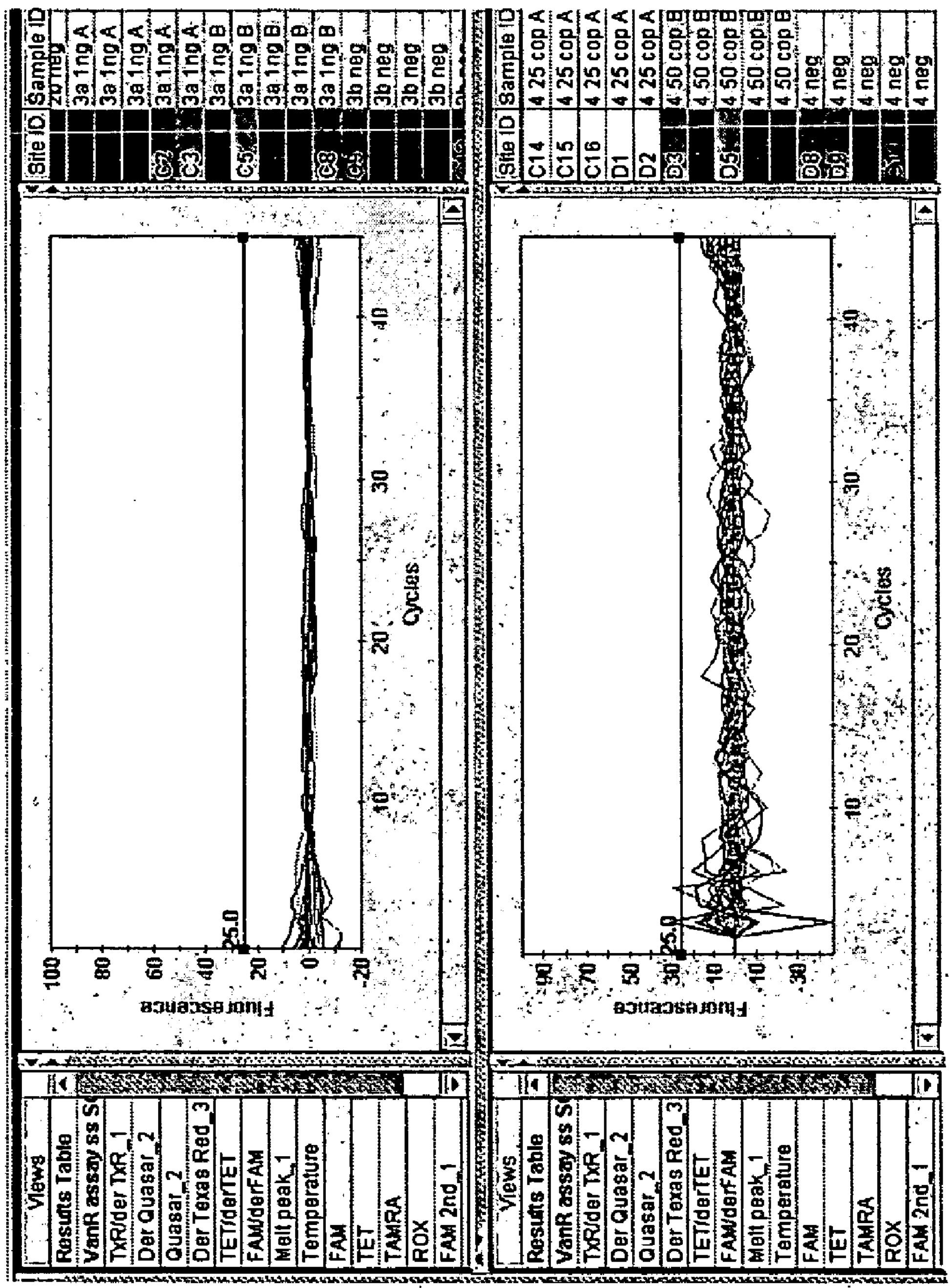

Views
Results Table
VanR assay ss S
TxR/der TxR_1
Der Quasar_2
Quasar_2
Der Texas Red_3
TET/derTET
FAM/derFAM
Melt peak_1
Temperature
FAM
TET
TAMRA
ROX
FAM 2nd_1
Fluorescence
Cycles
25.0
Site ID
Sample ID
3a 1ng A
3a 1ng B
3b neg
C14
C15
C16
D1
D2
4 25 cop A
4 50 cop B
4 neg

Figure 19A

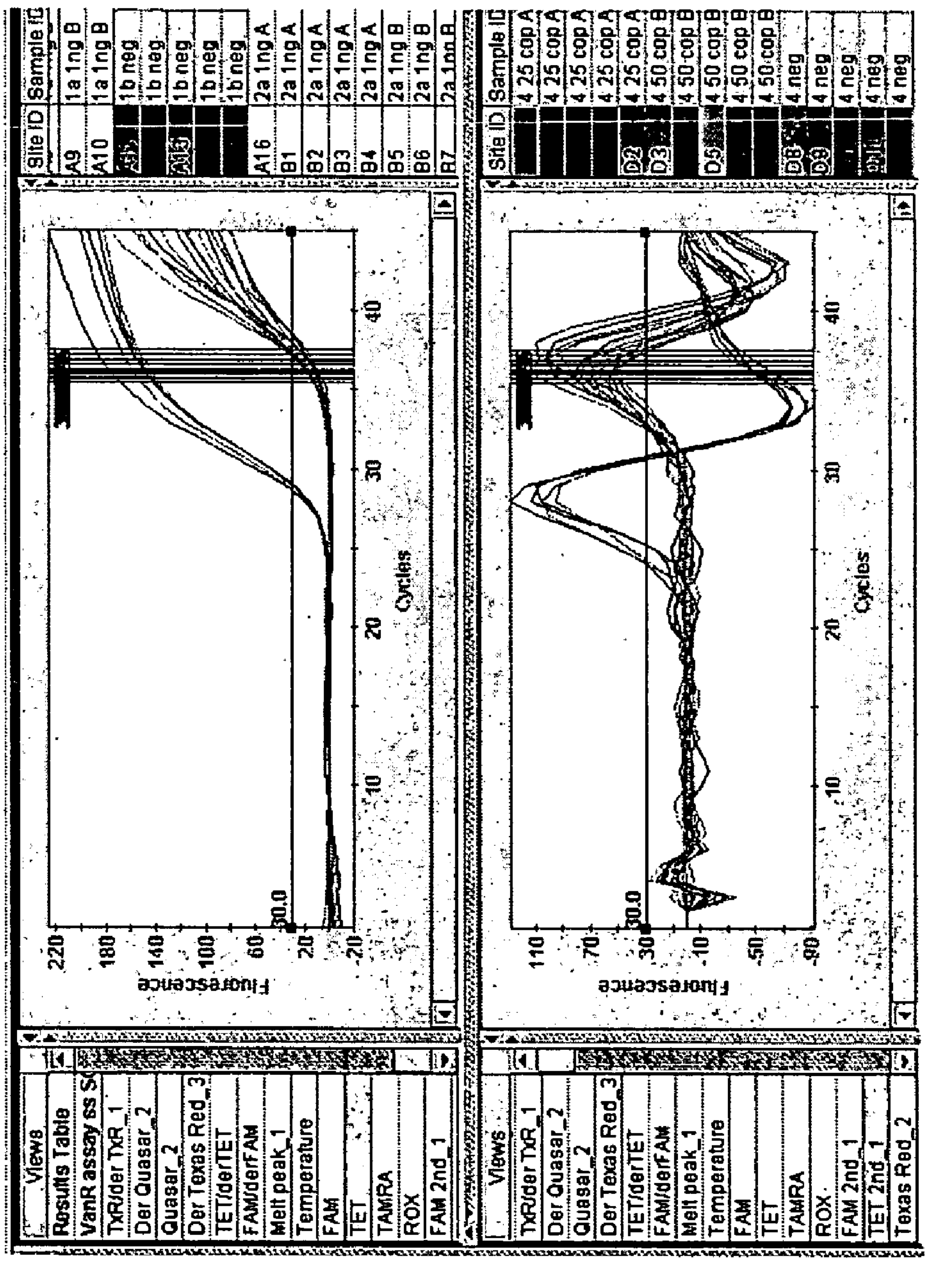


Figure 19B

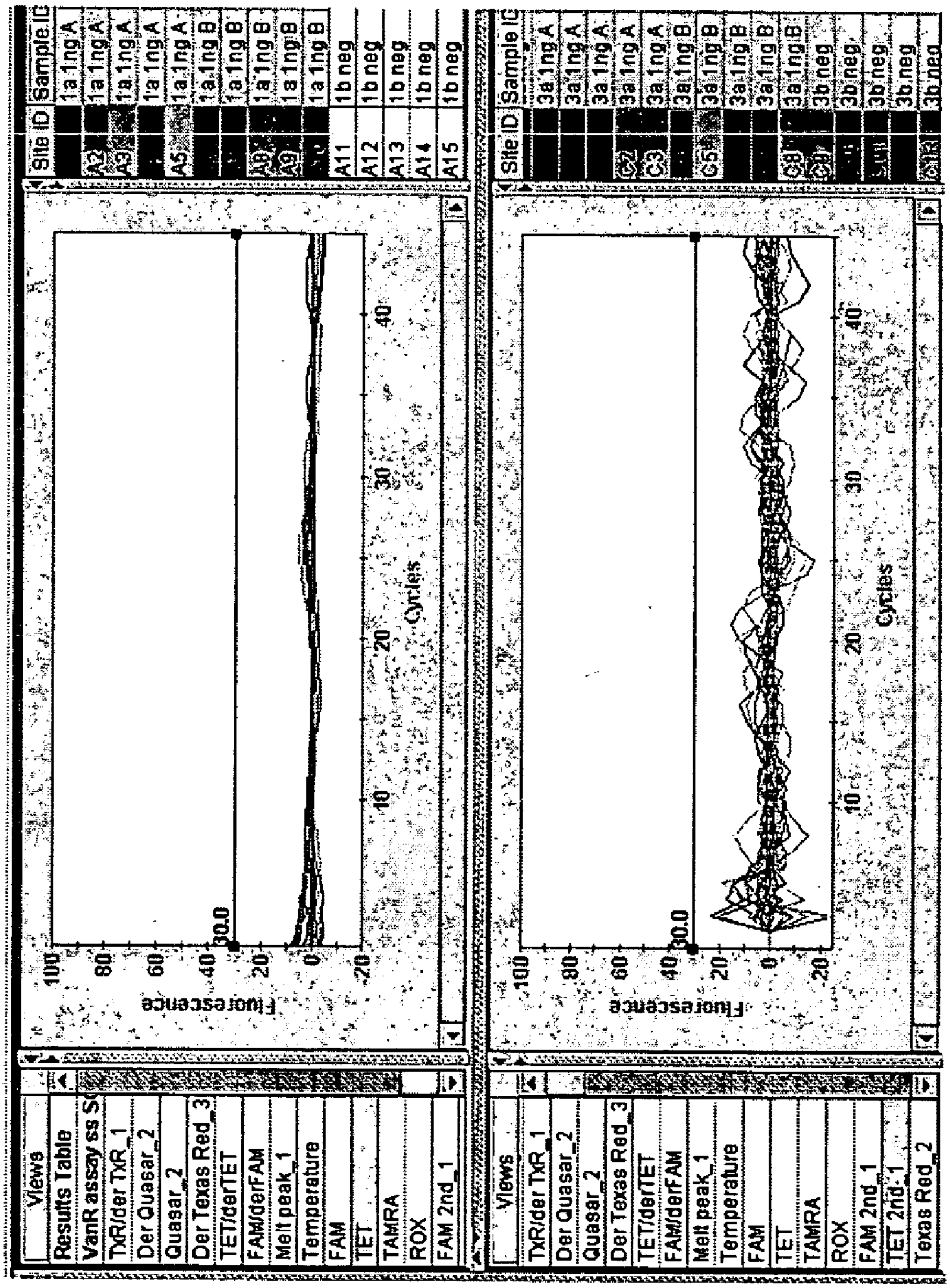

Views
Results Table
VanR assay ss S
TxR/der TxR_1
Der Quasar_2
Quasar_2
Der Texas Red_3
TET/derTET
FAM/derFAM
Melt peak_1
Temperature
FAM
TET
TAMRA
ROX
FAM 2nd_1
TET 2nd_1
Texas Red_2
Fluorescence
Cycles
30.0
Site ID
Sample ID
1a 1ng A
1a 1ng B
1b neg
3a 1ng A
3a 1ng B
3b neg
A2
A3
A5
A9
A11
A12
A13
A14
A15
C2
C3
C5
C8

Figure 20A

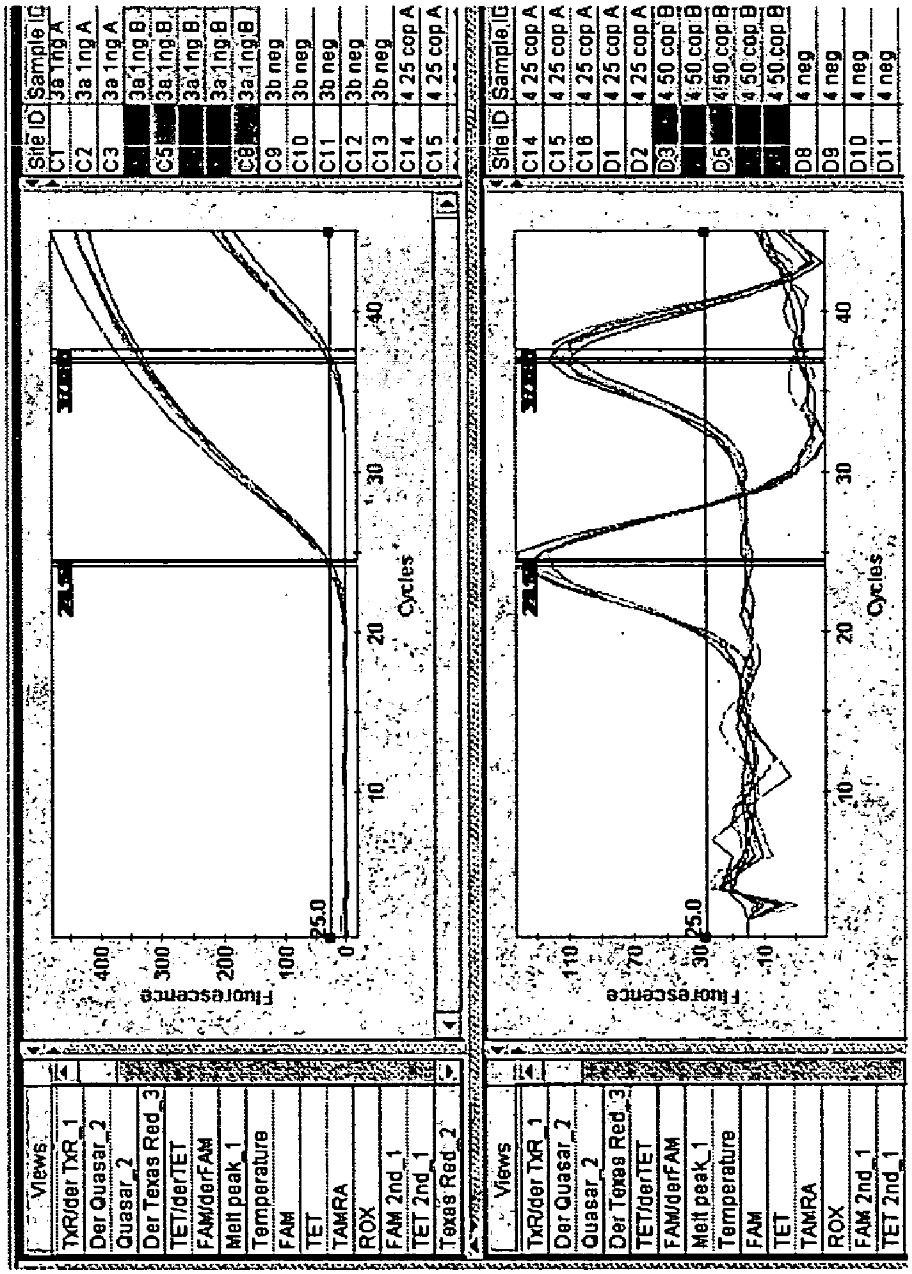

Views
TxR/der TxR_1
Der Quasar_2
Quasar_2
Der Texas Red_3
TET/derTET
FAM/derFAM
Melt peak_1
Temperature
FAM
TET
TAMRA
ROX
FAM 2nd_1
TET 2nd_1
Texas Red_2
Fluorescence
Cycles
Site ID
Sample ID
3a 1ng A
3b neg
4 25 cop A
4 50 cop B
4 neg

Figure 20B

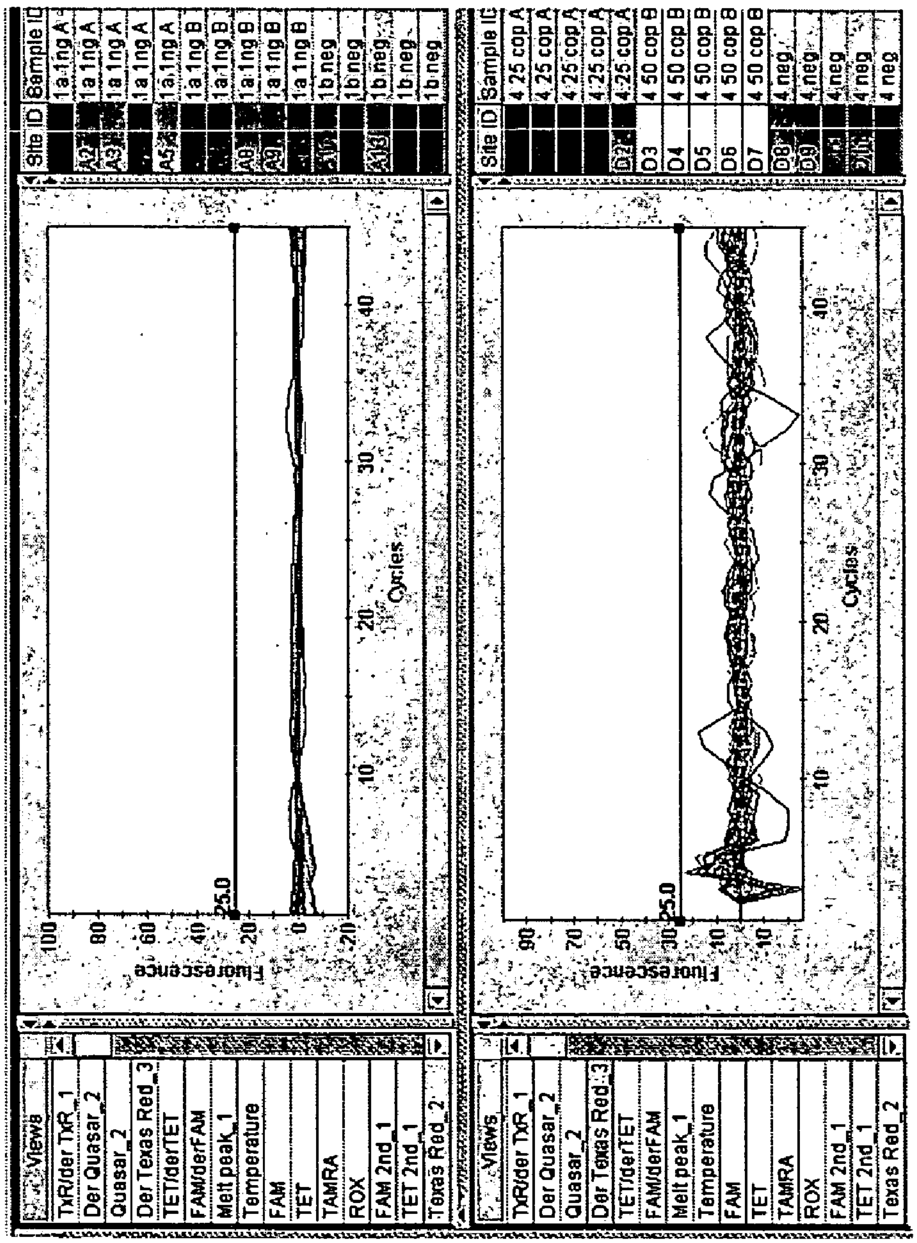

Views
TxR/der TxR_1
Der Quasar_2
Quasar_2
Der Texas Red_3
TET/derTET
FAM/derFAM
Melt peak_1
Temperature
FAM
TET
TAMRA
ROX
FAM 2nd_1
TET 2nd_1
Texas Red_2
Fluorescence
Cycles
25.0
Site ID
Sample ID
1a 1ng A
1a 1ng B
1b neg
4 25 cop A
4 50 cop B
4 neg

Figure 21

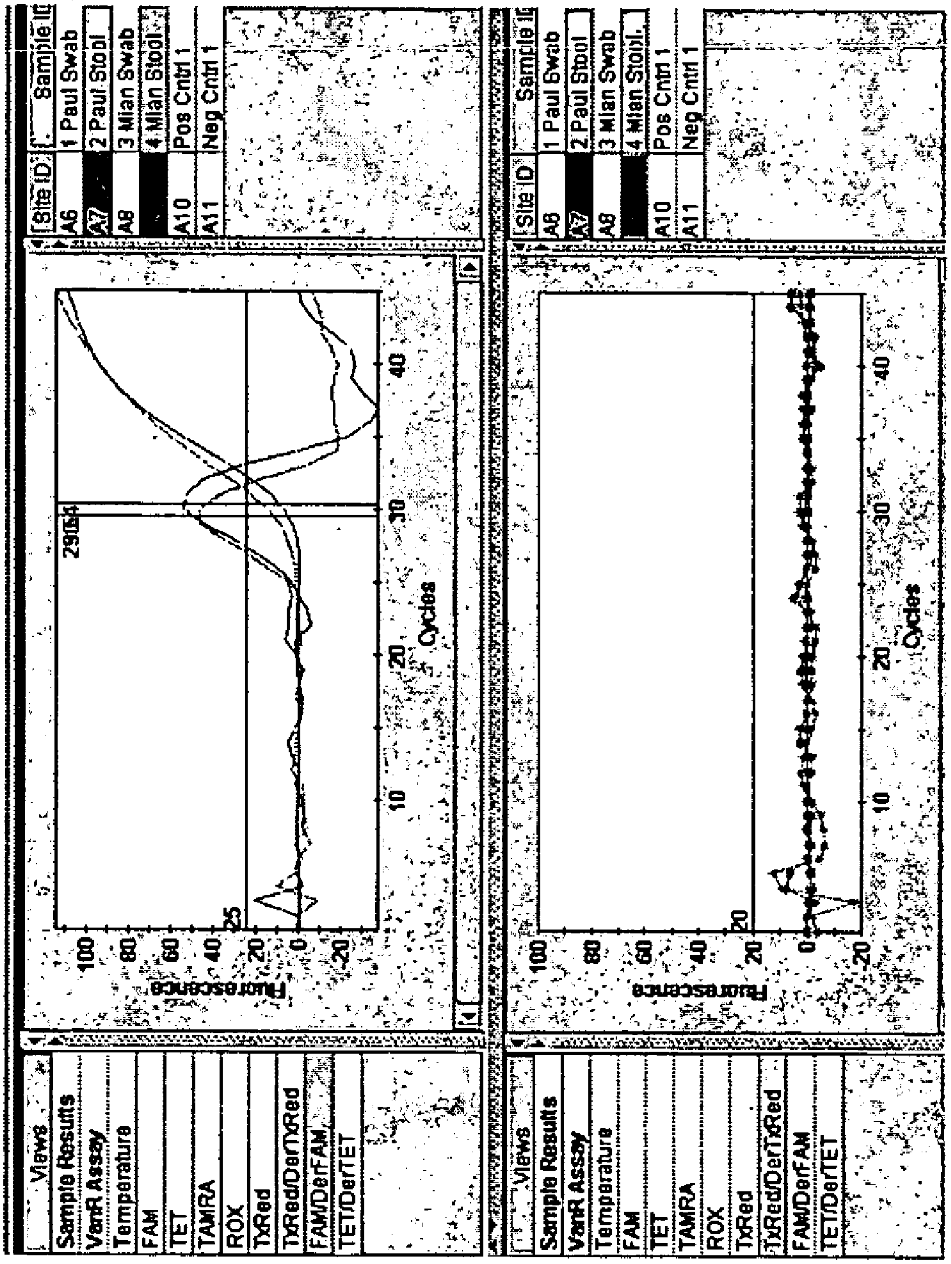

Views
Sample Results
VanR Assay
Temperature
FAM
TET
TAMRA
ROX
TxRed
TxRed/DerTxRed
FAM/DerFAM
TET/DerTET
Site ID
Sample ID
A6
1 Paul Swab
A7
2 Paul Stool
A8
3 Mian Swab
4 Mian Stool
A10
Pos Cntrl 1
A11
Neg Cntrl 1
Fluorescence
Cycles
100
80
60
40
20
0
-20
10
20
30
40

Figure 22

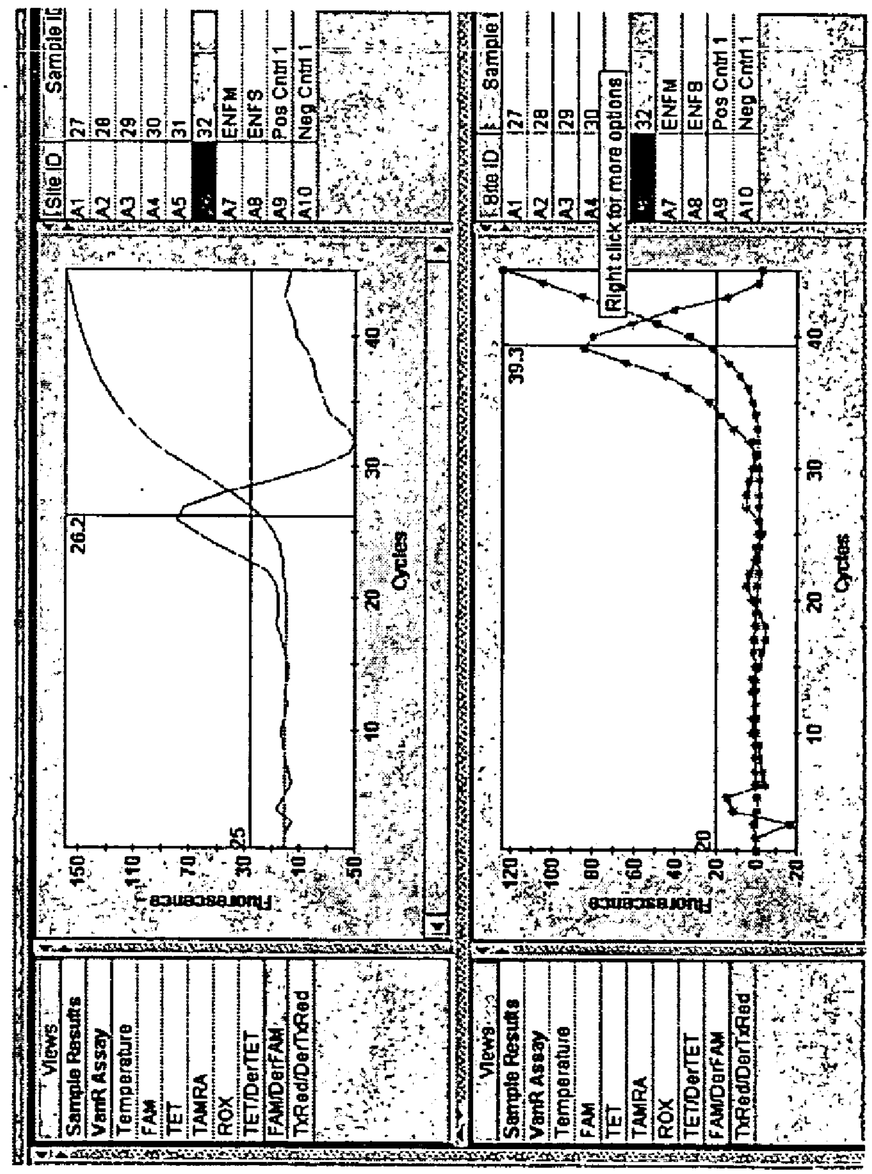

Views
Sample Results
VanR Assay
Temperature
FAM
TET
TAMRA
ROX
TET/DerTET
FAM/DerFAM
TxRed/DerTxRed
Fluorescence
Cycles
26.2
39.3
Site ID
Sample ID
Right click for more options
ENFM
ENFS
ENFB
Pos Cntrl 1
Neg Cntrl 1

Figure 23

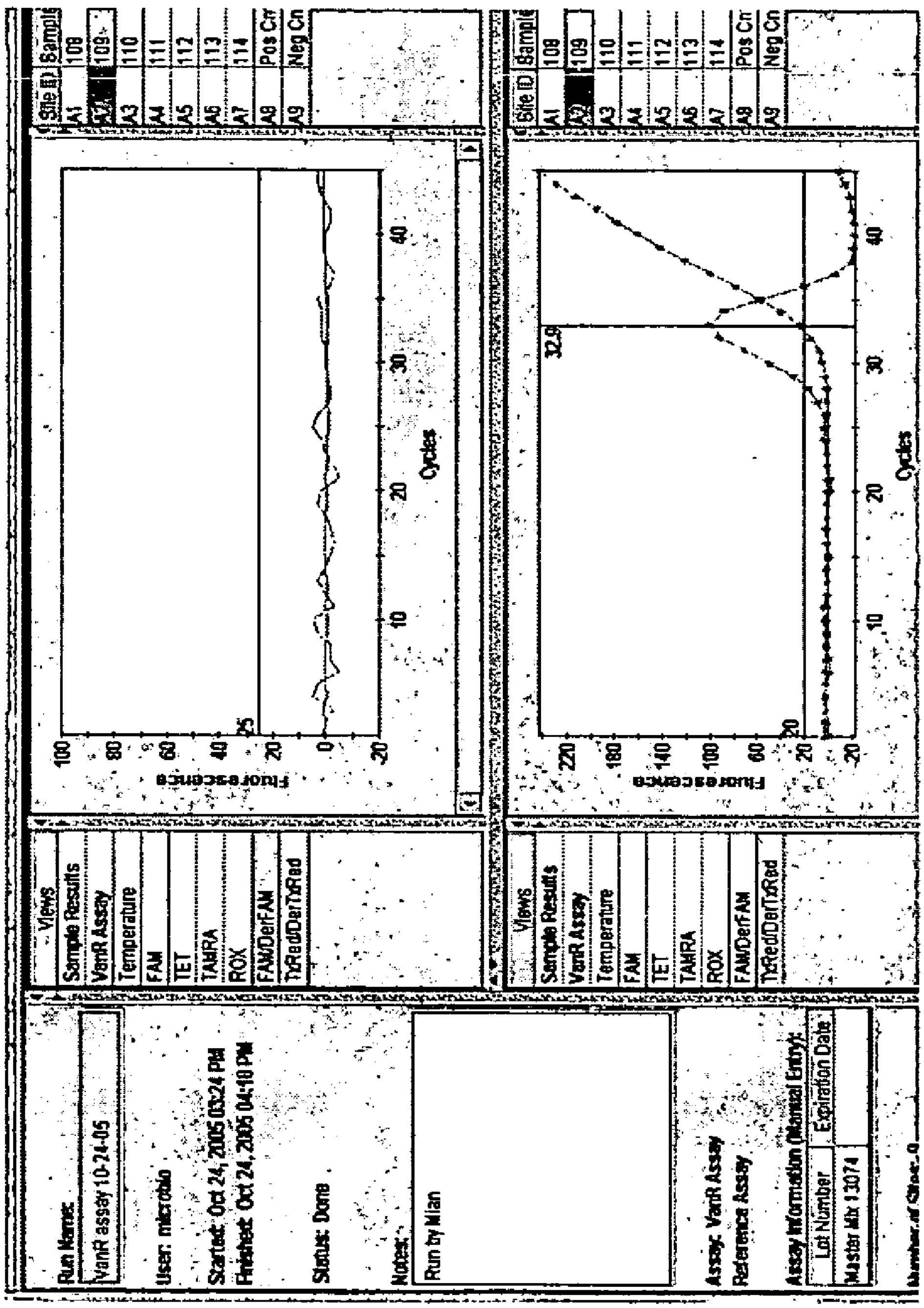

Run Name:
VanR assay 10-24-05
User: microbio
Started: Oct 24, 2005 03:24 PM
Finished: Oct 24, 2005 04:10 PM
Status: Done
Notes:
Run by Milan
Assay: VanR Assay
Reference Assay
Assay Information (Manual Entry):
Lot Number
Expiration Date
Master Mix 13074
Views
Sample Results
VanR Assay
Temperature
FAM
TET
TAMRA
ROX
FAM/DerFAM
TxRed/DerTxRed
Fluorescence
Cycles
32.9
Site ID
Sample
A1
A2
A3
A4
A5
A6
A7
A8
A9
108
109
110
111
112
113
114
Pos Cn
Neg Cn

Figure 24

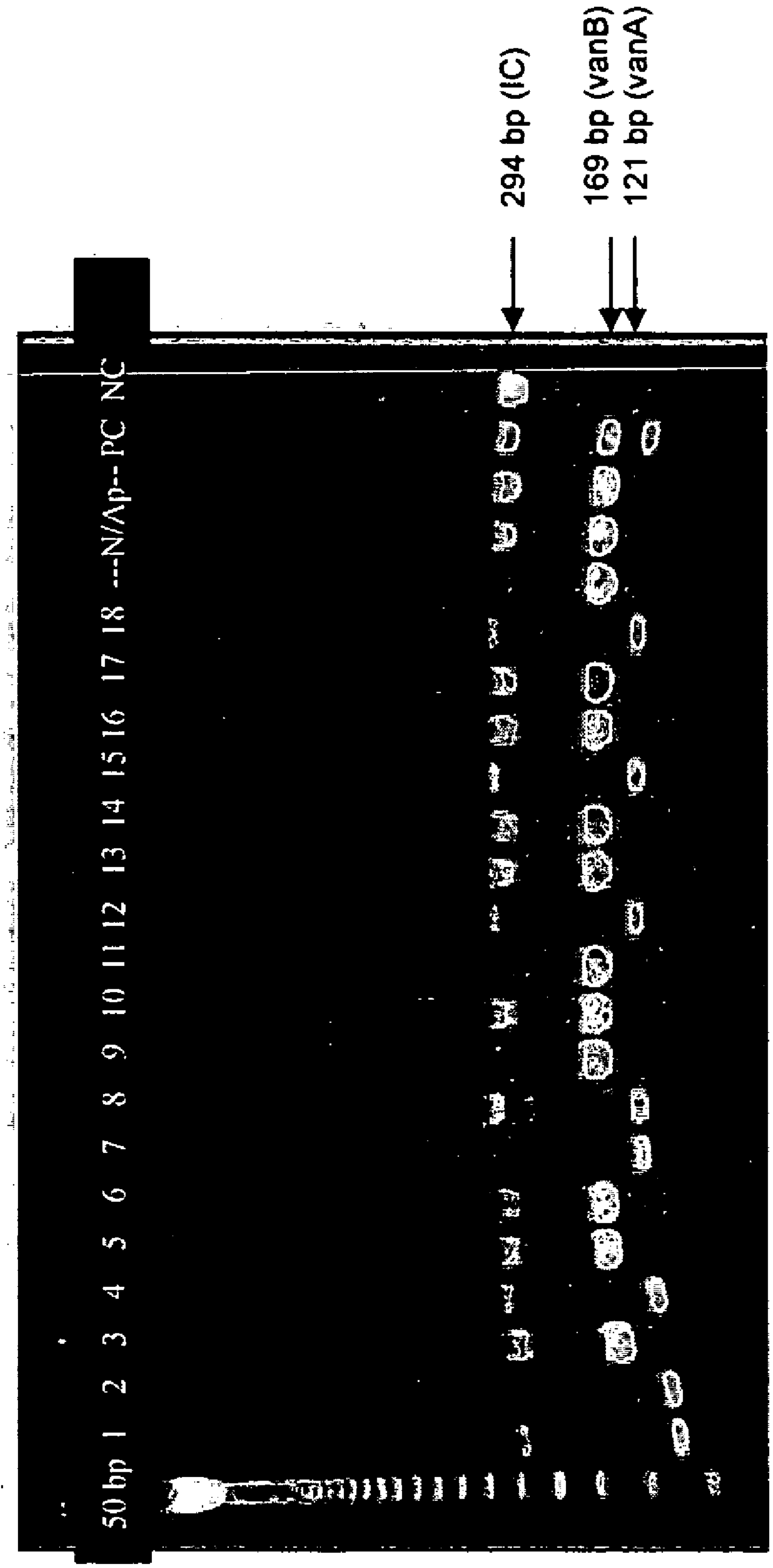
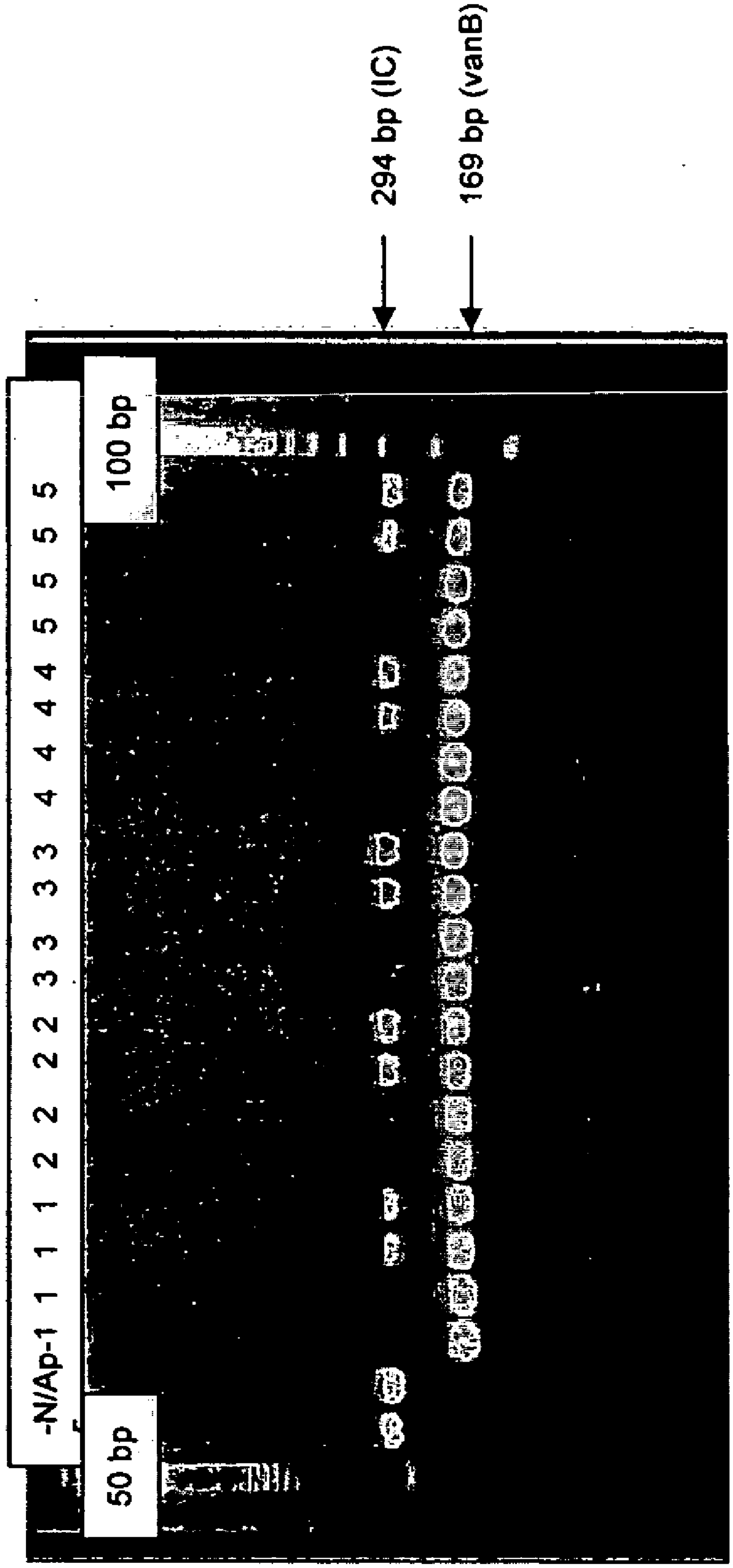

294 bp (IC)
169 bp (vanB)
121 bp (vanA)
50 bp 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 ---N/Ap-- PC NC

Figure 25



HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE PROBES AND PRIMERS FOR DETECTION OF MICROORGANISMS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of application Ser. No. 11/236,785, filed Sep. 27, 2005, which is a continuation of Ser. No. 10/089,177, filed Mar. 27, 2002, which is the U.S. national phase under 35 U.S.C. §371 of prior PCT International Application No. PCT/CA00/01150, filed Sep. 28, 2000, which claims the benefit of Canada Application No. 2037010 filed May 19, 2000, and Canada Application No. 2283458, filed Sep. 28, 1999, the disclosures of which are hereby expressly incorporated by reference in their entireties. This application is also a continuation-in-part of application Ser. No. 10/753,169, filed Jan. 7, 2004, which is a continuation of application Ser. No. 09/989,643, filed Nov. 20, 2001 which is a continuation of application Ser. No. 09/297,539 filed May 3, 1999 which is a national phase of PCT/CA97/00829 filed Nov. 4, 1997, which claims priority to application Ser. No. 08/743,637, filed Nov. 4, 1996, the disclosures of which are hereby expressly incorporated by reference in their entireties.

SEQUENCE LISTING

[0002] The present application is being filed along with duplicate copies of a CD-ROM marked "Copy 1" and "Copy 2" containing a Sequence Listing in electronic format. The duplicate copies of the CD-ROM each contain a file entitled GENOM.048NPCCP1.TXT created on Sep. 14, 2006 and is 1,929,380 bytes in size. The information on these duplicate CD-ROMs is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Classical Methods for the Identification of Microorganisms

[0003] Microorganisms are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, generally two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan™ system from Dade Behring and the Vitek™ system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these faster systems always require the primary isolation of the bacteria or fungi as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. So, the shortest time from sample reception to identification of the pathogen is around 24 hours. Moreover, fungi other than yeasts are often difficult or very slow to grow from clinical specimens. Identification must rely on labor-intensive techniques such as direct microscopic examination of the specimens and by direct and/or indirect immunological assays. Cultivation of most parasites is impractical in the clinical laboratory. Hence, microscopic examination of the specimen, a few immunological tests and clinical symptoms are often the only methods used for an identification that frequently remains presumptive.

[0004] The fastest bacterial identification system, the autoSCAN-Walk-Away™ system (Dade Behring) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5 to 6 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than Enterobacteriaceae (Croizé J., 1995, Lett. Infectiol. 10:109-113; York et al., 1992, J. Clin. Microbiol. 30:2903-2910). For Enterobacteriaceae, the percentage of non-conclusive identifications was 2.7 to 11.4%. The list of microorganisms identified by commercial systems based on classical identification methods is given in Table 15.

[0005] A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the main organisms associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

Clinical Specimens Tested in Clinical Microbiology Laboratories

[0006] Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and antibiotic susceptibility.

Conventional Pathogen Identification from Clinical Specimens

Urine Specimens

[0007] The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 μL of urine is streaked on agar plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial count of 10^7 CFU/L or more in urine. However, infections with less than 10^7 CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. 311:560-564). Importantly, approximately 80%

of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10^7 CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

[0008] Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koening et al., 1992, J. Clin. Microbiol. 30:342-345; Pezzlo et al., 1992, J. Clin. Microbiol. 30:640-684).

Blood Specimens

[0009] The Blood Specimens Received In The Microbiology Laboratory Are Always Submitted For Culture. Blood Culture Systems May Be Manual, Semi-Automated Or Completely Automated. The BACTEC™ System (From Becton Dickinson) And The Bactalert™ System (From Organon Teknika Corporation) Are The Two Most Widely Used Automated Blood Culture Systems. These Systems Incubate Blood Culture Bottles Under Optimal Conditions For Growth Of Most Bacteria. Bacterial Growth Is Monitored Continuously To Detect Early Positives By Using Highly Sensitive Bacterial Growth Detectors. Once Growth Is Detected, A Gram Stain Is Performed Directly From The Blood Culture And Then Used To Inoculate Nutrient Agar Plates. Subsequently, Bacterial Identification And Susceptibility Testing Are Carried Out From Isolated Bacterial Colonies With Automated Systems As Described Previously. Blood Culture Bottles Are Normally Reported As Negative If No Growth Is Detected After An Incubation Of 6 To 7 Days. Normally, The Vast Majority Of Blood Cultures Are Reported Negative. For Example, The Percentage Of Negative Blood Cultures At The Microbiology Laboratory Of The CHUL For The Period February 1994-January 1995 Was 93.1% (Table 3).

Other Clinical Samples

[0010] Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial, pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3). In all these normally sterile sites, tests for the universal detection of algae, archaea, bacteria, fungi and parasites would be very useful.

[0011] Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial or fungal pathogens potentially associated with the infection are grown and separated from the colonizing microbes using selective methods and then identified as described previously. Of course, the DNA-based universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non-sterile sites. On the other hand, DNA-based assays for species or genus or family or group detection and identification as well as for the detection of antimicrobial agents resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

DNA-Based Assays with any Specimen

[0012] There is an obvious need for rapid and accurate diagnostic tests for the detection and identification of algae, archaea, bacteria, fungi and parasites directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Bergeron and Ouellette, 1995, Infection 23:69-72; Bergeron and Ouellette, 1998, J Clin Microbiol. 36:2169-72). The DNA probes and amplification primers which are objects of the present invention are applicable for the detection and identification of algae, archaea, bacteria, fungi, and parasites directly from any clinical specimen such as blood, urine, sputum, cerebrospinal fluid, pus, genital and gastro-intestinal tracts, skin or any other type of specimens (Table 3). These assays are also applicable to detection from microbial cultures (e.g. blood cultures, bacterial or fungal colonies on nutrient agar, or liquid cell cultures in nutrient broth). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since these tests can be performed in one hour or less, they provide the clinician with new diagnostic tools which should contribute to a better management of patients with infectious diseases. Specimens from sources other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock, food products, environment such as water or soil, and others) may also be tested with these assays.

A High Percentage of Culture-Negative Specimens

[0013] Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of normally sterile clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would also be desirable, in addition to identify bacteria at the species or genus or family or group level in a given specimen, to screen out the high proportion of negative clinical specimens with a DNA-based test detecting the presence of any bacterium (i.e. universal bacterial detection). As disclosed in the present invention, such a screening test may be based on DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for any bacterium would give a positive amplification signal. Similarly, highly conserved genes of fungi and parasites could serve not only to identify particular species or genus or family or group but also to detect the presence of any fungi or parasite in the specimen.

Towards the Development of Rapid DNA-Based Diagnostic Tests

[0014] A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antimicrobial agents resistance genes from clinical samples (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, Mass.). There is no need for culture of the pathogens, hence the organisms can be detected directly from clinical samples, thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for microbial identification than currently used phenotypic identification systems which are based on biochemical tests and/or microscopic examination. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as *Mycobacterium tuberculosis, Chlamydia trachomatis, Neisseria gonorrhoeae* as well as for the detection of a variety of viruses (Tang Y. and Persing D. H., Molecular detection and identification of microorganisms, In: P. Murray et al., 1999, Manual of Clinical Microbiology, ASM press, 7th edition, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

[0015] Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention, for example: *Staphylococcus* sp. (U.S. Pat. No. 5,437,978), *Neisseria* sp. (U.S. Pat. No. 5,162,199 and European patent serial no. 0,337,896,131) and *Listeria monocytogenes* (U.S. Pat. Nos. 5,389,513 and 5,089,386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from those described in the present invention. To our knowledge there are only four patents published by others mentioning the use of any of the four highly conserved gene targets described in the present invention for diagnostic purposes (PCT international publication number WO92/03455 and WO00/14274, European patent publication number 0 133 671 B1, and European patent publication number 0 133 288 A2). WO92/03455 is focused on the inhibition of *Candida* species for therapeutic purposes. It describes antisense oligonucleotide probes hybridizing to *Candida* messenger RNA. Two of the numerous mRNA proposed as targets are coding for translation elongation factor 1 (tef1) and the beta subunit of ATPase. DNA amplification or hybridization are not under the scope of their invention and although diagnostic use is briefly mentioned in the body of the application, no specific claim is made regarding diagnostics. WO00/14274 describes the use of bacterial recA gene for identification and speciation of bacteria of the *Burkholderia cepacia* complex. Specific claims are made on a method for obtaining nucleotide sequence information for the recA gene from the target bacteria and a following comparison with a standard library of nucleotide sequence information (claim **1**), and on the use of PCR for amplification of the recA gene in a sample of interest (claims **4** to **7**, and **13**). However, the use of a discriminatory restriction enzyme in a RFLP procedure is essential to fulfill the speciation and WO00/14274 did not mention that multiple recA probes could be used simultaneously. Patent EP 0 133 288 A2 describes and claims the use of bacterial tuf (and fus) sequence for diagnostics based on hybridization of a tuf (or fus) probe with bacterial DNA. DNA amplification is not under the scope of EP 0 133 288 A2. Nowhere it is mentioned that multiple tuf (or fus) probes could be used simultaneously. No mention is made regarding speciation using tuf (or fus) DNA nucleic acids and/or sequences. The sensitivities of the tuf hybridizations reported are 1×10^6 bacteria or 1-100 ng of DNA. This is much less sensitive than what is achieved by our assays using nucleic acid amplification technologies.

[0016] Although there are phenotypic identification methods which have been used for more than 125 years in clinical microbiology laboratories, these methods do not provide information fast enough to be useful in the initial management of patients. There is a need to increase the speed of the diagnosis of commonly encountered bacterial, fungal and parasitical infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the microbial genotype (e.g. DNA level) is more stable than the phenotype (e.g. physiologic level).

[0017] Bacteria, fungi and parasites encompass numerous well-known microbial pathogens. Other microorganisms could also be pathogens or associated with human diseases. For example, achlorophylious algae of the *Prototheca* genus can infect humans. Archae, especially methanogens, are present in the gut flora of humans (Reeve, J. H., 1999, J. Bacteriol. 181:3613-3617). However, methanogens have been associated to pathologic manifestations in the colon, vagina, and mouth (Belay et al., 1988, Appl. Enviro. Microbiol. 54:600-603; Belay et al., 1990, J. Clin. Microbiol. 28:1666-1668; Weaver et al., 1986, Gut 27:698-704).

[0018] In addition to the identification of the infectious agent, it is often desirable to identify harmful toxins and/or to monitor the sensitivity of the microorganism to antimicrobial agents. As revealed in this invention, genetic identification of the microorganism could be performed simultaneously with toxin and antimicrobial agents resistance genes. Alternatively, assays to identify toxin and/or antimicrobial resistance genes can be performed separately and independently from assays for identification of infectious agents.

[0019] Knowledge of the genomic sequences of algal, archaeal, bacterial, fungal and parasitical species continuously increases as testified by the number of sequences available from public databases such as GenBank. From the sequences readily available from those public databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial, fungal and parasitical pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iii) the family-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens; (iv) the group-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (v) the universal detection of algal, archaeal, bacterial, fungal or parasitical pathogens, and/or (vi) the specific detection and identification of antimicrobial agents resistance genes, and/or (vii) the specific detection and identification of bacterial toxin genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

[0020] In our assigned U.S. Pat. No. 6,001,564 and our WO98/20157 patent publication, we described DNA sequences suitable for (i) the species-specific detection and identification of clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of antimicrobial agents resistance genes.

[0021] The WO98/20157 patent publication describes proprietary tuf DNA sequences as well as tuf sequences selected from public databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from these sequences. All the nucleic acid sequences described in that patent publication can enter in the composition of diagnostic kits or products and methods capable of a) detecting the presence of bacteria and fungi b) detecting specifically at the species, genus, family or group levels, the presence of bacteria and fungi and antimicrobial agents resistance genes associated with these pathogens. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and associated antimicrobial agents resistance genes and toxins genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antimicrobial agents resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent applications.

[0022] The present invention improves the assigned application by disclosing new proprietary tuf nucleic acids and/or sequences as well as describing new ways to obtain tuf nucleic acids and/or sequences. In addition we disclose new proprietary atpD and recA nucleic acids and/or sequences. In addition, new uses of tuf, atpD and recA DNA nucleic acids and/or sequences selected from public databases (Table 11) are disclosed.

Highly Conserved Genes for Identification and Diagnostics

[0023] Highly conserved genes are useful for identification of microorganisms. For bacteria, the most studied genes for identification of microorganisms are the universally conserved ribosomal RNA genes (rRNA). Among those, the principal targets used for identification purposes are the small subunit (SSU) ribosomal 16S rRNA genes (in prokaryotes) and 18S rRNA genes (in eukaryotes) (Relman and Persing, Genotyping Methods for Microbial Identification, In: D. H. Persing, 1996, PCR Protocols for Emerging Infectious Diseases, ASM Press, Washington D.C.). The rRNA genes are also the most commonly used targets for universal detection of bacteria (Chen et al., 1988, FEMS Microbiol. Lett. 57:19-24; McCabe et al., 1999, Mol. Genet. Metabol. 66:205-211) and fungi (Van Burik et al., 1998, J. Clin. Microbiol. 36:1169-1175).

[0024] However, it may be difficult to discriminate between closely related species when using primers derived from the 16S rRNA. In some instances, 16S rRNA sequence identity may not be sufficient to guarantee species identity (Fox et al., 1992, Int. J. Syst. Bacteriol. 42:166-170) and it has been shown that inter-operon sequence variation as well as strain to strain variation could undermine the application of 16S rRNA for identification purposes (Clayton et al., 1995, Int. J. Syst. Bacteriol. 45:595-599). The heat shock proteins (HSP) are another family of very conserved proteins. These ubiquitous proteins in bacteria and eukaryotes are expressed in answer to external stress agents. One of the most described of these HSP is HSP 60. This protein is very conserved at the amino acid level, hence it has been useful for phylogenetic studies. Similar to 16S rRNA, it would be difficult to discriminate between species using the HSP 60 nucleotide sequences as a diagnostic tool. However, Goh et al. identified a highly conserved region flanking a variable region in HSP 60, which led to the design of universal primers amplifying this variable region (Goh et al., U.S. Pat. No. 5,708,160). The sequence variations in the resulting amplicons were found useful for the design of species-specific assays.

SUMMARY OF THE INVENTION

[0025] Disclosed herein are compositions and methods for the detection and quantitation of antibiotic resistant organisms from a sample.

[0026] Some embodiments relate to compositions for the detection of a vancomycin resistant pathogen in a sample using a nucleic acid amplification assay. The composition can include at least one primer pair, comprising a forward and a reverse oligonucleotide, wherein the forward and reverse oligonucleotides each includes a binding region that is complementary to primer binding sites present on opposite strands of the pathogen's DNA, wherein the primer pair is adapted to amplify a vanA or vanB gene of the pathogen's DNA between and including said primer binding sites to produce a detectable amplification product. The binding regions of the oligonucleotides can correspond to, or be fully complementary to, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 consecutive nucleotides of at least two of the following sequences: SEQ ID NO: 1090, SEQ ID NO: 1091, SEQ ID NO: 1095, SEQ ID NO: 2298 and SEQ ID NO: 1096.

[0027] In some embodiments, the composition includes a primer pair wherein the primers comprise, consist essentially of, or consist of at least 10 consecutive nucleotides of SEQ ID NO: 1095 and SEQ ID NO: 1096. Preferred compositions include a primer pair wherein the primers comprises, consists essentially of, or consist of the sequences of SEQ ID NO: 1095 and SEQ ID NO: 1096.

[0028] Preferably, the composition comprises at least four primers, wherein the each of the four primers comprises, consists essentially of, or consists of at least 10 consecutive nucleotides of SEQ ID NOs: 1090, 1091, 1096 and 2298, or the complements thereof. In other preferred embodiments, the four primers comprise, consist essentially of, or consist of the SEQ ID NO: 1090, 1091, 1096, and 2298.

[0029] In some embodiments, the composition can also include at least one internal hybridization probe, wherein the internal hybridization probe can hybridize under stringent condition to the vanA or vanB amplification products produced by the compositions described above. Preferably, the internal hybridization probe is a molecular beacon. In more preferred embodiments, the molecular beacon can include the sequence of SEQ ID NO: 2299 or SEQ ID NO: 2300.

[0030] In further embodiments, the compositions can also include at least one internal control DNA, which can be amplified to produce an internal control amplicon under the same conditions and using the same oligonucleotides of the compositions described above. In further embodiments, the compositions can include an internal control probe that can hybridize under stringent conditions to the internal control amplicon. In some embodiments, the internal control DNA comprises the sequence of SEQ ID NO: 2302. Preferably, the internal control probe is a molecular beacon. In some embodiments, the internal control probe includes at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2301.

[0031] Also provided herein are kits that include the compositions described herein.

[0032] Methods to detect the presence of vancomycin-resistant organisms in a sample are also provided. In some embodiments, the method can include the step of annealing the nucleic acids of the sample with at least one probe and/or primer, wherein each of the primers and/or probes include nucleic acid sequences that correspond to, or are fully complementary to, at least 10 consecutive nucleotides of at least two of the following sequences: SEQ ID NO: 1090, SEQ ID NO: 1091, SEQ ID NO: 1095 and SEQ ID NO: 1096. The presence and/or amount of primer or probe that is annealed to said sample nucleic acid can be detected. In some embodiments, the primers and/or probes can include the nucleic acid sequences of SEQ ID NO's 1090, 1091, 1096 and 2297, or the complements thereof.

[0033] Preferably, the primers and/or probes are placed in the same physical enclosure.

[0034] In some embodiments, wherein at least one pair of primers is annealed to the sample DNA, and wherein said primer pair include nucleic acid sequences that correspond to, or are fully complementary to, at least 10 consecutive nucleotides of SEQ ID NO: 1090 and 1091 or SEQ ID NO: 1095 and 1096, the methods also include a step of amplifying the sample DNA with the annealed primer pair(s). For example, in some embodiments, the amplification step can include a method selected from the group consisting of:

[0035] (a) polymerase chain reaction (PCR),

[0036] (b) ligase chain reaction,

[0037] (c) nucleic acid sequence-based amplification,

[0038] (d) self-sustained sequence replication,

[0039] (e) strand displacement amplification,

[0040] (f) branched DNA signal amplification,

[0041] (g) nested PCR, and

[0042] (h) multiplex PCR.

[0043] Preferably, the amplification step includes a PCR amplification step.

[0044] In some embodiments that include an amplification step, the sample can also be contacted with at least one probe that hybridizes to an amplification product produced from at least one of the primer pairs. In preferred embodiments, the probe includes at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2299 or 2300. For example, in some embodiments, the at least one probe includes the sequence of SEQ ID NO: 2299 or 2300.

[0045] It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

[0046] from any algal, archaeal, bacterial, fungal or parasitical species in any sample suspected of containing said nucleic acids, and optionally,

[0047] from specific microbial species or genera selected from the group consisting of the species or genera listed in Table 4, and optionally,

[0048] from an antimicrobial agents resistance gene selected from the group consisting of the genes listed in Table 5, and optionally,

[0049] from a toxin gene selected from the group consisting of the genes listed in Table 6,

[0050] wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probes or primers;

[0051] said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any microbial species, specific microbial species or genus or family or group and antimicrobial agents resistance gene and/or toxin gene.

[0052] In a specific embodiment, a similar method directed to each specific microbial species or genus or family or group detection and identification, antimicrobial agents resistance genes detection, toxin genes detection, and universal bacterial detection, separately, is provided.

[0053] In a more specific embodiment, the method makes use of DNA fragments from conserved genes (proprietary sequences and sequences obtained from public databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted algal, archaeal, bacterial, fungal or parasitical nucleic acids.

[0054] In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers. To be a good diagnostic candidate, an oligonucleotide of at least 12 nucleotides should be capable of hybridizing with nucleic acids from given microorganism(s), and with substantially all strains and representatives of said microorganism(s); said oligonucleotide being species-, or genus-, or family-, or group-specific or universal.

[0055] In another particularly preferred embodiment, oligonucleotides primers and probes of at least 12 nucleotides in length are designed for their specificity and ubiquity based upon analysis of our databases of tuf, atpD and recA sequences. These databases are generated using both proprietary and public sequence information. Altogether, these databases form a sequence repertory useful for the design of primers and probes for the detection and identification of algal, archaeal, bacterial, fungal and parasitical microorganisms. The repertory can also be subdivided into subrepertories for sequence analysis leading to the design of various primers and probes.

[0056] The tuf, atpD and recA sequences databases as a product to assist the design of oligonucleotides primers and probes for the detection and identification of algal, archaeal, bacterial, fungal and parasitical microorganisms are also covered.

[0057] The proprietary oligonucleotides (probes and primers) are also another object of this invention.

[0058] Diagnostic kits comprising probes or amplification primers such as those for the detection of a microbial species or genus or family or phylum or group selected from the following list consisting of *Abiotrophia adiacens, Acinetobacter baumanii, Actinomycetae, Bacteroides, Cytophaga and Flexibacter phylum, Bacteroides fragilis, Bordetella pertussis, Bordetella* sp., *Campylobacter jejuni* and *C. coli, Candida albicans, Candida dubliniensis, Candida glabrata, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Candida zeylanoides, Candida* sp., *Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium* sp., *Corynebacterium* sp., *Crypococcus neoformans, Cryptococcus* sp., *Cryptosporidium parvum, Entamoeba* sp., Enterobacteriaceae group, *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Enterococcus* sp., *Escherichia coli* and *Shigella* sp. group, *Gemella* sp., *Giardia* sp., *Haemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Legionella* sp., *Leish-*

mania sp., Mycobacteriaceae family, *Mycoplasma pneumoniae, Neisseria gonorrhoeae*, platelets contaminants group (see Table 14), *Pseudomonas aeruginosa*, Pseudomonads group, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Staphylococcus* sp., *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus* sp., *Trypanosoma brucei, Trypanosoma cruzi, Trypanosoma* sp., Trypanosomatidae family, are also objects of the present invention.

[0059] Diagnostic kits further comprising probes or amplification primers for the detection of an antimicrobial agents resistance gene selected from the group listed in Table 5 are also objects of this invention.

[0060] Diagnostic kits further comprising probes or amplification primers for the detection of a toxin gene selected from the group listed in Table 6 are also objects of this invention.

[0061] Diagnostic kits further comprising probes or amplification primers for the detection of any other algal, archaeal, bacterial, fungal or parasitical species than those specifically listed herein, comprising or not comprising those for the detection of the specific microbial species or genus or family or group listed above, and further comprising or not comprising probes and primers for the antimicrobial agents resistance genes listed in Table 5, and further comprising or not comprising probes and primers for the toxin genes listed in Table 6 are also objects of this invention.

[0062] In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or genus or family or group; or universal detection of algae, archaea, bacteria, fungi or parasites; or antimicrobial agents resistance genes; or toxin genes; or for the detection of any microorganism (algae, archaea, bacteria, fungi or parasites).

[0063] In the above methods and kits, probes and primers are not limited to nucleic acids and may include, but are not restricted to analogs of nucleotides such as: inosine, 3-nitropyrrole nucleosides (Nichols et al., 1994, Nature 369:492-493), Linked Nucleic Acids (LNA) (Koskin et al., 1998, Tetrahedron 54:3607-3630), and Peptide Nucleic Acids (PNA) (Egholm et al., 1993, Nature 365:566-568).

[0064] In the above methods and kits, amplification reactions may include but are not restricted to: a) polymerase chain reaction (PCR), b) ligase chain reaction (LCR), c) nucleic acid sequence-based amplification (NASBA), d) self-sustained sequence replication (3SR), e) strand displacement amplification (SDA), f) branched DNA signal amplification (bDNA), g) transcription-mediated amplification (TMA), h) cycling probe technology (CPT), i) nested PCR, j) multiplex PCR, k) solid phase amplification (SPA), 1) nuclease dependent signal amplification (NDSA), m) rolling circle amplification technology (RCA), n) Anchored strand displacement amplification, o) Solid-phase (immobilized) rolling circle amplification.

[0065] In the above methods and kits, detection of the nucleic acids of target genes may include real-time or post-amplification technologies. These detection technologies can include, but are not limited to, fluorescence resonance energy transfer (FRET)-based methods such as adjacent hybridization to FRET probes (including probe-probe and probe-primer methods), TaqMan, Molecular Beacons, scorpions, nanoparticle probes and Sunrise (Amplifluor). Other detection methods include target genes nucleic acids detection via immunological methods, solid phase hybridization methods on filters, chips or any other solid support, whether the hybridization is monitored by fluorescence, chemiluminescence, potentiometry, mass spectrometry, plasmon resonance, polarimetry, colorimetry, or scanometry. Sequencing, including sequencing by dideoxy termination or sequencing by hybridization, e.g. sequencing using a DNA chip, is another possible method to detect and identify the nucleic acids of target genes.

[0066] In a preferred embodiment, a PCR protocol is used for nucleic acid amplification, in diagnostic method as well as in method of construction of a repertory of nucleic acids and deduced sequences.

[0067] In a particularly preferred embodiment, a PCR protocol is provided, comprising, an initial denaturation step of 1-3 minutes at 95° C., followed by an amplification cycle including a denaturation step of one second at 95° C. and an annealing step of 30 seconds at 45-65° C., without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with most selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific, antimicrobial agents resistance gene and toxin gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

[0068] It is also an object of the present invention that tuf, atpD and recA sequences could serve as drug targets and these sequences and means to obtain them revealed in the present invention can assist the screening, design and modeling of these drugs.

[0069] It is also an object of the present invention that tuf, atpD and recA sequences could serve for vaccine purposes and these sequences and means to obtain them revealed in the present invention can assist the screening, design and modeling of these vaccines.

[0070] We aim at developing a universal DNA-based test or kit to screen out rapidly samples which are free of algal, archaeal, bacterial, fungal or parasitical cells. This test could be used alone or combined with more specific identification tests to detect and identify the above algal and/or archaeal and/or bacterial and/or fungal and/or parasitical species and/or genera and/or family and/or group and to determine rapidly the bacterial resistance to antibiotics and/or presence of bacterial toxins. Although the sequences from the selected antimicrobial agents resistance genes are available from public databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current diagnostic methods based on bacterial cultures. Using an amplification method for the simultaneous or independent or sequential microbial detection-identification and antimicrobial resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure should save lives by optimizing treatment, should diminish antimicrobial agents resistance because less antibiotics will be prescribed, should reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and side effects of drugs, and decrease the time and costs associated with clinical laboratory testing.

[0071] In another embodiment, sequence repertories and ways to obtain them for other gene targets are also an object of this invention, such is the case for the hexA nucleic acids and/or sequences of Streptococci.

[0072] In yet another embodiment, for the detection of mutations associated with antibiotic resistance genes, we built repertories to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. Such repertories and ways to obtain them for pbp1a, pbp2b and pbp2x genes of sensitive and penicillin-resistant *Streptococcus pneumoniae* and also for gyrA and parC gene fragments from various bacterial species are also an object of the present invention.

[0073] The diagnostic kits, primers and probes mentioned above can be used to identify algae, archaea, bacteria, fungi, parasites, antimicrobial agents resistance genes and toxin genes on any type of sample, whether said diagnostic kits, primers and probes are used for in vitro or in situ applications. The said samples may include but are not limited to: any clinical sample, any environment sample, any microbial culture, any microbial colony, any tissue, and any cell line.

[0074] It is also an object of the present invention that said diagnostic kits, primers and probes can be used alone or in conjunction with any other assay suitable to identify microorganisms, including but not limited to: any immunoassay, any enzymatic assay, any biochemical assay, any lysotypic assay, any serological assay, any differential culture medium, any enrichment culture medium, any selective culture medium, any specific assay medium, any identification culture medium, any enumeration culture medium, any cellular stain, any culture on specific cell lines, and any infectivity assay on animals.

[0075] In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from proprietary fragments or from public databases. DNA fragments selected from public databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

[0076] In another embodiment, the amino acid sequences translated from the repertory of tuf, atpD and recA nucleic acids and/or sequences are also an object of the present invention.

[0077] It is clear to the individual skilled in the art that other oligonucleotide sequences appropriate for (i) the universal detection of algae, archaea, bacteria, fungi or parasites, (ii) the detection and identification of the above microbial species or genus or family or group, and (iii) the detection of antimicrobial agents resistance genes, and (iv) the detection of toxin genes, other than those listed in Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV may also be derived from the proprietary fragments or selected public database sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from public databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific, family-specific, group-specific, resistance gene-specific, toxin gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annexes I to III, XXI to XXII, XXXII to XXXVII, XXXIX to XLI, and XLIII to LIV which are suitable for diagnostic purposes. When a proprietary fragment or a public databases sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

[0078] Since a high percentage of clinical specimens are negative for bacteria (Table 3), DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and public database sequences. The amplification primers were selected from genes highly conserved in algae, archaea, bacteria, fungi and parasites, and are used to detect the presence of any algal, archaeal, bacterial, fungal or parasitical pathogen in clinical specimens in order to determine rapidly whether it is positive or negative for algae, archaea, bacteria, fungi or parasites. The selected genes, designated tuf, fus, atpD and recA, encode respectively 2 proteins (elongation factors Tu and G) involved in the translational process during protein synthesis, a protein (beta subunit) responsible for the catalytic activity of proton pump ATPase and a protein responsible for the homologous recombination of genetic material. The alignments of tuf, atpD and recA sequences used to derive the universal primers include both proprietary and public database sequences. The universal primer strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for microbiological testing.

[0079] Table 4 provides a list of the archaeal, bacterial, fungal and parasitical species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are revealed in the present invention. Tables 5 and 6 provide a list of antimicrobial agents resistance genes and toxin genes selected for diagnostic purposes. Table 7 provides the origin of tuf, atpD and recA nucleic acids and/or sequences listed in the sequence listing. Tables 8-10 and 12-14 provide lists of species used to test the specificity, ubiquity and sensitivity of some assays described in the examples. Table 11 provides a list of microbial species for which tuf and/or atpD and/or recA sequences are available in public databases. Table 15 lists the microorganisms identified by commercial systems. Tables 16-18 are part of Example 42, whereas Tables 19-20 are part of Example 43. Tables 21-22 illustrate Example 44, whereas Tables 23-25 illustrate Example 45.

BRIEF DESCRIPTION OF THE DRAWINGS

[0080] FIGS. **1** and **2** illustrate the principal subdivisions of the tuf and atpD sequences repertories, respectively. For the design of primers and probes, depending on the needs, one may want to use the complete data set illustrated on the top of the pyramid or use only a subset illustrated by the different branching points. Smaller subdivisions, representing groups, families, genus and species, could even be made to extend to the bottom of the pyramid. Because the tuf and atpD sequences are highly conserved and evolved with each species, the design of primers and probes does not need to include all the sequences within the database or its subdivisions. As illustrated in Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII, depending on the use, sequences from a limited number of species can be carefully selected to represent: i) only the main phylogenetic branches from which the intended probes and primers need to be differentiating, and ii) only the species for which they need to be matching. However, for ubiquity purposes, and especially for primers and probes identifying large groups of species (genus, family, group or universal, or sequencing primers), the more data is included into the sequence analysis, the better the probes and primers will be suitable for each particular intended use. Similarly, for specificity purposes, a larger data set (or repertory) ensures optimal primers and probes design by reducing the chance of employing nonspecific oligonucleotides.

[0081] FIG. **3** illustrates the approach used to design specific amplification primers from fusA as well as from the region between the end of fusA and the beginning of tuf in the streptomycin (str) operon (referred to as the fusA-tuf intergenic spacer in Table 7). Shown is a schematic organization of universal amplification primers (SEQ ID NOs. 1221-1229) in the str operon. Amplicon sizes are given in bases pairs. Drawing not to scale, as the fusA-tuf intergenic spacer size varies depending on the bacterial species. Indicated amplicon lengths are for *E. coli*.

[0082] FIGS. **4** to **6** are illustrations to Example 42, whereas FIGS. **7** to **10** illustrate Example 43. FIGS. **11** and **12** illustrate Example 44.

[0083] FIG. **4**. Abridged multiple amino acid sequence alignment of the partial tuf gene products from selected species illustrated using the program Alscript. Residues highly conserved in bacteria are boxed in grey and gaps are represented with dots. Residues in reverse print are unique to the enterococcal tufB as well as to streptococcal and lactococcal tuf gene products. Numbering is based on *E. coli* EF-Tu and secondary structure elements of *E. coli* EF-Tu are represented by cylinders (α-helices) and arrows (β-strands).

[0084] FIG. **5**. Distance matrix tree of bacterial EF-Tu based on amino acid sequence homology. The tree was constructed by the neighbor-joining method. The tree was rooted using archeal and eukaryotic EF-1α genes as the outgroup. The scale bar represents 5% changes in amino acid sequence, as determined by taking the sum of all of the horizontal lines connecting two species.

[0085] FIG. **6**. Southern hybridization of BglII/XbaI digested genomic DNAs of some enterococci (except for *E. casseliflavus* and *E. gallinarum* whose genomic DNA was digested with BamHI/PvuII) using the tufA gene fragment of *E. faecium* as probes. The sizes of hybridizing fragments are shown in kilobases. Strains tested are listed in Table 16.

[0086] FIG. **7**. *Pantoea* and *Tatumella* species specific signature indel in atpD genes. The nucleotide positions given are for *E. coli* atpD sequence (GenBank accession no. V00267). Numbering starts from the first base of the initiation codon.

[0087] FIG. **8**: Trees based on sequence data from tuf (left side) and atpD (right side). The phylogenetic analysis was performed using the Neighbor-Joining method calculated using the Kimura two-parameter method. The value on each branch indicates the occurrence (%) of the branching order in 750 bootstrapped trees.

[0088] FIG. **9**: Phylogenetic tree of members of the family Enterobacteriaceae based on tuf (a), atpD (b), and 16S rDNA (c) genes. Trees were generated by neighbor-joining method calculated using the Kimura two-parameter method. The value on each branch is the percentage of bootstrap replications supporting the branch. 750 bootstrap replications were calculated.

[0089] FIG. **10**: Plot of tuf distances versus 16S rDNA distances (a), atpD distances versus 16S rDNA distances (b), and atpD distances versus tuf distances (c). Symbols: ○, distances between pairs of strains belonging to the same species; ●, distances between *E. coli* strains and *Shigella* strains; □, distances between pairs belonging to the same genus; ■, distances between pairs belonging to different genera; Δ, distances between pairs belonging to different families.

[0090] FIG. **11** depicts a multiple nucleic acid sequence alignment of the vanA gene from the indicated GenBank nucleotide accession numbers. Above the alignment of the GenBank sequences is a consensus sequence, derived from the alignment of the nucleic acid sequences below. Below the alignment of the GenBank sequences and shaded in grey are the sequences of oligonucleotides (SEQ ID NOs: 1090 and 1091) and the position of a molecular beacon probe (SEQ ID NO: 2299) that hybridizes to the amplification product of SEQ ID NOs: 1090 and 1091.

[0091] FIG. **12** depicts a multiple nucleic acid sequence alignment of the vanB gene from the indicated GenBank nucleotide accession numbers. Above the alignment of the GenBank sequences is a consensus sequence, derived from the alignment of the nucleic acid sequences below. Below the alignment of the GenBank sequences and shaded in grey are the sequences of oligonucleotides (SEQ ID NOs: 1096 and 2298) and the position of a molecular beacon probe (SEQ ID NO: 2300) that hybridizes to the amplification product of SEQ ID NOs: 1096 and 2298.

[0092] FIGS. **13**A and **13**B shows a graphical depictions of PCR amplification curves measured from reactions containing molecular beacon probes. Reactions contained 0, 0.5, 2.5, 5. 10, or 20 copies of vanA resistant *E. faecium* (FIG. **13**A) or vanB resistant *E. faecalis* (FIG. **13**B) template DNA, as well as 3.5 copies of internal control DNA. Molecular beacon probes (SEQ ID NO: 2299 and 2300) were added to each reaction and the fluorescence of the reactions was measured (FIGS. **13**A and **13**B, respectively). SEQ ID NO: 2299 is labeled with FAM. SEQ ID NO: 2300 is labeled with Texas Red. SEQ ID NO: 2301 is labeled with TET.

[0093] FIG. **14** shows an agarose gel of the DNA amplification products from PCR using the template DNA sources listed in Table 29. The numbers above the lanes correspond to the numbers in Table 29.

[0094] FIG. **15** shows an agarose gel of the DNA amplification products from PCR using template DNA sources listed in Table 30. The numbers above the lanes correspond to the numbers in Table 30.

[0095] FIG. **16** shows an agarose gel of the DNA amplification products from PCR using template DNA sources listed in Table 31. The numbers above the lanes correspond to the numbers in Table 31.

[0096] FIG. **17** shows an agarose gel of the DNA amplification products from PCR using template DNA sources listed in Table 32. The numbers above the lanes correspond to the numbers in Table 32.

[0097] FIGS. **18**A and **18**B show the fluorescence signal readout obtained in the FAM channel when vanA template

(FIG. **18**A) or non-specific template (FIG. **18**B) DNA was used in PCR according to Example 23.

[0098] FIGS. **19**A and **19**B show the fluorescence signal readout obtained in the TET channel when internal control (IC) template (FIG. **19**A) or non-specific template (FIG. **19**B) DNA was used in PCR according to Example 23

[0099] FIGS. **20**A and **20**B show the fluorescence signal readout obtained in the Texas Red channel when vanB template (FIG. **20**A) or non-specific template (FIG. **20**B) DNA was used in the PCR according to Example 23.

[0100] FIG. **21** shows the fluorescent signal readout obtained in the vanR assay for a vanA positive clinical specimen. The top panel shows the fluorescent readout from the FAM channel, and the bottom panel shows the fluorescent readout in the Texas Red channel, designed to detect the vanB probe.

[0101] FIG. **22** shows the fluorescent signal readout obtained in the vanR assay for a clinical specimen that is both vanA and vanB positive. The top panel shows the readout from the FAM channel (vanA) and the bottom panel a shows the readout from the Texas Red channel (vanB).

[0102] FIG. **23** shows the fluorescent signal readout obtained in the vanR assay for a clinical specimen that is vanB positive. The top panel shows the FAM channel (vanA) and the bottom channel shows the fluorescent readout from the Texas Red channel (vanB).

[0103] FIG. **24** shows an agarose gel of the DNA amplification products from PCR using the template DNA sources listed in Table 36. The numbers above the lanes correspond to the numbers in Table 36.

[0104] FIG. **25** shows an agarose gel of the DNA amplification products from PCR using the template DNA sources listed in Table 37. The numbers above the lanes correspond to the numbers in Table 37.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0105] The present inventors reasoned that comparing the published *Haemophilus influenzae* and *Mycoplasma genitalium* genomes and searching for conserved genes could provide targets to develop useful diagnostic primers and probes. This sequence comparison is highly informative as these two bacteria are distantly related and most genes present in the minimal genome of *M. genitalium* are likely to be present in every bacterium. Therefore genes conserved between these two bacteria are likely to be conserved in all other bacteria.

[0106] Following the genomic comparison, it was found that several protein-coding genes were conserved in evolution. Highly conserved proteins included the translation elongation factors G (EF-G) and Tu (EF-Tu) and the β subunit of F_0F_1 type ATP-synthase, and to a lesser extent, the RecA recombinase. These four proteins coding genes were selected amongst the 20 most conserved genes on the basis that they all possess at least two highly conserved regions suitable for the design of universal amplification and sequencing primers. Moreover, within the fragment amplified by these primers, highly conserved and more variable regions are also present hence suggesting it might be possible to rapidly obtain sequence information from various microbial species to design universal as well as species-, genus-, family-, or group-specific primers and probes of potential use for the detection and identification and/or quantification of microorganisms.

[0107] Translation elongation factors are members of a family of GTP-binding proteins which intervene in the interactions of tRNA molecules with the ribosome machinery during essential steps of protein synthesis. The role of elongation factor Tu is to facilitate the binding of aminoacylated tRNA molecules to the A site of the ribosome. The eukaryotic, archaeal (archaebacterial) and algal homolog of EF-Tu is called elongation factor 1 alpha (EF-1α). All protein synthesis factors originated from a common ancestor via gene duplications and fusions (Cousineau et al., 1997, J. Mol. Evol. 45:661-670). In particular, elongation factor G (EF-G), although having a functional role in promoting the translocation of aminoacyl-tRNA molecules from the A site to the P site of the ribosome, shares sequence homologies with EF-Tu and is thought to have arisen from the duplication and fusion of an ancestor of the EF-Tu gene.

[0108] In addition, EF-Tu is known to be the target for antibiotics belonging to the elfamycin's group as well as to other structural classes (Anborgh and Parmeggiani, 1991, EMBO J. 10:779-784; Luiten et al., 1992, European patent application serial No. EP 0 466 251 A1). EF-G for its part, is the target of the antibiotic fusidic acid. In addition to its crucial activities in translation, EF-Tu has chaperone-like functions in protein folding, protection against heat denaturation of proteins and interactions with unfolded proteins (Caldas et al., 1998, J. Biol. Chem 273:11478-11482). Interestingly, a form of the EF-Tu protein has been identified as a dominant component of the periplasm of *Neisseria gonorrhoeae* (Porcella et al., 1996, Microbiology 142:2481-2489), hence suggesting that at least in some bacterial species, EF-Tu might be an antigen with vaccine potential.

[0109] F_0F_1 type ATP-synthase belongs to a superfamily of proton-translocating ATPases divided in three major families: P, V and F (Nelson and Taiz, 1989, TIBS 14:113-116). P-ATPases (or E_1-E_2 type) operate via a phosphorylated intermediate and are not evolutionarily related to the other two families. V-ATPases (or V_0V_1 type) are present on the vacuolar and other endomembranes of eukaryotes, on the plasma membrane of archaea (archaebacteria) and algae, and also on the plasma membrane of some eubacteria especially species belonging to the order Spirochaetales as well as to the Chlamydiaceae and Deinococcaceae families. F-ATPases (or F_0F_1 type) are found on the plasma membrane of most eubacteria, on the inner membrane of mitochondria and on the thylakoid membrane of chloroplasts. They function mainly in ATP synthesis. They are large multimeric enzymes sharing numerous structural and functional features with the V-ATPases. F and V-type ATPases have diverged from a common ancestor in an event preceding the appearance of eukaryotes. The β subunit of the F-ATPases is the catalytic subunit and it possesses low but significant sequence homologies with the catalytic A subunit of V-ATPases.

[0110] The translation elongation factors EF-Tu, EF-G and EF-1α and the catalytic subunit of F or V-types ATP-synthase, are highly conserved proteins sometimes used for phylogenetic analysis and their genes are also known to be highly conserved (Iwabe et al., 1989, Proc. Natl. Acad. Sci. USA 86:9355-9359, Gogarten et al., 1989, Proc. Natl. Acad. Sci. USA 86:6661-6665, Ludwig et al., 1993, Antonie van Leeuwenhoek 64:285-305).

[0111] A recent BLAST (Altschul et al., 1997, J. Mol. Biol. 215:403-410) search performed by the present inventors on the GenBank, European Molecular Biology Laboratory (EMBL), DNA Database of Japan (DDBJ) and specific genome project databases indicated that throughout bacteria, the EF-Tu and the β subunit of F_0F_1 type ATP-synthase genes may be more conserved than other genes that are well conserved between *H. influenzae* and *M. genitalium.*

[0112] The RecA recombinase is a multifunctional protein encoded by the recA gene. It plays a central role in homologous recombination, it is critical for the repair of DNA damage and it is involved in the regulation of the SOS system by promoting the proteolytic digestion of the LexA repressor. It is highly conserved in bacteria and could serve as a useful genetic marker to reconstruct bacterial phylogeny (Miller and Kokjohn, 1990, Annu. Rev. Microbiol. 44:365-394). Although RecA possesses some highly conserved sequence segments that we used to design universal primers aimed at sequencing the recA fragments, it is clearly not as well conserved EF-G, EF-Tu and (3 subunit of F_0F_1 type ATP-synthase. Hence, RecA may not be optimal for universal detection of bacteria with high sensitivity but it was chosen because preliminary data indicated that EF-G, EF-Tu and 1 subunit of F_0F_1 type ATP-synthase may sometimes be too closely related to find specific primer pairs that could discriminate between certain very closely related species and genera. While RecA, EF-G, EF-Tu and β subunit of F_0F_1 type ATP-synthase genes, possesses highly conserved regions suitable for the design of universal sequencing primers, the less conserved region between primers should be divergent enough to allow species-specific and genus-specific primers in those cases.

[0113] Thus, as targets to design primers and probes for the genetic detection of microorganisms, the present inventors have focused on the genes encoding these four proteins: tuf, the gene for elongation factor Tu (EF-Tu); fus, the gene for the elongation factor G (EF-G); atpD, the gene for β subunit of F_0F_1 type ATP-synthase; and recA, the gene encoding the RecA recombinase. In several bacterial genomes tuf is often found in two highly similar duplicated copies named tufA and tufB (Filer and Furano, 1981, J. Bacteriol. 148:1006-1011, Sela et al., 1989, J. Bacteriol. 171:581-584). In some particular cases, more divergent copies of the tuf genes can exist in some bacterial species such as some actinomycetes (Luiten et al. European patent application publication No. EP 0 446 251 A 1; Vijgenboom et al., 1994, Microbiology 140:983-998) and, as revealed as part of this invention, in several enterococcal species. In several bacterial species, tuf is organized in an operon with its homolog gene for the elongation factor G (EF-G) encoded by the fusA gene (FIG. **3**). This operon is often named the str operon. The tuf, fus, atpD and recA genes were chosen as they are well conserved in evolution and have highly conserved stretches as well as more variable segments. Moreover, these four genes have eukaryotic orthologs which are described in the present invention as targets to identify fungi and parasites. The eukaryotic homolog of elongation factor Tu is called elongation factor 1-alpha (EF-1α) (gene name: tef, tef1, ef1, ef-1 or EF-1). In fungi, the gene for EF-1α occurs sometimes in two or more highly similar duplicated copies (often named tef1, tef 2, tef3 . . .). In addition, eukaryotes have a copy of elongation factor Tu which is originating from their organelle genome ancestry (gene name: tuf1, tufM or tufA). For the purpose of the current invention, the genes for these four functionally and evolutionarily linked elongation factors (bacterial EF-Tu and EF-G, eukaryotic EF-1α, and organellar EF-Tu) will hereafter be designated as <<tuf nucleic acids and/or sequences>>. The eukaryotic (mitochondrial) F_0F_1 type ATP-synthase beta subunit gene is named atp2 in yeast. For the purpose of the current invention, the genes of catalytic sub-unit of either F or V-type ATP-synthase will hereafter be designated as <<atpD nucleic acids and/or sequences>>. The eukaryotic homologs of RecA are distributed in two families, typified by the Rad51 and Dmc1 proteins. Archaeal homologs of RecA are called RadA. For the purpose of the current invention, the genes corresponding to the latter proteins will hereafter be designated as <<recA nucleic acids and/or sequences>>.

[0114] In the description of this invention, the terms <<nucleic acids>> and <<sequences>> might be used interchangeably. However, <<nucleic acids>> are chemical entities while <<sequences>> are the pieces of information derived from (inherent to) these <<nucleic acids>>. Both nucleic acids and sequences are equivalently valuable sources of information for the matter pertaining to this invention.

[0115] Analysis of multiple sequence alignments of tuf and atpD sequences permitted the design of oligonucleotide primers (and probes) capable of amplifying (or hybridizing to) segments of tuf (and/or fus) and atpD genes from a wide variety of bacterial species (see Examples 1 to 4, 24 and 26, and Table 7). Sequencing and amplification primer pairs for tuf nucleic acids and/or sequences are listed in Annex I and hybridization probes are listed in Annexes III and XLVII. Sequencing and amplification primer pairs for atpD nucleic acids and/or sequences are listed in Annex II. Analysis of the main subdivisions of tuf and atpD sequences (see FIGS. **1** and **2**) permitted to design sequencing primers amplifying specifically each of these subdivisions. It should be noted that these sequencing primers could also be used as universal primers. However, since some of these sequencing primers include several variable sequence (degenerated) positions, their sensitivity could be lower than that of universal primers developed for diagnostic purposes. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

[0116] Similarly, analysis of multiple sequence alignments of recA sequences present in the public databases permitted the design of oligonucleotide primers capable of amplifying segments of recA genes from a wide variety of bacterial species. Sequencing and amplification primer pairs for recA sequences are listed in Annex XXI. The main subdivisions of recA nucleic acids and/or sequences comprise recA, radA, rad51 and dmc1. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

[0117] The present inventor's strategy is to get as much sequence data information from the four conserved genes (tuf, fus, atpD and recA). This ensemble of sequence data forming a repertory (with subrepertories corresponding to each target gene and their main sequence subdivisions) and then using the sequence information of the sequence repertory (or subrepertories) to design primer pairs that could permit either universal detection of algae or archaea or bacteria or fungi or parasites, detection of a family or group of microorganism (e.g. Enterobacteriaceae), detection of a genus (e.g. *Streptococcus*) or finally a specific species (e.g. *Staphylococcus aureus*). It should be noted that for the purpose of the present invention a group of microorganisms is defined depending on the needs of the particular diagnostic test. It does not need to respect a particular taxonomical grouping or phylum. See Example 12 where primers were designed to amplify a group a bacteria consisting of the 17 major bacterial species encountered as contaminants of platelet concentrates. Also remark that in that Example, the primers are not only able to sensitively and rapidly detect at least the 17 important bacterial species, but could also detect other species as well, as shown in Table 14. In these circumstances the primers shown in Example 12 are considered universal for platelet-contaminating bacteria. To develop an assay specific for the latter, one or more primers or probes specific to each species could be designed. Another example of primers and/or probes for group detection is given by the Pseudomonad group primers. These primers were designed based upon alignment of tuf sequences from real *Pseudomonas* species as well as from former *Pseudomonas* species such as *Stenotrophomonas maltophilia*. The resulting primers are able to amplify all *Pseudomonas* species tested as well as several species belonging to different genera, hence as being specific for a group including *Pseudomonas* and other species, we defined that group as Pseudomonads, as several members were former *Pseudomonas.*

[0118] For certain applications, it may be possible to develop a universal, group, family or genus-specific reaction and to proceed to species identification using sequence information within the amplicon to design species-specific internal probes or primers, or alternatively, to proceed directly by sequencing the amplicon. The various strategies will be discussed further below.

[0119] The ensembles formed by public and proprietary tuf, atpD and recA nucleic acids and/or sequences are used in a novel fashion so they constitute three databases containing useful information for the identification of microorganisms.

[0120] Sequence repertories of other gene targets were also built to solve some specific identification problems especially for microbial species genetically very similar to each other such as *E. coli* and *Shigella* (see Example 23). Based on tuf, atpD and recA sequences, *Streptococcus pneumoniae* is very difficult to differentiate from the closely related species *S. oralis* and *S. mitis*. Therefore, we elected to built a sequence repertory from hexA sequences (Example 19), a gene much more variable than our highly conserved tuf, atpD and recA nucleic acids and/or sequences.

[0121] For the detection of mutations associated with antibiotic resistance genes, we also built repertories to distinguish between point mutations reflecting only gene diversity and point mutations involved in resistance. This was done for pbp1a, pbp2b and pbp2x genes of penicillin-resistant and sensitive *Streptococcus pneumoniae* (Example 18) and also for gyrA and parC gene fragments of various bacterial species for which quinolone resistance is important to monitor.

Oligonucleotide Primers and Probes Design and Synthesis

[0122] The tuf, fus, atpD and recA DNA fragments sequenced by us and/or selected from public databases (GenBank and EMBL) were used to design oligonucleotides primers and probes for diagnostic purposes. Multiple sequence alignments were made using subsets of the tuf or atpD or recA sequences repertory. Subsets were chosen to encompass as much as possible of the targeted microorganism(s) DNA sequence data and also include sequence data from phylogenetically related microorganisms from which the targeted microorganism(s) should be distinguished. Regions suitable for primers and probes should be conserved for the targeted microorganism(s) and divergent for the microorganisms from which the targeted microorganism(s) should be distinguished. The large amount of tuf or atpD or recA sequences data in our repertory permits to reduce trial and errors in obtaining specific and ubiquitous primers and probes. We also relied on the corresponding peptide sequences of tuf, fus, atpD and recA nucleic acids and/or sequences to facilitate the identification of regions suitable for primers and probes design. As part of the design rules, all oligonucleotides (probes for hybridization and primers for DNA amplification by PCR) were evaluated for their suitability for hybridization or PCR amplification by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo™ 5.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Oligonucleotide probes and amplification primers were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division).

[0123] The oligonucleotide sequence of primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of algae or archaea or bacteria or fungi or parasites, (ii) the species-specific detection and identification of any microorganism, including but not limited to: *Abiotrophia adiacens, Bacteroides fragilis, Bordetella pertussis, Candida albicans, Candida dubliniensis, Candida glabrata, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Candida zeylanoides, Campylobacter jejuni* and *C. coli, Chlamydia pneumoniae, Chlamydia trachomatis, Cryptococcus neoformans, Cryptosporidium parvum, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Escherichia coli, Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Trypanosoma brucei, Trypanosoma cruzi*, (iii) the genus-specific detection of *Bordetella* species, *Candida species, Clostridium species, Corynebacterium* species, *Cryptococcus* species, *Entamoeba species, Enterococcus* species, *Gemella* species, *Giardia* species, *Legionella* species, *Leishmania* species, *Staphylococcus* species, *Streptococcus* species, *Trypanosoma* species, (iv) the family-specific detection of Enterobacteriaceae family members, Mycobacteriaceae family members, Trypanosomatidae family members, (v) the detection of *Enterococcus casseliflavus-flavescens-gallinarum* group, *Enterococcus, Gemella* and *Abiotrophia adiacens* group, Pseudomonads extended group, Platelet-contaminating bacteria group, (vi) the detection of clinically important antimicrobial agents resistance genes listed in Table 5, (vii) the detection of clinically important toxin genes listed in Table 6.

[0124] Variants for a given target microbial gene are naturally occurring and are attributable to sequence variation within that gene during evolution (Watson et al., 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, Calif.; Lewin, 1989, Genes IV, John Wiley & Sons, New York, N.Y.). For example, different strains of the same microbial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant algal, archaeal, bacterial, fungal or parasitical DNA nucleic acids and/or sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target nucleic acids and/or sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant microbial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

Sequencing of tuf Nucleic Acids and/or Sequences from a Variety of Archaeal, Bacterial, Fungal and Parasitical Species

[0125] The nucleotide sequence of a portion of tuf nucleic acids and/or sequences was determined for a variety of archaeal, bacterial, fungal and parasitical species. The amplification primers (SEQ ID NOs. 664 and 697), which amplify a tuf gene portion of approximately 890 bp, were used along with newly designed sequencing primer pairs (See Annex I for the sequencing primers for tuf nucleic acids and/or sequences). Most primer pairs can amplify different copies of tuf genes (tufA and tufB). This is not surprising since it is known that for several bacterial species these two genes are nearly identical. For example, the entire tufA and tufB genes from *E. coli* differ at only 13 nucleotide positions (Neidhardt et al., 1996, *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed., American Society for Microbiology Press, Washington, D.C.). Similarly, some fungi are known to have two nearly identical copies of tuf nucleic acids and/or sequences (EF-1□). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of tuf nucleic acids and/or sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The tuf sequencing primers even sometimes amplified highly divergent copies of tuf genes (tufC) as illustrated in the case of some enterococcal species (SEQ ID NOs.: 73, 75, 76, 614 to 618, 621 and 987 to 989). To prove this, we have determined the enterococcal tuf nucleic acids and/or sequences from PCR amplicons cloned into a plasmid vector. Using the sequence data from the cloned amplicons, we designed new sequencing primers specific to the divergent (tufC) copy of enterococci (SEQ ID NOs.: 658-659 and 661) and then sequenced directly the tufC amplicons. The amplification primers (SEQ ID NOs.: 543, 556, 557, 643-645, 660, 664, 694, 696 and 697) could be used to amplify the tuf nucleic acids and/or sequences from any bacterial species. The amplification primers (SEQ ID NOs.: 558, 559, 560, 653, 654, 655, 813, 815, 1974-1984, 1999-2003) could be used to amplify the tuf (EF-1 □ genes from any fungal and/or parasitical species. The amplification primers SEQ ID NOs. 1221-1228 could be used to amplify bacterial tuf nucleic acids and/or sequences of the EF-G subdivision (fusA) (FIG. 3). The amplification primers SEQ ID NOs. 1224, and 1227-1229 could be used to amplify bacterial tuf nucleic acids and/or sequences comprising the end of EF-G (fusA) and the beginning of EF-Tu (tuf), including the intergenic region, as shown in FIG. 3.

[0126] Most tuf fragments to be sequenced were amplified using the following amplification protocol: One μl of cell suspension (or of purified genomic DNA 0.1-100 ng/μl) was transferred directly to 19 μl of a PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 1 μM of each of the 2 primers, 200 μM of each of the four dNTPs, 0.5 unit of Taq DNA polymerase (Promega Corp., Madison, Wis.). PCR reactions were subjected to cycling using a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 94-96° C. followed by 30-45 cycles of 1 min at 95° C. for the denaturation step, 1 min at 50-55° C. for the annealing step and 1 min at 72° C. for the extension step. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The amplicons were then visualized by staining with methylene blue (Flores et al., 1992, Biotechniques, 13:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, Calif.). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the tuf genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 377) with their Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif.). The sequencing reactions were performed by using the same amplification primers and 10 ng/100 by of the gel-purified amplicon per reaction. For the sequencing of long amplicons such as those of eukaryotic tuf (EF-1 □ nucleic acids and/or sequences, we designed internal sequencing primers (SEQ ID NOs.: 654, 655 and 813) to be able to obtain sequence data on both strands for most of the fragment length. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artefacts, we have sequenced two preparations of the gel-purified tuf amplification product originating from two independent PCR amplifications. For most target microbial species, the sequences determined for both amplicon preparations were identical. In case of discrepancies, amplicons from a third independent PCR amplification were sequenced. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The tuf nucleic acids and/or sequences determined using the above strategy are described in the Sequence Listing. Table 7 gives the originating microbial species and the source for each tuf sequence in the Sequence Listing.

[0127] The alignment of the tuf sequences determined by us or selected from databases revealed clearly that the length of the sequenced portion of the tuf genes is variable. There may be insertions or deletions of several amino acids. In addition, in several fungi introns were observed. Intron nucleic acids and/or sequences are part of tuf nucleic acids and/or sequences and could be useful in the design of species-specific primers and probes. This explains why the size of the sequenced tuf amplification products was variable from one fungal species to another. Consequently, the nucleotide positions indicated on top of each of Annexes IV to XX, XXIII to XXXI, XXXVIII and XLII do not correspond for sequences having insertions or deletions.

[0128] It should also be noted that the various tuf nucleic acids and/or sequences determined by us occasionally contain base ambiguities. These degenerated nucleotides correspond to sequence variations between tufA and tufB genes (or copies of the EF-G subdivision of tuf nucleic acids and/or sequences, or copies of EF-1 □ subdivision of tuf nucleic acids and/or sequences for fungi and parasites) because the amplification primers amplify both tuf genes. These nucleotide variations were not attributable to nucleotide misincorporations by the Taq DNA polymerase because the sequence of both strands was identical and also because the sequences determined with both preparations of the gel-purified tuf amplicons obtained from two independent PCR amplifications were identical.

The Selection of Amplification Primers from Tuf Nucleic Acids and/or Sequences

[0129] The tuf sequences determined by us or selected from public databases were used to select PCR primers for universal detection of bacteria, as well as for genus-specific, species-specific family-specific or group-specific detection and identification. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various tuf sequences. For more details about the selection of PCR primers from tuf sequences please refer to Examples 5, 7-14, 17, 22, 24, 28, 30-31, 33, 36, and 38-40, and to Annexes VI-IX, XI-XIX and XXV.

Sequencing of atpD and recA Nucleic Acids and/or Sequences from a Variety of Archaeal, Bacterial, Fungal and Parasitical Species

[0130] The method used to obtain atpD and recA nucleic acids and/or sequences is similar to that described above for tuf nucleic acids and/or sequences.

The Selection of Amplification Primers from atpD or recA Nucleic Acids and/or Sequences

[0131] The comparison of the nucleotide sequence for the atpD or recA genes from various archaeal, bacterial, fungal and parasitical species allowed the selection of PCR primers (refer to Examples 6, 13, 29, 34 and 37, and to Annexes IV, V, X, and XX).

DNA Amplification

[0132] For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo™ 5.0 software to verify that they were good candidates for PCR amplification.

[0133] During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the microbial genome are used to amplify exponentially in vitro the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing et al, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

[0134] Briefly, the PCR protocols were as follows: Treated clinical specimens or standardized bacterial or fungal or parasitical suspensions (see below) or purified genomic DNA from bacteria, fungi or parasites were amplified in a 20 μl PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, 0.4 μM of each primer, 200 μM of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, Calif.). The TaqStart™ antibody, which is a neutralizing monoclonal antibody to Taq DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg et al., 1994, Biotechniques 16:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the microbial cells and eliminate or neutralize PCR inhibitors. For amplification from bacterial or fungal or parasitical cultures or from purified genomic DNA, the samples were added directly to the PCR amplification mixture without any pre-treatment step. An internal control was derived from sequences not found in the target microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. Alternatively, an internal control derived from rRNA was also useful to monitor the efficiency of microbial lysis protocols.

[0135] PCR reactions were then subjected to thermal cycling (3 min at 94-96° C. followed by 30 cycles of 1 second at 95° C. for the denaturation step and 30 seconds at 50-65° C. for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.). The number of cycles performed for the PCR assays varies according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are probably required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal or parasitical cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

[0136] The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase amplification (SPA), rolling circle amplification technology (RCA), solid phase RCA, anchored SDA and nuclease dependent signal amplification (NDSA) (Lee et al., 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, Mass.; Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Westin et al., 2000, Nat. Biotechnol. 18:199-204). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase the sensitivity and/or the rapidity of nucleic acid-based diagnostic tests. The scope of the present invention also covers the use of any nucleic acids amplification and detection technology including real-time or post-amplification detection technologies, any amplification technology combined with detection, any hybridization nucleic acid chips or arrays technologies, any amplification chips or combination of amplification and hybridization chips technologies. Detection and identification by any sequencing method is also under the scope of the present invention.

[0137] Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR or for DNA hybridization which are derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antimicrobial agents resistance or toxin gene sequences included in this document are also under the scope of this invention.

Detection of Amplification Products

[0138] Classically, detection of amplification is performed by standard ethidium bromide-stained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after or during amplification. One simple method for monitoring amplified DNA is to measure its rate of formation by measuring the increase in fluorescence of intercalating agents such as ethidium bromide or SYBR® Green I (Molecular Probes). If more specific detection is required, fluorescence-based technologies can monitor the appearance of a specific product during the reaction. The use of dual-labeled fluorogenic probes such as in the TaqMan™ system (Applied Biosystems) which utilizes the 5'-3' exonuclease activity of the Taq polymerase is a good example (Livak K. J. et al. 1995, PCR Methods Appl. 4:357-362). TaqMan™ can be performed during amplification and this "real-time" detection can be done in a single closed tube hence eliminating post-PCR sample handling and consequently preventing the risk of amplicon carryover. Several other fluorescence-based detection methods can be performed in real-time. Fluorescence resonance energy transfer (FRET) is the principle behind the use of adjacent hybridization probes (Wittwer, C. T. et al. 1997. BioTechniques 22:130-138), molecular beacons (Tyagi S. and Kramer F. R. 1996. Nature Biotechnology 14:303-308) and scorpions (Whitcomb et al. 1999. Nature Biotechnology 17:804-807). Adjacent hybridization probes are designed to be internal to the amplification primers. The 3' end of one probe is labelled with a donor fluorophore while the 5' end of an adjacent probe is labelled with an acceptor fluorophore. When the two probes are specifically hybridized in closed proximity (spaced by 1 to 5 nucleotides) the donor fluorophore which has been excited by an external light source emits light that is absorbed by a second acceptor that emit more fluorescence and yields a FRET signal. Molecular beacons possess a stem-and-loop structure where the loop is the probe and at the bottom of the stem a fluorescent moiety is at one end while a quenching moiety is at the other end. The beacons undergo a fluorogenic conformational change when they hybridize to their targets hence separating the fluorochrome from its quencher. The FRET principle is also used in an air thermal cycler with a built-in fluorometer (Wittwer, C. T. et al. 1997. BioTechniques 22:130-138). The amplification and detection are extremely rapid as reactions are performed in capillaries: it takes only 18 min to complete 45 cycles. Those techniques are suitable especially in the case where few pathogens are searched for. Boehringer-Roche Inc. sells the LightCycler™, and Cepheid makes the SmartCycler. These two apparatus are capable of rapid cycle PCR combined with fluorescent SYBR® Green I or FRET detection. We recently demonstrated in our laboratory, real-time detection of 10 CFU in less than 40 minutes using adjacent hybridization probes on the LightCycler™. Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated.

[0139] Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any sequence from our repertory and designed to specifically hybridize to DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus or family or group detection and identification may be derived from the amplicons produced by a universal, family-, group-, genus- or species-specific amplification assay(s). The oligonucleotide probes may be labeled with biotin or with digoxigenin or with any other reporter molecule (for more details see below the section on hybrid capture). Hybridization on a solid support is amendable to miniaturization.

[0140] At present the oligonucleotide nucleic acid microarray technology is appealing. Currently, available low to medium density arrays (Heller et al., An integrated microelectronics hybridization system for genomic research and diagnostic applications. In: Harrison, D. J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.) could specifically capture fluorescent-labelled amplicons. Detection methods for hybridization are not limited to fluorescence; potentiometry, colorimetry and plasmon resonance are some examples of alternative detection methods. In addition to detection by hybridization, nucleic acid microarrays could be used to perform rapid sequencing by hybridization. Mass spectrometry could also be applicable for rapid identification of the amplicon or even for sequencing of the amplification products (Chiu and Cantor, 1999, Clinical Chemistry 45:1578; Berkenkamp et al., 1998, Science 281:260).

[0141] For the future of our assay format, we also consider the major challenge of molecular diagnostics tools, i.e.: integration of the major steps including sample preparation, genetic amplification, detection, data analysis and presentation (Anderson et al., Advances in integrated genetic analysis. In: Harrison, D. J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.).

[0142] To ensure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y.). The concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and $MgCl_2$ are 0.1-1.5 μM and 1.0-10.0 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples.

Hybrid Capture and Chemiluminescence Detection of Amplification Products

[0143] Hybridization and detection of amplicons by chemiluminescence were adapted from Nikiforov et al. (1994, PCR Methods and Applications 3:285-291 and 1995, Anal. Biochem. 227:201-209) and from the DIG™ system protocol of Boehringer Mannheim. Briefly, 0.50 µl of a 25 picomoles solution of capture probe diluted in EDC {1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) are immobilized in each well of 96-wells plates (Microlite™ 2, Dynex) by incubation overnight at room temperature. The next day, the plates are incubated with a solution of 1% BSA diluted into TNTw (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.05% Tween™. 20) for 1 hour at 37° C. The plates are then washed on a Wellwash Ascent™ (Labsystems) with TNTw followed by Washing Buffer (100 mM maleic acid pH7.5; 150 mM NaCl; 0.3% Tween™ 20).

[0144] The amplicons were labelled with DIG-11-dUTP during PCR using the PCR DIG Labelling Mix from Boehringer Mannheim according to the manufacturer's instructions. Hybridization of the amplicons to the capture probes is performed in triplicate at stringent temperature (generally, probes are designed to allow hybridization at 55° C., the stringent temperature) for 30 minutes in 1.5 M NaCl; 10 mM EDTA. It is followed by two washes in 2×SSC; 0.1% SDS, then by four washes in 0.1×SSC; 0.1% SDS at the stringent temperature (55° C.). Detection with 1,2 dioxetane chemiluminescent alkaline phosphatase substrates like CSPD® (Tropix Inc.) is performed according to the manufacturer's instructions but with shorter incubations times and a different antibody concentration. The plates are agitated at each step, the blocking incubation is performed for only 5 minutes, the anti-DIG-AP1 is used at a 1:1000 dilution, the incubation with antibody lasts 15 minutes, the plates are washed twice for only 5 minutes. Finally, after a 2 minutes incubation into the detection buffer, the plates are incubated 5 minutes with CSPD® at room temperature followed by a 10 minutes incubation at 37° C. without agitation. Luminous signal detection is performed on a Dynex Microtiter Plate Luminometer using RLU (Relative Light Units).

Specificity, Ubiquity and Sensitivity Tests for Oligonucleotide Primers and Probes

[0145] The specificity of oligonucleotide primers and probes was tested by amplification of DNA or by hybridization with bacterial or fungal or parasitical species selected from a panel comprising closely related species and species sharing the same anatomo-pathological site (see Annexes and Examples). All of the bacterial, fungal and parasitical species tested were likely to be pathogens associated with infections or potential contaminants which can be isolated from clinical specimens. Each target DNA could be released from microbial cells using standard chemical and/or physical treatments to lyse the cells (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2^{nd} ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) or alternatively, genomic DNA purified with the GNOME™ DNA kit (Bio101, Vista, Calif.) was used. Subsequently, the DNA was subjected to amplification with the primer pairs. Specific primers or probes amplified only the target microbial species, genus, family or group.

[0146] Oligonucleotides primers found to amplify specifically the target species, genus, family or group were subsequently tested for their ubiquity by amplification (i.e. ubiquitous primers amplified efficiently most or all isolates of the target species or genus or family or group). Finally, the sensitivity of the primers or probes was determined by using 10-fold or 2-fold dilutions of purified genomic DNA from the targeted microorganism. For most assays, sensitivity levels in the range of 1-100 copies were obtained. The specificity, ubiquity and sensitivity of the PCR assays using the selected amplification primer pairs were tested either directly from cultures of microbial species or from purified microbial genomic DNA.

[0147] Probes were tested in hybrid capture assays as described above. An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus or family or group from which it was selected. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes detected efficiently most or all isolates of the target species or genus or family or group) by hybridization to microbial DNAs from different clinical isolates of the species or genus or family or group of interest including ATCC reference strains. Similarly, oligonucleotide primers and probes could be derived from antimicrobial agents resistance or toxin genes which are objects of the present invention.

Reference Strains

[0148] The reference strains used to build proprietary tuf, atpD and recA sequence data subrepertories, as well as to test the amplification and hybridization assays were obtained from (i) the American Type Culture Collection (ATCC), (ii) the Laboratoire de santé publique du Québec (LSPQ), (iii) the Centers for Disease Control and Prevention (CDC), (iv) the National Culture Type Collection (NCTC) and (v) several other reference laboratories throughout the world. The identity of our reference strains was confirmed by phenotypic testing and reconfirmed by analysis of tuf, atpD and recA sequences (see Example 13).

Antimicrobial Agents Resistance Genes

[0149] Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of microbial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal algal, archaeal, bacterial, fungal or parasitical detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians also need timely information about the ability of the microbial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly microbial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antimicrobial agents resistance genes (i.e. DNA-based tests for the specific detection of antimicrobial agents resistance genes). Since the sequence from the most important and common antimicrobial agents resistance genes are available from public databases, our strategy is to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the antimicrobial agents resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 5; descriptions of the designed amplification primers and internal probes are given in Annexes XXXIV-XXXVII, XXXIX, XLV, and L-LI. Our approach is unique because the antimicrobial agents resistance genes detection and the microbial detection and identification can be performed simultaneously, or independently, or sequentially in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Toxin Genes

[0150] Toxin identification is often very important to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians sometimes need timely information about the ability of certain bacterial pathogens to produce toxins. Since the sequence from the most important and common bacterial toxin genes are available from public databases, our strategy is to use the sequence from a portion or from the entire toxin gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of sensitive and rapid DNA-based tests. The list of each of the bacterial toxin genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in Table 6; descriptions of the designed amplification primers and internal probes are given in Annexes XXII, XXXII and XXXIII. Our approach is unique because the toxin genes detection and the bacterial detection and identification can be performed simultaneously, or independently, or sequentially, in multiplex or parallel or sequential assays under uniform PCR amplification conditions. These amplifications can also be done separately.

Universal Bacterial Detection

[0151] In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture. Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screening out the numerous negative specimens is thus useful as it reduces costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the tuf, atpD and recA nucleic acids and/or sequences. The universal primers selection was based on a multiple sequence alignment constructed with sequences from our repertory.

[0152] All computer analysis of amino acid and nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for the universal amplification of bacteria were selected with the help of the Oligo™ program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of base ambiguities in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y.).

[0153] The amplification conditions with the universal primers are very similar to those used for the species- and genus-specific amplification assays except that the annealing temperature is slightly lower. The original universal PCR assay described in our assigned WO98/20157 (SEQ ID NOs. 23-24 of the latter application) was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species as well as genomic DNA from *Leishmania donovani, Saccharomyces cerevisiae* and human lymphocytes. None of the above eukaryotic DNA preparations could be amplified by the universal assay, thereby suggesting that this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Table 4. We found that at least 104 of these strains could be amplified. However, the assay could be improved since bacterial species which could not be amplified with the original tuf nucleic acids and/or sequences-based assay included species belonging to the following genera: *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). Sequencing of the tuf genes from these bacterial species and others has been performed in the scope of the present invention in order to improve the universal assay. This sequencing data has been used to select new universal primers which may be more ubiquitous and more sensitive. Also, we improved our primer and probes design strategy by taking into consideration the phylogeny observed in analysing our repertory of tuf, atpD and recA sequences. Data from each of the 3 main subrepertories (tuf, atpD and recA) was subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group, inc.). This analysis indicated the main branches or phyla reflecting the relationships between sequences. Instead of trying to design primers or probes able to hybridize to all phyla, we designed primers or probes able to hybridize to the main phyla while trying to use the largest phylum possible. This strategy should allow less degenerated primers hence improving sensitivity and by combining primers in a multiplex assay, improve ubiquity. Universal primers SEQ ID NOs. 643-645 based on tuf sequences have been designed to amplify most pathogenic bacteria except Actinomyceteae, Clostridiaceae and the Cytophaga, Flexibacter and *Bacteroides* phylum (pathogenic bacteria of this phylum include mostly *Bacteroides, Porphyromonas* and *Prevotella* species). Primers to fill these gaps have been designed for Actinomyceteae (SEQ ID NOs. 646-648), Clostridiaceae (SEQ ID NOs. 796-797, 808-811), and the Cytophaga, Flexibacter and *Bacteroides* phylum (SEQ ID NOs. 649-651), also derived from tuf nucleic acids and/or sequences. These primers sets could be used alone or in conjunction to render the universal assay more ubiquitous.

[0154] Universal primers derived from atpD sequences include SEQ ID NOs. 562-565. Combination of these primers does not amplify human DNA but should amplify almost all pathogenic bacterial species except proteobacteria belonging to the epsilon subdivision (*Campylobacter* and *Helicobacter*), the bacteria from the Cytophaga, Flexibacter and

Bacteroides group and some actinomycetes and *corynebacteria*. By analysing atpD sequences from the latter species, primers and probes to specifically fill these gaps could be designed and used in conjunction with primers SEQ ID NOs. 562-565, also derived from atpD nucleic acids and/or sequences.

[0155] In addition, universality of the assay could be expanded by mixing atpD sequences-derived primers with tuf sequences-derived primers. Ultimately, even recA sequences-derived primers could be added to fill some gaps in the universal assay.

[0156] It is important to note that the 95 bacterial species selected to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

Amino Acid Sequences Derived from tuf, atpD and recA Nucleic Acids and/or Sequences

[0157] The amino acid sequences translated from the repertory of tuf, atpD and recA nucleic acids and/or sequences are also an object of the present invention. The amino acid sequence data will be particularly useful for homology modeling of three-dimensional (3D) structure of the elongation factor Tu, elongation factor G, elongation factor 1α, ATPase subunit beta and RecA recombinase. For all these proteins, at least one structure model has been published using X-ray diffraction data from crystals. Based on those structural informations it is possible to use computer software to build 3D model structures for any other protein having peptide sequence homologies with the known structure (Greer, 1991, Methods in Enzymology, 202:239-252; Taylor, 1994, Trends Biotechnol., 12(5):154-158; Sali, 1995, Curr. Opin. Biotechnol. 6:437-451; Sanchez and Sali, 1997, Curr. Opin. Struct. Biol. 7:206-214; Fischer and Eisenberg, 1999, Curr. Opin. Struct. Biol. 9:208-211; Guex et al., 1999, Trends Biochem. Sci. 24: 364-367). Model structures of target proteins are used for the design or to predict the behavior of ligands and inhibitors such as antibiotics. Since EF-Tu and EF-G are already known as antibiotic targets (see above) and since the beta subunit of ATPase and RecA recombinase are essential to the survival of the microbial cells in natural conditions of infection, all four proteins could be considered antibiotic targets. Sequence data, especially the new data generated by us could be very useful to assist the creation of new antibiotic molecules with desired spectrum of activity. In addition, model structures could be used to improve protein function for commercial purposes such as improving antibiotic production by microbial strains or increasing biomass.

[0158] The following detailed embodiments and appended drawings are provided as illustrative examples of his invention, with no intention to limit the scope thereof.

EXAMPLES AND ANNEXES

[0159] For sake of clarity, here is a list of Examples and Annexes:

[0160] Example 1: Sequencing of bacterial atpD (F-type and V-type) gene fragments.

[0161] Example 2: Sequencing of eukaryotic atpD (F-type and V-type) gene fragments.

[0162] Example 3: Sequencing of eukaryotic tuf (EF-1) gene fragments.

[0163] Example 4: Sequencing of eukaryotic tuf (organelle origin, M) gene fragments.

[0164] Example 5: Specific detection and identification of *Streptococcus agalactiae* using tuf sequences.

[0165] Example 6: Specific detection and identification of *Streptococcus agalactiae* using atpD sequences.

[0166] Example 7: Development of a PCR assay for detection and identification of staphylococci at genus and species levels.

[0167] Example 8: Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis.*

[0168] Example 9: Specific detection and identification of *Entamoeba histolytica.*

[0169] Example 10: Sensitive detection and identification of *Chlamydia trachomatis.*

[0170] Example 11: Genus-specific detection and identification of enterococci.

[0171] Example 12: Detection and identification of the major bacterial platelets contaminants using tuf sequences with a multiplex PCR test.

[0172] Example 13: The resolving power of the tuf and atpD sequences databases is comparable to the biochemical methods for bacterial identification.

[0173] Example 14: Detection of group B streptococci from clinical specimens.

[0174] Example 15: Simultaneous detection and identification of *Streptococcus pyogenes* and its pyrogenic exotoxin A.

[0175] Example 16: Real-time detection and identification of Shiga toxin-producing bacteria.

[0176] Example 17: Development of a PCR assay for the detection and identification of staphylococci at genus and species levels and its associated mecA gene.

[0177] Example 18: Sequencing of pbp1a, pbp2b and pbp2x genes of *Streptococcus pneumoniae.*

[0178] Example 19: Sequencing of hexA genes of *Streptococcus* species.

[0179] Example 20: Development of a multiplex PCR assay for the detection of *Streptococcus pneumoniae* and its penicillin resistance genes.

[0180] Example 21: Sequencing of the vancomycin resistance vanA, vanC1, vanC2 and vanC3 genes.

[0181] Example 22: Development of a PCR assay for the detection and identification of enterococci at genus and species levels and its associated resistance genes vanA and vanB.

[0182] Example 23: Development of a multiplex PCR assay for detection and identification of vancomycin-resistant *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Enterococcus casseliflavus*, and *Enterococcus flavescens.*

[0183] Example 24: Universal amplification involving the EF-G (fusA) subdivision of tuf sequences.

[0184] Example 25: DNA fragment isolation from *Staphylococcus saprophyticus* by arbitrarily primed PCR.

[0185] Example 26: Sequencing of prokaryotic tuf gene fragments.

[0186] Example 27: Sequencing of procaryotic recA gene fragments.

[0187] Example 28: Specific detection and identification of *Escherichia coli/Shigella* sp. using tuf sequences.

[0188] Example 29: Specific detection and identification of *Klebsiella pneumoniae* using atpD sequences.

[0189] Example 30: Specific detection and identification of *Acinetobacter* baumanii using tuf sequences.

[0190] Example 31: Specific detection and identification of *Neisseria gonorrhoeae* using tuf sequences.

[0191] Example 32: Sequencing of bacterial gyrA and parC gene fragments.

[0192] Example 33: Development of a PCR assay for the specific detection and identification of *Staphylococcus aureus* and its quinolone resistance genes gyrA and parC.

[0193] Example 34: Development of a PCR assay for the detection and identification of *Klebsiella pneumoniae* and its quinolone resistance genes gyrA and parC.

[0194] Example 35: Development of a PCR assay for the detection and identification of *Streptococcus pneumoniae* and its quinolone resistance genes gyrA and parC.

[0195] Example 36: Detection of extended-spectrum TEM-type β-lactamases in *Escherichia coli*.

[0196] Example 37: Detection of extended-spectrum SHV-type β-lactamases in *Klebsiella pneumoniae*.

[0197] Example 38: Development of a PCR assay for the detection and identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene tetM.

[0198] Example 39: Development of a PCR assay for the detection and identification of *Shigella* sp. and their associated trimethoprim resistance gene dhfrIa.

[0199] Example 40: Development of a PCR assay for the detection and identification of *Acinetobacter* baumanii and its associated aminoglycoside resistance gene aph(3')-VIa.

[0200] Example 41: Specific detection and identification of *Bacteroides fragilis* using atpD (V-type) sequences.

[0201] Example 42: Evidence for horizontal gene transfer in the evolution of the elongation factor Tu in Enterococci.

[0202] Example 43: Elongation factor Tu (tuf) and the F-ATPase beta-subunit (atpD) as phylogenetic tools for species of the family Enterobacteriaceae.

[0203] Example 44: Testing new pairs of PCR primers selected from two species-specific genomic DNA fragments which are objects of U.S. Pat. No. 6,001,564.

[0204] Example 45: Testing modified versions of PCR primers derived from the sequence of several primers which are objects of U.S. Pat. No. 6,001,564.

[0205] The various Annexes show the strategies used for the selection of a variety of DNA amplification primers, nucleic acid hybridization probes and molecular beacon internal probes:

- **[0206]** (i) Annex I shows the amplification primers used for nucleic acid amplification from tuf sequences.
- **[0207]** (ii) Annex II shows the amplification primers used for nucleic acid amplification from atpD sequences.
- **[0208]** (iii) Annex III shows the internal hybridization probes for detection of tuf sequences.
- **[0209]** (iv) Annex IV illustrates the strategy used for the selection of the amplification primers specific for atpD sequences of the F-type.
- **[0210]** (v) Annex V illustrates the strategy used for the selection of the amplification primers specific for atpD sequences of the V-type.
- **[0211]** (vi) Annex VI illustrates the strategy used for the selection of the amplification primers specific for the tuf sequences of organelle lineage (M, the letter M is used to indicate that in most cases, the organelle is the mitochondria).
- **[0212]** (vii) Annex VII illustrates the strategy used for the selection of the amplification primers specific for the tuf sequences of eukaryotes (EF-1).

[0213] (viii) Annex VIII illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from tuf sequences.

[0214] (ix) Annex IX illustrates the strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from tuf sequences.

[0215] (x) Annex X illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from atpD sequences.

[0216] (xi) Annex XI illustrates the strategy for the selection from tuf sequences of *Candida albicans*/dubliniensis-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe.

[0217] (xii) Annex XII illustrates the strategy for the selection of *Staphylococcus*-specific amplification primers from tuf sequences.

[0218] (xiii) Annex XIII illustrates the strategy for the selection of the *Staphylococcus*-specific hybridization probe from tuf sequences.

[0219] (xiv) Annex XIV illustrates the strategy for the selection of *Staphylococcus saprophyticus*-specific and *Staphylococcus haemolyticus*-specific hybridization probes from tuf sequences.

[0220] (xv) Annex XV illustrates the strategy for the selection of *Staphylococcus aureus*-specific and *Staphylococcus epidermidis*-specific hybridization probes from tuf sequences.

[0221] (xvi) Annex XVI illustrates the strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from tuf sequences.

[0222] (xvii) Annex XVII illustrates the strategy for the selection of the *Enterococcus*-specific amplification primers from tuf sequences.

[0223] (xviii) Annex XVIII illustrates the strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from tuf sequences.

[0224] (xix) Annex XIX illustrates the strategy for the selection of primers from tuf sequences for the identification of platelets contaminants.

[0225] (xx) Annex XX illustrates the strategy for the selection of the universal amplification primers from atpD sequences.

[0226] (xxi) Annex XXI shows the amplification primers used for nucleic acid amplification from recA sequences.

[0227] (xxii) Annex XXII shows the specific and ubiquitous primers for nucleic acid amplification from speA sequences.

[0228] (xxiii) Annex XXIII illustrates the first strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from speA sequences.

[0229] (xxiv) Annex XXIV illustrates the second strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from speA sequences.

[0230] (xxv) Annex XXV illustrates the strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from tuf sequences.

[0231] (xxvi) Annex XXVI illustrates the strategy for the selection of stx_1-specific amplification primers and hybridization probe.

[0232] (xxvii) Annex XXVII illustrates the strategy for the selection of stx_2-specific amplification primers and hybridization probe.

[0233] (xxviii) Annex XXVIII illustrates the strategy for the selection of vanA-specific amplification primers from van sequences.

[0234] (xxix) Annex XXIX illustrates the strategy for the selection of vanB-specific amplification primers from van sequences.

[0235] (xxx) Annex XXX illustrates the strategy for the selection of vanC-specific amplification primers from vanC sequences.

[0236] (xxxi) Annex XXXI illustrates the strategy for the selection of *Streptococcus pneumoniae*-specific amplification primers and hybridization probes from pbp1a sequences.

[0237] (xxxii) Annex XXXII shows the specific and ubiquitous primers for nucleic acid amplification from toxin gene sequences.

[0238] (xxxiii) Annex XXXIII shows the molecular beacon internal hybridization probes for specific detection of toxin sequences.

[0239] (xxxiv) Annex XXXIV shows the specific and ubiquitous primers for nucleic acid amplification from van sequences.

[0240] (xxxv) Annex XXXV shows the internal hybridization probes for specific detection of van sequences.

[0241] (xxxvi) Annex XXXVI shows the specific and ubiquitous primers for nucleic acid amplification from pbp sequences.

[0242] (xxxvii) Annex XXXVII shows the internal hybridization probes for specific detection of pbp sequences.

[0243] (xxxviii) Annex XXXVIII illustrates the strategy for the selection of vanAB-specific amplification primers and vanA- and vanB-specific hybridization probes from van sequences.

[0244] (xxxix) Annex XXXIX shows the internal hybridization probe for specific detection of mecA.

[0245] (xl) Annex XL shows the specific and ubiquitous primers for nucleic acid amplification from hexA sequences.

[0246] (xli) Annex XLI shows the internal hybridization probe for specific detection of hexA.

[0247] (xlii) Annex XLII illustrates the strategy for the selection of *Streptococcus pneumoniae* species-specific amplification primers and hybridization probe from hexA sequences.

[0248] (xliii) Annex XLIII shows the specific and ubiquitous primers for nucleic acid amplification from pcp sequences.

[0249] (xliv) Annex XLIV shows specific and ubiquitous primers for nucleic acid amplification of *S. saprophyticus* sequences of unknown coding potential.

[0250] (xlv) Annex XLV shows the molecular beacon internal hybridization probes for specific detection of antimicrobial agents resistance gene sequences.

[0251] (xlvi) Annex XLVI shows the molecular beacon internal hybridization probe for specific detection of *S. aureus* gene sequences of unknown coding potential.

[0252] (xlvii) Annex XLVII shows the molecular beacon hybridization internal probe for specific detection of tuf sequences.

[0253] (xlviii) Annex XLVIII shows the molecular beacon internal hybridization probes for specific detection of ddl and mtl sequences.

[0254] (xlix) Annex XLIX shows the internal hybridization probe for specific detection of *S. aureus* sequences of unknown coding potential.

[0255] (l) Annex L shows the amplification primers used for nucleic acid amplification from antimicrobial agents resistance genes sequences.

[0256] (li) Annex LI shows the internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.

[0257] (lii) Annex LII shows the molecular beacon internal hybridization probes for specific detection of atpD sequences.

[0258] (liii) Annex LIII shows the internal hybridization probes for specific detection of atpD sequences.

[0259] (liv) Annex LIVI shows the internal hybridization probes for specific detection of ddl and mtl sequences.

[0260] As shown in these Annexes, the selected amplification primers may contain inosines and/or base ambiguities. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degeneracies in the amplification primers allows mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y.).

EXAMPLES

Example 1

Sequencing of Bacterial atpD (F-Type and V-Type) Gene Fragments

[0261] As shown in Annex IV, the comparison of publicly available atpD (F-type) sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify atpD sequences (F-type) from a wide range of bacterial species. Using primers pairs SEQ ID NOs. 566 and 567, 566 and 814, 568 and 567, 570 and 567, 572 and 567, 569 and 567, 571 and 567, 700 and 567, it was possible to amplify and sequence atpD sequences SEQ ID NOs. 242-270, 272-398, 673-674, 737-767, 866-867, 942-955, 1245-1254, 1256-1265, 1527, 1576, 1577, 1600-1604, 1640-1646, 1649, 1652, 1655, 1657, 1659-1660, 1671, 1844-1845, and 1849-1865.

[0262] Similarly, Annex V shows the strategy to design the PCR primers able to amplify atpD sequences of the V-type from a wide range of archaeal and bacterial species. Using primers SEQ ID NOs. 681-683, it was possible to amplify and sequence atpD sequences SEQ ID NOs. 827-832, 929-931, 958 and 966. As the gene was difficult to amplify for several species, additional amplification primers were designed inside the original amplicon (SEQ ID NOs. 1203-1207) in order to obtain sequence information for these species. Other primers (SEQ ID NO. 1212, 1213, 2282-2285) were also designed to amplify regions of the atpD gene (V-type) in archaebacteria.

Example 2

Sequencing of Eukaryotic atpD (F-Type and V-Type) Gene Fragments

[0263] The comparison of publicly available atpD (F-type) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify atpD sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 568 and 573, 574 and 573, 574 and 708, and 566 and 567, it was possible to amplify and sequence atpD sequences SEQ ID NOs. 458-497, 530-538, 663, 667, 676, 678-680, 768-778, 856-862, 889-896, 941, 1638-1639, 1647, 1650-1651, 1653-1654, 1656, 1658, 1684, 1846-1848, and 2189-2192.

[0264] In the same manner, the primers described in Annex V (SEQ ID NOs. 681-683) could amplify the atpD (V-type) gene from various fungal and parasitical species. This strategy allowed to obtain SEQ ID NOs. 834-839, 956-957, and 959-965.

Example 3

Sequencing of Eukaryotic tuf (EF-1) Gene Fragments

[0265] As shown in Annex VII, the comparison of publicly available tuf (EF-1) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify tuf sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NOs. 558 and 559, 813 and 559, 558 and 815, 560 and 559, 653 and 559, 558 and 655, and 654 and 559, 1999 and 2000, 2001 and 2003, 2002 and 2003, it was possible to amplify and sequence tuf sequences SEQ ID NOs. 399-457, 509-529, 622-624, 677, 779-790, 840-842, 865, 897-903, 1266-1287, 1561-1571 and 1685.

Example 4

Sequencing of Eukaryotic tuf (Organelle Origin, M) Gene Fragments

[0266] As shown in Annex VI, the comparison of publicly available tuf (organelle origin, M) sequences from a variety of fungal and parasitical organelles revealed conserved regions allowing the design of PCR primers able to amplify tuf sequences of several organelles belonging to a wide range fungal and parasitical species. Using primers pairs SEQ ID NOs. 664 and 652, 664 and 561, 911 and 914, 912 and 914, 913 and 915, 916 and 561, 664 and 917, it was possible to amplify and sequence tuf sequences SEQ ID NOs. 498-508, 791-792, 843-855, 904-910, 1664, 1666-1667, 1669-1670, 1673-1683, 1686-1689, 1874-1876, 1879, 1956-1960, and 2193-2199.

Example 5

Specific Detection and Identification of *Streptococcus agalactiae* Using tuf Sequences

[0267] As shown in Annex VIII, the comparison of tuf sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. The multiple sequence alignment includes the tuf sequences of four bacterial strains from the target species as well as tuf sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species and genera, especially from the closely related species, thereby permitting the species-specific, ubiquitous and sensitive detection and identification of the target bacterial species.

[0268] The chosen primer pair, oligos SEQ ID NO. 549 and SEQ ID NO. 550, gives an amplification product of 252 bp. Standard PCR was carried out using 0.4 μM of each primer, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1× Taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0.5 U Taq DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 1 μl of genomic DNA sample in a final volume of 20 μl using a PTC-200 thermocycler (MJ Research Inc.). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 62° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide.

[0269] Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the bacterial species listed in Table 8. Efficient amplification was observed only for the 5 *S. agalactiae* strains listed. Of the other bacterial species, including 32 species representative of the vaginal flora and 27 other streptococcal species, only *S. acidominimus* yielded amplification. The signal with 0.1 ng of *S. acidominimus* genomic DNA was weak and the detection limit for this species was 10 pg (corresponding to more than 4000 genome copies) while the detection limit for *S. agalactiae* was 2.5 fg (corresponding to one genome copy) of genomic DNA.

[0270] To increase the specificity of the assay, internal probes were designed for FRET (Fluorescence Resonance Energy Transfer) detection using the LightCycler™ (Idaho Technology). As illustrated in Annex IX, a multiple sequence alignment of streptococcal tuf sequence fragments corresponding to the 252 by region amplified by primers SEQ ID NO. 549 and SEQ ID NO. 550, was used for the design of internal probes TSagHF436 (SEQ ID NO. 582) and TSagHF465 (SEQ ID NO. 583). The region of the amplicon selected for internal probes contained sequences unique and specific to *S. agalactiae*. SEQ ID NO. 583, the more specific probe, is labelled with fluorescein in 3', while SEQ ID NO. 582, the less discriminant probe, is labelled with CY5 in 5' and blocked in 3' with a phosphate group. However, since the FRET signal is only emitted if both probes are adjacently hybridized on the same target amplicon, detection is highly specific.

[0271] Real-time detection of PCR products using the LightCycler™ was carried out using 0.4 μM of each primer (SEQ ID NO. 549-550), 0.2 μM of each probe (SEQ ID NO. 582-583), 2.5 mM $MgCl_2$, BSA 450 μg/ml, 1×PC2 Buffer (AB Peptides, St-Louis, dNTP 0.2 mM (Pharmacia), 0.5 U KlenTaq1™ DNA polymerase (AB Peptides) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 0.7 μl of genomic DNA sample in a final volume of 7 μl using a LightCycler thermocycler (Idaho Technology). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 94° C. for initial denaturation, then forty cycles of three steps consisting of 0 second (this setting meaning the LightCycler will reach the target temperature and stay at it for its minimal amount of time) at 94° C., 10 seconds at 64° C., 20 seconds at 72° C. Amplification was monitored during each annealing steps using the fluorescence ratio. The streptococcal species having close sequence homologies with the tuf sequence of *S. agalactiae* (*S. acidominimus, S. anginosus, S. bovis, S. dysgalactiae, S. equi, S. ferus, S. gordonii, S. intermedius, S. parasanguis, S. parauberis, S. salivarius, S. sanguis, S. suis*) as well as *S. agalactiae* were tested in the LightCycler with 0.07 ng of genomic DNA per reaction. Only *S. agalactiae* yielded an amplification signal, hence demonstrating that the assay is species-specific. With the LightCycler™ assay using the internal FRET probes, the detection limit for *S. agalactiae* was 1-2 genome copies of genomic DNA.

Example 6

Specific Detection and Identification of *Streptococcus agalactiae* Using atpD Sequences

[0272] As shown in Annex X, the comparison of atpD sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The primer design strategy is similar to the strategy described in the preceding Example except that atpD sequences were used in the alignment.

[0273] Four primers were selected, ASag42 (SEQ ID NO. 627), ASag52 (SEQ ID NO. 628), ASag206 (SEQ ID NO. 625) and ASag371 (SEQ ID NO. 626). The following combinations of these four primers give four amplicons; SEQ ID NO. 627+SEQ ID NO. 625=190 bp, SEQ ID NO. 628+SEQ ID NO. 625=180 bp, SEQ ID NO. 627+SEQ ID NO. 626=355 bp, and SEQ ID NO. 628+SEQ ID NO. 626=345 bp.

[0274] Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc) using 0.4 μM of each primers pair, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1× taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 0.5 U Taq DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto), 1 μl of genomic DNA sample in a final volume of 20 μL. The optimal cycling conditions for maximum sensitivity and specificity were adjusted for each primer pair. Three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at the optimal annealing temperature specified below were followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. Since atpD sequences are relatively more specific than tuf sequences, only the most closely related species namely, the streptococcal species listed in Table 9, were tested.

[0275] All four primer pairs only amplified the six *S. agalactiae* strains. With an annealing temperature of 63° C., the primer pair SEQ ID NO. 627+SEQ ID NO. 625 had a sensitivity of 1-5 fg (equivalent to 1-2 genome copies). At 55° C., the primer pair SEQ ID NO. 628+SEQ ID NO. 625 had a sensitivity of 2.5 fg (equivalent to 1 genome copy). At 60° C., the primer pair SEQ ID NO. 627+SEQ ID NO. 626 had a sensitivity of 10 fg (equivalent to 4 genome copies). At 58° C., the primer pair SEQ ID NO. 628+SEQ ID NO. 626 had a sensitivity of 2.5-5 fg (equivalent to 1-2 genome copies). This proves that all four primer pairs can detect *S. agalactiae* with high specificity and sensitivity. Together with Example 5, this example demonstrates that both tuf and atpD sequences are suitable and flexible targets for the identification of microorganisms at the species level. The fact that 4 different primer pairs based on atpD sequences led to efficient and specific amplification of *S. agalactiae* demonstrates that the challenge is to find target genes suitable for diagnostic purposes, rather than finding primer pairs from these target sequences.

Example 7

Development of a PCR Assay for Detection and Identification of Staphylococci at Genus and Species Levels

Materials and Methods

[0276] Bacterial strains. The specificity of the PCR assay was verified by using a panel of ATCC (America Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH; German Collection of Microorganisms and Cell Cultures) reference strains consisting of 33 gram-negative and 47 gram-positive bacterial species (Table 12). In addition, 295 clinical isolates representing 11 different species of staphylococci from the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL) (Step-Foy, Québec, Canada) were also tested to further validate the *Staphylococcus*-specific PCR assay. These strains were all identified by using (i) conventional methods or (ii) the automated MicroScan Autoscan-4 system equipped with the Positive BP Combo Panel Type 6 (Dade Diagnostics, Mississauga, Ontario, Canada). Bacterial strains from frozen stocks kept at −80° C. in brain heart infusion (BHI) broth containing 10% glycerol were cultured on sheep blood agar or in BHI broth (Quelab Laboratories Inc, Montréal, Québec, Canada).

[0277] PCR primers and internal probes. Based on multiple sequence alignments, regions of the tuf gene unique to staphylococci were identified. *Staphylococcus*-specific PCR primers TStaG422 (SEQ ID NO. 553) and TStaG765 (SEQ ID NO. 575) were derived from these regions (Annex XII). These PCR primers are displaced by two nucleotide positions compared to original *Staphylococcus*-specific PCR primers described in our patent publication WO98/20157 (SEQ ID NOs. 17 and 20 in the said patent publication). These modifications were done to ensure specificity and ubiquity of the primer pair, in the light of new tuf sequence data revealed in the present patent application for several additional staphylococcal species and strains.

[0278] Similarly, sequence alignment analysis were performed to design genus and species-specific internal probes (see Annexes XIII to XVI). Two internal probes specific for *Staphylococcus* (SEQ ID NOs. 605-606), five specific for *S. aureus* (SEQ ID NOs. 584-588), five specific for *S. epidermidis* (SEQ ID NO. 589-593), two specific for *S. haemolyticus* (SEQ ID NOs. 594-595), three specific for *S. hominis* (SEQ ID NOs. 596-598), four specific for *S. saprophyticus* (SEQ ID NOs. 599-601 and 695), and two specific for coagulase-negative *Staphylococcus* species including *S. epidermidis, S. hominis, S. saprophyticus, S. auricularis, S. capitis, S. haemolyticus, S. lugdunensis, S. simulans, S. cohnii* and *S. warneri* (SEQ ID NOs. 1175-1176) were designed. The range of mismatches between the *Staphylococcus*-specific 371-bp amplicon and each of the 20-mer species-specific internal probes was from 1 to 5, in the middle of the probe when possible. No mismatches were present in the two *Staphylococcus*-specific probes for the 11 species analyzed: *S. aureus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. simulans* and *S. warneri*. In order to verify the intra-specific sequence conservation of the nucleotide sequence, sequences were obtained for the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the species *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*. The Oligo™ (version 5.0) primer analysis software (National Biosciences, Plymouth, Minn.) was used to confirm the absence of self-complementary regions within and between the primers or probes. When required, the primers contained inosines or degenerated nucleotides at one or more variable positions. Oligonucleotide primers and probes were synthesized on a model 394 DNA synthesizer (Applied Biosystems, Mississauga, Ontario, Canada). Detection of the hybridization was performed with the DIG-labeled dUTP incorporated during amplification with the *Staphylococcus*-specific PCR assay, and the hybridization signal was detected with a luminometer (Dynex Technologies) as described above in the section on luminescent detection of amplification products. Annexes XIII to XVI illustrate the strategy for the selection of several internal probes.

[0279] PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA or from a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 bacteria per ml. One nanogram of genomic DNA or 1 □l of the standardized bacterial suspension was transferred directly to a 19 □l PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 □M (each) of the two *Staphylococcus* genus-specific primers (SEQ ID NOs. 553 and 575), 200 □M (each) of the four deoxynucleoside triphosphates (Pharmacia Biotech), 3.3 □g/□l bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), and 0.5 U Taq polymerase (Promega) coupled with TaqStart™ Antibody (Clontech). The PCR amplification was performed as follows: 3 min. at 94° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 55° C., plus a terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

[0280] For determination of the sensitivities of the PCR assays, two-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Results

[0281] Amplifications with the *Staphylococcus* genus-specific PCR assay. The specificity of the assay was assessed by performing 30-cycle and 40-cycle PCR amplifications with the panel of gram-positive (47 species from 8 genera) and gram-negative (33 species from 22 genera) bacterial species listed in Table 12. The PCR assay was able to detect efficiently 27 of 27 staphylococcal species tested in both 30-cycle and 40-cycle regimens. For 30-cycle PCR, all bacterial species tested other than staphylococci were negative. For 40-cycle PCR, *Enterococcus faecalis* and *Macrococcus caseolyticus* were slightly positive for the *Staphylococcus*-specific PCR assay. The other species tested remained negative. Ubiquity tests performed on a collection of 295 clinical isolates provided by the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), including *Staphylococcus aureus* (n=34), *S. auricularis* (n=2), *S. capitis* (n=19), *S. cohnii* (n=5), *S. epidermidis* (n=18), *S. haemolyticus* (n=21), *S. hominis* (n=73), *S. lugdunensis* (n=17), *S. saprophyticus* (n=6), *S. simulans* (n=3), *S. warneri* (n=32) and *Staphylococcus* sp. (n=65), showed a uniform amplification signal with the 30-cycle PCR assays and a perfect relation between the genotype and classical identification schemes.

[0282] The sensitivity of the *Staphylococcus*-specific assay with 30-cycle and 40-cycle PCR protocols was determined by using purified genomic DNA from the 11 staphylococcal species previously mentioned. For PCR with 30 cycles, a detection limit of 50 copies of genomic DNA was consistently obtained. In order to enhance the sensitivity of the assay, the number of cycles was increased. For 40-cycle PCR assays, the detection limit was lowered to a range of 5-10 genome copies, depending on the staphylococcal species tested.

[0283] Hybridization between the *Staphylococcus*-specific 371-bp amplicon and species-specific or genus-specific internal probes. Inter-species polymorphism was sufficient to generate species-specific internal probes for each of the principal species involved in human diseases (*S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*). In order to verify the intra-species sequence conservation of the nucleotide sequence, sequence comparisons were performed on the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the 5 principal staphylococcal species: *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*. Results showed a high level of conservation of nucleotide sequence between different unrelated strains from the same species. This sequence information allowed the development of staphylococcal species identification assays using species-specific internal probes hybridizing to the 371-bp amplicon. These assays are specific and ubiquitous for those five staphylococcal species. In addition to the species-specific internal probes, the genus-specific internals probes were able to recognize all or most *Staphylococcus* species tested.

Example 8

Differentiating Between the Two Closely Related Yeast Species *Candida albicans* and *Candida dubliniensis*

[0284] It is often useful for the clinician to be able to differentiate between two very closely related species of microorganisms. *Candida albicans* is the most important cause of invasive human mycose. In recent years, a very closely related species, *Candida dubliniensis*, was isolated in immunosuppressed patients. These two species are difficult to distinguish by classic biochemical methods. This example demonstrates the use of tuf sequences to differentiate *Candida albicans* and *Candida dubliniensis*. PCR primers SEQ ID NOs. 11-12, from previous patent publication WO98/20157, were selected for their ability to specifically amplify a tuf (elongation factor 1 alpha type) fragment from both species (see Annex XI for primer positions). Within this tuf fragment, a region differentiating *C. albicans* and *C. dubliniensis* by two nucleotides was selected and used to design two internal probes (see Annex XI for probe design, SEQ ID NOs. 577 and 578) specific for each species. Amplification of genomic DNA from *C. albicans* and *C. dubliniensis* was carried out using DIG-11-dUTP as described above in the section on chemiluminescent detection of amplification products. Internal probes SEQ ID NOs. 577 and 578 were immobilized on the bottom of individual microtiter plates and hybridization was carried out as described above in the above section on chemiluminescent detection of amplification products. Luminometer data showed that the amplicon from *C. albicans* hybridized only to probe SEQ ID NO. 577 while the amplicon from *C. dubliniensis* hybridized only to probe SEQ ID NO. 578, thereby demonstrating that each probe was species-specific.

Example 9

Specific Identification of *Entamoeba histolytica*

[0285] Upon analysis of tuf (elongation factor 1 alpha) sequence data, it was possible to find four regions where *Entamoeba histolytica* sequences remained conserved while other parasitical and eukaryotic species have diverged. Primers TEntG38 (SEQ ID NO. 703), TEntG442 (SEQ ID NO. 704), TEntG534 (SEQ ID NO. 705), and TEntG768 (SEQ ID NO. 706) were designed so that SEQ ID NO. 703 could be paired with the three other primers. On PTC-200 thermocyclers (MJ Research), the cycling conditions for initial sensitivity and specificity testing were 3 min. at 94° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 55° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. The three primer pairs could detect the equivalent of less than 200 *E. histolytica* genome copies. Specificity was tested using 0.5 ng of purified genomic DNA from a panel of microorganisms including *Babesia bovis, Babesia microtti, Candida albicans, Crithidia fasciculata, Leishmania major, Leishmania hertigi* and *Neospora caninum*. Only *E. histolytica* DNA could be amplified, thereby suggesting that the assay was species-specific.

Example 10

Sensitive Identification of *Chlamydia trachomatis*

[0286] Upon analysis of tuf sequence data, it was possible to find two regions where *Chlamydia trachomatis* sequences remained conserved while other species have diverged. Primers Ctr82 (SEQ ID NO. 554) and Ctr249 (SEQ ID NO. 555) were designed. With the PTC-200 thermocyclers (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 60° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. The assay could detect the equivalent of 8 *C. trachomatis* genome copies. Specificity was tested with 0.1 ng of purified genomic DNA from a panel of microorganisms including 22 species commonly encountered in the vaginal flora (*Bacillus subtilis, Bacteroides fragilis, Candida albicans, Clostridium difficile, Corynebacterium cervicis, Corynebacterium urealyticum, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus influenzae, Klebsiella oxytoca, Lactobacillus acidophilus, Peptococcus niger, Peptostreptococcus prevotii, Porphyromonas asaccharolytica, Prevotella melaninogenica, Propionibacterium acnes, Staphylococcus aureus, Streptococcus* acidominimus, and *Streptococcus agalactiae*). Only *C. trachomatis* DNA could be amplified, thereby suggesting that the assay was species-specific.

Example 11

Genus-Specific Detection and Identification of Enterococci

[0287] Upon analysis of tuf sequence data and comparison with the repertory of tuf sequences, it was possible to find two regions where *Enterococcus* sequences remained conserved while other genera have diverged (Annex XVII). Primer pair Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) was tested for its specificity by using purified genomic DNA from a panel of bacteria listed in Table 10. Using the PTC-200 thermocycler (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 55° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm. The 18 enterococcal species listed in Table 10 were all amplified efficiently. The only other species amplified were *Abiotrophia adiacens, Gemella haemolysans* and *Gemella morbillorum*, three gram-positive species. Sensitivity tested with several strains of *E. casseliflavus, E. faecium, E. faecalis, E. flavescens* and *E. gallinarum* and with one strain of each other *Enterococcus* species listed in Table 10 ranged from 1 to 10 copies of genomic DNA. The sequence variation within the 308-bp amplicon was sufficient so that internal probes could be used to speciate the amplicon and differentiate enterococci from *Abiotrophia adiacens, Gemella haemolysans* and *Gemella morbillorum*, thereby allowing to achieve excellent specificity. Species-specific internal probes were generated for each of the clinically important species, *E. faecalis* (SEQ ID NO. 1174), *E. faecium* (SEQ ID NO. 602), and the group including *E. casseliflavus, E. flavescens* and *E. gallinarum* (SEQ ID NO. 1122) (Annex XVIII). The species-specific internal probes were able to differentiate their respective *Enterococcus* species from all other *Enterococcus* species. These assays are sensitive, specific and ubiquitous for those five *Enterococcus* species.

Example 12

Identification of the Major Bacterial Platelets Contaminants Using Tuf Sequences with a Multiplex PCR Test

[0288] Blood platelets preparations need to be monitored for bacterial contaminations. The tuf sequences of 17 important bacterial contaminants of platelets were aligned. As shown in Annex XIX, analysis of these sequences allowed the design of PCR primers. Since in the case of contamination of platelet concentrates, detecting all species (not just the more frequently encountered ones) is desirable, perfect specificity of primers was not an issue in the design. However, sensitivity is important. That is why, to avoid having to put too much degeneracy, only the most frequent contaminants were included in primer design, knowing that the selected primers would anyway be able to amplify more species than the 17 used in the design because they target highly conserved regions of tuf sequences. Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelet concentrates were chosen (oligos Tplaq 769 and Tplaq 991, respectively SEQ ID NOs. 636 and 637) thereby permitting the detection of these bacterial species. However, sensitivity was slightly deficient with staphylococci. To ensure maximal sensitivity in the detection of all the more frequent bacterial contaminants, a multiplex assay also including oligonucleotide primers targeting the *Staphylococcus* genera (oligos Stag 422, SEQ ID NO. 553; and Stag 765, SEQ ID NO. 575) was developed. The bacterial species detected with the assay are listed in Table 14.

[0289] The primer pairs, oligos SEQ ID NO. 636 and SEQ ID NO. 637 that give an amplification product of 245 pb, and oligos SEQ ID NO. 553 and SEQ ID NO. 575 that give an amplification product of 368 pb, were used simultaneously in the multiplex PCR assay. Detection of these PCR products was made on the LightCycler thermocycler (Idaho Technology) using SYBR® Green I (Molecular Probe Inc.). SYBR® Green I is a fluorescent dye that binds specifically to double-stranded DNA.

[0290] Fluorogenic detection of PCR products with the LightCycler was carried out using 1.0 μM of both Tplaq primers (SEQ ID NOs. 636-637) and 0.4 μM of both TStaG primers (SEQ ID NOs. 553 and 575), 2.5 mM $MgCl_2$, BSA 7.5 μM, dNTP 0.2 mM (Pharmacia), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 U Taq DNA polymerase (Boerhinger Mannheim) coupled with TaqStart™ antibody (Clontech), and 0.07 ng of genomic DNA sample in a final volume of 7 μl. The optimal cycling conditions for maximum sensitivity and specificity were 1 minute at 94° C. for initial denaturation, then forty-five cycles of three steps consisting of 0 second at 95° C., 5 seconds at 60° C. and 9 seconds at 72° C. Amplification was monitored during each elongation cycle by measuring the level of SYBR® Green I. However, real analysis takes place after PCR. Melting curves are done for each sample and transformation of the melting peak allows determination of Tm. Thus primer-dimer and specific PCR product are discriminated. With this assay, all prominent bacterial contaminants of platelet concentrates listed in Annex XIX and Table 14 were detected. Sensitivity tests were performed on the 9 most frequent bacterial contaminants of platelets. The detection limit was less than 20 genome copies for *E. cloacae, B. cereus, S. choleraesuis* and *S. marcescens*; less than 15 genome copies for *P. aeruginosa*; and 2 to 3 copies were detected for *S. aureus, S. epidermidis, E. coli* and *K. pneumoniae*. Further refinements of assay conditions should increase sensitivity levels.

Example 13

The Resolving Power of the tuf and atpD Sequences Databases is Comparable to the Biochemical Methods for Bacterial Identification

[0291] The present gold standard for bacterial identification is mainly based on key morphological traits and batteries of biochemical tests. Here we demonstrate that the use of tuf and atpD sequences combined with simple phylogenetic analysis of databases formed by these sequences is comparable to the gold standard. In the process of acquiring data for the tuf sequences, we sequenced the tuf gene of a strain that was given to us labelled as *Staphylococcus hominis* ATCC 35982. That tuf sequence (SEQ ID NO. 192) was incorporated into the tuf sequences database and subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group). This analysis indicated that SEQ ID NO. 192 is not associated with other *S. hominis* strains but rather with the *S. warneri* strains. The ATCC 35982 strain was sent to the reference laboratory of the Laboratoire de santé publique du Québec (LSPQ). They used the classic identification scheme for staphylococci (Kloos and Schleifer, 1975., J. Clin. Microbiol. 1:82-88). Their results shown that although the colonial morphology could correspond to *S. hominis*, the more precise biochemical assays did not. These assays included discriminant mannitol, mannose and ribose acidification tests as well as rapid and dense growth in deep thioglycolate agar. The LSPQ report identified strain ATCC 35982 as *S. warneri* which confirms our database analysis. The same thing happened for *S. warneri* (SEQ ID NO. 187) which had initially been identified as *S. haemolyticus* by a routine clinical laboratory using a low resolving power automated system (MicroScan, AutoScan-4™). Again, the tuf and LSPQ analysis agreed on its identification as *S. warneri*. In numerous other instances, in the course of acquiring tuf and atpD sequence data from various species and genera, analysis of our tuf and/or atpD sequence databases permitted the exact identification of mislabeled or erroneously identified strains. These results clearly demonstrate the usefulness and the high resolving power of our sequence-based identification assays using the tuf and atpD sequences databases.

Example 14

Detection of Group B Streptococci from Clinical Specimens

Introduction

[0292] *Streptococcus agalactiae*, the group B *streptococcus* (GBS), is responsible for a severe illness affecting neonate infants. The bacterium is passed from the healthy carrier mother to the baby during delivery. To prevent this infection, it is recommended to treat expectant mothers susceptible of carrying GBS in their vaginal/anal flora. Carrier status is often a transient condition and rigorous monitoring requires cultures and classic bacterial identification weeks before delivery. To improve the detection and identification of GBS we developed a rapid, specific and sensitive PCR test fast enough to be performed right at delivery.

Materials and Methods

[0293] GBS clinical specimens. A total of 66 duplicate vaginal/anal swabs were collected from 41 consenting pregnant women admitted for delivery at the Centre Hospitalier Universitaire de Québec, Pavillon Saint-Francois d'Assise following the CDC recommendations. The samples were obtained either before or after rupture of membranes. The swab samples were tested at the Centre de Recherche en Infectiologie de l'Université Laval within 24 hours of collection. Upon receipt, one swab was cut and then the tip of the swab was added to GNS selective broth for identification of group B streptococci (GBS) by the standard culture methods recommended by the CDC. The other swab was processed following the instruction of the IDI DNA extraction kit (Infectio Diagnotics (IDI) Inc.) prior to PCR amplification.

[0294] Oligonucleotides. PCR primers, Tsag340 (SEQ ID NO. 549) and Tsag552 (SEQ ID NO. 550) complementary to the regions of the tuf gene unique for GBS were designed based upon a multiple sequence alignment using our repertory of tuf sequences. Oligo primer analysis software (version 5.0) (National Biosciences) was used to analyse primers annealing temperature, secondary structure potential as well as mispriming and dimerization potential. The primers were synthesized using a model 391 DNA synthesizer (Applied Biosystems).

[0295] A pair of fluorescently labeled adjacent hybridization probes Sag465-F (SEQ ID NO. 583) and Sag436-C (SEQ ID NO. 582) were synthesized and purified by Operon Technologies. They were designed to meet the recommendations of the manufacturer (Idaho Technology) and based upon multiple sequence alignment analysis using our repertory of tuf sequences to be specific and ubiquitous for GBS. These adjacent probes, which are separated by one nucleotide, allow fluorescence resonance energy transfer (FRET), generating an increased fluorescence signal when both hybridized simultaneously to their target sequences. The probe SEQ ID NO. 583 was labeled with FITC in 3 prime while SEQ ID NO. 582 was labeled with Cy5 in 5 prime. The Cy5-labeled probes contained a 3'-blocking phosphate group to prevent extension of the probes during the PCR reactions.

[0296] PCR amplification. Conventional amplifications were performed either from 2 µl of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 20 µl PCR mixture contained 0.4 µM of each GBS-specific primer (SEQ ID NOs. 549-550), 200 µM of each deoxyribonucleotide (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 3.3 mg/ml bovine serum albumin (BSA) (Sigma), and 0.5 U of Taq polymerase (Promega) combined with the TaqStart™ antibody (Clontech). The TaqStart™ antibody, which is a neutralizing monoclonal antibody of Taq DNA polymerase, was added to all PCR reactions to enhance the efficiency of the amplification. The PCR mixtures were subjected to thermal cycling (3 min at 95° C. and then 40 cycles of 1 s at 95° C., and 30 s at 62° C. with a 2-min final extension at 72° C.) with a PTC-200 DNA Engine thermocycler (MJ research). The PCR-amplified reaction mixture was resolved by agarose gel electrophoresis.

[0297] The LightCycler™ PCR amplifications were performed with 1 µl of a purified genomic DNA preparation or cell lysates of vaginal/anal specimens. The 10 µl amplification mixture consisted of 0.4 µM each GBS-specific primer (SEQ ID NOs. 549-550), 200 µM each dNTP, 0.2 µM each fluorescently labeled probe (SEQ ID NOs. 582-583), 300 µg/ml BSA (Sigma), and 1 µl of 10×PC2 buffer (containing 50 mM Tris-HCl (pH 9.1), 16 mM ammonium sulfate, 3.5 mM Mg^{2+}, and 150 µg/ml BSA) and 0.5 U KlenTaq1™ (AB Peptides) coupled with TaqStart™ antibody (Clontech). KlenTaq1™ is a highly active and more heat-stable DNA polymerase without 5'-exonuclease activity. This prevents hydrolysis of hybridized probes by the 5' to 3' exonuclease activity. A volume of 7 µl of the PCR mixture was transferred into a composite capillary tube (Idaho Technology). The tubes were then centrifuged to move the reaction mixture to the tips of the capillaries and then cleaned with optical-grade methanol. Subsequently the capillaries were loaded into the carousel of a LC32 LightCycler™ (Idaho Technology), an instrument that combines rapid-cycle PCR with fluorescence analysis for continuous monitoring during amplification. The PCR reaction mixtures were subjected to a denaturation step at 94° C. for 3 min followed by 45 cycles of 0 s at 94° C., 20 s at 64° C. and 10 s at 72° C. with a temperature transition rate of 20° C./s. Fluorescence signals were obtained at each cycle by sequentially positioning each capillary on the carousel at the focus of optical elements affiliated to the built-in fluorimeter for 100 milliseconds. Complete amplification and analysis required about 35 min.

[0298] Specificity and sensitivity tests. The specificity of the conventional and LightCycler™ PCR assays was verified by using purified genomic DNA (0.1 ng/reaction) from a battery of ATCC reference strains representing 35 clinically relevant gram-positive species (*Abiotrophia defectiva* ATCC 49176, *Bifidobacterium breve* ATCC 15700, *Clostridium difficile* ATCC 9689, *Corynebacterium urealyticum* ATCC 43042, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus durans* ATCC 19432, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus gallinarum* ATCC 49573, *Enterococcus raffinosus* ATCC 49427, *Lactobacillus reuteri* ATCC 23273, *Lactococcus lactis* ATCC 19435, *Listeria monocytogenes* ATCC 15313, Peptococcus niger ATCC 27731, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus* prevotii ATCC 9321, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus agalactiae* ATCC 27591, *Streptococcus* anginosus ATCC 33397, *Streptococcus bovis* ATCC 33317, *Streptococcus constellatus* ATCC 27823, *Streptococcus* dysgalactiae ATCC 43078, *Streptococcus gordonii* ATCC 10558, *Streptococcus mitis* ATCC 33399, *Streptococcus mutans* ATCC 25175, *Streptococcus oralis* ATCC 35037, *Streptococcus* parauberis ATCC 6631, *Streptococcus pneumoniae* ATCC 6303, *Streptococcus pyogenes* ATCC 19615, *Streptococcus* salivarius ATCC 7073, *Streptococcus sanguinis* ATCC 10556, *Streptococcus uberis* ATCC 19436). These microbial species included 15 species of streptococci and many members of the normal vaginal and anal floras. In addition, 40 GBS isolates of human origin, whose identification was confirmed by a latex agglutination test (Streptex, Murex), were also used to evaluate the ubiquity of the assay.

[0299] For determination of the sensitivities (i.e., the minimal number of genome copies that could be detected) for conventional and LightCycler™ PCR assays, serial 10-fold or 2-fold dilutions of purified genomic DNA from 5 GBS ATCC strains were used. Results

[0300] Evaluation of the GBS-specific conventional and LightCycler™ PCR assays. The specificity of the two assays demonstrated that only DNAs from GBS strains could be amplified. Both PCR assays did not amplify DNAs from any other bacterial species tested including 14 streptococcal species other than GBS as well as phylogenetically related species belonging to the genera *Enterococcus, Peptostreptococcus* and *Lactococcus*. Important members of the vaginal or anal flora, including coagulase-negative staphylococci, *Lactobacillus* sp., and *Bacteriodes* sp. were also negative with the GBS-specific PCR assay. The LightCycler™ PCR assays detected only GBS DNA by producing an increased fluorescence signal which was interpreted as a positive PCR result.

Both PCR methods were able to amplify all of 40 GBS clinical isolates, showing a perfect correlation with the phenotypic identification methods.

[0301] The sensitivity of the assay was determined by using purified genomic DNA from the 5 ATCC strains of GBS. The detection limit for all of these 5 strains was one genome copy of GBS. The detection limit of the assay with the LightCycler™ was 3.5 fg of genomic DNA (corresponding to 1-2 genome copies of GBS). These results confirmed the high sensitivity of our GBS-specific PCR assay.

[0302] Direct Detection of GBS from vaginal/anal specimens. Among 66 vaginal/anal specimens tested, 11 were positive for GBS by both culture and PCR. There was one sample positive by culture only. The sensitivity of both PCR methods with vaginal/anal specimens for identifying colonization status in pregnant women at delivery was 91.7% when compared to culture results. The specificity and positive predictive values were both 100% and the negative predictive value was 97.8%. The time for obtaining results was approximately 45 min for LightCycler™ PCR, approximately 100 min for conventional PCR and 48 hours for culture.

Conclusion

[0303] We have developed two PCR assays (conventional and LightCycler™) for the detection of GBS, which are specific (i.e., no amplification of DNA from a variety of bacterial species other than GBS) and sensitive (i.e., able to detect around 1 genome copy for several reference ATCC strains of GBS). Both PCR assays are able to detect GBS directly from vaginal/anal specimens in a very short turnaround time. Using the real-time PCR assay on LightCycler™, we can detect GBS carriage in pregnant women at delivery within 45 minutes.

Example 15

Simultaneous Detection and Identification of *Streptococcus pyogenes* and its Pyrogenic Exotoxin A

[0304] The rapid detection of *Streptococcus pyogenes* and of its pyrogenic exotoxin A is of clinical importance. We developed a multiplex assay which permits the detection of strains of *S. pyogenes* carrying the pyrogenic toxin A gene, which is associated with scarlet fever and other pathologies. In order to specifically detect *S. pyogenes*, nucleotide sequences of the pyrrolidone carboxylyl peptidase (pcp) gene were aligned to design PCR primers Spy291 (SEQ ID NO. 1211) and Spy473 (SEQ ID NO. 1210). Next, we designed primers for the specific detection of the pyrogenic exotoxin A. Nucleotide sequences of the speA gene, carried on the bacteriophage T12, were aligned as shown in Annex XXIII to design PCR primers Spytx814 (SEQ ID NO. 994) and Spytx 927 (SEQ ID NO. 995).

[0305] The primer pairs: oligos SEQ ID NOs. 1210-1211, yielding an amplification product of 207 bp, and oligos SEQ ID NOs. 994-995, yielding an amplification product of 135 bp, were used in a multiplex PCR assay.

[0306] PCR amplification was carried out using 0.4 μM of both pairs of primers, 2.5 mM $MgCl_2$, BSA 0.05 μM, dNTP 0.2 μM (Pharmacia), 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), and 1 μl of genomic DNA sample in a final volume of 20 PCR amplification was performed using a PTC-200 thermal cycler (MJ Research). The optimal cycling conditions for maximum specificity and sensitivity were 3 minutes at 94° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 63° C., followed by a final step of 2 minutes at 72° C. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

[0307] The detection limit was less than 5 genome copies for both *S. pyogenes* and its pyrogenic exotoxin A. The assay was specific for pyrogenic exotoxin A-producing *S. pyogenes*: strains of the 27 other species of *Streptococcus* tested, as well as 20 strains of various gram-positive and gram-negative bacterial species were all negative.

[0308] A similar approach was used to design an alternative set of speA-specific primers (SEQ ID NOs. 996 to 998, see Annex XXIV). In addition, another set of primers based on the tuf gene (SEQ ID NOs. 999 to 1001, see Annex XXV) could be used to specifically detect *Streptococcus pyogenes.*

Example 16

Real-Time Detection and Identification of Shiga Toxin-Producing Bacteria

[0309] Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* cause bloody diarrhea. Currently, identification relies mainly on the phenotypic identification of *S. dysenteriae* and *E. coli* serotype O157:H7. However, other serotypes of *E. coli* are increasingly found to be producers of type 1 and/or type 2 Shiga toxins. Two pairs of PCR primers targeting highly conserved regions present in each of the Shiga toxin genes stx_1 and stx_2 were designed to amplify all variants of those genes (see Annexes XXVI and XXVII). The first primer pair, oligonucleotides 1SLT224 (SEQ ID NO. 1081) and 1SLT385 (SEQ ID NO. 1080), yields an amplification product of 186 by from the six, gene. For this amplicon, the 1SLTB1-Fam (SEQ ID NO. 1084) molecular beacon was designed for the specific detection of six, using the fluorescent label 6-carboxy-fluorescein. The 1 SltS1-FAM (SEQ ID NO. 2012) molecular scorpion was also designed as an alternate way for the specific detection of stx_1. A second pair of PCR primers, oligonucleotides 2SLT537 (SEQ ID NO. 1078) and 2SLT678b (SEQ ID NO. 1079), yields an amplification product of 160 by from the stx_2 gene. Molecular beacon 2SLTB1-Tet (SEQ ID NO. 1085) was designed for the specific detection of stx_2 using the fluorescent label 5-tetrachloro-fluorescein. Both primer pairs were combined in a multiplex PCR assay.

[0310] PCR amplification was carried out using 0.8 μM of primer pair SEQ ID NOs. 1080-1081, 0.5 μM of primer pair SEQ ID NOs. 1078-1079, 0.3 μM of each molecular beacon, 8 mM $MgCl_2$, 490 μg/mL BSA, 0.2 mM dNTPs (Pharmacia), 50 mM Tris-HCl, 16 mM NH_4SO_4, 1× TaqMaster (Eppendorf), 2.5 U KlenTaq1 DNA polymerase (AB Peptides) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), and 1 μl of genomic DNA sample in a final volume of 25 PCR amplification was performed using a SmartCycler thermal cycler (Cepheid). The optimal cycling conditions for maximum sensitivity and specificity were 60 seconds at 95° C. for initial denaturation, then 45 cycles of three steps consisting of 10 seconds at 95° C., 15 seconds at 56° C. and 5 seconds at 72° C. Detection of the PCR products was made in real-time by measuring the fluorescent signal emitted by the molecular beacon when it hybridizes to its target at the end of the annealing step at 56° C.

[0311] The detection limit was the equivalent of less than 5 genome copies. The assay was specific for the detection of both toxins, as demonstrated by the perfect correlation between PCR results and the phenotypic characterization performed using antibodies specific for each Shiga toxin type. The assay was successfully performed on several Shiga toxin-producing strains isolated from various geographic areas of the world, including 10 O157:H7 *E. coli,* 5 non-O157:H7 *E. coli* and 4 *S. dysenteriae.*

Example 17

Development of a PCR Assay for the Detection and Identification of Staphylococci at Genus and Species Levels and its Associated mecA Gene

[0312] The *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) were used in multiplex with the mecA-specific PCR primers and the *S. aureus*-specific primers described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 261 and 262 for mecA and SEQ ID NOs. 152 and 153 for *S. aureus* in the said patent). Sequence alignment analysis of 10 publicly available mecA gene sequences allowed to design an internal probe specific to mecA (SEQ ID NO. 1177). An internal probe was also designed for the *S. aureus*-specific amplicon (SEQ ID NO 1234). PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μM (each) of the two *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μM (each) of the mecA-specific primers and 0.4 μM (each) of the *S. aureus*-specific primers were used in the PCR mixture. The specificity of the multiplex assay with 40-cycle PCR protocols was verified by using purified genomic DNA from five methicillin-resistant and fifteen methicillin-sensitive staphylococcal strains. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from twenty-three methicillin-resistant and twenty-eight methicillin-sensitive staphylococcal strains. The detection limit was 2 to 10 genome copies of genomic DNA, depending on the staphylococcal species tested. Furthermore, the mecA-specific internal probe, the *S. aureus*-specific internal probe and the coagulase-negative staphylococci-specific internal probe (described in Example 7) were able to recognize twenty-three methicillin-resistant staphylococcal strains and twenty-eight methicillin-sensitive staphylococcal strains with high sensitivity and specificity.

[0313] The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1232 for detection of the *S. aureus*-specific amplicon, SEQ ID NO. 1233 for detection of coagulase-negative staphylococci and SEQ ID NO. 1231 for detection of mecA.

[0314] Alternatively, a multiplex PCR assay containing the *Staphylococcus*-specific PCR primers described in Example 7 (SEQ ID NOs. 553 and 575) and the mecA-specific PCR primers described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were developed. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 7, with the exception that 0.4 μM (each) of the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) and 0.4 μM (each) of the mecA-specific primers described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 261 and 262 in the said patent) were used in the PCR mixture. The sensitivity of the multiplex assay with 40-cycle PCR protocols was determined by using purified genomic DNA from two methicillin-resistant and five methicillin-sensitive staphylococcal strains. The detection limit was 2 to 5 copies of genomic DNA, depending on the staphylococcal species tested. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with two strains of methicillin-resistant *S. aureus*, two strains of methicillin-sensitive *S. aureus* and seven strains of methicillin-sensitive coagulase-negative staphylococci. The mecA-specific internal probe (SEQ ID NO. 1177) and the *S. aureus*-specific internal probe (SEQ ID NO. 587) described in Example 7 were able to recognize all the strains with high specificity showing a perfect correlation with susceptibility to methicillin. The sensitivity of the PCR assay coupled with capture-probe hybridization was tested with one strain of methicillin-resistant *S. aureus*. The detection limit was around 10 copies of genomic DNA.

Example 18

Sequencing of pbp1a, pbp2b and pbp2x Genes of *Streptococcus pneumoniae*

[0315] Penicillin resistance in *Streptococcus pneumoniae* involves the sequential alteration of up to five penicillin-binding proteins (PBPs) 1A, 1B, 2A, 2X and 2B in such a way that their affinity is greatly reduce toward the antibiotic molecule. The altered PBP genes have arisen as the result of interspecies recombination events from related streptococcal species. Among the PBPs usually found in *S. pneumoniae*, PBPs 1A, 2B, and 2X play the most important role in the development of penicillin resistance. Alterations in PBP 2B and 2X mediate low-level resistance to penicillin while additional alterations in PBP 1A plays a significant role in full penicillin resistance.

[0316] In order to generate a database for pbp sequences that can be used for design of primers and/or probes for the specific and ubiquitous detection of β-lactam resistance in *S. pneumoniae*, pbp1a, pbp2b and pbp2x DNA fragments sequenced by us or selected from public databases (GenBank and EMBL) from a variety of *S. pneumoniae* strains were used to design oligonucleotide primers. This database is essential for the design of specific and ubiquitous primers and/or probes for detection of β-lactam resistance in *S. pneumoniae* since the altered PBP 1A, PBP 2B and PBP 2X of β-lactam resistant *S. pneumoniae* are encoded by mosaic genes with numerous sequence variations among resistant isolates. The PCR primers were located in conserved regions of pbp genes and were able to amplify pbp1a, pbp2b, and pbp2x sequences of several strains of *S. pneumoniae* having various levels of resistance to penicillin and third-generation cephalosporins. Using primer pairs SEQ ID NOs. 1125 and 1126, SEQ ID NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, it was possible to amplify and determine pbp1a sequences SEQ ID NOs. 1004-1018, 1648, 2056-2060 and 2062-2064, pbp2b sequences SEQ ID NOs. 1019-1033, and pbp2x sequences SEQ ID NOs. 1034-1048. Six other PCR primers (SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) were also designed and used to complete the sequencing of pbp1a, pbp2b and pbp2x amplification products. The described primers (SEQ ID NOs. 1125 and 1126, SEQ ID

NOs. 1142 and 1143, SEQ ID NOs. 1146 and 1147, SEQ ID NOs. 1127-1128, 1144-1145, 1148-1149) represent a powerful tool for generating new pbp sequences for design of primers and/or probes for detection of β-lactam resistance in *S. pneumoniae.*

Example 19

Sequencing of hexA Genes of *Streptococcus* Species

[0317] The hexA sequence of *S. pneumoniae* described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NO. 31 in the said patent, SEQ ID NO. 1183 in the present application) allowed the design of a PCR primer (SEQ ID NO. 1182) which was used with primer Spn1401 described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NO. 156 in the said patent, SEQ ID NO. 1179 in the present application) to generate a database for hexA sequences that can be used to design primers and/or probes for the specific identification and detection of *S. pneumoniae* (Annex XLII). Using primers SEQ ID NO. 1179 and SEQ ID NO. 1182 (Annex XLII), it was possible to amplify and determine the hexA sequence from *S. pneumoniae* (4 strains) (SEQ ID NOs. 1184-1187), *S. mitis* (three strains) (SEQ ID NOs. 1189-1191) and *S. oralis* (SEQ ID NO. 1188).

Example 20

Development of Multiplex PCR Assays Coupled with Capture Probe Hybridization for the Detection and Identification of *Streptococcus pneumoniae* and its Penicillin Resistance Genes

[0318] Two different assays were developed to identify *S. pneumoniae* and its susceptibility to penicillin.

Assay I:

[0319] Bacterial strains. The specificity of the multiplex PCR assay was verified by using a panel of ATCC (American Type Culture Collection) reference strains consisting of 33 gram-negative and 67 gram-positive bacterial species (Table 13). In addition, a total of 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis* from the American Type Culture Collection, the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL), (Step-Foy, Québec, Canada), the Laboratoire de santé publique du Québec, (Sainte-Anne-de-Bellevue, Québec, Canada), the Sunnybrook and Women's College Health Sciences Centre (Toronto, Canada), the Infectious Diseases Section, Department of Veterans Affairs Medical Center, (Houston, USA) were also tested to further validate the *Streptococcus pneumoniae*-specific PCR assay. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

[0320] PCR primers and internal probes. The analysis of hexA sequences from a variety of streptococcal species from the publicly available hexA sequence and from the database described in Example 19 (SEQ ID NOs. 1184-1191) allowed the selection of a PCR primer specific to *S. pneumoniae*, SEQ ID NO. 1181. This primer was used with the *S. pneumoniae*-specific primer SEQ ID NO. 1179 to generate an amplification product of 241 by (Annex XLII). The PCR primer SEQ ID NO. 1181 is located 127 nucleotides downstream on the hexA sequence compared to the original *S. pneumoniae*-specific PCR primer Spn1515 described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NO. 157 in the said patent). These modifications were done to ensure the design of the *S. pneumoniae*-specific internal probe according to the new hexA sequences of several streptococcal species from the database described in Example 19 (SEQ ID NOs. 1184-1191).

[0321] The analysis of pbp1a sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the identification of amino acid substitutions Ile-459 to Met and Ser-462 to Ala that occur in isolates with high-level penicillin resistance (MICs≧1 μg/ml), and amino acid substitutions Ser-575 to Thr, Gln-576 to Gly and Phe-577 to Tyr that are common to all penicillin-resistant isolates with MICs≧0.25 μg/ml. As shown in Annex XXXI, PCR primer pair SEQ ID NOs. 1130 and 1131 were designed to detect high-level penicillin resistance (MICs≧1 μg/ml), whereas PCR primer pair SEQ ID NOs. 1129 and 1131 were designed to detect intermediate- and high-level penicillin resistance (MICs≧0.25 μg/ml).

[0322] The analysis of hexA sequences from the publicly available hexA sequence and from the database described in Example 19 allowed the design of an internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) (Annex XLII). The range of mismatches between the *S. pneumoniae*-specific 241-bp amplicon was from 2 to 5, in the middle of the 19-bp probe. The analysis of pbp1a sequences from public databases and from the database described in Example 18 allowed the design of five internal probes containing all possible mutations to detect the high-level penicillin resistance 383-bp amplicon (SEQ ID NOs. 1197, 1217-1220). Alternatively, two other internal probes (SEQ ID NOs. 2024-2025) can also be used to detect the high-level penicillin resistance 383-bp amplicon. Five internal probes containing all possible mutations to detect the 157-bp amplicon which includes intermediate- and high-level penicillin resistance were also designed (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). Design and synthesis of primers and probes, and detection of the probe hybridization were performed as described in Example 7. Annex XXXI illustrates one of the internal probe for detection of the high-level penicillin resistance 383-bp amplicon (SEQ ID NO. 1197) and one of the internal probe for detection of the intermediate- and high-level penicillin resistance 157-bp amplicon (SEQ ID NO. 1193).

[0323] PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μl of genomic DNA at 0.1 ng/μl, or 1 μl of a bacterial lysate, was transferred to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (H 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.1 μM (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.2 μM of primer SEQ ID NO. 1129, 0.7 μM of primer SEQ ID NO. 1131, and 0.6 μM of primer SEQ ID NO. 1130, 0.05 mM bovine serum albumin (BSA), and 0.5 U Taq polymerase (Promega) coupled with TaqStart™ antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1×PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

[0324] For determination of the sensitivity of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

[0325] Capture probe hybridization. The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

Results

[0326] Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17 genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. There was a perfect correlation between PCR and standard susceptibility testing for 33 penicillin-sensitive isolates. Among 12 *S. pneumoniae* isolates with intermediate penicillin resistance based on susceptibility testing, 11 had intermediate resistance based on PCR, but one *S. pneumoniae* isolate with penicillin MIC of 0.25 μg/ml showed a high-level penicillin resistance based on genotyping. Among 53 isolates with high-level penicillin resistance based on susceptibility testing, 51 had high-level penicillin resistance based on PCR but two isolates with penicillin MIC>1 μg/ml showed an intermediate penicillin resistance based on genotyping. In general, there was a good correlation between the genotype and classical culture method for bacterial identification and susceptibility testing.

[0327] The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

[0328] Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneumoniae* strains but did not hybridize to the *S. mitis* and *S. oralis* amplicons. The five internal probes specific to the high-level resistance amplicon (SEQ ID NOs. 1197, 1217-1220) detected all amplification patterns corresponding to high-level resistance. The two *S. pneumoniae* strains with penicillin MIC>1 μg/ml that showed an intermediate penicillin resistance based on PCR amplification were also intermediate resistance based on probe hybridization. Similarly, among 12 strains with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with the five internal probes specific to the intermediate and high-level resistance amplicon (SEQ ID NOs. 1094, 1192-1193, 1214 and 1216). The strain described above having a penicillin MIC of 0.25 μg/ml which was high-level penicillin resistance based on PCR amplification was also high-level resistance based on probe hybridization. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae.*

Assay II:

[0329] Bacterial strains. The specificity of the multiplex PCR assay was verified by using the same strains as those used for the development of Assay I. The penicillin MICs (minimal inhibitory concentrations) were measured by the broth dilution method according to the recommended protocol of NCCLS.

[0330] PCR primers and internal probes. The analysis of pbp1a sequences from *S. pneumoniae* strains with various levels of penicillin resistance from public databases and from the database described in Example 18 allowed the design of two primers located in the constant region of pbp1a. PCR primer pair (SEQ ID NOs. 2015 and 2016) was designed to amplify a 888-bp variable region of pbp1a from all *S. pneumoniae* strains. A series of internal probes were designed for identification of the pbp1a mutations associated with penicillin resistance in *S. pneumoniae*. For detection of high-level penicillin resistance (MICs≧1 μg/ml), three internal probes were designed (SEQ ID NOs. 2017-2019). Alternatively, ten other internal probes were designed that can also be used for detection of high-level resistance within the 888-bp pbp1a amplicon: (1) three internal probes for identification of the amino acid substitutions Thr-371 to Ser or Ala within the motif S370TMK (SEQ ID NOs. 2031-2033); (2) two internal probes for detection of the amino acid substitutions Ile-459 to Met and Ser-462 to Ala near the motif S428RN (SEQ ID NOs. 1135 and 2026); (3) two internal probes for identification of the amino acid substitutions Asn-443 to Asp (SEQ ID NOs. 1134 and 2027); and (4) three internal probes for detection of all sequence variations within another region (SEQ ID NOs. 2028-2030). For detection of high-level and intermediate penicillin resistance (MICs 0.25 μg/ml), four internal probes were designed (SEQ ID NOs. 2020-2023). Alternatively, six other internal probes were designed for detection of the four consecutive amino acid substitutions T574SQF to A574TGY near the motif K557TG (SEQ ID NOs. 2034-2039) that can also be used for detection of intermediate- and high-level resistance within the 888-bp pbp1a amplicon.

[0331] PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA using a PTC-200 thermocycler (MJ Research). 1 μl of genomic DNA at 0.1 ng/μl, or 1 μl of a bacterial lysate, was transferred to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.08 μM (each) of the *S. pneumoniae*-specific primers SEQ ID NO. 1179 and SEQ ID NO. 1181, 0.4 μM of the pbp1a-specific primer SEQ ID NO. 2015, 1.2 μM of pbp1a-specific primer SEQ ID NO. 2016, 0.05 mM bovine serum albumin (BSA), and 0.5 U Taq polymerase (Promega) coupled with TaqStart™ antibody. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1×PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

[0332] For determination of the sensitivities of the PCR assays, 10-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

[0333] Capture probe hybridization. The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates as described for Assay I.

Results

[0334] Amplifications with the multiplex PCR assay. The specificity of the assay was assessed by performing 40-cycle PCR amplifications with the panel of gram-positive (67 species from 12 genera) and gram-negative (33 species from 17 genera) bacterial species listed in Table 13. All bacterial species tested other than *S. pneumoniae* were negative except *S. mitis* and *S. oralis*. Ubiquity tests were performed using a collection of 98 *S. pneumoniae* strains including high-level penicillin resistance (n=53), intermediate resistance (n=12) and sensitive (n=33) strains. All the above *S. pneumoniae* strains produced the 888-bp amplicon corresponding to pbp1a and the 241-bp fragment corresponding to hexA.

[0335] The sensitivity of the *S. pneumoniae*-specific assay with 40-cycle PCR protocols was determined by using purified genomic DNA from 9 isolates of *S. pneumoniae*. The detection limit was around 10 copies of genomic DNA for all of them.

[0336] Post-PCR hybridization with internal probes. The specificity of the multiplex PCR assay coupled with capture-probe hybridization was tested with 98 strains of *S. pneumoniae*, 16 strains of *S. mitis* and 3 strains of *S. oralis*. The internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) detected all 98 *S. pneumoniae* strains but did not hybridize to the S. mills and *S. oralis* amplicons. The three internal probes (SEQ ID NOs 2017-2019) specific to high-level resistance detected all the 43 strains with high-level penicillin resistance based on susceptibility testing. Among 12 isolates with intermediate-penicillin resistance based on susceptibility testing, 11 showed intermediate-penicillin resistance based on hybridization with 4 internal probes (SEQ ID NOs. 2020-2023) and one strain having penicillin MIC of 0.25 μg/ml was misclassified as high-level penicillin resistance. In summary, the combination of the multiplex PCR and hybridization assays results in a highly specific test for the detection of penicillin-resistant *Streptococcus pneumoniae*.

Example 21

Sequencing of the Vancomycin Resistance vanA, vanC1, vanC2 and vanC3 Genes

[0337] The publicly available sequences of the vanH-vanA-vanX-vanY locus of transposon Tnl546 from *E. faecalis*, vanC1 sequence from one strain of *E. gallinarum*, vanC2 and vanC3 sequences from a variety of *E. casseliflavus* and *E. flavescens* strains, respectively, allowed the design of PCR primers able to amplify the vanA, vanC1, vanC2 and vanC3 sequences of several *Enterococcus* species. Using primer pairs van6877 and van9106 (SEQ ID NOs. 1150 and 1155), vanC1-122 and vanC1-1315 (SEQ ID NOs. 1110 and 1109), and vanC2C3-1 and vanC2C3-1064 (SEQ ID NOs. 1108 and 1107), it was possible to amplify and determine vanA sequences SEQ ID NOs. 1049-1057, vanC1 sequences SEQ ID NOs. 1058-1059, vanC2 sequences SEQ ID NOs. 1060-1063 and vanC3 sequences SEQ ID NOs. 1064-1066, respectively. Four other PCR primers (SEQ ID NOs. 1151-1154) were also designed and used to complete the sequencing of vanA amplification products.

Example 22

Development of a PCR Assay for the Detection and Identification of Enterococci at Genus and Species Levels and its Associated Resistance Genes vanA and vanB

[0338] The comparison of vanA and vanB sequences revealed conserved regions allowing the design of PCR primers specific to both vanA and vanB sequences (Annex XXXVIII). The PCR primer pair vanAB459 and vanAB830R (SEQ ID NOs. 1112 and 1111) was used in multiplex with the *Enterococcus*-specific primers Encg313dF and Encg599c (SEQ ID NOs. 1137 and 1136) described in Example 11. Sequence alignment analysis of vanA and vanB sequences revealed regions suitable for the design of internal probes specific to vanA (SEQ ID NO. 1170) and vanB (SEQ ID NO. 1171). PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 11. The optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94° C., followed by forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 62° C., plus a terminal extension at 72° C. for 2 minutes. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 nanogram of purified genomic DNA from a panel of bacteria listed in Table 10. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of *E. casseliflavus*, eight strains of *E. gallinarum*, two strains of *E. flavescens*, two vancomycin-resistant strains of *E. faecalis* and one vancomycin-sensitive strain of *E. faecalis*, three vancomycin-resistant strains of *E. faecium*, one vancomycin-sensitive strain of *E. faecium* and one strain of each of the other enterococcal species listed in Table 10. The detection limit was 1 to 10 copies of genomic DNA, depending on the enterococcal species tested. The vanA- and vanB-specific internal probes (SEQ ID NOs. 1170 and 1171), as well as the *E. faecalis*- and *E. faecium*-specific internal probes (SEQ ID NOs. 1174 and 602) and the internal probe specific to the group including *E. casseliflavus, E. gallinarum* and *E. flavescens* (SEQ ID NO. 1122) described in Example 11, were able to recognize vancomycin-resistant enterococcal species with high sensitivity, specificity and ubiquity showing a perfect correlation between the genotypic and phenotypic analysis.

[0339] The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1236 for the detection of *E. faecalis*, SEQ ID NO. 1235 for the detection of *E. faecium*, SEQ ID NO. 1240 for the detection of vanA, and SEQ ID NO. 1241 for the detection of vanB.

Example 23

Development of a Multiplex PCR Assay for Detection and Identification of Vancomycin-Resistant Organisms Including *Enterococcus faecalis, Enterococcus faecium* and the Group Including *Enterococcus gallinarum, Enterococcus Casseliflavus*, and *Enterococcus flavescens*.

[0340] The vanA and vanB genes encode the major glycopeptide resistance phenotypes in vancomycin resistant microorganisms. To design an assay to detect vancomycin resistant microorganisms, the nucleotide sequence of the vanA and vanB genes were analyzed. FIGS. **13** and **14** show a nucleotide sequence alignment of the vanA and vanB genes from the bacterial strains listed in Tables 26 and 27, respectively. Shown above the sequence alignments is a consensus DNA sequence derived from the multiple sequences. The analysis of vanA and vanB sequences revealed conserved regions allowing design of PCR primer pairs (SEQ ID NOs. 1089 and 1090 and SEQ ID NOs: 1090 and 1091) specific to vanA sequences (Annex XXVIII) and PCR primer pairs (SEQ ID NOs. 1095 and 1096 and SEQ ID NOs: 2298 and 1096) specific to vanB sequences (Annex XXIX). Shown below the sequence alignments in FIGS. **13** and **14** is the sequence and location of the vanA primers SEQ ID NO: 1090 and 1091 and the vanB primers SEQ ID NOs: 2298 and 1096, respectively. Also shown are the sequence and location of molecular beacon probes, SEQ ID NOs: 2299, 2300, designed for the detection of the vanA and vanB amplification products, respectively. SEQ ID NO: 2299. The vanA molecular beacon (SEQ ID NO: 2299) contains a 5' carboxyfluorescein (FAM) fluorophore. The vanB molecular beacon (SEQ ID NO: 2300) contains a sulforhodamine active ester (Texas Red) at its 5' end. Both beacons contain the nonfluorescent quencher moiety dabcyl chloride (DABCYL) at their 3' ends.

[0341] An internal control DNA, pERVd (SEQ ID NO: 2302) was designed for the vanR assay. The primers of SEQ ID NO: 1090 and SEQ ID NO: 1096 can hybridize to produce and amplification product from the internal control DNA. A molecular beacon probe (SEQ ID NO: 2301) was designed to detect the amplification product of the internal control DNA. SEQ ID NO: 2301 contains a 5' tetrachlorofluorescein (TET) fluorophore and the DABCYL quencher moiety at its 3' end.

[0342] In a first experiment, the vanA-specific PCR primer pair (SEQ ID. NOs. 1089 and 1090) was used in multiplex with the vanB-specific PCR primer pair described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent). The comparison of vanC1, vanC2 and vanC3 sequences revealed conserved regions allowing design of PCR primers (SEQ ID NOs. 1101 and 1102) able to generate a 158-bp amplicon specific to the group including *E. gallinarum, E. casseliflavus* and *E. flavescens* (Annex XXX). The vanC-specific PCR primer pair (SEQ ID NOs. 1101 and 1102) was used in multiplex with the *E. faecalis*-specific PCR primer pair described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94° C., followed by forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 58° C., plus a terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. The vanA-specific PCR primer pair (SEQ ID NOs. 1089 and 1090), the vanB-specific primer pair (SEQ ID NOs. 1095 and 1096) and the vanC-specific primer pair (SEQ ID NOs. 1101 and 1102) were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of 5 vancomycin-sensitive *Enterococcus* species, 3 vancomycin-resistant *Enterococcus* species, 13 other gram-positive bacteria and one gram-negative bacterium. Specificity tests were performed with the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication) and with the *E. faecalis*-specific PCR primer pair described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) on a panel of 37 gram-positive bacterial species. All *Enterococcus* strains were amplified with high specificity showing a perfect correlation between the genotypic and phenotypic analysis. The sensitivity of the assays was determined for several strains of *E. gallinarum, E. casseliflavus, E. flavescens* and vancomycin-resistant *E. faecalis* and *E. faecium*. Using each of the *E. faecalis*- and *E. faecium*-specific PCR primer pairs as well as vanA-, vanB- and vanC-specific PCR primers used alone or in multiplex as described above, the sensitivity ranged from 1 to 10 copies of genomic DNA.

[0343] The format of the assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 1238 for the detection of *E. faecalis*, SEQ ID NO. 1237 for the detection of *E. faecium*, SEQ ID NO. 1239 for the detection of vanA, and SEQ ID NO. 1241 for the detection of vanB.

[0344] Another PCR assay was developed for the detection of vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis*. This assay included two multiplex: (1) the first multiplex contained the vanA-specific primer pair (SEQ ID NOs. 1090-1091) and the vanB-specific PCR primer pair described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 1095 and 1096 in the present patent and SEQ ID NOs. 231 and 232 in the said patent), and (2) the second multiplex contained the *E. faecalis*-specific PCR primer pair described in our assigned U.S. Pat. No. 5,994,066 (SEQ ID NOs. 40 and 41 in the said patent) and the *E. faecium*-specific PCR primer pair described in our patent publication WO98/20157 (SEQ ID NOs. 1 and 2 in the said publication). For both multiplexes, the optimal cycling conditions for maximum sensitivity and specificity were found to be 3 min. at 94° C., followed by forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 58° C., plus a terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. The two multiplexes were tested for their specificity by using 0.1 nanogram of purified genomic DNA from a panel of two vancomycin-sensitive *E. faecalis* strains, two vancomycin-resistant *E. faecalis* strains, two vancomycin-sensitive *E. faecium* strains, two vancomycin-resistant *E. faecium* strains, 16 other enterococcal species and 31 other gram-positive bacterial species. All the *E. faecium* and *E. faecalis* strains were amplified with high specificity showing a perfect correlation between the genotypic analysis and the susceptibility to glycopeptide antibiotics (vancomycin and teicoplanin). The sensitivity of the assay was determined for two vancomycin-resistant *E. faecalis* strains and two vancomycin-resistant *E. faecium* strains. The detection limit was 5 copies of genomic DNA for all the strains.

[0345] This multiplex PCR assay was coupled with capture-probe hybridization. Four internal probes were designed: one specific to the vanA amplicon (SEQ ID NO. 2292), one specific to the vanB amplicon (SEQ ID NO. 2294), one specific to the *E. faecalis* amplicon (SEQ ID NO. 2291) and one specific to the *E. faecium* amplicon (SEQ ID NO.

2287). Each of the internal probes detected their specific amplicons with high specificity and sensitivity.

The VanR Assay

Development

[0346] In a next set of experiments, a multiplex real-time PCR reaction (the VanR assay) using the vanA primer pair SEQ ID NO: 1090 and 1091, the vanB primer pair SEQ ID NO: 1096 and 2298, the internal control DNA SEQ ID NO: 2302, and the molecular beacon probes SEQ ID NOs: 2299, 2300, and 2301 was performed.

[0347] For the template DNA, purified genomic DNA from vanA resistant *Enterococcus faecium* or vanB resistant *Enterococcus faecalis* was obtained from strains grown on blood agar plates under standard conditions. Genomic DNA was extracted from the cultures using the GNOME kit (QBIOgene, Carlsbad, Calif.) according to the manufacturer's instructions. Template DNA samples were treated with RNase prior to quantitation. Genomic DNA concentration was determined and the quality of the genomic DNA preparations was confirmed using conventional methods.

[0348] Lyophilized reagents listed in Table 24 were used for the VanR assay. The lyophilized reagents were reconstituted with 225 μl diluent (116 mM Tris-HCl, pH 8.3, 11.6 mM KCl, 3.48 mM $MgCl_2$, 5.8 mM NH_2SO_4) and subsequently divided into 25 μl aliquots. 0.5, 2.5, 5, 10 or 20 copies of template DNA was added to each of 5 replicate reactions.

[0349] The VanR assay PCR was run in a SMART CYCLER™ PCR machine under the following conditions: 60° C. for 6 sec, followed by 95° C. for 900 sec, followed by 45 cycles of 95° C. for 5 seconds, 63° C. for 10 sec and 72° C. for 20 sec. The fluorescent readout from the FAM channel (vanA) and the Texas Red channel (vanB) of the reactions is shown in FIGS. **13**A and **13**B, respectively. The VanR assay is capable of detecting 5 copies of vanA target DNA in a sample (FIG. **13**A) and of detecting 10 copies of vanB target DNA in a sample (FIG. **13**B). For each concentration of template DNA, the number of positive assay results out of each of the five replicates was recorded (Table 15).

Specificity

[0350] To demonstrate the specificity of the VanR assay, vanC, vanD, vanE, and vanG resistant enterococci, other closely related bacteria, normal and pathogenic anal or fecal flora, or human DNA as listed in Tables 16-18 were assayed as follows: VanR assay lyophilized reagents (Table 38) were reconstituted with 225 μl diluent and aliquoted as described above. Template DNA was prepared using conventional methods and diluted in sample preparation buffer to a final concentration of 0.33 ng/μl. 3 μl template DNA was added to each master mix, and the reaction was carried out as described above in the SMART CYCLER™ PCR machine. 41 electrophoresis loading buffer was added to the reactions upon completion, and 15 μl of each reaction was run on an agarose gel to view the PCR amplification products, as shown in FIGS. **14-17**.

[0351] To assess the specificity of the molecular beacons in the VanR assay, PCR assays were preformed to test cross-reactivity with amplified vanA and vanB target DNA and internal control DNA. The reaction components listed in Table 34 were each added to premix in Table 33. For each reaction, 25.8 μl of the final mix was combined with 3 μl template DNA (either 25 copies vanA DNA or 50 copies vanB DNA) or TE buffer (1×), as indicated. The reactions were performed in the SMART CYCLER™ PCR machine using the VanR assay conditions as described above. Table 35 shows the results of the experiment. Positive results were obtained in the FAM channel in every reaction that contained vanA template DNA and the vanA primers. Positive results were obtained in the Texas Red channel in every reaction that contained vanB template DNA and vanB primers. Positive results obtained in the TET channel in every reaction that contained internal control template DNA and internal control primers.

[0352] Negative results were obtained in each channel for every reaction that did not contain a target DNA that could be amplified and detected using the indicated molecular beacon probe. In other words, the molecular beacon probes did not cross-react with non-specific DNAs. FIGS. **18**A-**20**B show the fluorescent readouts of the experiments.

Validation with Clinical Samples

[0353] To validate the VanR assay, the sensitivity of the VanR assay was performed on clinical samples. Briefly, the VanR assay lyophilized reagents listed in Table 38 were reconstituted in 225 μl diluent and aliquoted as described above. DNA isolation and quantitation was performed using conventional techniques. The samples were processed in a SMART CYCLER' PCR machine using the VanR reaction conditions described above.

[0354] FIG. **21** shows the fluorescent readout in the FAM (vanA) channel and the Texas Red channel (vanB) from a specimen that is vanA resistant. The reaction gave a positive result in the FAM channel but not the Texas Red channel. FIG. **22** shows the fluorescent readout in the FAM and Texas Red channels from a combination of a vanA resistant specimen and a vanB resistant specimen. Positive results were obtained in both the FAM and the Texas Red channel. FIG. **23** shows the fluorescent readout in the FAM and Texas Red channels from a vanB positive specimen. The results were negative in the FAM channel, and positive in the Texas Red channel.

Sensitivity

[0355] To assess the sensitivity of the VanR assay, template DNA isolated from enterococci from various geographic regions, and on template DNA from vanB resistant bacterial species other than enterococci was isolated and tested in the VanR assay as described above. Table 36 lists the enterococcal strains from various regions in the world tested in the assay. The table identifies the vanA and vanB phenotype of each strain. PCR amplification products were detected by running a sample of each reaction on an agarose gel, shown in FIG. **24**. vanA or vanB PCR amplification products were detectable in each of the strains tested. Table 37 lists non-enterococcal, vanB resistant strains tested in the VanR assay. The PCR amplification products are shown in FIG. **25**. The reaction produced an amplification product of the expected size for the vanB amplicon.

[0356] These results demonstrate that the VanR assay is sensitive and specific.

Example 24

Universal Amplification Involving the EF-G (fusA) Subdivision of tuf Sequences

[0357] As shown in FIG. **3**, primers SEQ ID NOs. 1228 and 1229 were designed to amplify the region between the end of fusA and the beginning of tuf genes in the str operon.

Genomic DNAs from a panel of 35 strains were tested for PCR amplification with those primers. In the initial experiment, the following strains showed a positive result: *Abiotrophia adiacens* ATCC 49175*, Abiotrophia defectiva* ATCC 49176, *Bacillus subtilis* ATCC 27370*, Clostridium difficile* ATCC 9689, *Enterococcus avium* ATCC 14025, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus cecorum* ATCC 43198, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus flavescens* ATCC 49996, *Enterococcus gallinarum* ATCC 49573, *Enterococcus solitarius* ATCC 49428, *Escherichia coli* ATCC 11775, *Haemophilus influenzae* ATCC 9006, *Lactobacillus acidophilus* ATCC 4356, Peptococcus niger ATCC 27731, *Proteus mirabilis* ATCC 25933, *Staphylococcus aureus* ATCC 43300, *Staphylococcus auricularis* ATCC 33753, *Staphylococcus capitis* ATCC 27840, *Staphylococcus epidemidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus hominis* ATCC 27844, *Staphylococcus lugdunensis* ATCC 43809, *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus simulans* ATCC 27848, and *Staphylococcus warneri* ATCC 27836. This primer pair could amplify additional bacterial species; however, there was no amplification for some species, suggesting that the PCR cycling conditions could be optimized or the primers modified. For example, SEQ ID NO. 1227 was designed to amplify a broader range of species.

[0358] In addition to other possible primer combinations to amplify the region covering fusA and tuf, FIG. **3** illustrates the positions of amplification primers SEQ ID NOs. 1221-1227 which could be used for universal amplification of fusA segments. All of the above mentioned primers (SEQ ID NOs. 1221-1229) could be useful for the universal and/or the specific detection of bacteria.

[0359] Moreover, different combinations of primers SEQ ID NOs. 1221-1229, sometimes in combination with tuf sequencing primer SEQ ID NO. 697, were used to sequence portions of the str operon, including the intergenic region. In this manner, the following sequences were generated: SEQ ID NOs. 1518-1526, 1578-1580, 1786-1821, 1822-1834, 1838-1843, 2184, 2187, 2188, 2214-2249, and 2255-2269.

Example 25

DNA Fragment Isolation from *Staphylococcus saprophyticus* by Arbitrarily Primed PCR

[0360] DNA sequences of unknown coding potential for the species-specific detection and identification of *Staphylococcus saprophyticus* were obtained by the method of arbitrarily primed PCR (AP-PCR).

[0361] AP-PCR is a method which can be used to generate specific DNA probes for microorganisms (Fani et al., 1993, Molecular Ecology 2:243-250). A description of the AP-PCR protocol used to isolate a species-specific genomic DNA fragment from *Staphylococcus saprophyticus* follows. Twenty different oligonucleotide primers of 10 nucleotides in length (all included in the AP-PCR kit OPAD (Operon Technologies, Inc., Alameda, Calif.)) were tested systematically with DNAs from 5 bacterial strains of *Staphylococcus saprophyticus* as well as with bacterial strains of 27 other staphylococcal (non-*S. saprophyticus*) species. For all bacterial species, amplification was performed directly from one μL (0.1 ng/μL) of purified genomic DNA. The 25 μL PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 1.2 μM of only one of the 20 different AP-PCR primers OPAD, 200 μM of each of the four dNTPs, 0.5 U of Taq DNA polymerase (Promega Corp., Madison, Wis.) coupled with TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, Calif.). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler as follows: 3 min at 96° C. followed by 42 cycles of 1 min at 94° C. for the denaturation step, 1 min at 31° C. for the annealing step and 2 min at 72° C. for the extension step. A final extension step of 7 min at 72° C. followed the 42 cycles to ensure complete extension of PCR products. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis on a 1.5% agarose gel containing 0.25 μg/ml of ethidium bromide. The size of the amplification products was estimated by comparison with a 50-bp molecular weight ladder.

[0362] Amplification patterns specific for *Staphylococcus saprophyticus* were observed with the AP-PCR primer OPAD-16 (sequence: 5'-AACGGGCGTC-3'). Amplification with this primer consistently showed a band corresponding to a DNA fragment of approximately 380 by for all *Staphylococcus saprophyticus* strains tested but not for any of the other staphylococcal species tested.

[0363] The band corresponding to the 380 by amplicon, specific and ubiquitous for *S. saprophyticus* based on AP-PCR, was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc.). The gel-purified DNA fragment was cloned into the T/A cloning site of the pCR 2.1™ plasmid vector (Invitrogen Inc.) using T4 DNA ligase (New England BioLabs). Recombinant plasmids were transformed into *E. coli* DH5α competent cells using standard procedures. All reactions were performed according to the manufacturer's instructions. Plasmid DNA isolation was done by the method of Birnboim and Doly (Nucleic Acid Res., 1979, 7:1513-1523) for small-scale preparations. All plasmid DNA preparations were digested with the EcoRI restriction endonuclease to ensure the presence of the approximately 380 by AP-PCR insert into the plasmid. Subsequently, a large-scale and highly purified plasmid DNA preparation was performed from two selected clones shown to carry the AP-PCR insert by using the QIAGEN plasmid purification kit (midi format). These large-scale plasmid preparations were used for automated DNA sequencing.

[0364] The 380 by nucleotide sequence was determined for three strains of *S. saprophyticus* (SEQ ID NOs. 74, 1093, and 1198). Both strands of the AP-PCR insert from the two selected clones were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers by using the Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase® Terminator Double-stranded DNA Sequencing Kit (Applied Biosystems, Foster City, Calif.).

[0365] Optimal species-specific amplification primers (SEQ ID NOs. 1208 and 1209) have been selected from the sequenced AP-PCR *Staphylococcus saprophyticus* DNA fragments with the help of the primer analysis software Oligo™ 5.0 (National BioSciences Inc). The selected primers were tested in PCR assays to verify their specificity and ubiquity. Data obtained with DNA preparations from reference ATCC strains of 49 gram-positive and 31 gram-negative bacterial species, including 28 different staphylococcal species, indicate that the selected primer pairs are specific for *Staphylococcus saprophyticus* since no amplification signal has been observed with DNAs from the other staphylococcal or bacterial species tested. This assay was able to amplify efficiently DNA from all 60 strains of *S. saprophyticus* from various origins tested. The sensitivity level achieved for three *S. saprophyticus* reference ATCC strains was around 6 genome copies.

Example 26

Sequencing of Prokaryotic tuf Gene Fragments

[0366] The comparison of publicly available tuf sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify tuf sequences from a wide range of bacterial species. Using primer pair SEQ ID NOs. 664 and 697, it was possible to amplify and determine tuf sequences SEQ ID NOs.: 1-73, 75-241, 607-618, 621, 662, 675, 717-736, 868-888, 932, 967-989, 992, 1002, 1572-1575, 1662-1663, 1715-1733, 1835-1837, 1877-1878, 1880-1881, 2183, 2185, 2200, 2201, and 2270-2272.

Example 27

Sequencing of Procaryotic recA Gene Fragments

[0367] The comparison of publicly available recA sequences from a variety of bacterial species revealed conserved regions, allowing the design of PCR primers able to amplify recA sequences from a wide range of bacterial species. Using primer pairs SEQ ID NOs. 921-922 and 1605-1606, it was possible to amplify and determine recA sequences SEQ ID NOs.: 990-991, 1003, 1288-1289, 1714, 1756-1763, 1866-1873 and 2202-2212.

Example 28

Specific Detection and Identification of *Escherichia coli/Shigella* Sp. Using tuf Sequences

[0368] The analysis of tuf sequences from a variety of bacterial species allowed the selection of PCR primers (SEQ ID NOs. 1661 and 1665) and of an internal probe (SEQ ID NO. 2168) specific to *Escherichia coli/Shigella* sp. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. The multiple sequence alignment included the tuf sequences of *Escherichia coli/Shigella* sp. as well as tuf sequences from other species and bacterial genera, especially representatives of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from the closely related species, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

[0369] The chosen primer pair, oligos SEQ ID NOs. 1661 and 1665, gives an amplification product of 219 bp. Standard PCR was carried out using 0.4 μM of each primer, 2.5 mM $MgCl_2$, BSA 0.05 mM, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, dNTPs 0.2 mM (Pharmacia), 0.5 U Taq DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories Inc.), 1 μl of genomic DNA sample in a final volume of 20 μl using a PTC-200 thermocycler (MJ Research). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 60° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. Visualization of the PCR products was made under UV at 254 nm.

[0370] Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Escherichia coli* (7 strains), *Shigella sonnei, Shigella flexneri, Shigella dysenteriae, Salmonella typhimyurium, Salmonella typhi, Salmonella enteritidis, Tatumella ptyseos, Klebsiella pneumoniae* (2 strains), *Enterobacter aerogenes, Citrobacter farmeri, Campylobacter jejuni, Serratia marcescens*. Amplification was observed only for the *Escherichia coli* and *Shigella* sp. strains listed and *Escherichia fergusonii*. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *E. coli* and three strains of *Shigella* sp. The detection limit for *E. coli* and *Shigella* sp. was 1 to 10 copies of genomic DNA, depending on the strains tested.

Example 29

Specific Detection and Identification of *Klebsiella pneumoniae* Using atpD Sequences

[0371] The analysis of atpD sequences from a variety of bacterial species allowed the selection of PCR primers specific to *K. pneumoniae*. The primer design strategy is similar to the strategy described in Example 28 except that atpD sequences were used in the alignment.

[0372] Two *K. pneumoniae*-specific primers were selected, (SEQ ID NOs. 1331 and 1332) which give an amplification product of 115 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 55° C., followed by terminal extension at 72° C. for 2 minutes.

[0373] Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Klebsiella pneumoniae* (2 strains), *Klebsiella ornitholytica, Klebsiella oxytoca* (2 strains), *Klebsiella planticola, Klebsiella terrigena, Citrobacter freundii, Escherichia coli, Salmonella cholerasuis typhi, Serratia marcescens, Enterobacter aerogenes, Proteus vulgaris, Kluyvera ascorbata, Kluyvera georgiana, Kluyvera cryocrescens* and *Yersinia enterolitica*. Amplification was detected for the two *K. pneumoniae* strains, *K. planticola, K. terrigena* and the three *Kluyvera* species tested. Analysis of the multiple alignment sequence of the atpD gene allowed the design of an internal probe SEQ ID NO. 2167 which can discriminate *Klebsiella pneumoniae* from other *Klebsiella* sp. and *Kluyvera* sp. The sensitivity of the assay with 40-cycle PCR was verified with one strain of *K. pneumoniae*. The detection limit for *K. pneumoniae* was around 10 copies of genomic DNA.

Example 30

Specific Detection and Identification of *Acinetobacter baumannii* Using atpD Sequences

[0374] The analysis of atpD sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Acinetobacter baumannii*. The primer design strategy is similar to the strategy described in Example 28.

[0375] Two *A. baumannii*-specific primers were selected, SEQ ID NOs. 1690 and 1691, which give an amplification product of 233 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 60° C., followed by terminal extension at 72° C. for 2 minutes.

[0376] Specificity of the assay was tested by adding to the PCR reactions 0.1 ng of genomic DNA from each of the following bacterial species: *Acinetobacter* baumannii (3 strains), *Acinetobacter anitratus, Acinetobacter lwoffi, Serratia marcescens, Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Psychrobacter phenylpyruvicus, Neisseria gonorrheoae, Haemophilus haemoliticus, Yersinia enterolitica, Proteus vulgaris, Eikenella corrodens, Escherichia coli*. Amplification was detected only for *A. baumannii, A. anitratus* and *A. lwoffi*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *A. baumannii*. The detection limit for the two *A. baumannii* strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the atpD gene allowed the design of a *A. baumannii*-specific internal probe (SEQ ID NO. 2169).

Example 31

Specific Detection and Identification of *Neisseria gonorrhoeae* Using tuf Sequences

[0377] The analysis of tuf sequences from a variety of bacterial species allowed the selection of PCR primers specific to *Neisseria gonorrhoeae*. The primer design strategy is similar to the strategy described in Example 28.

[0378] Two *N. gonorrhoeae*-specific primers were selected, SEQ ID NOs. 551 and 552, which give an amplification product of 139 bp. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 65° C., followed by terminal extension at 72° C. for 2 minutes.

[0379] Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the following bacterial species: *Neisseria gonorrhoeae* (19 strains), *Neisseria meningitidis* (2 strains), *Neisseria lactamica, Neisseria flavescens, Neisseria animalis, Neisseria canis, Neisseria cuniculi, Neisseria elongata, Neisseria mucosa, Neisseria polysaccharea, Neisseria sicca, Neisseria subflava, Neisseria weaveri*. Amplification was detected only for *N. gonorrhoeae, N. sicca* and *N. polysaccharea*. The sensitivity of the assay with 40-cycle PCR was verified with two strains of *N. gonorrhoeae*. The detection limit for the *N. gonorrhoeae* strains tested was 5 copies of genomic DNA. Analysis of the multiple alignment sequence of the tuf gene allowed the design of an internal probe, SEQ ID NO. 2166, which can discriminate *N. gonorrhoeae* from *N. sicca* and *N. polysaccharea*.

Example 32

Sequencing of Bacterial gyrA and parC Gene Fragments. SEQUENCING of Bacterial gyrA and parC Fragments

[0380] One of the major mechanism of resistance to quinolone in various bacterial species is mediated by target changes (DNA gyrase and/or topoisomerase IV). These enzymes control DNA topology and are vital for chromosome function and replication. Each of these enzymes is a tetramer composed of two subunits: GyrA and GyrB forming A_2B_2 complex in DNA gyrase; and ParC and ParE forming and C_2E_2 complex in DNA topoisomerase IV. It has been shown that they are hotspots, called the quinolone-resistance-determining region (QRDR) for mutations within gyrA that encodes for the GyrA subunit of DNA gyrase and within parC that encodes the parC subunit of topoisomerase IV.

[0381] In order to generate a database for gyrA and parC sequences that can be used for design of primers and/or probes for the specific detection of quinolone resistance in various bacterial species, gyrA and parC DNA fragments selected from public database (GenBanK and EMBL) from a variety of bacterial species were used to design oligonucleotide primers.

[0382] Using primer pair SEQ ID NOs. 1297 and 1298, it was possible to amplify and determine gyrA sequences from *Klebsiella oxytoca* (SEQ ID NO. 1764), *Klebsiella pneumoniae* subsp. *ozaneae* (SEQ ID NO. 1765), *Klebsiella* planticola (SEQ ID NO. 1766), *Klebsiella pneumoniae* (SEQ ID NO. 1767), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1768-1769), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (SEQ ID NO. 1770), *Klebsiella* terrigena (SEQ ID NO. 1771), Kluyvera ascorbata (SEQ ID NO. 2013), Kluyvera georgiana (SEQ ID NO. 2014) and *Escherichia coli* (4 strains) (SEQ ID NOs. 2277-2280). Using primer pair SEQ ID NOs. 1291 and 1292, it was possible to amplify and determine gyrA sequences from *Legionella pneumophila* subsp. *pneumophila* (SEQ ID NO. 1772), *Proteus mirabilis* (SEQ ID NO. 1773), *Providencia rettgeri* (SEQ ID NO. 1774), *Proteus vulgaris* (SEQ ID NO. 1775) and *Yersinia* enterolitica (SEQ ID NO. 1776). Using primer pair SEQ ID NOs. 1340 and 1341, it was possible to amplify and determine gyrA sequence from *Staphylococcus aureus* (SEQ ID NO. 1255).

[0383] Using primers SEQ ID NOs. 1318 and 1319, it was possible to amplify and determine parC sequences from K oxytoca (two strains) (SEQ ID NOs. 1777-1778), *Klebsiella pneumoniae* subsp. *ozaenae* (SEQ ID NO. 1779), *Klebsiella* planticola (SEQ ID NO. 1780), *Klebsiella pneumoniae* (SEQ ID NO. 1781), *Klebsiella pneumoniae* subsp. *pneumoniae* (two strains) (SEQ ID NOs. 1782-1783), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (SEQ ID NO. 1784) and *Klebsiella* terrigena (SEQ ID NO. 1785).

Example 33

Development of a PCR Assay for the Specific Detection and Identification of *Staphylococcus aureus* and its Quinolone Resistance Genes gyrA and parC

[0384] The analysis of gyrA and parC sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of gyrA and parC from *Staphylococcus aureus*. PCR primer pair SEQ ID NOs. 1340 and 1341 was designed to amplify the gyrA sequence of *S. aureus*, whereas PCR primer pair SEQ ID NOs. 1342 and 1343 was designed to amplify *S. aureus* parC. The comparison of gyrA and parC sequences from *S. aureus* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-84 to Leu, Glu-88 to Gly or Lys in the GyrA subunit of DNA gyrase encoded by gyrA and amino acid changes Ser-80 to Phe or Tyr and Ala-116 to Glu in the ParC subunit of topoisomerase IV encoded by parC. These amino acid substitutions in GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *S. aureus* gyrA (SEQ ID NO. 1940) and wild-type *S. aureus* parC (SEQ ID NO. 1941) as well as internal probes for the specific detection of each of the gyrA (SEQ ID NOs. 1333-1335) and parC mutations identified in quinolone-resistant *S. aureus* (SEQ ID NOs. 1336-1339) were designed.

[0385] The gyrA- and parC-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343) were used in multiplex. PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.3, 0.3, 0.6 and 0.6 μM of each primers, respectively, as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 62° C., followed by terminal extension at 72° C. for 2 minutes. Detection of the PCR products was made by electrophoresis in agarose gels (2%) containing 0.25 μg/ml of ethidium bromide. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-positive bacteria. The list included the following: *Abiotrophia adiacens, Abiotrophia defectiva, Bacillus cereus, Bacillus mycoides, Enterococcus faecalis* (2 strains), *Enterococcus flavescens, Gemella morbillorum, Lactococcus lactis, Listeria innocua, Listeria monocytogenes, Staphylococcus aureus* (5 strains), *Staphylococcus auricalis, Staphylococcus capitis* subsp. *urealyticus, Staphylococcus carnosus, Staphylococcus chromogenes, Staphylococcus epidermidis* (3 strains), *Staphylococcus gallinarum, Staphylococcus haemolyticus* (2 strains), *Staphylococcus hominis, Staphylococcus hominis* subsp *hominis, Staphylococcuslentus, Staphylococcus lugdunensis, Staphylococcus saccharolyticus, Staphylococcus saprophyticus* (3 strains), *Staphylococcus simulans, Staphylococcus warneri, Staphylococcus xylosus, Streptococcus agalactiae, Streptococcus pneumoniae*. Strong amplification of both gyrA and parC genes was only detected for the *S. aureus* strains tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with one quinolone-sensitive and four quinolone-resistant strains of *S. aureus*. The detection limit was 2 to 10 copies of genomic DNA, depending on the strains tested.

[0386] Detection of the hybridization with the internal probes was performed as described in Example 7. The internal probes specific to wild-type gyrA and parC of *S. aureus* and to the gyrA and parC variants of *S. aureus* were able to recognize two quinolone-resistant and one quinolone-sensitive *S. aureus* strains showing a perfect correlation with the susceptibility to quinolones.

[0387] The complete assay for the specific detection of *S. aureus* and its susceptibility to quinolone contains the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7 and the multiplex containing the *S. aureus* gyrA- and parC-specific primer pairs (SEQ ID NOs. 1340-1341 and SEQ ID NOs. 1342-1343). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. aureus* (SEQ ID NO. 587) described in Example 7 and the internal probes specific to wild-type *S. aureus* gyrA and parC (SEQ ID NOs. 1940-1941) and to the *S. aureus* gyrA and parC variants (SEQ ID NOs. 1333-1338).

[0388] An assay was also developed for the detection of quinolone-resistant *S. aureus* using the SmartCycler (Cepheid). Real-time detection is based on the use of *S. aureus* parC-specific primers (SEQ ID NOs. 1342 and 1343) and the *Staphylococcus*-specific primers (SEQ ID NOs. 553 and 575) described in Example 7. Internal probes were designed for molecular beacon detection of the wild-type *S. aureus* parC (SEQ ID NO. 1939), for detection of the Ser-80 to Tyr or Phe amino acid substitutions in the ParC subunit encoded by *S. aureus* parC (SEQ ID NOs. 1938 and 1955) and for detection of *S. aureus* (SEQ ID NO. 2282).

Example 34

Development of a PCR Assay for the Detection and Identification of *Klebsiella pneumoniae* and its Quinolone Resistance Genes gyrA and parC

[0389] The analysis of gyrA and parC sequences from a variety of bacterial species from the public databases and from the database described in Example 32 revealed conserved regions allowing the design of PCR primers specific to the quinolone-resistance-determining region (QRDR) of gyrA and parC from *K. pneumoniae*. PCR primer pair SEQ ID NOs. 1936 and 1937, or pair SEQ ID NOs. 1937 and 1942, were designed to amplify the gyrA sequence of *K. pneumoniae*, whereas PCR primer pair SEQ ID NOs. 1934 and 1935 was designed to amplify *K. pneumoniae* parC sequence. An alternative pair, SEQ ID NOs. 1935 and 1936, can also amplify *K. pneumoniae* parC. The comparison of gyrA and parC sequences from *K. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-83 to Tyr or Phe and Asp-87 to Gly or Ala and Asp-87 to Asn in the GyrA subunit of DNA gyrase encoded by gyrA and amino acid changes Ser-80 to Ile or Arg and Glu-84 to Gly or Lys in the ParC subunit of topoisomerase IV encoded by parC. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of wild-type *K. pneumoniae* gyrA (SEQ ID NO. 1943) and wild-type *K. pneumoniae* parC (SEQ ID NO. 1944) as well as internal probes for the specific detection of each of the gyrA (SEQ ID NOs. 1945-1949) and parC mutations identified in quinolone-resistant *K. pneumoniae* (SEQ ID NOs. 1950-1953) were designed.

[0390] Two multiplex using the *K. pneumoniae* gyrA- and parC-specific primer pairs were used: the first multiplex contained *K. pneumoniae* gyrA-specific primers (SEQ ID NOs. 1937 and 1942) and *K. pneumoniae* parC-specific primers (SEQ ID NOs. 1934 and 1935) and the second multiplex contained *K. pneumoniae* gyrA/parC-specific primer (SEQ ID NOs. 1936), *K. pneumoniae* gyrA-specific primer (SEQ ID NO. 1937) and *K. pneumoniae* parC-specific primer (SEQ ID NO. 1935). Standard PCR was carried out on PTC-200 thermocyclers (MJ Research) using for the first multiplex 0.6, 0.6, 0.4, 0.4 μM of each primer, respectively, and for the second multiplex 0.8, 0.4, 0.4 μM of each primer, respectively. PCR amplification and agarose gel electrophoresis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 62° C., followed by terminal extension at 72° C. for 2 minutes. The specificity of the two multiplex assays with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of gram-negative bacteria. The list included: *Acinetobacter baumannii, Citrobacter freundii, Eikenella corrodens, Enterobacter aerogenes, Enterobacter cancerogenes, Enterobacter cloacae, Escherichia coli* (10 strains), *Haemophilus influenzae, Klebsiella pneumoniae, Klebsiella ornitholytica, Klebsiella oxytoca* (2 strains), *Klebsiella planticola, Klebsiella terrigena, Kluyvera ascorbata, Kluyvera cryocrescens, Kluyvera georgiana, Neisseria gonorrhoeae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella choleraesuis* subsp. *typhimurium, Salmonella enteritidis, Serratia liquefaciens, Serratia marcescens* and *Yersinia enterocolytica*. For both multiplex, strong amplification of both gyrA and parC was observed only for the *K. pneumoniae* strain tested. The sensitivity of the two multiplex assays with 40-cycle PCR was verified with one quinolone-sensitive strain of *K. pneumoniae*. The detection limit was around 10 copies of genomic DNA.

[0391] The complete assay for the specific detection of *K. pneumoniae* and its susceptibility to quinolone contains the *Klebsiella*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29 and either the multiplex containing the *K. pneumoniae* gyrA- and parC-specific primers (SEQ ID NOs. 1935, 1936, 1937) or the multiplex containing the *K. pneumoniae* gyrA- and parC-specific primers (SEQ ID NOs. 1934, 1937, 1939, 1942). Amplification is coupled with post-PCR hybridization with the internal probe specific to *K. pneumoniae* (SEQ ID NO. 2167) described in Example 29 and the internal probes specific to wild-type *K. pneumoniae* gyrA and parC (SEQ ID NOs. 1943, 1944) and to the *K. pneumoniae* gyrA and parC variants (SEQ ID NOs. 1945-1949 and 1950-1953).

[0392] An assay was also developed for the detection of quinolone-resistant *K. pneumoniae* using the SmartCycler (Cepheid). Real-time detection is based on the use of resistant *K. pneumoniae* gyrA-specific primers (SEQ ID NOs. 1936 and 1937) and the *K. pneumoniae*-specific primers (SEQ ID NOs. 1331 and 1332) described in Example 29. Internal probes were designed for molecular beacon detection of the wild-type *K. pneumoniae* gyrA (SEQ ID NO. 2251), for detection of the Ser-83 to Tyr or Phe and/or Asp-87 to Gly or Asn in the GyrA subunit of DNA gyrase encoded by gyrA (SEQ ID NOs. 2250) and for detection of *K. pneumoniae* (SEQ ID NO. 2281).

Example 35

Development of a PCR Assay for Detection and Identification of *S. pneumoniae* and its Quinolone Resistance Genes gyrA and parC

[0393] The analysis of gyrA and parC sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify the quinolone-resistance-determining region (QRDR) of gyrA and parC from all *S. pneumoniae* strains. PCR primer pair SEQ ID NOs. 2040 and 2041 was designed to amplify the QRDR of *S. pneumoniae* gyrA, whereas PCR primer pair SEQ ID NOs. 2044 and 2045 was designed to amplify the QRDR of *S. pneumoniae* parC. The comparison of gyrA and parC sequences from *S. pneumoniae* strains with various levels of quinolone resistance allowed the identification of amino acid substitutions Ser-81 to Phe or Tyr in the GyrA subunit of DNA gyrase encoded by gyrA and amino acid changes Ser-79 to Phe in the ParC subunit of topoisomerase IV encoded by parC. These amino acid substitutions in the GyrA and ParC subunits occur in isolates with intermediate- or high-level quinolone resistance. Internal probes for the specific detection of each of the gyrA (SEQ ID NOs. 2042 and 2043) and parC (SEQ ID NO. 2046) mutations identified in quinolone-resistant *S. pneumoniae* were designed.

[0394] For all bacterial species, amplification was performed from purified genomic DNA. 1 μl of genomic DNA at 0.1 ng/μL was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM (each) of the above primers SEQ ID NOs. 2040, 2041, 2044 and 2045, 0.05 mM bovine serum albumin (BSA) and 0.5 U Taq polymerase coupled with TaqStart™ antibody. The optimal cycling conditions for maximum sensitivity' and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 58° C., followed by terminal extension at 72° C. for 2 minutes. In order to generate Digoxigenin (DIG)-labeled amplicons for capture probe hybridization, 0.1×PCR DIG labeling four deoxynucleoside triphosphates mix (Boehringer Mannheim GmbH) was used for amplification.

[0395] The DIG-labeled amplicons were hybridized to the capture probes bound to 96-well plates. The plates were incubated with anti-DIG-alkaline phosphatase and the chemiluminescence was measured by using a luminometer (MLX, Dynex Technologies Inc.) after incubation with CSPD and recorded as Relative Light Unit (RLU). The RLU ratio of tested sample with and without captures probes was then calculated. A ratio 2.0 was defined as a positive hybridization signal. All reactions were performed in duplicate.

[0396] The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria listed in Table 13. Strong amplification of both gyrA and parC was detected only for the *S. pneumoniae* strains tested. Weak amplification of both gyrA and parC genes was detected for *Staphylococcus simulans*. The detection limit tested with purified genomic DNA from 5 strains of *S. pneumoniae* was 1 to 10 genome copies. In addition, 5 quinolone-resistant and 2 quinolone-sensitive clinical isolates of *S. pneumoniae* were tested to further validate the developed multiplex PCR coupled with capture probe hybridization assays. There was a perfect correlation between detection of *S. pneumoniae* gyrA and parC mutations and the susceptibility to quinolone.

[0397] The complete assay for the specific detection of *S. pneumoniae* and its susceptibility to quinolone contains the *S. pneumoniae*-specific primers (SEQ ID NOs. 1179 and 1181) described in Example 20 and the multiplex containing the *S. pneumoniae* gyrA-specific and parC-specific primer pairs (SEQ ID NOS. 2040 and 2041 and SEQ ID NOs. 2044 and 2045). Amplification is coupled with post-PCR hybridization with the internal probe specific to *S. pneumoniae* (SEQ ID NO. 1180) described in Example and the internal probes specific to each of the *S. pneumoniae* gyrA and parC variants (SEQ ID NOs. 2042, 2043 and 2046).

Example 36

Detection of Extended-Spectrum TEM-Type β-Lactamases in *Escherichia coli*

[0398] The analysis of TEM sequences which confer resistance to third-generation cephalosporins and to β-lactamase inhibitors allowed the identification of amino acid substitutions Met-69 to Ile or Leu or Val, Ser-130 to Gly, Arg-164 to Ser or His, Gly-238 to Ser, Glu-240 to Lys and Arg-244 to Ser or Cys or Thr or His or Leu. PCR primers SEQ ID NOs. 1907 and 1908 were designed to amplify TEM sequences. Internal probes for the specific detection of wild-type TEM (SEQ ID NO. 2141) and for each of the amino acid substitutions (SEQ ID NOs. 1909-1926) identified in TEM variants were designed to detect resistance to third-generation cephalosporins and to β-lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

[0399] For all bacterial species, amplification was performed from purified genomic DNA. One μl of genomic DNA at 0.1 ng/μl was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of the TEM-specific primers SEQ ID NOs. 1907 and 1908, 200 μM (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U Taq polymerase (Promega) coupled with TaqStart™ antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95° C., 30 seconds at 55° C. and 30 seconds at 72° C., followed by terminal extension at 72° C. for 2 minutes.

[0400] The specificity of the TEM-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β-lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). Amplification with the TEM-specific primers was detected only for strains containing TEM.

[0401] The sensitivity of the assay with 40-cycle PCR was verified with three *E. coli* strains containing TEM-1 or TEM-10 or TEM-49, one *K. pneumoniae* strain containing TEM-47 and one *P. mirabilis* strain containing TEM-39. The detection limit was 5 to 100 copies of genomic DNA, depending on the TEM-containing strains tested.

[0402] The TEM-specific primers SEQ ID NOs. 1907 and 1908 were used in multiplex with the *Escherichia coli/Shigella* sp.-specific primers SEQ ID NOs. 1661 and 1665 described in Example 28 to allow the complete identification of *Escherichia coli/Shigella* sp. and the susceptibility to β-lactams. PCR amplification with 0.4 μM of each of the primers and agarose gel analysis of the amplified products was performed as described above.

[0403] The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three third-generation cephalosporin-resistant *Escherichia coli* strains (one with TEM-10, one with TEM-28 and the other with TEM-49), two third-generation cephalosporin-sensitive *Escherichia coli* strain (one with TEM-1 and the other without TEM), one third-generation cephalosporin-resistant *Klebsiella pneumoniae* strain (with TEM-47), and one β-lactamase-inhibitor-resistant *Proteus mirabilis* strain (with TEM-39). The multiplex was highly specific to *Escherichia coli* strains containing TEM.

[0404] The complete assay for detection of TEM-type β-lactamases in *E. coli* includes PCR amplification using the multiplex containing the TEM-specific primers (SEQ ID NOs. 1907 and 1908) and the *Escherichia coli/Shigella* sp.-specific primers (SEQ ID NOs. 1661 and 1665) coupled with post PCR-hybridization with the internal probes specific to wild-type TEM (SEQ ID NO. 2141) and to the TEM variants (SEQ ID NOs. 1909-1926).

Example 37

Detection of Extended-Spectrum SHY-Type β-Lactamases in *Klebsiella pneumoniae*

[0405] The comparison of SHV sequences, which confer resistance to third-generation cephalosporins and to β-lactamase inhibitors, allowed the identification of amino acid substitutions Ser-130 to Gly, Asp-179 to Ala or Asn, Gly-238 to Ser, and Glu-240 to Lys. PCR primer pair SEQ ID NOs. 1884 and 1885 was designed to amplify SHV sequences. Internal probes for the specific identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants were designed to detect resistance to third-generation cephalosporins and to β-lactamase inhibitors. Design and synthesis of primers and probes, and detection of the hybridization were performed as described in Example 7.

[0406] For all bacterial species, amplification was performed from purified genomic DNA. One μl of genomic DNA at 0.1 ng/μl was transferred directly to a 19 μl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of the SHV-specific primers SEQ ID NO. 1884 and 1885, 200 μM (each) of the four deoxynucleoside triphosphates, 0.05 mM bovine serum albumin (BSA) and 0.5 U Taq polymerase (Promega) coupled with TaqStart™ antibody. PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 95° C. for initial denaturation, then forty cycles of three steps consisting of 5 seconds at 95° C., 30 seconds at 55° C. and 30 seconds at 72° C., followed by terminal extension at 72° C. for 2 minutes.

[0407] The specificity of the SHV-specific primers with 40-cycle PCR was verified by using 0.1 ng of purified genomic from the following bacteria: two third-generation cephalosporin-resistant *Klebsiella pneumoniae* strains (one with SHV-2a and the other with SHV-12), one third-generation cephalosporin-sensitive *Klebsiella pneumoniae* strain (with SHV-1), two third-generation cephalosporin-resistant *Escherichia coli* strains (one with SHV-8 and the other with SHV-7), and two third-generation cephalosporin-sensitive *Escherichia coli* strains (one with SHV-1 and the other without any SHV). Amplification with the SHV-specific primers was detected only for strains containing SHV.

[0408] The sensitivity of the assay with 40-cycle PCR was verified with four strains containing SHV. The detection limit was 10 to 100 copies of genomic DNA, depending on the SHV-containing strains tested.

[0409] The amplification was coupled with post-PCR hybridization with the internal probes specific for identification of wild-type SHV (SEQ ID NO. 1896) and for each of the amino acid substitutions (SEQ ID NOs. 1886-1895 and 1897-1898) identified in SHV variants. The specificity of the probes was verified with six strains containing various SHV enzymes, one *Klebsiella pneumoniae* strain containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one *Klebsiella pneumoniae* strain containing SHV-12, one *Escherichia coli* strain containing SHV-1, one *Escherichia coli* strain containing SHV-7 and one *Escherichia coli* strain containing SHV-8. The probes correctly detected each of the SHV genes and their specific mutations. There was a perfect correlation between the SHV genotype of the strains and the susceptibility to β-lactam antibiotics.

[0410] The SHV-specific primers SEQ ID NOs. 1884 and 1885 were used in multiplex with the *K. pneumoniae*-specific primers SEQ ID NOs. 1331 and 1332 described in Example 29 to allow the complete identification of *K. pneumoniae* and the susceptibility to β-lactams. PCR amplification with 0.4 μM of each of the primers and agarose gel analysis of the amplified products were performed as described above.

[0411] The specificity of the multiplex with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: three *K. pneumoniae* strains containing SHV-1, one *Klebsiella pneumoniae* strain containing SHV-2a, one *Klebsiella pneumoniae* strain containing SHV-12, one *K. rhinoscleromatis* strain containing SHV-1, one *Escherichia coli* strain without SHV. The multiplex was highly specific to *Klebsiella pneumoniae* strain containing SHV.

Example 38

Development of a PCR Assay for the Detection and Identification of *Neisseria gonorrhoeae* and its associated tetracycline resistance gene tetM

[0412] The analysis of publicly available tetM sequences revealed conserved regions allowing the design of PCR primers specific to tetM sequences. The PCR primer pair SEQ ID NOs. 1588 and 1589 was used in multiplex with the *Neisseria gonorrhoeae*-specific primers SEQ ID NOs. 551 and 552 described in Example 31. Sequence alignment analysis of tetM sequences revealed regions suitable for the design of an internal probe specific to tetM (SEQ ID NO. 2254). PCR amplification was carried out on PTC-200 thermocyclers (MJ Research) using 0.4 μM of each primer pair as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follow: three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 60° C., followed by terminal extension at 72° C. for 2 minutes.

[0413] The specificity of the multiplex PCR assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from the following bacteria: two tetracycline-resistant *Escherichia coli* strains (one containing the tetracycline-resistant gene tetB and the other containing the tetracycline-resistant gene tetC), one tetracycline-resistant *Pseudomonas aeruginosa* strain (containing the tetracycline-resistant gene tetA), nine tetracycline-resistant *Neisseria gonorrhoeae* strains, two tetracycline-sensitive *Neisseria meningitidis* strains, one tetracycline-sensitive *Neisseria polysaccharea* strain, one tetracycline-sensitive *Neisseria sicca* strain and one tetracycline-sensitive *Neisseria* subflava strain. Amplification with both the tetM-specific and *Neisseria gonorrhoeae*-specific primers was detected only for *N. gonorrhoeae* strains containing tetM. There was a weak amplification signal using *Neisseria gonorrhoeae*-specific primers for the following species: *Neisseria sicca, Neisseria polysaccharea* and *Neisseria meningitidis*. There was a perfect correlation between the tetM genotype and the tetracycline susceptibility pattern of the *Neisseria gonorrhoeae* strains tested. The internal probe specific to *N. gonorrhoeae* SEQ ID NO. 2166 described in Example 31 can discriminate *Neisseria gonorrhoeae* from the other *Neisseria* sp.

[0414] The sensitivity of the assay with 40-cycle PCR was verified with two tetracycline resistant strains of *N. gonorrhoeae*. The detection limit was 5 copies of genomic DNA for both strains.

Example 39

Development of a PCR Assay for the Detection and Identification of *Shigella* Sp. and their Associated Trimethoprim Resistance Gene dhfrIa

[0415] The analysis of publicly available dhfrIa and other dhfr sequences revealed regions allowing the design of PCR primers specific to dhfrIa sequences. The PCR primer pair (SEQ ID NOs. 1459 and 1460) was used in multiplex with the *Escherichia coli/Shigella* sp.-specific primers SEQ ID NOs. 1661 and 1665 described in Example 28. Sequence alignment analysis of dhfrIa sequences revealed regions suitable for the design of an internal probe specific to dhfrIa (SEQ ID NO. 2253). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28 with an annealing temperature of 60° C. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria. The list included the following trimethoprim-sensitive strains, *Salmonella typhimyurium, Salmonella typhi, Salmonella enteritidis, Tatumella ptyseos, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter farmeri, Campylobacter jejuni, Serratia marcescens, Shigella dysenteriae, Shigella flexneri, Shigella sonnei*, six trimethoprim-resistant *Escherichia coli* strains (containing dhfrIa or dhfrV or dhfrVII or dhfrXII or dhfrXIII or dhfrXV), four trimethoprim-resistant strains containing dhfrIa (*Shigella sonnei, Shigella flexneri, Shigella dysenteriae* and *Escherichia coli*). There was a perfect correlation between the dhfrIa genotype and the trimethoprim susceptibility pattern of the *Escherichia coli* and *Shigella* sp. strains tested. The dhfrIa primers were specific to the dhfrIa gene and did not amplify any of the other trimethoprim-resistant dhfr genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with three strains of trimethoprim-resistant strains of *Shigella* sp. The detection limit was 5 to 10 genome copies of DNA, depending on the *Shigella* sp. strains tested.

Example 40

Development of a PCR Assay for the Detection and Identification of *Acinetobacter baumannii* and its Associated Aminoglycoside Resistance Gene aph (3')-VIa

[0416] The comparison of publicly available aph(3')-VIa sequence revealed regions allowing the design of PCR primers specific to aph(3)-VIa. The PCR primer pair (SEQ ID NOs. 1404 and 1405) was used in multiplex with the *Acinetobacter baumannii*-specific primers SEQ ID NOs. 1692 and 1693 described in Example 30. Analysis of the aph(3)-VIa sequence revealed region suitable for the design of an internal probe specific to aph(3')-VIa (SEQ ID NO. 2252). PCR amplification and agarose gel analysis of the amplified products were performed as described in Example 28. The specificity of the multiplex assay with 40-cycle PCR was verified by using 0.1 ng of purified genomic DNA from a panel of bacteria including: two aminoglycoside-resistant *A. baumanni* strains (containing aph(3)-VIa), one aminoglycoside-sensitive *A. baumani* strain, one of each of the following aminoglycoside-resistant bacteria, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene aacC1, one *Serratia marcescens* strain containing the aminoglycoside-resistant gene aacC4, one *Enterobacter cloacae* strain containing the aminoglycoside-resistant gene aacC2, one *Enterococcus faecalis* containing the aminoglycoside-resistant gene aacA-aphD, one *Pseudomonas aeruginosa* strain containing the aminoglycoside-resistant gene aac6IIa and one of each of the following aminoglycoside-sensitive bacterial species, *Acinetobacter anitratus, Acinetobacter lwoffi, Psychobbacter phenylpyruvian, Neisseria gonorrhoeae, Haemophilus haemolyticus, Haemophilus influenzae, Yersinia enterolitica, Proteus vulgaris, Eikenella corrodens, Escherichia coli*. There was a perfect correlation between the aph(3')-VIa genotype and the aminoglycoside susceptibility pattern of the *A. baumannii* strains tested. The aph(3)-VIa-specific primers were specific to the aph(3)-VIa gene and did not amplify any of the other aminoglycoside-resistant genes tested. The sensitivity of the multiplex assay with 40-cycle PCR was verified with two strains of aminoglycoside-resistant strains of *A. baumannii*. The detection limit was 5 genome copies of DNA for both *A. baumannii* strains tested.

Example 41

Specific Identification of *Bacteroides fragilis* Using atpD (V-Type) Sequences

[0417] The comparison of atpD (V-type) sequences from a variety of bacterial species allowed the selection of PCR primers for *Bacteroides fragilis*. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various atpD sequences from *B. fragilis*, as well as atpD sequences from the related species *B. dispar*, bacterial genera and archaea, especially representatives with phylogenetically related atpD sequences. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species, especially from closely related species *B. dispar,* thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

[0418] The chosen primer pair, SEQ ID NOs. 2134-2135, produces an amplification product of 231 bp. Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc.) using 0.4 μM of each primers pair as described in Example 28. The optimal cycling conditions for maximum sensitivity and specificity were as follows: three minutes at 95° C. for initial denaturation, then forty cycles of two steps consisting of 1 second at 95° C. and 30 seconds at 60° C., followed by terminal extension at 72° C. for 2 minutes.

[0419] The format of this assay is not limited to the one described above. A person skilled in the art could adapt the assay for different formats such as PCR with real-time detection using molecular beacon probes. Molecular beacon probes designed to be used in this assay include, but are not limited to, SEQ ID NO. 2136 for the detection of the *B. fragilis* amplicon.

Example 42

Evidence for Horizontal Gene Transfer in the Evolution of the Elongation Factor Tu in Enterococci

Overview

[0420] The elongation factor Tu, encoded by tuf genes, is a GTP binding protein that plays a central role in protein synthesis. One to three tuf genes per genome are present depending on the bacterial species. Most low G+C gram-positive bacteria carry only one tuf gene. We have designed degenerate PCR primers derived from consensus sequences of the tuf gene to amplify partial tuf sequences from 17 enterococcal species and other phylogenetically related species. The amplified DNA fragments were sequenced either by direct sequencing or by sequencing cloned inserts containing putative amplicons. Two different tuf genes (tufA and tufB) were found in 11 enterococcal species, including *Enterococcus avium, E. casseliflavus, E. dispar, E. durans, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium*, and *E. raffinosus*. For the other six enterococcal species (*E. cecorum, E. columbae, E. faecalis, E. sulfureus, E. saccharolyticus*, and *E. solitarius*), only the tufA gene was present. Based on 16S rRNA gene sequence analysis, the 11 species having two tuf genes all share a common ancestor, while the six species having only one copy diverged from the enterococcal lineage before that common ancestor. The presence of one or two copies of the tuf gene in enterococci was confirmed by Southern hybridization. Phylogenetic analysis of tuf sequences demonstrated that the enterococcal tufA gene branches with the *Bacillus, Listeria* and *Staphylococcus* genera, while the enterococcal tufB gene clusters with the genera *Streptococcus* and *Lactococcus*. Primary structure, analysis showed that four amino acid residues within the sequenced regions are conserved and unique to the enterococcal tufB genes and the tuf genes of streptococci and *L. lactis*. The data suggest that an ancestral *streptococcus* or a *streptococcus*-related species may have horizontally transferred a tuf gene to the common ancestor of the 11 enterococcal species which now carry two tuf genes.

Introduction

[0421] The elongation factor Tu (EF-Tu) is a GTP binding protein playing a central role in protein synthesis. It mediates the recognition and transport of aminoacyl-tRNAs and their positioning to the A-site of the ribosome. The highly conserved function and ubiquitous distribution render the elongation factor a valuable phylogenetic marker among eubacteria and even throughout the archaebacterial and eukaryotic kingdoms. The tuf genes encoding elongation factor Tu are present in various copy numbers per bacterial genome. Most gram-negative bacteria contain two tuf genes. As found in *Escherichia coli*, the two genes, while being almost identical in sequence, are located in different parts of the bacterial chromosome. However, recently completed microbial genomes revealed that only one tuf gene is found in *Helicobacter pylori* as well as in some obligate parasitic bacteria, such as *Borrelia burgdorferi, Rickettsia prowazekii*, and *Treponema pallidum*, and in some cyanobacteria. In most gram-positive bacteria studied so far, only one tuf gene was found. However, Southern hybridization showed that there are two tuf genes in some clostridia as well as in *Streptomyces coelicolor* and *S. lividans*. Up to three tuf-like genes have been identified in *S. ramocissimus*.

[0422] Although massive prokaryotic gene transfer is suggested to be one of the factors responsible for the evolution of bacterial genomes, the genes encoding components of the translation machinery are thought to be highly conserved and difficult to be transferred horizontally due to the complexity of their interactions. However, a few recent studies demonstrated evidence that horizontal gene transfer has also occurred in the evolution of some genes coding for the translation apparatus, namely, 16S rRNA and some aminoacyl-tRNA synthetases. No further data suggest that such a mechanism is involved in the evolution of the elongation factors. Previous studies concluded that the two copies of tuf genes in the genomes of some bacteria resulted from an ancient event of gene duplication. Moreover, a study of the tuf gene in *R. prowazekii* suggested that intrachromosomal recombination has taken place in the evolution of the genome of this organism.

[0423] To date, little is known about the tuf genes of enterococcal species. In this study, we analyzed partial sequences of tuf genes in 17 enterococcal species, namely, *E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. solitarius*, and *E. sulfureus*. We report here the presence of two divergent copies of tuf genes in 11 of these enterococcal species. The 6 other species carried a single tuf gene. The evolutionary implications are discussed.

Materials and Methods

[0424] Bacterial strains. Seventeen enterococcal strains and other gram-positive bacterial strains obtained from the American Type Culture Collection (ATCC, Manassas, Va.) were used in this study (Table 16). All strains were grown on sheep blood agar or in brain-heart infusion broth prior to DNA isolation.

[0425] DNA isolation. Bacterial DNAs were prepared using the G NOME DNA extraction kit (Bio101, Vista, Calif.) as previously described.

[0426] Sequencing of putative tuf genes. In order to obtain the tuf gene sequences of enterococci and other gram-positive bacteria, two sequencing approaches were used: 1) sequencing of cloned PCR products and 2) direct sequencing of PCR products. A pair of degenerate primers (SEQ ID NOs. 664 and 697) were used to amplify an 886-bp portion of the tuf genes from enterococcal species and other gram-positive bacteria as previously described. For *E. avium, E. casseliflavus*, E. dispar, *E. durans, E. faecium, E. gallinarum, E. hirae, E. mundtii, E. pseudoavium*, and *E. raffinosus*, the amplicons were cloned using the Original TA cloning kit (Invitrogen, Carlsbad, Calif.) as previously described. Five clones for each species were selected for sequencing. For *E. cecorum, E. faecalis, E. saccharolyticus*, and *E. solitarius* as well as the other gram-positive bacteria, the sequences of the 886-bp amplicons were obtained by direct sequencing. Based on the results obtained from the earlier rounds of sequencing, two pairs of primers were designed for obtaining the partial tuf sequences from the other enterococcal species by direct sequencing. One pair of primers (SEQ ID NOs. 543 and 660) were used to amplify the enterococcal tuf gene fragments from E. columbae, *E. malodoratus*, and E. sulfureus. Another pair of primers (SEQ ID NOs. 664 and 661) were used to amplify the second tuf gene fragments from *E. avium, E. malodoratus*, and *E. pseudoavium.*

[0427] Prior to direct sequencing, PCR products were electrophoresed on 1% agarose gel at 120V for 2 hours. The gel was then stained with 0.02% methylene blue for 30 minutes and washed twice with autoclaved distilled water for 15 minutes. The gel slices containing PCR products of the expected sizes were cut out and purified with the QIAquick gel extraction kit (QIAgen Inc., Mississauga, Ontario, Canada) according to the manufacturer's instructions. PCR mixtures for sequencing were prepared as described previously. DNA sequencing was carried out with the Big Dye™ Terminator Ready Reaction cycle sequencing kit using a 377 DNA sequencer (PE Applied Biosystems, Foster City, Calif.). Both strands of the amplified DNA were sequenced. The sequence data were verified using the Sequencer™ 3.0 software (Gene Codes Corp., Ann Arbor, Mich.).

[0428] Sequence analysis and phylogenetic study. Nucleotide sequences of the tuf genes and their respective flanking regions for *E. faecalis, Staphylococcus aureus*, and *Streptococcus pneumoniae*, were retrieved from the TIGR microbial genome database and *S. pyogenes* from the University of Oklahoma database. DNA sequences and deduced protein sequences obtained in this study were compared with those in all publicly available databases using the BLAST and FASTA programs. Unless specified, sequence analysis was conducted with the programs from GCG package (Version 10; Genetics Computer Group, Madison, Wis.). Sequence alignment of the tuf genes from 74 species representing all three kingdoms of life (Tables 16 and 17) were carried out by use of Pileup and corrected upon visual analysis. The N- and C-termini extremities of the sequences were trimmed to yield a common block of 201 amino acids sequences and equivocal residues were removed. Phylogenetic analysis was performed with the aid of PAUP 4.0b4 written by Dr. David L. Swofford (Sinauer Associates, Inc., Publishers, Sunderland, Mass.). The distance matrix and maximum parsimony were used to generate phylogenetic trees and bootstrap resampling procedures were performed using 500 and 100 replications in each analysis, respectively.

[0429] Protein structure analysis. The crystal structures of (i)*Thermus aquaticus* EF-Tu in complex with Phe-tRNA^{Phe} and a GTP analog and (ii) *E. coli* EF-Tu in complex with GDP served as templates for constructing the equivalent models for enterococcal EF-Tu. Homology modeling of protein structure was performed using the SWISS-MODEL server and inspected using the SWISS-PDB viewer version 3.1.

[0430] Southern hybridization. In a previous study, we amplified and cloned an 803-bp PCR product of the tuf gene fragment from *E. faecium*. Two divergent sequences of the inserts, which we assumed to be tufA and tufB genes, were obtained. The recombinant plasmid carrying either tufA or tufB sequence was used to generate two probes labeled with Digoxigenin (DIG)-11-dUTP by PCR incorporation following the instructions of the manufacturer (Boehringer Mannheim, Laval, Québec, Canada). Enterococcal genomic DNA samples (1-2 µg) were digested to completion with restriction endonucleases BglII and XbaI as recommended by the supplier (Amersham Pharmacia Biotech, Mississauga, Ontario, Canada). These restriction enzymes were chosen because no restriction sites were observed within the amplified tuf gene fragments of most enterococci. Southern blotting and filter hybridization were performed using positively charged nylon membranes (Boehringer Mannheim) and QuikHyb hybridization solution (Stratagene Cloning Systems, La Jolla, Calif.) according to the manufacturers' instructions with modifications. Twenty μl of each digestion were electrophoresed for 2 h at 120V on a 0.8% agarose gel. The DNA fragments were denatured with 0.5 M NaOH and transferred by Southern blotting onto a positively charged nylon membrane (Boehringer Mannheim). The filters were pre-hybridized for 15 min and then hybridized for 2 h in the QuikHyb solution at 68° C. with either DIG-labeled probe. Posthybridization washings were performed twice with 0.5×SSC, 1% SDS at room temperature for 15 min and twice in the same solution at 60° C. for 15 min. Detection of bound probes was achieved using disodium 3-(4-methoxyspiro(1,2-dioxetane-3,2'-(5'-chloro) tricyclo(3,3.1.$1^{3.7}$) decan)-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim) as specified by the manufacturer.

[0431] GenBank submission. The GenBank accession numbers for partial tuf gene sequences generated in this study are given in Table 16.

Results

[0432] Sequencing and nucleotide sequence analysis. In this study, all gram-positive bacteria other than enterococci yielded a single tuf sequence of 886 by using primers SEQ ID NOs. 664 and 697 (Table 16). Each of four enterococcal species including E. cecorum, *E. faecalis*, E. saccharolyticus, and *E. solitarius* also yielded one 886-bp tuf sequence. On the other hand, for *E. avium, E. casseliflavus*, E. dispar, *E. durans, E. faecium, E. gallinarum, E. hirae, E. mundtii, E. pseudoavium*, and *E. raffinosus*, direct sequencing of the 886-bp fragments revealed overlapping peaks according to their sequence chromatograms, suggesting the presence of additional copies of the tuf gene. Therefore, the tuf gene fragments of these 10 species were cloned first and then sequenced. Sequencing data revealed that two different types of tuf sequences (tufA and tufB) are found in eight of these species including *E. casseliflavus, E. dispar, E. durans, E. faecium, E. gallinarum, E. hirae, E. mundtii*, and *E. raffinosus*. Five clones from *E. avium* and *E. pseudoavium* yielded only a single tuf sequence. These new sequence data allowed the design of new primers specific for the enterococcal tufA or tufB sequences. Primers SEQ ID NOs. 543 and 660 were designed to amplify only enterococcal tufA sequences and a 694-bp fragment was amplified from all 17 enterococcal species. The 694-bp sequences of tufA genes from E. columbae, *E. malodoratus*, and *E. sulfureus* were obtained by direct sequencing using these primers. Primers SEQ ID NOs. 664 and 661 were designed for the amplification of 730-bp portion of tufB genes and yielded the expected fragments from 11 enterococcal species, including *E. malodoratus* and the 10 enterococcal species in which heterogeneous tuf sequences were initially found. The sequences of the tufB fragments for *E. avium, E. malodoratus* and *E. pseudoavium* were determined by direct sequencing using the primers SEQ ID NOs. 664 and 661. Overall, tufA gene fragments were obtained from all 17 enterococcal species but tufB gene fragments were obtained with only 11 enterococcal species (Table 16).

[0433] The identities between tufA and tufB for each enterococcal species were 68-79% at the nucleotide level and 81 to 89% at the amino acid level. The tufA gene is highly conserved among all enterococcal species with identities varying from 87% to 99% for DNA and 93% to 99% for amino acid sequences, while the identities among tufB genes of enterococci varies from 77% to 92% for DNA and 91% to 99% for amino acid sequences, indicating their different origins and evolution (Table 18). Since *E. solitarius* has been transferred to the genus *Tetragenococcus*, which is also a low G+C gram-positive bacterium, our sequence comparison did not include this species as an *enterococcus*. G+C content of enterococcal tufA sequences ranged from 40.8% to 43.1%, while that of enterococcal tufB sequences varied from 37.8% to 46.3%. Based on amino acid sequence comparison, the enterococcal tufA gene products share higher identities with those of *Abiotrophia adiacens, Bacillus subtilis, Listeria monocytogenes, S. aureus*, and *S. epidermidis*. On the other hand, the enterococcal tufB gene products share higher percentages of amino acid identity with the tuf genes of *S. pneumoniae, S. pyogenes* and *Lactococcus* lactis (Table 18).

[0434] In order to elucidate whether the two enterococcal tuf sequences encode genuine EF-Tu, the deduced amino acid sequences of both genes were aligned with other EF-Tu sequences available in SWISSPROT (Release 38). Sequence alignment demonstrated that both gene products are highly conserved and carry all conserved residues present in this portion of prokaryotic EF-Tu (FIG. **4**). Therefore, it appears that both gene products could fulfill the function of EF-Tu. The partial tuf gene sequences encode the portion of EF-Tu from residues 117 to 317, numbered as in *E. coli*. This portion makes up of the last four α-helices and two β-strands of domain I, the entire domain II and the N-terminal part of domain III on the basis of the determined structures of *E. coli* EF-Tu.

[0435] Based on the deduced amino acid sequences, the enterococcal tufB genes have unique conserved residues Lys129, Leu140, Ser230, and Asp234 (*E. coli* numbering) that are also conserved in streptococci and *L. lactis*, but not in the other bacteria (FIG. **4**). All these residues are located in loops except for Ser230. In other bacteria the residue Ser230 is substituted for highly conserved Thr, which is the 5^{th} residue of the third β-strand of domain II. This region is partially responsible for the interaction between the EF-Tu and aminoacyl-tRNA by the formation of a deep pocket for any of the 20 naturally occurring amino acids. According to our three-dimensional model (data not illustrated), the substitution Thr230→Ser in domain II of EF-Tu may have little impact on the capability of the pocket to accommodate any amino acid. However, the high conservation of Thr230 comparing to the unique Ser substitution found only. in streptococci and 11 enterococci could suggest a subtle functional role for this residue.

[0436] The tuf gene sequences obtained for *E. faecalis, S. aureus, S. pneumoniae* and *S. pyogenes* were compared with their respective incomplete genome sequence. Contigs with more than 99% identity were identified. Analysis of the *E. faecalis* genome data revealed that the single *E. faecalis* tuf gene is located within an str operon where tuf is preceded by fus that encodes the elongation factor G. This str operon is present in *S. aureus* and *B. subtilis* but not in the two streptococcal genomes examined. The 700-bp or so sequence upstream the *S. pneumoniae* tuf gene has no homology with any known gene sequences. In *S. pyogenes*, the gene upstream of tuf is similar to a cell division gene, ftsW, suggesting that the tuf genes in streptococci are not arranged in a str operon.

[0437] Phylogenetic analysis. Phylogenetic analysis of the tuf amino acid sequences with representatives of eubacteria, archeabacteria, and eukaryotes using neighbor-joining and maximum parsimony methods showed three major clusters representing the three kingdoms of life. Both methods gave similar topologies consistent with the rRNA gene data (data not shown). Within the bacterial clade, the tree is polyphyletic but tufA genes from all enterococcal species always clustered with those from other low G+C gram-positive bacteria (except for streptococci and lactococci), while the tufB genes of the 11 enterococcal species form a distinct cluster with streptococci and *L. lactis* (FIG. **5**). Duplicated genes from the same organism do not cluster together, thereby not suggesting evolution by recent gene duplication.

[0438] Southern hybridization. Southern hybridization of BglII/XbaI digested genomic DNA from 12 enterococcal species tested with the tufA probe (DIG-labeled tufA fragment from *E. faecium*) yielded two bands of different sizes in 9 species, which also carried two divergent tuf sequences according to their sequencing data. For *E. faecalis* and *E. solitarius*, a single band was observed indicating that one tuf gene is present (FIG. **6**). A single band was also found when digested genomic DNA from *S. aureus, S. pneumoniae*, and *S. pyogenes* were hybridized with the tufA probe (data not shown). For *E. faecium*, the presence of three bands can be explained by the existence of a XbaI restriction site in the middle of the tufA sequence, which was confirmed by sequencing data. Hybridization with the tufB probe (DIG-labeled tufB fragment of *E. faecium*) showed a banding profile similar to the one obtained with the tufA probe (data not shown).

Discussion

[0439] In this study, we have shown that two divergent copies of genes encoding the elongation factor Tu are present in some enterococcal species. Sequence data revealed that both genes are highly conserved at the amino acid level. One copy (tufA) is present in all enterococcal species, while the other (tufB) is present only in 11 of the 17 enterococcal species studied. Based on 16S rRNA sequence analysis, these 11 species are members of three different enterococcal subgroups (*E. avium, E. faecium*, and *E. gallinarum* species groups) and a distinct species (E. dispar). Moreover, 16S rDNA phylogeny suggests that these 11 species possessing 2 tuf genes all share a common ancestor before they further evolved to become the modern species. Since the six other species having only one copy diverged from the enterococcal lineage before that common ancestor, it appears that the presence of one tuf gene in these six species is not attributable to gene loss.

[0440] Two clusters of low G+C gram-positive bacteria were observed in the phylogenetic tree of the tuf genes: one contains a majority of low G+C gram-positive bacteria and the other contains lactococci and streptococci. This is similar to the finding on the basis of phylogenetic analysis of the 16S rRNA gene and the hrcA gene coding for a unique heat-shock regulatory protein. The enterococcal tufA genes branched with most of the low G+C gram-positive bacteria, suggesting that they originated from a common ancestor. On the other hand, the enterococcal tufB genes branched with the genera *Streptococcus* and *Lactococcus* that form a distinct lineage separated from other low G+C gram-positive bacteria (FIG. **5**). The finding that these EF-Tu proteins share some conserved amino acid residues unique to this branch also supports the idea that they may share a common ancestor. Although these conserved residues might result from convergent evolution upon a specialized function, such convergence at the sequence level, even for a few residues, seems to be rare, making it an unlikely event. Moreover, no currently known selective pressure, if any, would account for keeping one versus two tuf genes in bacteria. The G+C contents of enterococcal tufA and tufB sequences are similar, indicating that they both originated from low G+C gram-positive bacteria, in accordance with the phylogenetic analysis.

[0441] The tuf genes are present in various copy numbers in different bacteria. Furthermore, the two tuf genes are normally associated with characteristic flanking genes. The two tuf gene copies commonly encountered within gram-negative bacteria are part of the bacterial str operon and tRNA-tufB operon, respectively. The arrangement of tufA in the str operon was also found in a variety of bacteria, including *Thermotoga maritima*, the most ancient bacteria sequenced so far, *Aquifex aeolicus*, cyanobacteria, *Bacillus* sp., *Micrococcus luteus, Mycobacterium tuberculosis*, and *Streptomyces* sp. Furthermore, the tRNA-tufB operon has also been identified in *Aquifex aeolicus, Thermus thermophilus*, and *Chlamydia trachomatis*. The two widespread tuf gene arrangements argue in favor of their ancient origins. It is noteworthy that most obligate intracellular parasites, such as *Mycoplasma* sp., *R. prowazekii, B. burgdorferi*, and *T. pallidum*, contain only one tuf gene. Their flanking sequences are distinct from the two conserved patterns as a result of selection for effective propagation by an extensive reduction in genome size by intragenomic recombination and rearrangement.

[0442] Most gram-positive bacteria with low G+C content sequenced to date contain only a single copy of the tuf gene as a part of the str operon. This is the case for *B. subtilis, S. aureus* and *E. faecalis*. PCR amplification using a primer targeting a conserved region of the fus gene and the tufA-specific primer SEQ ID NO. 660, but not the tufB-specific primer SEQ ID NO. 661, yielded the expected amplicons for all 17 enterococcal species tested, indicating the presence of the fus-tuf organization in all enterococci (data not shown). However, in the genomes of *S. pneumoniae* and *S. pyogenes*, the sequences flanking the tuf genes varies although the tuf gene itself remains highly conserved. The enterococcal tufB genes are clustered with streptococci, but at present we do not have enough data to identify the genes flanking the enterococcal tufB genes. Furthermore, the functional role of the enterococcal tufB genes remains unknown. One can only postulate that the two divergent gene copies are expressed under different conditions.

[0443] The amino acid sequence identities between the enterococcal tufA and tufB genes are lower than either i) those between the enterococcal tufA and the tuf genes from other low G+C gram-positive bacteria (streptococci and lactococci excluded) or ii) those between the enterococcal tufB and streptococcal and lactococcal tuf genes. These findings suggest that the enterococcal tufA genes share a common ancestor with other low G+C gram-positive bacteria via the simple scheme of vertical evolution, while the enterococcal tufB genes are more closely related to those of streptococci and lactococci. The facts that some enterococci possess an additional tuf gene and that the single streptococcal tuf gene is nut clustered with other low G+C gram-positive bacteria cannot be explained by the mechanism of gene duplication or intrachromosomal recombination. According to sequence and phylogenetic analysis, we propose that the presence of the additional copy of the tuf genes in 11 enterococcal species is due to horizontal gene transfer. The common ancestor of the 11 enterococcal species now carrying tufB genes acquired a tuf gene from an ancestral *streptococcus* or a *streptococcus*-related species during enterococcal evolution through gene transfer before the diversification of modern enterococci.

Further study of the flanking regions of the gene may provide more clues for the origin and function of this gene in enterococci.

[0444] Recent studies of genes and genomes have demonstrated that considerable horizontal transfer occurred in the evolution of aminoacyl-tRNA synthetases in all three kingdoms of life. The heterogeneity of 16S rRNA is also attributable to horizontal gene transfer in some bacteria, such as *Streptomyces, Thermomonospora chromogena* and *Mycobacterium celatum*. In this study, we provide the first example in support of a likely horizontal transfer of the tuf gene encoding the elongation factor Tu. This may be an exception since stringent functional constraints do not allow for frequent horizontal transfer of the tuf gene as with other genes. However, enterococcal tuf genes should not be the only such exception as we have noticed that the phylogeny of *Streptomyces* tuf genes is equally or more complex than that of enterococci. For example, the three tuf-like genes in a high G+C gram-positive bacterium, *S. ramocissimus,* branched with the tuf genes of phylogenetically divergent groups of bacteria (FIG. **5**). Another example may be the tuf genes in clostridia, which represent a phylogenetically very broad range of organisms and form a plethora of lines and groups of various complexities and depths. Four species belonging to three different clusters within the genus *Clostridium* have been shown by Southern hybridization to carry two copies of the tuf gene. Further sequence data and phylogenetic analysis may help interpreting the evolution of the elongation factor Tu in these gram-positive bacteria. Since the tuf genes and 16S rRNA genes are often used for phylogenetic study, the existence of duplicate genes originating from horizontal gene transfer may alter the phylogeny of microorganisms when the laterally acquired copy of the gene is used for such analysis. Hence, caution should be taken in interpreting phylogenetic data. In addition, the two tuf genes in enterococci have evolved separately and are distantly related to each other phylogenetically. The enterococcal tufB genes are less conserved and unique to the 11 enterococcal species only. We previously demonstrated that the enterococcal tufA genes could serve as a target to develop a DNA-based assay for identification of enterococci. The enterococcal tufB genes would also be useful in identification of these 11 enterococcal species.

Example 43

Elongation Factor Tu (tuf) and the F-ATPase Beta-Subunit (atpD) as Phylogenetic Tools for Species of the Family Enterobacteriaceae

Summary

[0445] The phylogeny of enterobacterial species commonly found in clinical samples was analyzed by comparing partial sequences of their elongation factor Tu (tuf) genes and their F-ATPase beta-subunit (atpD) genes. A 884-bp fragment for tuf and a 884- or 871-bp fragment for atpD were sequenced for 88 strains of 72 species from 25 enterobacterial genera. The atpD sequence analysis revealed a specific indel to *Pantoea* and Tatumella species showing for the first time a tight phylogenetic affiliation between these two genera. Comprehensive tuf and atpD phylogenetic trees were constructed and are in agreement with each other. Monophyletic genera are *Yersinia, Pantoea, Edwardsiella, Cedecea, Salmonella, Serratia, Proteus*, and *Providencia*. Analogous trees were obtained based on available 16S rDNA sequences from databases. tuf and atpD phylogenies are in agreement with the 16S rDNA analysis despite the smaller resolution power for the latter. In fact, distance comparisons revealed that tuf and atpD genes provide a better resolution for pairs of species belonging to the family Enterobacteriaceae. However, 16S rDNA distances are better resolved for pairs of species belonging to different families. In conclusion, tuf and atpD conserved genes are sufficiently divergent to discriminate different species inside the family Enterobacteriaceae and offer potential for the development of diagnostic tests based on DNA to identify enterobacterial species.

Introduction

[0446] Members of the family Enterobacteriaceae are facultatively anaerobic gram-negative rods, catalase-positive and oxidase-positive (Brenner, 1984). They are found in soil, water, plants, and in animals from insects to man. Many enterobacteria are opportunistic pathogens. In fact, members of this family are responsible for about 50% of nosocomial infections in the United States (Brenner, 1984). Therefore, this family is of considerable clinical importance.

[0447] Major classification studies on the family Enterobacteriaceae are based on phenotypic traits (Brenner et al., 1999; Brenner et al., 1980; Dickey & Zumoff, 1988; Farmer III et al., 1980; Farmer III et al., 1985b; Farmer III et al., 1985a) such as biochemical reactions and physiological characteristics. However, phenotypically distinct strains may be closely related by genotypic criteria and may belong to the same genospecies (Bercovier et al., 1980; Hartl & Dykhuizen, 1984). Also, phenotypically close strains (biogroups) may belong to different genospecies, like *Klebsiella pneumoniae* and *Enterobacter aerogenes* (Brenner, 1984) for example. Consequently, identification and classification of certain species may be ambiguous with techniques based on phenotypic tests (Janda et al., 1999; Kitch et al., 1994; Sharma et al., 1990).

[0448] More advances in the classification of members of the family Enterobacteriaceae have come from DNA-DNA hybridization studies (Brenner et al., 1993; Brenner et al., 1986; Brenner, et al., 1980; Farmer III, et al., 1980; Farmer III, et al., 1985b; Izard et al., 1981; Steigerwalt et al., 1976). Furthermore, the phylogenetic significance of bacterial classification based on 16S rDNA sequences has been recognized by many workers (Stackebrandt & Goebel, 1994; Wayne et al., 1987). However, members of the family Enterobacteriaceae have not been subjected to extensive phylogenetic analysis of 16S rDNA (Sproer et al., 1999). In fact, this molecule was not thought to solve taxonomic problems concerning closely related species because of its very high degree of conservation (Brenner, 1992; Sproer, et al., 1999). Another drawback of the 16S rDNA gene is that it is found in several copies within the genome (seven in *Escherichia coli* and *Salmonella typhimurium*) (Hill & Harnish, 1981). Due to sequence divergence between the gene copies, direct sequencing of PCR products is often not suitable to achieve a representative sequence (Cilia et al., 1996; Hill & Harnish, 1981). Other genes such as gap and ompA (Lawrence et al., 1991), rpoB (Mollet et al., 1997), and infB (Hedegaard et al., 1999) were used to resolve the phylogeny of enterobacteria. However, none of these studies covered an extensive number of species.

[0449] tuf and atpD are the genes encoding the elongation factor Tu (EF-Tu) and the F-ATPase beta-subunit, respectively. EF-Tu is involved in peptide chain formation (Ludwig et al., 1990). The two copies of the tuf gene (tufA and tufB) found in enterobacteria (Sela et al., 1989) share high identity level (99%) in *Salmonella typhimurium* and in *E. coli*. The recombination phenomenon could explain sequence homogenization between the two copies (Abdulkarim & Hughes, 1996; Grunberg-Manago, 1996). F-ATPase is present on the plasma membranes of eubacteria (Nelson & Taiz, 1989). It functions mainly in ATP synthesis (Nelson & Taiz, 1989) and the beta-subunit contains the catalytic site of the enzyme. EF-Tu and F-ATPase are highly conserved throughout evolution and shows functional constancy (Amann et al., 1988; Ludwig, et al., 1990). Recently, phylogenies based on protein sequences from EF-Tu and F-ATPase beta-subunit showed good agreement with each other and with the rDNA data (Ludwig et al., 1993).

[0450] We elected to sequence 884-bp fragments of tuf and atpD from 88 clinically relevant enterobacterial strains representing 72 species from 25 genera. These sequences were used to create phylogenetic trees that were compared with 16S rDNA trees. These trees revealed good agreement with each others and demonstrated the high resolution of tuf and atpD phylogenies at the species level.

Materials and Methods

[0451] Bacterial strains and genomic material. All bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC) or the Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (DSMZ). These enterobacteria can all be recovered from clinical specimens, but not all are pathogens. Whenever possible, we choose type strains. Identification of all strains was confirmed by classical biochemical tests using the automated system MicroScan WalkAway-96 system equipped with a Negative BP Combo Panel Type 15 (Dade Behring Canada). Genomic DNA was purified using the G NOME DNA kit (Bio 101). Genomic DNA from *Yersinia pestis* was kindly provided by Dr. Robert R. Brubaker. Strains used in this study and their descriptions are shown in Table 19.

[0452] PCR primers. The eubacterial tuf and atpD gene sequences available from public databases were analyzed using the GCG package (version 8.0) (Genetics Computer Group). Based on multiple sequence alignments, two highly conserved regions were chosen for each genes, and PCR primers were derived from these regions with the help of Oligo primer analysis software (version 5.0) (National Biosciences). A second 5' primer was design to amplify the gene atpD for few enterobacteria difficult to amplify with the first primer set. When required, the primers contained inosines or degeneracies to account for variable positions. Oligonucleotide primers were synthesized with a model 394 DNA/RNA synthesizer (PE Applied Biosystems). PCR primers used in this study are listed in Table 20.

[0453] DNA sequencing. An 884-bp portion of the tuf gene and an 884-bp portion (or alternatively an 871-bp portion for a few enterobacterial strains) of the atpD gene were sequenced for all enterobacteria listed in the first strain column of Table 19. Amplification was performed with 4 ng of genomic DNA. The 40-μl PCR mixtures used to generate PCR products for sequencing contained 1.0 μM each primer, 200 μM each deoxyribonucleoside triphosphate (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0 at 25° C.), 50 mM KCl, 0.1% (w/v) Triton X-100, 2.5 mM $MgCl_2$, 0.05 mM BSA, 0.3 U of Taq DNA polymerase (Promega) coupled with TaqStart™ antibody (Clontech Laboratories). The TaqStart™ neutralizing monoclonal antibody for Taq DNA polymerase was added to all PCR mixtures to enhance efficiency of amplification (Kellogg et al., 1994). The PCR mixtures were subjected to thermal cycling (3 min at 95° C. and then 35 cycles of 1 min at 95° C., 1 min at 55° C. for tuf or 50° C. for atpD, and 1 min at 72° C., with a 7-min final extension at 72° C.) using a PTC-200 DNA Engine thermocycler (MJ Research). PCR products having the predicted sizes were recovered from an agarose gel stained for 15 min with 0.02% of methylene blue followed by washing in sterile distilled water for 15 min twice (Flores et al., 1992). Subsequently, PCR products having the predicted sizes were recovered from gels using the QIAquick gel extraction kit (QIAGEN).

[0454] Both strands of the purified amplicons were sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) on an automated DNA sequencer (Model 377). Amplicons from two independent PCR amplifications sequenced for each strain to ensure the absence of sequencing errors attributable to nucleotide miscorporations by the Taq DNA polymerase. Sequence assembly was performed with the aid of Sequencher 3.0 software (Gene Codes).

[0455] Phylogenetic analysis. Multiple sequence alignments were performed using PileUp from the GCG package (Version 10.0) (Genetics Computer Group) and checked by eye with the editor SeqLab to edit sequences if necessary and to note which regions were to be excluded for phylogenetic analysis. *Vibrio cholerae* and *Shewanella putrefaciens* were used as outgroups. Bootstrap subsets (750 sets) and phylogenetic trees were generated with the Neighbor Joining algorithm from Dr. David Swofford's PAUP (Phylogenetic Analysis Using Parsimony) Software version 4.0b4 (Sinauer Associates) and with tree-bisection branch-swapping. The distance model used was Kimura (1980) two-parameter. Relative rate test was performed with the aid of Phyltest program version 2.0 (c).

Results and Discussion

DNA Amplification, Sequencing And Sequence Alignments

[0456] A PCR product of the expected size of 884 by was obtained for tuf and of 884 or 871 by for atpD from all bacterial strains tested. After subtracting for biased primer regions and ambiguous single strand data, sequences of at least 721 by for tuf and 713 by for atpD were submitted to phylogenetic analyses. These sequences were aligned with tuf and atpD sequences available in databases to verify that the nucleotide sequences indeed encoded a part of tested genes. Gaps were excluded to perform phylogenetic analysis.

Signature Sequences

[0457] From the sequence alignments obtained from both tested genes, only one insertion was observed. This five amino acids insertion is located between the positions 325 and 326 of atpD gene of *E. coli* strain K-12 (Saraste et al., 1981) and can be considered a signature sequence of Tatumella ptyseos and *Pantoea* species (FIG. **7**). The presence of a conserved indel of defined length and sequence and flanked by conserved regions could suggest a common ancestor, particularly when members of a given taxa share this indel (Gupta, 1998). To our knowledge, high relatedness between the genera *Tatumella* and *Pantoea* is demonstrated for the first time.

[0458] *Enterobacter agglomerans* ATCC 27989 sequence does not possess the five amino acid indel (FIG. **7**). This indel could represent a useful marker to help resolve the *Enterobacter agglomerans* and *Pantoea* classification. Indeed, the transfer of *Enterobacter agglomerans* to *Pantoea* agglomerans was proposed in 1989 by Gavini et al. (Gavini et al., 1989). However, some strains are provisionally classified as *Pantoea* sp. until their interrelatedness is elucidated (Gavini, et al., 1989). Since the transfer was proposed, the change of nomenclature has not yet been made for all *Enterobacter agglomerans* in the ATCC database. The absence of the five amino acids indel suggests that some strains of *Enterobacter agglomerans* most likely do not belong to the genus *Pantoea*.

Phylogenetic Trees Based on Partial Tuf Sequences, Atpd Sequences, and Published 16S Rdna Data of Members of the Enterobacteriaceae.

[0459] Representative trees constructed from tuf and atpD sequences with the neighbor-joining method are shown in FIG. **8**. The phylogenetic trees generated from partial tuf sequences and atpD sequences are very similar. Nevertheless, atpD tree shows more monophyletic groups corresponding to species that belong to the same genus. These groups are more consistent with the actual taxonomy. For both genes, some genera are not monophyletic. These results support previous phylogenies based on the genes gap and ompA (Lawrence, et al., 1991), rpoB (Mollet, et al., 1997), and infB (Hedegaard, et al., 1999) which all showed that the genera *Escherichia* and *Klebsiella* are polyphyletic. There were few differences in branching between tuf and atpD genes.

[0460] Even though *Pantoea agglomerans* and *Pantoea dispersa* indels were excluded for phylogenetic analysis, these two species grouped together and were distant from *Enterobacter agglomerans* ATCC 27989, adding another evidence that the latter species is heterogenous and that not all members of this species belong to the genus *Pantoea*. In fact, the *E. agglomerans* strain ATCC 27989 exhibits branch lengths similar to others *Enterobacter* species with both genes. Therefore, we suggest that this strain belong to the genus *Enterobacter* until further reclassification of that genus.

[0461] tuf and atpD trees exhibit very short genetic distances between taxa belonging to the same genetic species including species segregated for clinical considerations. This first concern *E. coli* and *Shigella* species that were confirmed to be the same genetic species by hybridization studies (Brenner et al., 1972; Brenner et al., 1972; Brenner et al., 1982) and phylogenies based on 16S rDNA (Wang et al., 1997) and rpoB genes (Mollet, et al., 1997). Hybridization studies (Bercovier, et al., 1980) and phylogeny based on 16S rDNA genes (Ibrahim et al., 1994) demonstrated also that *Yersinia pestis* and *Y. pseudotuberculosis* are the same genetic species. Among *Yersinia pestis* and *Y. pseudotuberculosis*, the three *Klebsiella pneumoniae subspecies, E. coli*-Shigella species, and *Salmonella* choleraesuis subspecies, *Salmonella* is a less tightly knit species than the other genetic species. The same is true for *E. coli* and *Shigella* species.

[0462] *Escherichia fergusonii* is very close to *E. coli*-Shigella genetic species. This observation is corroborated by 16S rDNA phylogeny (McLaughlin et al., 2000) but not by DNA hybridization values. In fact, *E. fergusonii* is only 49% to 63% related to *E. coli-Shigella* (Farmer III, et al., 1985b). It was previously observed that very recently diverged species may not be recognizable based on 16S rDNA sequences although DNA hybridization established them as different species (Fox et al., 1992). Therefore, *E. fergusonii* could be a new "quasi-species".

[0463] atpD phylogeny revealed *Salmonella* subspecies *divisions* consistent with the actual taxonomy. This result was already observed by Christensen et al. (Christensen & Olsen, 1998). Nevertheless, tuf partial sequences discriminate less than atpD between *Salmonella* subspecies.

[0464] Overall, tuf and atpD phylogenies exhibit enough divergence between species to ensure efficient discrimination. Therefore, it could be easy to distinguish phenotypically close enterobacteria belonging to different genetic species such as *Klebsiella pneumoniae* and *Enterobacter aerogenes*.

[0465] Phylogenetic relationships between *Salmonella, E. coli* and *C. freundii* are not well defined. 16S rDNA and 23S rDNA sequence data reveals a closer relationship between *Salmonella* and *E. coli* than between *Salmonella* and *C. freundii* (Christensen et al., 1998), while DNA homology studies (Selander et al., 1996) and infB phylogeny (Hedegaard, et al., 1999) showed that *Salmonella* is more closely related to *C. freundii* than to *E. coli*. In that regard, tuf and atpD phylogenies are coherent with 16S rDNA and 23S rDNA sequence analysis.

[0466] Phylogenetic analyses were also performed using amino acids sequences. tuf tree based on amino acids is characterized by a better resolution between taxa outgroup and taxa ingroup (enterobacteria) than tree based on nucleic acids whereas atpD trees based on amino acids and nucleic acids give almost the same resolution between taxa outgroup and ingroup (data not shown).

[0467] Relative rate test (or two cluster test (Takezaki et al., 1995)) evaluates if evolution is constant between two taxa. Before to apply the test, the topology of a tree is determined by tree-building method without the assumption of rate constancy. Therefore, two taxa (or two groups of taxa) are compared with a third taxon that is an outgroup of the first two taxa (Takezaki, et al., 1995). Few pairs of taxa that exhibited a great difference between their branch lengths at particular nodes were chosen to perform the test. This test reveals that tuf and atpD are not constant in their evolution within the family Enterobacteriaceae. For tuf, for example, the hypothesis of rate constancy is rejected (Z value higher than 1.96) between *Yersinia* species. The same is true for *Proteus* species. For atpD, for example, evolution is not constant between *Proteus* species, between *Proteus* species and *Providencia* species, and between *Yersinia species* and *Escherichia coli*. For 16S rDNA, for example, evolution is not constant between two *E. coli*, between *E. coli* and *Enterobacter aerogenes*, and between *E. coli* and *Proteus vulgaris*. These results suggest that tuf, atpD and 16S rDNA could not serve as a molecular clock for the entire family Enterobacteriaceae.

[0468] Since the number and the nature of taxa can influence topology of trees, phylogenetic trees from tuf and atpD were reconstructed using sequences corresponding to strains for which 16S rDNA genes were published in GenEMBL. These trees were similar to those generated using 16S rDNA (FIG. **9**). Nevertheless, 16S rDNA tree gave poorer resolution power than tuf and atpD gene trees. Indeed, these latter exhibited less multifurcation (polytomy) than the 16S rDNA tree. Comparison of Distances Based on tuf, atpD, and 16S rDNA data.

[0469] tuf, atpD, and 16S rDNA distances (i.e. the number of differences per nucleotide site) were compared with each other for each pair of strains. We found that the tuf and atpD distances were respectively 2.268±0.965 and 2.927±0.896 times larger than 16S rDNA distances (FIGS. **10***a* and *b*). atpD distances were 1.445±0.570 times larger than tuf distances (FIG. **10***c*). FIG. **10** also shows that the tuf, atpD, and 16S rDNA distances between members of different species of the same genus (0.053±0.034, 0.060±0.020, and 0.024±0.010, respectively) were in mean smaller than the distances between members of different genera belonging to the same family (0.103±0.053, 0.129±0.051, and 0.044±0.013, respectively). However, the overlap exhibits with standard deviations add to a focus of evidences that some enterobacterial genera are not well defined (Brenner, 1984). In fact, many distances for pairs of species especially belonging to the genera *Escherichia, Shigella, Enterobacter, Citrobacter, Klebsiella*, and Kluyvera overlap distances for pairs of species belonging to the same genus (FIG. **10**). For example, distances for pairs composed by species of *Citrobacter* and species of *Klebsiella* overlap distances for pairs composed by two *Citrobacter* or by two *Klebsiella.*

[0470] Observing the distance distributions, 16S rDNA distances reveal a clear separation between the families Enterobacteriaceae and Vibrionaceae despite the fact that the family Vibrionaceae is genetically very close to the Enterobacteriaceae (FIGS. **10***a* and *b*). Nevertheless, tuf and atpD show higher discriminating power below the family level (FIGS. **10***a* and *b*).

[0471] There were some discrepancies in the relative distances for the same pairs of taxa between the two genes studied. First, distances between *Yersinia* species are at least two times lower for atpD than for tuf (FIG. **10***c*). Also, distances at the family level (between Enterobacteriaceae and Vibrionaceae) show that Enterobacteriaceae is a tightlier knit family with atpD gene (*Proteus* genus excepted) than with tuf gene. Both genes well delineate taxa belonging to the same species. There is one exception with atpD: *Klebsiella* planticola and *K. ornithinolithica* belong to the same genus but fit with taxa belonging to the same species (FIGS. **10***a* and *c*). These two species are also very close genotypically with tuf gene. This suggest that *Klebsiella* planticola and *K. ornithinolithica* could be two newborn species. tuf and atpD genes exhibit little distances between *Escherichia fergusonii* and *E. coli*-Shigella species. Unfortunately, comparison with 16S rDNA could not be achieved because the *E. fergusonii* 16S rDNA sequence is not yet accessible in GenEMBL database. Therefore, the majority of phenotypically close enterobacteria could be easily discriminated genotypically using tuf and atpD gene sequences.

[0472] In conclusion, tuf and atpD genes exhibit phylogenies consistent with 16S rDNA genes phylogeny. For example, they reveal that the family Enterobacteriaceae is monophyletic. Moreover, tuf and atpD distances provide a higher discriminating power than 16S rDNA distances. In fact, tuf and atpD genes discriminate well between different genospecies and are conserved between strains of the same genetic species in such a way that primers and molecular probes for diagnostic purposes could be designed. Preliminary studies support these observations and diagnostic tests based on tuf and atpD sequence data to identify enterobacteria are currently under development.

Example 44

Testing New Pairs of PCR Primers Selected from Two Species-Specific Genomic DNA Fragments which are Objects of Our Assigned U.S. Pat. No. 6,001,564

[0473] Objective. The goal of these experiments is to demonstrate that it is relatively easy for a person skilled in the art to find other PCR primer pairs from the species-specific fragments used as targets for detection and identification of a variety of microorganisms. In fact, we wish to prove that the PCR primers previously tested by our group and which are objects of the present patent application are not the only possible good choices for diagnostic purposes. For this example, we used diagnostic targets described in our assigned U.S. Pat. No. 6,001,564.

[0474] Experimental strategy. We have selected randomly two species-specific genomic DNA fragments for this experiment. The first one is the 705-bp fragment specific to *Staphylococcus epidermidis* (SEQ ID NO: 36 from U.S. Pat. No. 6,001,564) while the second one is the 466-bp fragment specific to *Moraxella catarrhalis* (SEQ ID NO: 29 from U.S. Pat. No. 6,001,564). Subsequently, we have selected from these two fragments a number of PCR primer pairs other than those previously tested. We have chosen 5 new primer pairs from each of these two sequences which are well dispersed along the DNA fragment (FIGS. **11** and **12**). We have tested these primers for their specificity and compared them with the original primers previously tested. For the specificity tests, we have tested all bacterial species closely related to the target species based on phylogenetic analysis with three conserved genes (rRNA genes, tuf and atpD). The rational for selecting a restricted number of bacterial species to evaluate the specificity of the new primer pairs is based on the fact that the lack of specificity of a DNA-based assay is attributable to the detection of closely related species which are more similar at the nucleotide level. Based on the phylogenetic analysis, we have selected (i) species from the closely related genus *Staphylococcus, Enterococcus, Streptococcus* and *Listeria* to test the specificity of the *S. epidermidis*-specific PCR assays and (ii) species from the closely related genus *Moraxella, Kingella* and *Neisseria* to test the specificity of the *M. catarrhalis*-specific PCR assays.

Materials and Methods

[0475] Bacterial strains. All bacterial strains used for these experiments were obtained from the American Type Culture Collection (ATCC, Rockville, Md.).

[0476] Genomic DNA isolation. Genomic DNA was purified from the ATCC reference strains by using the G-nome DNA kit (Bio 101 Inc., Vista, Calif.).

[0477] Oligonucleotide design and synthesis. PCR primers were designed with the help of the Oligo™ primer analysis software Version 4.0 (National Biosciences Inc., Plymouth, Minn.) and synthesized using a model 391 DNA synthesizer (Applied Biosystems, Foster City, Calif.).

[0478] PCR assays. All PCR assays were performed by using genomic DNA purified from reference strains obtained from the ATCC. One μl of purified DNA preparation (containing 0.01 to 1 ng of DNA per μl) was added directly into the PCR reaction mixture. The 20 μL PCR reactions contained final concentrations of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 μM of each primer, 200 μM of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega, Madison, Wis.) combined with the TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, Calif.). An internal control was integrated into all amplification reactions to verify the efficiency of the amplification reaction as well as to ensure that significant PCR inhibition was absent. Primers amplifying a region of 252 by from a control plasmid added to each amplification reaction were used to provide the internal control. PCR reactions were then subjected to thermal cycling (3 min at 95° C. followed by 30 cycles of 1 second at 95° C. for the denaturation step and 30 seconds at 50 to 65° C. for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.). PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 0.25 μg/mL of ethidium bromide under UV at 254 nm.

Results

[0479] Tables 21 and 22 show the results of specificity tests with the 5 new primer pairs selected from SEQ ID NO: 29 (specific to *M. catarrhalis* from U.S. Pat. No. 6,001,564) and SEQ ID NO: 36 (specific to *S. epidermidis* from U.S. Pat. No. 6,001,564), respectively. In order to evaluate the performance of these new primers pairs, we compared them in parallel with the original primer pairs previously tested.

[0480] For *M. catarrhalis*, all of the 5 selected PCR primer pairs were specific for the target species because none of the closely related species could be amplified (Table 21). In fact, the comparison with the original primer pair SEQ ID NO: 118+SEQ ID NO: 119 (from U.S. Pat. No. 6,001,564) revealed that all new pairs showed identical results in terms of specificity and sensitivity thereby suggesting their suitability for diagnostic purposes.

[0481] For *S. epidermidis,* 4 of the 5 selected PCR primer pairs were specific for the target species (Table 22). It should be noted that for 3 of these four primer pairs the annealing temperature had to be increased from 55° C. to 60 or 65° C. to attain specificity for *S. epidermidis*. Again the comparison with the original primer pair SEQ ID NO: 145+SEQ ID NO: 146 (from U.S. Pat. No. 6,001,564) revealed that these four primer pairs were as good as the original pair. Increasing the annealing temperature for the PCR amplification is well known by persons skilled in the art to be a very effective way to improve the specificity of a PCR assay (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, Mass.). In fact, those skilled in the art are well aware of the fact that the annealing temperature is critical for the optimization of PCR assays. Only the primer pair VBsep3+VBsep4 amplified bacterial species other than *S. epidermidis* including the staphylococcal species *S. capitis, S. cohnii, S. aureus, S. haemolyticus* and *S. hominis* (Table 22). For this non-specific primer pair, increasing the annealing temperature from 55 to 65° C. was not sufficient to attain the desired specificity. One possible explanation for the fact that it appears slightly easier to select species-specific primers for M catarrhalis than for *S. epidermidis* is that M catarrhalis is more isolated in phylogenetic trees than *S. epidermidis*. The large number of coagulase negative staphylococcal species such as *S. epidermidis* is largely responsible for this phylogenetic clustering.

Conclusion

[0482] These experiment clearly show that it is relatively easy for a person skilled in the art to select, from the species-specific DNA fragments selected as target for identification, PCR primer pairs suitable for diagnostic purposes other than those previously tested. The amplification conditions can be optimize by modifying critical variables such as the annealing temperature to attain the desired specificity and sensitivity. Consequently, we consider that it is legitimate to claim any possible primer sequences selected from the species-specific fragment and that it would be unfair to grant only the claims dealing with the primer pairs previously tested. By extrapolation, these results strongly suggest that it is also relatively easy for a person skilled in the art to select, from the species-specific DNA fragments, DNA probes suitable for diagnostic purposes other than those previously tested.

Example 45

Testing Modified Versions of PCR Primers Derived from the Sequence of Several Primers which are Objects of U.S. Pat. No. 6,001,564.

[0483] Objective. The purpose of this project is to verify the efficiency of amplification by modified PCR primers derived from primers previously tested. The types of primer modifications to be tested include (i) variation of the sequence at one or more nucleotide positions and (ii) increasing or reducing the length of the primers. For this example, we used diagnostic targets described in U.S. Pat. No. 6,001,564.

Experimental Strategy:

[0484] Testing Primers with Nucleotide Changes

[0485] We have designed 13 new primers which are derived from the *S. epidermidis*-specific SEQ ID NO: 146 from U.S. Pat. No. 6,001,564 (Table 23). These primers have been modified at one or more nucleotide positions. As shown in Table 23, the nucleotide changes were introduced all along the primer sequence. Furthermore, instead of modifying the primer at any nucleotide position, the nucleotide changes were introduced at the third position of each codon to better reflect potential genetic variations in vivo. It should be noted that no nucleotide changes were introduced at the 3' end of the oligonucleotide primers because those skilled in the art are well aware of the fact that mismatches at the 3' end should be avoided (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). All of these modified primers were tested in PCR assays in combination with SEQ ID NO: 145 from U.S. Pat. No. 6,001,564 and the efficiency of the amplification was compared with the original primer pair SEQ ID NO: 145+SEQ ID NO: 146 previously tested in U.S. Pat. No. 6,001,564.

Testing Shorter or Longer Versions of Primers

[0486] We have designed shorter and longer versions of the original *S. epidermidis*-specific PCR primer pair SEQ ID NO: 145+146 from U.S. Pat. No. 6,001,564 (Table 24) as well as shorter versions of the original *P. aeruginosa*-specific primer pair SEQ ID NO: 83+84 from U.S. Pat. No. 6,001,564 (Table 25). As shown in Tables 24 and 25, both primers of each pair were shortened or lengthen to the same length. Again, those skilled in the art know that the melting temperature of both primers from a pair should be similar to avoid preferential binding at one primer binding site which is detrimental in PCR (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, Mass.). All of these shorter or longer primer versions were tested in PCR assays and the efficiency of the amplification was compared with the original primer pair SEQ ID NOs 145 and 146.

Materials and Methods

[0487] See the Materials and methods section of Example 44.

Results

[0488] Testing Primers with Nucleotide Changes

[0489] The results of the PCR assays with the 13 modified versions of SEQ ID NO: 146 from U.S. Pat. No. 6,001,564 are shown in Table 23. The 8 modified primers having a single nucleotide variation showed an efficiency of amplification identical to the original primer pair based on testing with 3 different dilutions of genomic DNA. The four primers having two nucleotide variations and primer VBmut12 having 3 nucleotide changes also showed PCR results identical to those obtained with the original pair. Finally, primer VBmut13 with four nucleotide changes showed a reduction in sensitivity by approximately one log as compared with the original primer pair. However, reducing the annealing temperature from 55 to 50° C. gave an efficiency of amplification very similar to that observed with the original primer pair (Table 23). In fact, reducing the annealing temperature of PCR cycles represents an effective way to reduce the stringency of hybridization for the primers and consequently allows the binding of probes with mismatches (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Subsequently, we have confirmed the specificity of the PCR assays with each of these 13 modified versions of SEQ ID NO: 146 from U.S. Pat. No. 6,001,564 by performing amplifications from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

Testing Shorter or Longer Versions of Primers

[0490] For these experiments, two primer pairs were selected: i) SEQ ID NO: 145+146 from U.S. Pat. No. 6,001,564 (specific to *S. epidermidis*) which are AT rich and ii) SEQ ID NO: 83+84 (specific to *P. aeruginosa*) which are GC rich. For the AT rich sequence, primers of 15 to 30 nucleotide in length were designed (Table 24) while for the GC rich sequences, primers of 13 to 19 nucleotide in length were designed (Table 25).

[0491] Table 24 shows that, for an annealing temperature of 55° C., the 30-25-, 20- and 17-nucleotide versions of SEQ ID NO: 145 and 146 from U.S. Pat. No. 6,001,564 all showed identical results as compared with the original primer pair except that the 17-nucleotide version amplified slightly less efficiently the *S. epidermidis* DNA. Reducing the annealing temperature from 55 to 45° C. for the 17-nucleotide version allowed to increase the amplification efficiency to a level very similar to that with the original primer pair (SEQ ID NO: 145+146 from U.S. Pat. No. 6,001,564). Regarding the 15-nucleotide version, there was amplification of *S. epidermidis* DNA only when the annealing temperature was reduced to 45° C. Under those PCR conditions the assay remained *S. epidermidis*-specific but the amplification signal with *S. epidermidis* DNA was slightly lower as compared with the original primer pair. Subsequently, we have further confirmed the specificity of the shorter or longer versions by amplifying DNA from all bacterial species closely related to *S. epidermidis* which are listed in Table 22.

[0492] Table 25 shows that, for an annealing temperature of 55° C., all shorter versions of SEQ ID NO: 83 and 84 from U.S. Pat. No. 6,001,564 showed identical PCR results as compared with the original primer pair. As expected, these results show that it is simpler to reduce the length of GC rich as compared with AT rich. This is attributable to the fact that GC binding is more stable than AT binding.

Conclusion

[0493] Testing Primers with Nucleotide Changes

[0494] The above experiments clearly show that PCR primers may be modified at one or more nucleotide positions without affecting the specificity and the sensitivity of the PCR assay. These results strongly suggest that a given oligonucleotide can detect variant genomic sequences from the target species. In fact, the nucleotide changes in the selected primers were purposely introduced at the third position of each codon to mimic nucleotide variation in genomic DNA. Thus we conclude that it is justified to claim "a variant thereof" for i) the SEQ IDs of the fragments and oligonucleotides which are object of the present patent application and ii) genomic variants of the target species.

Testing Shorter or Longer Versions of Primers

[0495] The above experiments clearly show that PCR primers may be shorter or longer without affecting the specificity and the sensitivity of the PCR assay. We have showed that oligonucleotides ranging in sizes from 13 to 30 nucleotides may be as specific and sensitive as the original primer pair from which they were derived. Consequently, these results suggest that it is not exaggerated to claim sequences having at least 12 nucleotide in length.

[0496] This invention has been described herein above, and it is readily apparent that modifications can be made thereto without departing from the spirit of this invention. These modifications are under the scope of this invention, as defined in the appended claims.

TABLE 1

Distribution (%) of nosocomial pathogens for various human infections in USA (1990-1992)[1].

Pathogen	UTI[2]	SSI[3]	BSI[4]	Pneumonia	CSF[5]
Escherichia coli	27	9	5	4	2
Staphylococcus aureus	2	21	17	21	2
Staphylococcus epidermidis	2	6	20	0	1
Enterococcus faecalis	16	12	9	2	0
Enterococcus faecium	1	1	0	0	0
Pseudomonas aeruginosa	12	9	3	18	0
Klebsiella pneumoniae	7	3	4	9	0
Proteus mirabilis	5	3	1	2	0
Streptococcus pneumoniae	0	0	3	1	18
Group B *Streptococci*	1	1	2	1	6
Other *streptococci*	3	5	2	1	3
Haemophilus influenzae	0	0	0	6	45
Neisseria meningitidis	0	0	0	0	14
Listeria monocytogenes	0	0	0	0	3
Other *enterococci*	1	1	0	0	0
Other *staphylococci*	2	8	13	2	0
Candida albicans	9	3	5	5	0
Other *Candida*	2	1	3	1	0
Enterobacter sp.	5	7	4	12	2
Acinetobacter sp.	1	1	2	4	2
Citrobacter sp.	2	1	1	1	0
Serratia marcescens	1	1	1	3	1
Other *Klebsiella*	1	1	1	2	1
Others	0	6	4	5	0

[1]Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6: 428-442).
[2]Urinary tract infection.
[3]Surgical site infection.
[4]Bloodstream infection.
[5]Cerebrospinal fluid.

TABLE 2

Distribution (%) of bloodstream infection pathogens in Quebec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

			UK[3]		
Organism	Quebec[1]	Canada[2]	Community-acquired	Hospital-acquired	USA[4] Hospital-acquired
E. coli	15.6	53.8	24.8	20.3	5.0
S. epidermidis and other CoNS[5]	25.8	—	0.5	7.2	31.0
S. aureus	9.6	—	9.7	19.4	16.0
S. pneumoniae	6.3	—	22.5	2.2	—
E. faecalis	3.0	—	1.0	4.2	—
E. faecium	2.6	—	0.2	0.5	—
Enterococcus sp.	—	—	—	—	9.0
H. influenzae	1.5	—	3.4	0.4	—
P. aeruginosa	1.5	8.2	1.0	8.2	3.0
K. pneumoniae	3.0	11.2	3.0	9.2	4.0
P. mirabilis	—	3.9	2.8	5.3	1.0
S. pyogenes	—	—	1.9	0.9	—
Enterobacter sp.	4.1	5.5	0.5	2.3	4.0
Candida sp.	8.5	—	—	1.0	8.0
Others	18.5	17.4	28.7	18.9	19.0

[1]Data obtained for 270 isolates collected at the Centre Hospitalier del'Université Laval (CHUL) during a 5 month period (May to October 1995).
[2]Data from 10 hospitals throughout Canada representing 941 gram-negative isolates. (Chamberland et al., 1992, Clin. Infect. Dis., 15: 615-628).
[3]Data from a 20-year study (1969-1988) for nearly 4000 isolates. (Eykyn et al., 1990, J. Antimicrob. Chemother., Suppl. C, 25: 41-58).
[4]Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6: 428-442).
[5]Coagulase-negative *staphylococci*.

TABLE 3

Distribution of positive and negative clinical specimens tested at the microbiology laboratory of the CHUL (February 1994-January 1995).

Clinical specimens and/or sites	No. of samples tested (%)	% of positive specimens	% of negative specimens
Urine	17,981 (54.5)	19.4	80.6
Blood culture/marrow	10,010 (30.4)	6.9	93.1
Sputum	1,266 (3.8)	68.4	31.6
Superficial pus	1,136 (3.5)	72.3	27.7
Cerebrospinal fluid	553 (1.7)	1.0	99.0
Synovial fluid	523 (1.6)	2.7	97.3
Respiratory tract	502 (1.5)	56.6	43.4
Deep pus	473 (1.4)	56.8	43.2
Ears	289 (0.9)	47.1	52.9
Pleural and pericardial fluid	132 (0.4)	1.0	99.0
Peritoneal fluid	101 (0.3)	28.6	71.4
Total:	32,966 (100.0)	20.0	80.0

TABLE 4

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention.
Bacterial species

Abiotrophia adiacens
Abiotrophia defectiva
Achromobacter xylosoxidans subsp. *denitrificans*
Acetobacterium woodi
Acetobacter aceti
Acetobacter altoacetigenes
Acetobacter polyoxogenes
Acholeplasma laidlawii
Acidothermus cellulolyticus

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention.
Bacterial species

Acidiphilum facilis
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Actinomyces meyeri
Aerococcus viridans
Aeromonas hydrophila
Aeromonas salmonicida
Agrobacterium radiobacter
Agrobacterium tumefaciens
Alcaligenes faecalis subsp. *faecalis*
Allochromatium vinosum
Anabaena variabilis
Anacystis nidulans
Anaerorhabdus furcosus
Aquifex aeolicus
Aquifex pyrophilus
Arcanobacterium haemolyticum
Archaeoglobus fulgidus
Azotobacter vinelandii
Bacillus anthracis
Bacillus cereus
Bacillus firmus
Bacillus halodurans
Bacillus megaterium
Bacillus mycoides
Bacillus pseudomycoides
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacillus weihenstephanensis
Bacteroides distasonis
Bacteroides fragilis
Bacteroides forsythus

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Bacteroides ovatus
Bacteroides vulgatus
Bartonella henselae
Bifidobacterium adolescentis
Bifidobacterium breve
Bifidobacterium dentium
Bifidobacterium longum
Blastochloris viridis
Borrelia burgdorferi
Bordetella pertussis
Bordetella bronchiseptica
Brucella abortus
Brevibacterium linens
Brevibacterium flavum
Brevundimonas diminuta
Buchnera aphidicola
Budvicia aquatica
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Buttiauxella agrestis
Butyrivibrio fibrisolvens
Campylobacter coli
Campylobacter curvus
Campylobacter fetus subsp. *fetus*
Campylobacter fetus subsp. *venerealis*
Campylobacter gracilis
Campylobacter jejuni
Campylobacter jejuni subsp. *doylei*
Campylobacter jejuni subsp. *jejuni*
Campylobacter lari
Campylobacter rectus
Campylobacter sputorum subsp. *sputorum*
Campylobacter upsaliensis
Cedecea davisae
Cedecea lapagei
Cedecea neteri
Chlamydia pneumoniae
Chlamydia psittaci
Chlamydia trachomatis
Chlorobium vibrioforme
Chloroflexus aurantiacus
Chryseobacterium meningosepticum
Citrobacter amalonaticus
Citrobacter braakii
Citrobacter farmeri
Citrobacter freundii
Citrobacter koseri
Citrobacter sedlakii
Citrobacter werkmanii
Citrobacter youngae
Clostridium acetobutylicum
Clostridium beijerinckii
Clostridium bifermentans
Clostridium botulinum
Clostridium difficile
Clostridium innocuum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium perfringens
Clostridium ramosum
Clostridium tertium
Clostridium tetani
Comamonas acidovorans
Corynebacterium accolens
Corynebacterium bovis
Corynebacterium cervicis
Corynebacterium diphtheriae
Corynebacterium flavescens
Corynebacterium genitalium
Corynebacterium glutamicum

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Corynebacterium jeikeium
Corynebacterium kutscheri
Corynebacterium minutissimum
Corynebacterium mycetoides
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudogenitalium
Corynebacterium pseudotuberculosis
Corynebacterium renale
Corynebacterium striatum
Corynebacterium ulcerans
Corynebacterium urealyticum
Corynebacterium xerosis
Coxiella burnetii
Cytophaga lytica
Deinococcus radiodurans
Deinonema sp.
Edwardsiella hoshinae
Edwardsiella tarda
Ehrlichia canis
Ehrlichia risticii
Eikenella corrodens
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter amnigenus
Enterobacter asburiae
Enterobacter cancerogenus
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter hormaechei
Enterobacter sakazakii
Enterococcus avium
Enterococcus casseliflavus
Enterococcus cecorum
Enterococcus columbae
Enterococcus dispar
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Enterococcus gallinarum
Enterococcus hirae
Enterococcus malodoratus
Enterococcus mundtii
Enterococcus pseudoavium
Enterococcus raffinosus
Enterococcus saccharolyticus
Enterococcus solitarius
Enterococcus sulfureus
Clostridium sordellii
Erwinia amylovora
Erwinia carotovora
Escherichia coli
Escherichia fergusonii
Escherichia hermannii
Escherichia vulneris
Eubacterium lentum
Eubacterium nodatum
Ewingella americana
Francisella tularensis
Frankia alni
Fervidobacterium islandicum
Fibrobacter succinogenes
Flavobacterium ferrigeneum
Flexistipes sinusarabici
Fusobacterium gonidiaformans
Fusobacterium necrophorum subsp. *necrophorum*
Fusobacterium nucleatum subsp. *polymorphum*
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Globicatella sanguis
Gloeobacter violaceus

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Gloeothece sp.
Gluconobacter oxydans
Haemophilus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus ducreyi
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Haemophilus segnis
Hafnia alvei
Halobacterium marismortui
Halobacterium salinarum
Haloferax volcanii
Helicobacter pylori
Herpetoshiphon aurantiacus
Kingella kingae
Klebsiella ornithinolytica
Klebsiella oxytoca
Klebsiella planticola
Klebsiella pneumoniae subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*
Klebsiella pneumoniae subsp. *rhinoscleromatis*
Klebsiella terrigena
Kluyvera ascorbata
Kluyvera cryocrescens
Kluyvera georgiana
Kocuria kristinae
Lactobacillus acidophilus
Lactobacillus garvieae
Lactobacillus paracasei
Lactobacillus casei subsp. *casei*
Lactococcus garvieae
Lactococcus lactis
Lactococcus lactis subsp. *lactis*
Legionella micdadei
Legionella pneumophila subsp. *pneumophila*
Leminorella grimontii
Leminorella richardii
Leptospira biflexa
Leptospira interrogans
Leuconostoc mesenteroides subsp. *dextranicum*
Listeria innocua
Listeria ivanovii
Listeria monocytogenes
Listeria seeligeri
Macrococcus caseolyticus
Magnetospirillum magnetotacticum
Megamonas hypermegale
Methanobacterium thermoautotrophicum
Methanococcus jannaschii
Methanococcus vannielii
Methanosarcina barkeri
Methanosarcina jannaschii
Methylobacillus flagellatum
Methylomonas clara
Micrococcus luteus
Micrococcus lylae
Mitsuokella multacidus
Mobiluncus curtisii subsp. *holmesii*
Moellerella thermoacetica
Moellerella wisconsensis
Moorella thermoacetica
Moraxella catarrhalis
Moraxella osloensis
Morganella morganii subsp. *morganii*
Mycobacterium avium
Mycobacterium bovis
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium leprae

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Mycobacterium terrae
Mycobacterium tuberculosis
Mycoplasma capricolum
Mycoplasma gallisepticum
Mycoplasma genitalium
Mycoplasma hominis
Mycoplasma pirum
Mycoplasma mycoides
Mycoplasma pneumoniae
Mycoplasma pulmonis
Mycoplasma salivarium
Myxococcus xanthus
Neisseria animalis
Neisseria canis
Neisseria cinerea
Neisseria cuniculi
Neisseria elongata subsp. *elongata*
Neisseria elongata subsp. *intermedia*
Neisseria flava
Neisseria flavescens
Neisseria gonorrhoeae
Neisseria lactamica
Leclercia adecarboxylata
Neisseria meningitidis
Neisseria mucosa
Neisseria perflava
Neisseria pharyngis var. *flava*
Neisseria polysaccharea
Neisseria sicca
Neisseria subflava
Neisseria weaveri
Obesumbacterium proteus
Ochrobactrum anthropi
Pantoea agglomerans
Pantoea dispersa
Paracoccus denitrificans
Pasteurella multocida
Pectinatus frisingensis
Peptococcus niger
Peptostreptococcus anaerobius
Peptostreptococcus asaccharolyticus
Peptostreptococcus prevotii
Phormidium ectocarpi
Pirellula marina
Planobispora rosea
Plesiomonas shigelloides
Plectonema boryanum
Porphyromonas asaccharolytica
Porphyromonas gingivalis
Pragia fontium
Prevotella buccalis
Prevotella melaninogenica
Prevotella oralis
Prevotella ruminocola
Prochlorothrix hollandica
Propionibacterium acnes
Propionigenium modestum
Proteus mirabilis
Proteus penneri
Proteus vulgaris
Providencia alcalifaciens
Providencia rettgeri
Providencia rustigianii
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Psychrobacter phenylpyruvicum
Pyrococcus abyssi
Rahnella aquatilis
Rickettsia prowazekii

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Rhizobium leguminosarum
Rhizobium phaseoli
Rhodobacter capsulatus
Rhodobacter sphaeroides Rhodopseudomonas palustris
Rhodospirillum rubrum
Ruminococcus albus
Ruminococcus bromii
Salmonella bongori
Salmonella choleraesuis subsp. *arizonae*
Salmonella choleraesuis subsp *choleraesuis*
Salmonella choleraesuis subsp. *diarizonae*
Salmonella choleraesuis subsp. *houtenae*
Salmonella choleraesuis subsp. *indica*
Salmonella choleraesuis subsp. *salamae*
Serpulina hyodysenteriae
Serratia ficaria
Serratia fonticola
Serratia grimesii
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Serratia plymuthica
Serratia rubidaea
Shewanella putrefaciens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Sinorhizobium meliloti
Spirochaeta aurantia
Staphylococcus aureus
Staphylococcus aureus subsp. *aureus*
Staphylococcus auricularis
Staphylococcus capitis subsp. *capitis*
Staphylococcus cohnii subsp. *cohnii*
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus hominis subsp. hominis
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus sciuri subsp. sciuri
Staphylococcus simulans
Staphylococcus warneri
Stigmatella aurantiaca
Stenotrophomonas maltophilia
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus cricetus
Streptococcus cristatus
Streptococcus downei
Streptococcus dysgalactiae
Streptococcus equi subsp. equi
Streptococcus ferus
Streptococcus gordonii
Streptococcus macacae
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus parasanguinis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivarius
Streptococcus salivarius subsp. *thermophilus*
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus suis
Streptococcus uberis

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention. Bacterial species
Streptococcus vestibularis
Streptomyces anbofaciens
Streptomyces aureofaciens
Streptomyces cinnamoneus
Streptomyces coelicolor
Streptomyces collinus
Streptomyces lividans
Streptomyces netropsis
Streptomyces ramocissimus
Streptomyces rimosus
Streptomyces venezuelae
Succinivibrio dextrinosolvens
Synechococcus sp.
Synechocystis sp.
Tatumella ptyseos
Taxeobacter occealus
Tetragenococcus halophilus
Thermoplasma acidophilum
Thermotoga maritima
Thermus aquaticus
Thermus thermophilus
Thiobacillus ferrooxidans
Thiomonas cuprina
Trabulsiella guamensis
Treponema pallidum
Ureaplasma urealyticum
Veillonella parvula
Vibrio alginolyticus
Vibrio anguillarum
Vibrio cholerae
Vibrio mimicus
Wolinella succinogenes
Xanthomonas citri
Xanthomonas oryzae
Xenorhabdus bovieni
Xenorhabdus nematophilus
Yersinia bercovieri
Yersinia enterocolitica
Yersinia frederiksensii
Yersinia intermedia
Yersinia pestis
Yersinia pseudotuberculosis
Yersinia rohdei
Yokenella regensburgei
Zoogloea ramigera

TABLE 4

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention (continued)
Fungal species
Absidia corymbifera
Absidia glauca
Alternaria alternate
Arxula adeninivorans
Aspergillus flavus
Aspergillus fumigatus
Aspergillus nidulans
Aspergillus niger
Aspergillus oryzae
Aspergillus terreus
Aspergillus versicolor
Aureobasidium pullulans
Basidiobolus ranarum
Bipolaris hawaiiensis
Bilophila wadsworthia

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention (continued)
Blastoschizomyces capitatus
Blastomyces dermatitidis
Candida albicans
Candida catenulata
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida haemulonii
Candida inconspicua
Candida kefyr
Candida krusei
Candida lambica
Candida lusitaniae
Candida norvegica
Candida norvegensis
Candida parapsilosis
Candida rugosa
Candida sphaerica
Candida tropicalis
Candida utilis
Candida viswanathii
Candida zeylanoides
Cladophialophora carrionii
Coccidioides immitis
Coprinus cinereus
Cryptococcus albidus
Cryptococcus humicolus
Cryptococcus laurentii
Cryptococcus neoformans
Cunninghamella bertholletiae
Curvularia lunata
Emericella nidulans
Emmonsia parva
Eremothecium gossypii
Exophiala dermatitidis
Exophiala jeanselmei
Exophiala moniliae
Exserohilum rostratum
Eremothecium gossypii
Fonsecaea pedrosoi
Fusarium moniliforme
Fusarium oxysporum
Fusarium solani
Geotrichum sp.
Histoplasma capsulatum
Hortaea werneckii
Issatchenkia orientalis Kudrjanzev
Kluyveromyces lactis
Malassezia furfur
Malassezia pachydermatis
Malbranchea filamentosa
Metschnikowia pulcherrima
Microsporum audouinii
Microsporum canis
Mucor circinelloides
Neurospora crassa
Paecilomyces lilacinus
Paracoccidioides brasiliensis
Penicillium marneffei
Phialaphora verrucosa
Pichia anomala
Piedraia hortai
Podospora anserina
Podospora curvicolla
Puccinia graminis
Pseudallescheria boydii
Reclinomonas americana
Rhizomucor racemosus
Rhizopus oryzae
Rhodotorula minuta
Rhodotorula mucilaginosa

TABLE 4-continued

Example of microbial species for which tuf and/or atpD and/or recA nucleic acids and/or sequences are used in the present invention (continued)
Saccharomyces cerevisiae
Saksenaea vasiformis
Schizosaccharomyces pombe
Scopulariopsis koningii
Sordaria macrospora
Sporobolomyces salmonicolor
Sporothrix schenckii
Stephanoascus ciferrii
Syncephalastrum racemosum
Trichoderma reesei
Trichophyton mentagrophytes
Trichophyton rubrum
Trichophyton tonsurans
Trichosporon cutaneum
Ustilago maydis
Wangiella dermatitidis
Yarrowia lipolytica
Parasitical species
Babesia bigemina
Babesia bovis
Babesia microti
Blastocystis hominis
Crithidia fasciculata
Cryptosporidium parvum
Entamoeba histolytica
Giardia lamblia
Kentrophoros sp.
Leishmania aethiopica
Leishmania amazonensis
Leishmania braziliensis
Leishmania donovani
Leishmania infantum
Leishmania enriettii
Leishmania gerbilli
Leishmania guyanensis
Leishmania hertigi
Leishmania major
Leishmania mexicana
Leishmania panamensis
Leishmania tarentolae
Leishmania tropica
Neospora caninum
Onchocerca volvulus
Plasmodium berghei
Plasmodium falciparum
Plasmodium knowlesi
Porphyra purpurea
Toxoplasma gondii
Treponema pallidum
Trichomonas tenax
Trichomonas vaginalis
Trypanosoma brucei
Trypanosoma brucei subsp. *brucei*
Trypanosoma congolense
Trypanosoma cruzi

TABLE 5

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
aac(3)-Ib[2]	Aminoglycosides	*Enterobacteriaceae* Pseudomonads	L06157	
aac(3)-IIb[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M97172	
aac(3)-IVa[2]	Aminoglycosides	*Enterobacteriaceae*	X01385	
aac(3)-VIa[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M88012	
aac(2')-1a[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	X04555	
aac(6')-aph(2'')[2]	Aminoglycosides	*Enterococcus* sp., *Staphylococcus* sp.		83-86[3]
aac(6')-Ia,[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M18967	
aac(6')-Ic[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	M94066	
aac(6')-IIa[2]	Aminoglycosides	Pseudomonads		112[4]
aadB [ant(2'')-Ia[2]]	Aminoglycosides	*Enterobacteriaceae*		53-54[3]
aacC1 [aac(3)-Ia[2]]	Aminoglycosides	Pseudomonads		55-56[3]
aacC2 [aac(3)-IIa[2]]	Aminoglycosides	Pseudomonads		57-58[3]
aacC3 [aac(3)-III[2]]	Aminoglycosides	Pseudomonads		59-60[3]
aacA4 [aac(6')-Ib[2]]	Aminoglycosides	Pseudomonads		65-66[3]
ant(3'')-Ia[2]	Aminoglycosides	*Enterobacteriaceae*, *Enterococcus* sp., *Staphylococcus* sp.	X02340 M10241	
ant(4')-Ia[2]	Aminoglycosides	*Staphylococcus* sp.	V01282	
aph(3')-Ia[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	J01839	
aph(3')-IIa[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	V00618	
aph(3')-IIIa[2]	Aminoglycosides	*Enterococcus* sp., *Staphylococcus* sp.	V01547	
aph(3')-VIa[2]	Aminoglycosides	*Enterobacteriaceae*, Pseudomonads	X07753	
rpsL[2]	Streptomycin	*M. tuberculosis*, *M. avium* complex	X80120 U14749 X70995 L08011	
bla_{OXA}[5,6]	β-lactams	*Enterobacteriaceae*, Pseudomonads	Y10693 AJ238349 AJ009819 X06046 X03037 X07260 U13880 X75562 AF034958 J03427 Z22590 U59183 L38523 U63835 AF043100 AF060206 U85514 AF043381 AF024602 AF064820	110[4]
bla_{ROB}[5]	β-lactams	*Haemophilus* sp.		45-48[3]
bla_{SHV}[5,6]	β-lactams	*Enterobacteriacea*, *Pseudomonas aeruginosa*	AF124984 AF148850 M59181 X98099 M33655 AF148851 X53433 L47119 AF074954 X53817 AF096930 X55640 Y11069	41-44[3]

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
			U20270	
			U92041	
			S82452	
			X98101	
			X98105	
			AF164577	
			AJ011428	
			AF116855	
			AB023477	
			AF293345	
			AF227204	
			AF208796	
			AF132290	
bla_{TEM}[5,6]	β-lactams	*Enterobacteriaceae*,	AF012911	37-40[3]
		Neisseria sp.,	U48775	
		Haemophilus sp.	AF093512	
			AF052748	
			X64523	
			Y13612	
			X57972	
			AF157413	
			U31280	
			U36911	
			U48775	
			V00613	
			X97254	
			AJ012256	
			X04515	
			AF126482	
			U09188	
			M88143	
			Y14574	
			AF188200	
			AJ251946	
			Y17581	
			Y17582	
			Y17583	
			M88143	
			U37195	
			Y17584	
			X64523	
			U95363	
			Y10279	
			Y10280	
			Y10281	
			AF027199	
			AF104441	
			AF104442	
			AF062386	
			X57972	
			AF047171	
			AF188199	
			AF157553	
			AF190694	
			AF190695	
			AF190693	
			AF190692	
bla_{SHV}[5,6]	β-lactams	*Enterobacteriacea*,	AF124984	41-44[3]
		Pseudomonas aeruginosa	AF148850	
			M59181	
			X98099	
			M33655	
			AF148851	
			X53433	
			L47119	
			AF074954	
			X53817	
			AF096930	
			X55640	
			Y11069	
			U20270	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
			U92041	
			S82452	
			X98101	
			X98105	
			AF164577	
			AJ011428	
			AF116855	
			AB023477	
			AF293345	
			AF227204	
			AF208796	
			AF132290	
bla$_{TEM}$[5,6]	β-lactams	*Enterobacteriaceae*,	AF012911	37-40[3]
		Neisseria sp.,	U48775	
		Haemophilus sp.	AF093512	
			AF052748	
			X64523	
			Y13612	
			X57972	
			AF157413	
			U31280	
			U36911	
			U48775	
			V00613	
			X97254	
			AJ012256	
			X04515	
			AF126482	
			U09188	
			M88143	
			Y14574	
			AF188200	
			AJ251946	
			Y17581	
			Y17582	
			Y17583	
			M88143	
			U37195	
			Y17584	
			X64523	
			U95363	
			Y10279	
			Y10280	
			Y10281	
			AF027199	
			AF104441	
			AF104442	
			AF062386	
			X57972	
			AF047171	
			AF188199	
			AF157553	
			AF190694	
			AF190695	
			AF190693	
			AF190692	
bla$_{CARB}$[5]	β-lactams	*Pseudomonas* sp.,	J05162	
		Enterobacteriaceae	S46063	
			M69058	
			U14749	
			D86225	
			D13210	
			Z18955	
			AF071555	
			AF153200	
			AF030945	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
$bla_{CTX\text{-}M\text{-}1}$[5]	β-lactams	*Enterobacteriaceae*	X92506	
$bla_{CTX\text{-}M\text{-}2}$[5]	β-lactams	*Enterobacteriaceae*	X92507	
$bla_{CMY\text{-}2}$[7]	β-lactams	*Enterobacteriaceae*	X91840	
			AJ007826	
			AJ011293	
			AJ011291	
			Y17716	
			Y16783	
			Y16781	
			Y15130	
			U77414	
			S83226	
			Y15412	
			X78117	
bla_{IMP}[5]	β-lactams	*Enterobacteriaceae*,	AJ223604	
		Pseudomonas aeruginosa	S71932	
			D50438	
			D29636	
			X98393	
			AB010417	
			D78375	
$bla_{PER\text{-}1}$[5]	β-lactams	*Enterobacteriaceae*,	Z21957	
		Pseudomodanaceae		
$bla_{PER\text{-}2}$[7]	β-lactams	*Enterobacteriaceae*	X93314	
blaZ[12]	β-lactams	*Enterococcus* sp.,		111[4]
		Staphylococcus sp.		
mecA[12]	β-lactams	*Staphylococcus* sp.		97-98[3]
pbp1a[13]	β-lactams	*Streptococcus pneumoniae*	M90527	1004-1018,
			X67872	1648, 2056-2064,
			AB006868	2273-2276
			AB006874	
			X67873	
			AB006878	
			AB006875	
			AB006877	
			AB006879	
			AF046237	
			AF046235	
			AF026431	
			AF046232	
			AF046233	
			AF046236	
			X67871	
			Z49095	
			AF046234	
			AB006873	
			X67866	
			X67868	
			AB006870	
			AB006869	
			AB006872	
			X67870	
			AB006871	
			X67867	
			X67869	
			AB006876	
			AF046230	
			AF046238	
			Z49094	
pbp2b[13]	β-lactams	*Streptococcus pneumoniae*	X16022	1019-1033
			M25516	
			M25518	
			M25515	
			U20071	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
			U20084 U20082 U20067 U20079 Z22185 U20072	
pbp2b[13]	β-lactams	*Streptococcus pneumoniae*	U20083 U20081 M25522 U20075 U20070 U20077 U20068 Z22184 U20069 U20078 M25521 M25525 M25519 Z21981 M25523 M25526 U20076 U20074 M25520 M25517 M25524 Z22230 U20073 U20080	
pbp2x[13]	β-lactams	*Streptococcus pneumoniae*	X16367 X65135 AB011204 AB011209 AB011199 AB011200 AB011201 AB011202 AB011198 AB011208 AB011205 AB015852 AB011210 AB015849 AB015850 AB015851 AB015847 AB015846 AB011207 AB015848 Z49096	1034-1048
int	β-lactams, trimethoprim	*Enterobacteriaceae*,		99-102[3]
sul	aminoglycosides, antiseptic, chloramphenicol	Pseudomonads		103-106[3]
ermA[14]	Macrolides, lincosamides, streptogramin B	*Staphylococcus* sp.		113[4]
ermB[14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae*, *Staphylococcus* sp. *Enterococcus* sp. *Streptococcus* sp.		114[4]
ermC[14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae*, *Staphylococcus* sp.		115[4]
ereA[12]	Macrolides	*Enterobacteriaceae*, *Staphylococcus* sp.	M11277 E01199 AF099140	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
ereB[12]	Macrolides	*Enterobacteriaceae*	A15097	
		Staphylococcus sp.	X03988	
msrA[12]	Macrolides	*Staphylococcus* sp.		77-80[3]
mefA, mefE[8]	Macrolides	*Streptococcus* sp.	U70055	
			U83667	
mphA[8]	Macrolides	*Enterobacteriaceae*,	D16251	
		Staphylococcus sp.	U34344	
			U36578	
linA/linA'[9]	Lincosamides	*Staphylococcus* sp.	J03947	
			M14039	
			A15070	
			E01245	
linB[10]	Lincosamides	*Enterococcus faecium*	AF110130	
			AJ238249	
vga[15]	Streptrogramin	*Staphylococcus* sp.	M90056	89-90[3]
			U82085	
vgb[15]	Streptrogramin	*Staphylococcus* sp.	M36022	
			M20219	
			AF015628	
vat[15]	Streptrogramin	*Staphylococcus* sp.	L07778	87-88[3]
vatB[15]	Streptrogramin	*Staphylococcus* sp.	U19459	
			L38809	
satA[15]	Streptrogramin	*Enterococcus faecium*	L12033	81-82[3]
mupA[12]	Mupirocin	*Staphylococcus aureus*	X75439	
			X59478	
			X59477	
gyrA[16]	Quinolones	Gram-positive and	X95718	1255, 1607-1608,
		gram-negative bacteria	X06744	1764-1776,
			X57174	2013-2014,
			X16817	2277-2280
			X71437	
			AF065152	
			AF060881	
			D32252	
parC/grlA[16]	Quinolones	Gram-positive and	AB005036	1777-1785
		gram-negative bacteria	AF056287	
			X95717	
			AF129764	
			AB017811	
			AF065152	
parE/grlB[16]	Quinolones	Gram-positive bacteria	X95717	
			AF065153	
			AF058920	
norA[16]	Quinolones	*Staphylococcus* sp.	D90119	
			M80252	
			M97169	
mexR (nalB)[16]	Quinolones	*Pseudomonas aeruginosa*	U23763	
nfxB[16]	Quinolones	*Pseudomonas aeruginosa*	X65646	
cat[12]	Chloramphenicol	Gram-positive and	M55620	
		gram-negative bacteria	X15100	
			A24651	
			M28717	
			A00568	
			A00569	
			X74948	
			Y00723	
			A24362	
			A00569	
			M93113	
			M62822	
			M58516	
			V01277	
			X02166	
			M77169	
			X53796	
			J01841	
			X07848	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
ppflo-like	Chloramphenicol		AF071555	
embB[17]	Ethambutol	*Mycobacterium tuberculosis*	U68480	
pncA[17]	Pyrazinamide	*Mycobacterium tuberculosis*	U59967	
rpoB[17]	Rifampin	*Mycobacterium tuberculosis*	AF055891	
			AF055892	
			S71246	
			L27989	
			AF055893	
inhA[17]	Isoniazid	*Mycobacterium tuberculosis*	AF106077	
			U02492	
vanA[12]	Vancomycin	*Enterococcus* sp.		67-70[3]
				1049-1057
vanB[12]	Vancomycin	*Enterococcus* sp.		116[4]
vanC1[12]	Vancomycin	*Enterococcus gallinarum*		117[4]
				1058-1059
vanC2[12]	Vancomycin	*Enterococcus casseliflavus*	U94521	1060-1063
			U94522	
			U94523	
			U94524	
			U94525	
			L29638	
vanC3[12]	Vancomycin	*Enterococcus flavescens*	L29639	1064-1066
			U72706	
vanD[18]	Vancomycin	*Enterococcus faecium*	AF130997	
vanE[12]	Vancomycin	*Enterococcus faecium*	AF136925	
tetB[19]	Tetracycline	Gram-negative bacteria	J01830	
			AF162223	
			AP000342	
			S83213	
			U81141	
			V00611	
tetM[19]	Tetracycline	Gram-negative and Gram-positive bacteria	X52632	
			AF116348	
			U50983	
			X92947	
			M211136	
			U08812	
			X04388	
sul II[20]	Sulfonamides	Gram-negative bacteria	M36657	
			AF017389	
			AF017391	
dhfrIa[20]	Trimethoprim	Gram-negative bacteria	AJ238350	
			X17477	
			K00052	
			U09476	
			X00926	
dhfrIb[20]	Trimethoprim	Gram-negative bacteria	Z50805	
			Z50804	
dhfrV[20]	Trimethoprim	Gram-negative bacteria	X12868	
dhfrVI[20]	Trimethoprim	Gram-negative bacteria	Z86002	
dhfrVII[20]	Trimethoprim	Gram-negative bacteria	U31119	
			AF139109	
			X58425	
dhfrVIII[20]	Trimethoprim	Gram-negative bacteria	U10186	
			U09273	
dhfrIX[20]	Trimethoprim	Gram-negative bacteria	X57730	
dhfrXII[20]	Trimethoprim	Gram-negative bacteria	Z21672	
			AF175203	
			AF180731	
			M84522	
dhfrXIII[20]	Trimethoprim	Gram-negative bacteria	Z50802	
dhfrXV[20]	Trimethoprim	Gram-negative bacteria	Z83331	
dhfrXVII[20]	Trimethoprim	Gram-negative bacteria	AF170088	
			AF180469	
			AF169041	
dfrA[20]	Trimethoprim	*Staphylococcus* sp.	AF045472	
			U40259	
			AF051916	
			X13290	

TABLE 5-continued

Antimicrobial agents resistance genes selected for diagnostic purposes z

Gene	Antimicrobial agent	Bacteria[1]	ACCESSION NO.	SEQ ID NO.
			Y07536	
			Z16422	
			Z48233	

[1]Bacteria having high incidence for the specified antibiotic resistance gene. The presence of the antibiotic resistance genes in other bacteria is not excluded.
[2]Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbial. Rev. 57: 138-163.
[3]Antibiotic resistance genes from our assigned U.S. Pat. No. 6,001,564 for which we have selected PCR primer pairs.
[4]These SEQ ID NOs. refer to a previous patent (publication WO98/20157).
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[6]Nucleotide mutations in bla_{SHV}, bla_{TEM}, and bla_{OXA}, are associated with extended-spectrum β-lactamase or inhibitor-resistant β-lactamase.
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[14]Jensen, L. B., N. Frimodt-Moller, F. M. Aarestrup. 1999. Presence of erm gene classes in Gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. 170: 151-158:
[15]Thal, L. A., and M. J. Zervos. 1999. Occurrence and epidemiology of resistance to virginimycin and streptrogramins. J. Antimicrob. Chemother. 43: 171-176.
[16]Martinez J. L., A. Alonso, J. M. Gomez-Gomez, and F. Baquero. 1998. Quinolone resistance by mutations in chromosomal gyrase genes. Just the tip of the iceberg? J. Antimicrob. Chemother. 42: 683-688
[17]Cockerill III, F. R. 1999. Genetic methods for assessing antimicrobial resistance. Antimicrob. Agents. Chemother. 43: 199-212.
[18]Casadewall, B. and P. Courvalin. 1999 Characterization of the vanD glycopeptide resistance gene cluster from *Enterococcus faecium* BM 4339. J. Bacteriol. 181: 3644-3648.
[19]Roberts, M. C. 1999. Genetic mobility and distribution of tetracycline resistance determinants. Ciba Found. Symp. 207: 206-222.
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TABLE 6

List of bacterial toxins selected for diagnostic purposes.

Organism	Toxin	Accession number
Actinobacillus actinomycetemcomitans	Cytolethal distending toxin (cdtA, cdtB, cdtC)	AF006830
	Leukotoxin (ltxA)	M27399
Actinomyces pyogenes	Hemolysin (pyolysin)	U84782
Aeromonas hydrophila	Aerolysin (aerA)	M16495
	Haemolysin (hlyA)	U81555
	Cytotonic enterotoxin (alt)	L77573
Bacillus anthracis	Anthrax toxin (cya)	M23179
Bacillus cereus	Enterotoxin (bceT)	D17312
		AF192766, AF192767
	Enterotoxic hemolysin BL	AJ237785
	Non-haemolytic enterotoxins A, B and C (nhe)	Y19005
Bacillus mycoides	Hemolytic enterotoxin HBL	AJ243150 to AJ243153
Bacillus pseudomycoides	Hemolytic enterotoxin HBL	AJ243154 to AJ243156
Bacteroides fragilis	Enterotoxin (bftP)	U67735
	Matrix metalloprotease/enterotoxin (fragilysin)	S75941, AF038459
	Metalloprotease toxin-2	U90931
		AF081785
	Metalloprotease toxin-3	AF056297
Bordetella bronchiseptica	Adenylate cyclase hemolysin (cyaA)	Z37112, U22953
	Dermonecrotic toxin (dnt)	U59687
		AB020025
Bordetella pertussis	Pertussis toxin (S1 subunit, tox)	AJ006151
		AJ006153
		AJ006155
		AJ006157
		AJ006159
		AJ007363
		M14378, M16494
		AJ007364
		M13223
		X16347

TABLE 6-continued

List of bacterial toxins selected for diagnostic purposes.

Organism	Toxin	Accession number
	Adenyl cyclase (cya)	18323
	Dermonecrotic toxin (dnt)	U10527
Campylobacter jejuni	Cytolethal distending toxin (cdtA, cdtB, cdtC)	U51121
Citrobacter freundii	Shiga-like toxin (slt-llcA)	X67514, S53206
Clostridium botulinum	Botulism toxin (BoNT) (A, B, E and F serotypes are neurotoxic for humans; the other serotypes have not been considered)	X52066, X52088
		X73423
		M30196
		X70814
		X70819
		X71343
		Z11934
		X70817
		M81186
		X70818
		X70815
		X62089
		X62683
		S76749
		X81714
		X70816
		X70820
		X70281
		L35496
		M92906
Clostridium difficile	A toxin (enterotoxin) (tcdA) (cdtA)	AB012304
		AF053400
		Y12616
		X51797
		X17194
		M30307
	B toxin (cytotoxin) (toxB) (cdtB)	Z23277
		X53138
Clostridium perfringens	Alpha (phospholipase C) (cpa)	L43545
		L43546
		L43547
		L43548
		X13608
		X17300
		D10248
	Beta (dermonecrotic protein) (cpb)	L13198
		X83275
		L77965
	Enterotoxin (cpe)	AJ000766
		M98037
		X81849
		X71844
		Y16009
	Enterotoxin pseudogene (not expressed)	AF037328
		AF037329
		AF037330
	Epsilon toxin (etxD)	M80837
		M95206
		X60694
	Iota (Ia and Ib)	X73562
	Lambda (metalloprotease)	D45904
	Theta (perfringolysin O)	M36704
Clostridium sordellii	Cytotoxin L	X82638
Clostridium tetani	Tetanos toxin	X06214
		X04436
Corynebacterium diphtheriae	Diphtheriae toxin	X00703
Corynebacterium pseudotuberculosis	Phospholipase C	A21336
Eikenella corrodens	lysine decarboxylase (cadA)	U89166
Enterobacter cloacae	Shiga-like toxin II	Z50754, U33502
Enterococcus faecalis	Cytolysin B (cylB)	M38052
Escherichia coli (EHEC)	Hemolysin toxin (hlyA and ehxA)	AF043471
		X94129
		X79839
		X86087
		AB011549
		AF074613

TABLE 7

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1	*Acinetobacter baumannii*	This patent	tuf
2	*Actinomyces meyeri*	This patent	tuf
3	*Aerococcus viridans*	This patent	tuf
4	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	tuf
5	*Anaerorhabdus furcosus*	This patent	tuf
6	*Bacillus anthracis*	This patent	tuf
7	*Bacillus cereus*	This patent	tuf
8	*Bacteroides distasonis*	This patent	tuf
9	*Enterococcus casseliflavus*	This patent	tuf
10	*Staphylococcus saprophyticus*	This patent	tuf
11	*Bacteroides ovatus*	This patent	tuf
12	*Bartonella henselae*	This patent	tuf
13	*Bifidobacterium adolescentis*	This patent	tuf
14	*Bifidobacterium dentium*	This patent	tuf
15	*Brucella abortus*	This patent	tuf
16	*Burkholderia cepacia*	This patent	tuf
17	*Cedecea davisae*	This patent	tuf
18	*Cedecea neteri*	This patent	tuf
19	*Cedecea lapagei*	This patent	tuf
20	*Chlamydia pneumoniae*	This patent	tuf
21	*Chlamydia psittaci*	This patent	tuf
22	*Chlamydia trachomatis*	This patent	tuf
23	*Chryseobacterium meningosepticum*	This patent	tuf
24	*Citrobacter amalonaticus*	This patent	tuf
25	*Citrobacter braakii*	This patent	tuf
26	*Citrobacter koseri*	This patent	tuf
27	*Citrobacter farmeri*	This patent	tuf
28	*Citrobacter freundii*	This patent	tuf
29	*Citrobacter sedlakii*	This patent	tuf
30	*Citrobacter werkmanii*	This patent	tuf
31	*Citrobacter youngae*	This patent	tuf
32	*Clostridium perfringens*	This patent	tuf
33	*Comamonas acidovorans*	This patent	tuf
34	*Corynebacterium bovis*	This patent	tuf
35	*Corynebacterium cervicis*	This patent	tuf
36	*Corynebacterium flavescens*	This patent	tuf
37	*Corynebacterium kutscheri*	This patent	tuf
38	*Corynebacterium minutissimum*	This patent	tuf
39	*Corynebacterium mycetoides*	This patent	tuf
40	*Corynebacterium pseudogenitalium*	This patent	tuf
41	*Corynebacterium renale*	This patent	tuf
42	*Corynebacterium ulcerans*	This patent	tuf
43	*Corynebacterium urealyticum*	This patent	tuf
44	*Corynebacterium xerosis*	This patent	tuf
45	*Coxiella burnetii*	This patent	tuf
46	*Edwardsiella hoshinae*	This patent	tuf
47	*Edwardsiella tarda*	This patent	tuf
48	*Eikenella corrodens*	This patent	tuf
49	*Enterobacter aerogenes*	This patent	tuf
50	*Enterobacter agglomerans*	This patent	tuf
51	*Enterobacter amnigenus*	This patent	tuf
52	*Enterobacter asburiae*	This patent	tuf
53	*Enterobacter cancerogenus*	This patent	tuf
54	*Enterobacter cloacae*	This patent	tuf
55	*Enterobacter gergoviae*	This patent	tuf
56	*Enterobacter hormaechei*	This patent	tuf
57	*Enterobacter sakazakii*	This patent	tuf
58	*Enterococcus casseliflavus*	This patent	tuf
59	*Enterococcus cecorum*	This patent	tuf
60	*Enterococcus dispar*	This patent	tuf
61	*Enterococcus durans*	This patent	tuf
62	*Enterococcus faecalis*	This patent	tuf
63	*Enterococcus faecalis*	This patent	tuf
64	*Enterococcus faecium*	This patent	tuf
65	*Enterococcus flavescens*	This patent	tuf
66	*Enterococcus gallinarum*	This patent	tuf
67	*Enterococcus hirae*	This patent	tuf
68	*Enterococcus mundtii*	This patent	tuf
69	*Enterococcus pseudoavium*	This patent	tuf
70	*Enterococcus raffinosus*	This patent	tuf
71	*Enterococcus saccharolyticus*	This patent	tuf
72	*Enterococcus solitarius*	This patent	tuf

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
73	*Enterococcus casseliflavus*	This patent	tuf (C)
74	*Staphylococcus saprophyticus*	This patent	unknown
75	*Enterococcus flavescens*	This patent	tuf (C)
76	*Enterococcus gallinarum*	This patent	tuf (C)
77	*Ehrlichia canis*	This patent	tuf
78	*Escherichia coli*	This patent	tuf
79	*Escherichia fergusonii*	This patent	tuf
80	*Escherichia hermannii*	This patent	tuf
81	*Escherichia vulneris*	This patent	tuf
82	*Eubacterium lentum*	This patent	tuf
83	*Eubacterium nodatum*	This patent	tuf
84	*Ewingella americana*	This patent	tuf
85	*Francisella tularensis*	This patent	tuf
86	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	tuf
87	*Gemella haemolysans*	This patent	tuf
88	*Gemella morbillorum*	This patent	tuf
89	*Haemophilus actinomycetemcomitans*	This patent	tuf
90	*Haemophilus aphrophilus*	This patent	tuf
91	*Haemophilus ducreyi*	This patent	tuf
92	*Haemophilus haemolyticus*	This patent	tuf
93	*Haemophilus parahaemolyticus*	This patent	tuf
94	*Haemophilus parainfluenzae*	This patent	tuf
95	*Haemophilus paraphrophilus*	This patent	tuf
96	*Haemophilus segnis*	This patent	tuf
97	*Hafnia alvei*	This patent	tuf
98	*Kingella kingae*	This patent	tuf
99	*Klebsiella omithinolytica*	This patent	tuf
100	*Klebsiella oxytoca*	This patent	tuf
101	*Klebsiella planticola*	This patent	tuf
102	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	tuf
103	*Klebsiella pneumoniae pneumoniae*	This patent	tuf
104	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	tuf
105	*Kluyvera ascorbata*	This patent	tuf
106	*Kluyvera cryocrescens*	This patent	tuf
107	*Kluyvera georgiana*	This patent	tuf
108	*Lactobacillus casei* subsp. *casei*	This patent	tuf
109	*Lactococcus lactis* subsp. *lactis*	This patent	tuf
110	*Leclercia adecarboxylata*	This patent	tuf
111	*Legionella micdadei*	This patent	tuf
112	*Legionella pneumophila* subsp. *pneumophila*	This patent	tuf
113	*Leminorella grimontii*	This patent	tuf
114	*Leminorella richardii*	This patent	tuf
115	*Leptospira interrogans*	This patent	tuf
116	*Megamonas hypermegale*	This patent	tuf
117	*Mitsuokella multacidus*	This patent	tuf
118	*Mobiluncus curtisii* subsp. *holmesii*	This patent	tuf
119	*Moellerella wisconsensis*	This patent	tuf
120	*Moraxella catarrhalis*	This patent	tuf
121	*Morganella morganii* subsp. *morganii*	This patent	tuf
122	*Mycobacterium tuberculosis*	This patent	tuf
123	*Neisseria cinerea*	This patent	tuf
124	*Neisseria elongata* subsp. *elongata*	This patent	tuf
125	*Neisseria flavescens*	This patent	tuf
126	*Neisseria gonorrhoeae*	This patent	tuf
127	*Neisseria lactamica*	This patent	tuf
128	*Neisseria meningitidis*	This patent	tuf
129	*Neisseria mucosa*	This patent	tuf
130	*Neisseria sicca*	This patent	tuf
131	*Neisseria subflava*	This patent	tuf
132	*Neisseria weaveri*	This patent	tuf
133	*Ochrobactrum anthropi*	This patent	tuf
134	*Pantoea agglomerans*	This patent	tuf
135	*Pantoea dispersa*	This patent	tuf
136	*Pasteurella multocida*	This patent	tuf
137	*Peptostreptococcus anaerobius*	This patent	tuf
138	*Peptostreptococcus asaccharolyticus*	This patent	tuf
139	*Peptostreptococcus prevotii*	This patent	tuf
140	*Porphyromonas asaccharolytica*	This patent	tuf
141	*Porphyromonas gingivalis*	This patent	tuf
142	*Pragia fontium*	This patent	tuf
143	*Prevotella melaninogenica*	This patent	tuf
144	*Prevotella oralis*	This patent	tuf

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
145	*Propionibacterium acnes*	This patent	tuf
146	*Proteus mirabilis*	This patent	tuf
147	*Proteus penneri*	This patent	tuf
148	*Proteus vulgaris*	This patent	tuf
149	*Providencia alcalifaciens*	This patent	tuf
150	*Providencia rettgeri*	This patent	tuf
151	*Providencia rustigianii*	This patent	tuf
152	*Providencia stuartii*	This patent	tuf
153	*Pseudomonas aeruginosa*	This patent	tuf
154	*Pseudomonas fluorescens*	This patent	tuf
155	*Pseudomonas stutzeri*	This patent	tuf
156	*Psychrobacter phenylpyruvicum*	This patent	tuf
157	*Rahnella aquatilis*	This patent	tuf
158	*Salmonella choleraesuis* subsp. *arizonae*	This patent	tuf
159	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Choleraesuis	This patent	tuf
160	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	tuf
161	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Heidelberg	This patent	tuf
162	*Salmonella choleraesuis* subsp. *houtenae*	This patent	tuf
163	*Salmonella choleraesuis* subsp. *indica*	This patent	tuf
164	*Salmonella choleraesuis* subsp. *salamae*	This patent	tuf
165	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhi	This patent	tuf
166	*Serratia fonticola*	This patent	tuf
167	*Serratia liquefaciens*	This patent	tuf
168	*Serratia marcescens*	This patent	tuf
169	*Serratia odorifera*	This patent	tuf
170	*Serratia plymuthica*	This patent	tuf
171	*Serratia rubidaea*	This patent	tuf
172	*Shigella boydii*	This patent	tuf
173	*Shigella dysenteriae*	This patent	tuf
174	*Shigella flexneri*	This patent	tuf
175	*Shigella sonnei*	This patent	tuf
176	*Staphylococcus aureus*	This patent	tuf
177	*Staphylococcus aureus*	This patent	tuf
178	*Staphylococcus aureus*	This patent	tuf
179	*Staphylococcus aureus*	This patent	tuf
180	*Staphylococcus aureus* subsp. *aureus*	This patent	tuf
181	*Staphylococcus auricularis*	This patent	tuf
182	*Staphylococcus capitis* subsp. *capitis*	This patent	tuf
183	*Macrococcus caseolyticus*	This patent	tuf
184	*Staphylococcus cohnii* subsp. *cohnii*	This patent	tuf
185	*Staphylococcus epidermidis*	This patent	tuf
186	*Staphylococcus haemolyticus*	This patent	tuf
187	*Staphylococcus warneri*	This patent	tuf
188	*Staphylococcus haemolyticus*	This patent	tuf
189	*Staphylococcus haemolyticus*	This patent	tuf
190	*Staphylococcus haemolyticus*	This patent	tuf
191	*Staphylococcus hominis* subsp. *hominis*	This patent	tuf
192	*Staphylococcus warneri*	This patent	tuf
193	*Staphylococcus hominis*	This patent	tuf
194	*Staphylococcus hominis*	This patent	tuf
195	*Staphylococcus hominis*	This patent	tuf
196	*Staphylococcus hominis*	This patent	tuf
197	*Staphylococcus lugdunensis*	This patent	tuf
198	*Staphylococcus saprophyticus*	This patent	tuf
199	*Staphylococcus saprophyticus*	This patent	tuf
200	*Staphylococcus saprophyticus*	This patent	tuf
201	*Staphylococcus sciuri* subsp. *sciuri*	This patent	tuf
202	*Staphylococcus warneri*	This patent	tuf
203	*Staphylococcus warneri*	This patent	tuf
204	*Bifidobacterium longum*	This patent	tuf
205	*Stenotrophomonas maltophilia*	This patent	tuf
206	*Streptococcus acidominimus*	This patent	tuf
207	*Streptococcus agalactiae*	This patent	tuf
208	*Streptococcus agalactiae*	This patent	tuf
209	*Streptococcus agalactiae*	This patent	tuf
210	*Streptococcus agalactiae*	This patent	tuf
211	*Streptococcus anginosus*	This patent	tuf
212	*Streptococcus bovis*	This patent	tuf
213	*Streptococcus anginosus*	This patent	tuf
214	*Streptococcus cricetus*	This patent	tuf

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
215	*Streptococcus cristatus*	This patent	tuf
216	*Streptococcus downei*	This patent	tuf
217	*Streptococcus dysgalactiae*	This patent	tuf
218	*Streptococcus equi* subsp. *equi*	This patent	tuf
219	*Streptococcus ferus*	This patent	tuf
220	*Streptococcus gordonii*	This patent	tuf
221	*Streptococcus anginosus*	This patent	tuf
222	*Streptococcus macacae*	This patent	tuf
223	*Streptococcus gordonii*	This patent	tuf
224	*Streptococcus mutans*	This patent	tuf
225	*Streptococcus parasanguinis*	This patent	tuf
226	*Streptococcus ratti*	This patent	tuf
227	*Streptococcus sanguinis*	This patent	tuf
228	*Streptococcus sobrinus*	This patent	tuf
229	*Streptococcus suis*	This patent	tuf
230	*Streptococcus uberis*	This patent	tuf
231	*Streptococcus vestibularis*	This patent	tuf
232	*Tatumella ptyseos*	This patent	tuf
233	*Trabulsiella guamensis*	This patent	tuf
234	*Veillonella parvula*	This patent	tuf
235	*Yersinia enterocolitica*	This patent	tuf
236	*Yersinia frederiksenii*	This patent	tuf
237	*Yersinia intermedia*	This patent	tuf
238	*Yersinia pestis*	This patent	tuf
239	*Yersinia pseudotuberculosis*	This patent	tuf
240	*Yersinia rohdei*	This patent	tuf
241	*Yokenella regensburgei*	This patent	tuf
242	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	atpD
243	*Acinetobacter baumannii*	This patent	atpD
244	*Acinetobacter lwoffii*	This patent	atpD
245	*Staphylococcus saprophyticus*	This patent	atpD
246	*Alcaligenes faecalis* subsp. *faecalis*	This patent	atpD
247	*Bacillus anthracis*	This patent	atpD
248	*Bacillus cereus*	This patent	atpD
249	*Bacteroides distasonis*	This patent	atpD
250	*Bacteroides ovatus*	This patent	atpD
251	*Leclercia adecarboxylata*	This patent	atpD
252	*Stenotrophomonas maltophilia*	This patent	atpD
253	*Bartonella henselae*	This patent	atpD
254	*Bifidobacterium adolescentis*	This patent	atpD
255	*Brucella abortus*	This patent	atpD
256	*Cedecea davisae*	This patent	atpD
257	*Cedecea lapagei*	This patent	atpD
258	*Cedecea neteri*	This patent	atpD
259	*Chryseobacterium meningosepticum*	This patent	atpD
260	*Citrobacter amalonaticus*	This patent	atpD
261	*Citrobacter braakii*	This patent	atpD
262	*Citrobacter koseri*	This patent	atpD
263	*Citrobacter farmeri*	This patent	atpD
264	*Citrobacter freundii*	This patent	atpD
265	*Citrobacter koseri*	This patent	atpD
266	*Citrobacter sedlakii*	This patent	atpD
267	*Citrobacter werkmanii*	This patent	atpD
268	*Citrobacter youngae*	This patent	atpD
269	*Clostridium innocuum*	This patent	atpD
270	*Clostridium perfringens*	This patent	atpD
272	*Corynebacterium diphtheriae*	This patent	atpD
273	*Corynebacterium pseudodiphtheriticum*	This patent	atpD
274	*Corynebacterium ulcerans*	This patent	atpD
275	*Corynebacterium urealyticum*	This patent	atpD
276	*Coxiella burnetii*	This patent	atpD
277	*Edwardsiella hoshinae*	This patent	atpD
278	*Edwardsiella tarda*	This patent	atpD
279	*Eikenella corrodens*	This patent	atpD
280	*Enterobacter agglomerans*	This patent	atpD
281	*Enterobacter arnnigenus*	This patent	atpD
282	*Enterobacter asburiae*	This patent	atpD
283	*Enterobacter cancerogenus*	This patent	atpD
284	*Enterobacter cloacae*	This patent	atpD
285	*Enterobacter gergoviae*	This patent	atpD
286	*Enterobacter hormaechei*	This patent	atpD
287	*Enterobacter sakazakii*	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.			
SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
288	*Enterococcus avium*	This patent	atpD
289	*Enterococcus casseliflavus*	This patent	atpD
290	*Enterococcus durans*	This patent	atpD
291	*Enterococcus faecalis*	This patent	atpD
292	*Enterococcus faecium*	This patent	atpD
293	*Enterococcus gallinarum*	This patent	atpD
294	*Enterococcus saccharolyticus*	This patent	atpD
295	*Escherichia fergusonii*	This patent	atpD
296	*Escherichia hermannii*	This patent	atpD
297	*Escherichia vulneris*	This patent	atpD
298	*Eubacterium lentum*	This patent	atpD
299	*Ewingella americana*	This patent	atpD
300	*Francisella tularensis*	This patent	atpD
301	*Fusobacterium gonidiaformans*	This patent	atpD
302	*Fusobacterium necrophorum* subsp. *necrophorum*	This patent	atpD
303	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	atpD
304	*Gardnerella vaginalis*	This patent	atpD
305	*Gemella haemolysans*	This patent	atpD
306	*Gemella morbillorum*	This patent	atpD
307	*Haemophilus ducreyi*	This patent	atpD
308	*Haemophilus haemolyticus*	This patent	atpD
309	*Haemophilus parahaemolyticus*	This patent	atpD
310	*Haemophilus parainfluenzae*	This patent	atpD
311	*Hafnia alvei*	This patent	atpD
312	*Kingella kingae*	This patent	atpD
313	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	atpD
314	*Klebsiella omithinolytica*	This patent	atpD
315	*Klebsiella oxytoca*	This patent	atpD
316	*Klebsiella planticola*	This patent	atpD
317	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	atpD
318	*Kluyvera ascorbata*	This patent	atpD
319	*Kluyvera cryocrescens*	This patent	atpD
320	*Kluyvera georgiana*	This patent	atpD
321	*Lactobacillus acidophilus*	This patent	atpD
322	*Legionella pneumophila* subsp. *pneumophila*	This patent	atpD
323	*Leminorella grimontii*	This patent	atpD
324	*Listeria monocytogenes*	This patent	atpD
325	*Micrococcus lylae*	This patent	atpD
326	*Moellerella wisconsensis*	This patent	atpD
327	*Moraxella catarrhalis*	This patent	atpD
328	*Moraxella osloensis*	This patent	atpD
329	*Morganella morganii* subsp. *morganii*	This patent	atpD
330	*Pantoea agglomerans*	This patent	atpD
331	*Pantoea dispersa*	This patent	atpD
332	*Pasteurella multocida*	This patent	atpD
333	*Pragia fontium*	This patent	atpD
334	*Proteus mirabilis*	This patent	atpD
335	*Proteus vulgaris*	This patent	atpD
336	*Providencia alcalifaciens*	This patent	atpD
337	*Providencia rettgeri*	This patent	atpD
338	*Providencia rustigianii*	This patent	atpD
339	*Providencia stuartii*	This patent	atpD
340	*Psychrobacter phenylpyruvicum*	This patent	atpD
341	*Rahnella aquatilis*	This patent	atpD
342	*Salmonella choleraesuis* subsp. *arizonae*	This patent	atpD
343	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Choleraesuis	This patent	atpD
344	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	atpD
345	*Salmonella choleraesuis* subsp. *houtenae*	This patent	atpD
346	*Salmonella choleraesuis* subsp. *indica*	This patent	atpD
347	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	atpD
348	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi B	This patent	atpD
349	*Salmonella choleraesuis* subsp. *salamae*	This patent	atpD
350	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhi	This patent	atpD
351	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	This patent	atpD
352	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Virchow	This patent	atpD
353	*Serratia ficaria*	This patent	atpD
354	*Serratia fonticola*	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
355	*Serratia grimesii*	This patent	atpD
356	*Serratia liquefaciens*	This patent	atpD
357	*Serratia marcescens*	This patent	atpD
358	*Serratia odorifera*	This patent	atpD
359	*Serratia plymuthica*	This patent	atpD
360	*Serratia rubidaea*	This patent	atpD
361	*Pseudomonas putida*	This patent	atpD
362	*Shigella boydii*	This patent	atpD
363	*Shigella dysenteriae*	This patent	atpD
364	*Shigella flexneri*	This patent	atpD
365	*Shigella sonnei*	This patent	atpD
366	*Staphylococcus aureus*	This patent	atpD
367	*Staphylococcus auricularis*	This patent	atpD
368	*Staphylococcus capitis* subsp. *capitis*	This patent	atpD
369	*Staphylococcus cohnii* subsp. *cohnii*	This patent	atpD
370	*Staphylococcus epidermidis*	This patent	atpD
371	*Staphylococcus haemolyticus*	This patent	atpD
372	*Staphylococcus hominis* subsp. *hominis*	This patent	atpD
373	*Staphylococcus hominis*	This patent	atpD
374	*Staphylococcus lugdunensis*	This patent	atpD
375	*Staphylococcus saprophyticus*	This patent	atpD
376	*Staphylococcus simulans*	This patent	atpD
377	*Staphylococcus wameri*	This patent	atpD
378	*Streptococcus acidominimus*	This patent	atpD
379	*Streptococcus agalactiae*	This patent	atpD
380	*Streptococcus agalactiae*	This patent	atpD
381	*Streptococcus agalactiae*	This patent	atpD
382	*Streptococcus agalactiae*	This patent	atpD
383	*Streptococcus agalactiae*	This patent	atpD
384	*Streptococcus dysgalactiae*	This patent	atpD
385	*Streptococcus equi* subsp. *equi*	This patent	atpD
386	*Streptococcus anginosus*	This patent	atpD
387	*Streptococcus salivarius*	This patent	atpD
388	*Streptococcus suis*	This patent	atpD
389	*Streptococcus uberis*	This patent	atpD
390	*Tatumella ptyseos*	This patent	atpD
391	*Trabulsiella guamensis*	This patent	atpD
392	*Yersinia bercovieri*	This patent	atpD
393	*Yersinia enterocolitica*	This patent	atpD
394	*Yersinia frederiksenii*	This patent	atpD
395	*Yersinia intermedia*	This patent	atpD
396	*Yersinia pseudotuberculosis*	This patent	atpD
397	*Yersinia rohdei*	This patent	atpD
398	*Yokenella regensburgei*	This patent	atpD
399	*Yarrowia lipolytica*	This patent	tuf (EF-1)
400	*Absidia corymbifera*	This patent	tuf (EF-1)
401	*Alternaria alternata*	This patent	tuf (EF-1)
402	*Aspergillus flavus*	This patent	tuf (EF-1)
403	*Aspergillus fumigatus*	This patent	tuf (EF-1)
404	*Aspergillus fumigatus*	This patent	tuf (EF-1)
405	*Aspergillus niger*	This patent	tuf (EF-1)
406	*Blastoschizomyces capitatus*	This patent	tuf (EF-1)
407	*Candida albicans*	This patent	tuf (EF-1)
408	*Candida albicans*	This patent	tuf (EF-1)
409	*Candida albicans*	This patent	tuf (EF-1)
410	*Candida albicans*	This patent	tuf (EF-1)
411	*Candida albicans*	This patent	tuf (EF-1)
412	*Candida dubliniensis*	This patent	tuf (EF-1)
413	*Candida catenulata*	This patent	tuf (EF-1)
414	*Candida dubliniensis*	This patent	tuf (EF-1)
415	*Candida dubliniensis*	This patent	tuf (EF-1)
416	*Candida famata*	This patent	tuf (EF-1)
417	*Candida glabrata*	WO98/20157	tuf (EF-1)
418	*Candida guilliermondii*	This patent	tuf (EF-1)
419	*Candida haemulonii*	This patent	tuf (EF-1)
420	*Candida inconspicua*	This patent	tuf (EF-1)
421	*Candida kefyr*	This patent	tuf (EF-1)
422	*Candida krusei*	WO98/20157	tuf (EF-1)
423	*Candida lambica*	This patent	tuf (EF-1)
424	*Candida lusitaniae*	This patent	tuf (EF-1)
425	*Candida norvegensis*	This patent	tuf (EF-1)
426	*Candida parapsilosis*	WO98/20157	tuf (EF-1)

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
427	*Candida rugosa*	This patent	tuf (EF-1)
428	*Candida sphaerica*	This patent	tuf (EF-1)
429	*Candida tropicalis*	WO98/20157	tuf (EF-1)
430	*Candida utilis*	This patent	tuf (EF-1)
431	*Candida viswanathii*	This patent	tuf (EF-1)
432	*Candida zeylanoides*	This patent	tuf (EF-1)
433	*Coccidioides immitis*	This patent	tuf (EF-1)
434	*Cryptococcus albidus*	This patent	tuf (EF-1)
435	*Exophiala jeanselmei*	This patent	tuf (EF-1)
436	*Fusarium oxysporum*	This patent	tuf (EF-1)
437	*Geotrichum* sp.	This patent	tuf (EF-1)
438	*Histoplasma capsulatum*	This patent	tuf (EF-1)
439	*Issatchenkia orientalis* Kudrjanzev	This patent	tuf (EF-1)
440	*Malassezia furfur*	This patent	tuf (EF-1)
441	*Malassezia pachydermatis*	This patent	tuf (EF-1)
442	*Malbranchea filamentosa*	This patent	tuf (EF-1)
443	*Metschnikowia pulcherrima*	This patent	tuf (EF-1)
444	*Paecilomyces lilacinus*	This patent	tuf (EF-1)
445	*Paracoccidioides brasiliensis*	This patent	tuf (EF-1)
446	*Penicillium marneffei*	This patent	tuf (EF-1)
447	*Pichia anomala*	This patent	tuf (EF-1)
448	*Pichia anomala*	This patent	tuf (EF-1)
449	*Pseudallescheria boydii*	This patent	tuf (EF-1)
450	*Rhizopus oryzae*	This patent	tuf (EF-1)
451	*Rhodotorula minuta*	This patent	tuf (EF-1)
452	*Sporobolomyces salmonicolor*	This patent	tuf (EF-1)
453	*Sporothrix schenckii*	This patent	tuf (EF-1)
454	*Stephanoascus ciferrii*	This patent	tuf (EF-1)
455	*Trichophyton mentagrophytes*	This patent	tuf (EF-1)
456	*Trichosporon cutaneum*	This patent	tuf (EF-1)
457	*Wangiella dermatitidis*	This patent	tuf (EF-1)
458	*Aspergillus fumigatus*	This patent	atpD
459	*Blastoschizomyces capitatus*	This patent	atpD
460	*Candida albicans*	This patent	atpD
461	*Candida dubliniensis*	This patent	atpD
462	*Candida famata*	This patent	atpD
463	*Candida glabrata*	This patent	atpD
464	*Candida guilliermondii*	This patent	atpD
465	*Candida haemulonii*	This patent	atpD
466	*Candida inconspicua*	This patent	atpD
467	*Candida kefyr*	This patent	atpD
468	*Candida krusei*	This patent	atpD
469	*Candida lambica*	This patent	atpD
470	*Candida lusitaniae*	This patent	atpD
471	*Candida norvegensis*	This patent	atpD
472	*Candida parapsilosis*	This patent	atpD
473	*Candida rugosa*	This patent	atpD
474	*Candida sphaerica*	This patent	atpD
475	*Candida tropicalis*	This patent	atpD
476	*Candida utilis*	This patent	atpD
477	*Candida viswanathii*	This patent	atpD
478	*Candida zeylanoides*	This patent	atpD
479	*Coccidioides immitis*	This patent	atpD
480	*Cryptococcus albidus*	This patent	atpD
481	*Fusarium oxysporum*	This patent	atpD
482	*Geotrichum* sp.	This patent	atpD
483	*Histoplasma capsulatum*	This patent	atpD
484	*Malassezia furfur*	This patent	atpD
485	*Malassezia pachydermatis*	This patent	atpD
486	*Metschnikowia pulcherrima*	This patent	atpD
487	*Penicillium mameffei*	This patent	atpD
488	*Pichia anomala*	This patent	atpD
489	*Pichia anomala*	This patent	atpD
490	*Rhodotorula minuta*	This patent	atpD
491	*Rhodotorula mucilaginosa*	This patent	atpD
492	*Sporobolomyces salmonicolor*	This patent	atpD
493	*Sporothrix schenckii*	This patent	atpD
494	*Stephanoascus ciferrii*	This patent	atpD
495	*Trichophyton mentagrophytes*	This patent	atpD
496	*Wangiella dermatitidis*	This patent	atpD
497	*Yarrowia lipolytica*	This patent	atpD
498	*Aspergillus fumigatus*	This patent	tuf (M)

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
499	*Blastoschizomyces capitatus*	This patent	tuf (M)
500	*Candida rugosa*	This patent	tuf (M)
501	*Coccidioides immitis*	This patent	tuf (M)
502	*Fusarium oxysporum*	This patent	tuf (M)
503	*Histoplasma capsulatum*	This patent	tuf (M)
504	*Paracoccidioides brasiliensis*	This patent	tuf (M)
505	*Penicillium marneffei*	This patent	tuf (M)
506	*Pichia anomala*	This patent	tuf (M)
507	*Trichophyton mentagrophytes*	This patent	tuf (M)
508	*Yarrowia lipolytica*	This patent	tuf (M)
509	*Babesia bigemina*	This patent	tuf (EF-1)
510	*Babesia bovis*	This patent	tuf (EF-1)
511	*Crithidia fasciculata*	This patent	tuf (EF-1)
512	*Entamoeba histolytica*	This patent	tuf (EF-1)
513	*Giardia lamblia*	This patent	tuf (EF-1)
514	*Leishmania tropica*	This patent	tuf (EF-1)
515	*Leishmania aethiopica*	This patent	tuf (EF-1)
516	*Leishmania tropica*	This patent	tuf (EF-1)
517	*Leishmania donovani*	This patent	tuf (EF-1)
518	*Leishmania infantum*	This patent	tuf (EF-1)
519	*Leishmania enriettii*	This patent	tuf (EF-1)
520	*Leishmania gerbilli*	This patent	tuf (EF-1)
521	*Leishmania hertigi*	This patent	tuf (EF-1)
522	*Leishmania major*	This patent	tuf (EF-1)
523	*Leishmania amazonensis*	This patent	tuf (EF-1)
524	*Leishmania mexicana*	This patent	tuf (EF-1)
525	*Leishmania tarentolae*	This patent	tuf (EF-1)
526	*Leishmania tropica*	This patent	tuf (EF-1)
527	*Neospora caninum*	This patent	tuf (EF-1)
528	*Trichomonas vaginalis*	This patent	tuf (EF-1)
529	*Trypanosoma brucei* subsp. *brucei*	This patent	tuf (EF-1)
530	*Crithidia fasciculata*	This patent	atpD
531	*Leishmania tropica*	This patent	atpD
532	*Leishmania aethiopica*	This patent	atpD
533	*Leishmania donovani*	This patent	atpD
534	*Leishmania infantum*	This patent	atpD
535	*Leishmania gerbilli*	This patent	atpD
536	*Leishmania hertigi*	This patent	atpD
537	*Leishmania major*	This patent	atpD
538	*Leishmania amazonensis*	This patent	atpD
607	*Enterococcus faecalis*	WO98/20157	tuf
608	*Enterococcus faecium*	WO98/20157	tuf
609	*Enterococcus gallinarum*	WO98/20157	tuf
610	*Haemophilus influenzae*	WO98/20157	tuf
611	*Staphylococcus epidermidis*	WO98/20157	tuf
612	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	tuf
613	*Serratia ficaria*	This patent	tuf
614	*Enterococcus malodoratus*	This patent	tuf (C)
615	*Enterococcus durans*	This patent	tuf (C)
616	*Enterococcus pseudoavium*	This patent	tuf (C)
617	*Enterococcus dispar*	This patent	tuf (C)
618	*Enterococcus avium*	This patent	tuf (C)
619	*Saccharomyces cerevisiae*	Database	tuf (M)
621	*Enterococcus faecium*	This patent	tuf (C)
622	*Saccharomyces cerevisiae*	This patent	tuf (EF-1)
623	*Cryptococcus neoformans*	This patent	tuf (EF-1)
624	*Candida albicans*	WO98/20157	tuf (EF-1)
662	*Corynebacterium diphtheriae*	WO98/20157	tuf
663	*Candida catenulata*	This patent	atpD
665	*Saccharomyces cerevisiae*	Database	tuf (EF-1)
666	*Saccharomyces cerevisiae*	Database	atpD
667	*Trypanosoma cruzi*	This patent	atpD
668	*Corynebacterium glutamicum*	Database	tuf
669	*Escherichia coli*	Database	atpD
670	*Helicobacter pylori*	Database	atpD
671	*Clostridium acetobutylicum*	Database	atpD
672	*Cytophaga lytica*	Database	atpD
673	*Ehrlichia risticii*	This patent	atpD
674	*Vibrio cholerae*	This patent	atpD
675	*Vibrio cholerae*	This patent	tuf
676	*Leishmania enriettii*	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
677	*Babesia microti*	This patent	tuf (EF-1)
678	*Cryptococcus neoformans*	This patent	atpD
679	*Cryptococcus neoformans*	This patent	atpD
680	*Cunninghamella bertholletiae*	This patent	atpD
684	*Candida tropicalis*	Database	atpD (V)
685	*Enterococcus hirae*	Database	atpD (V)
686	*Chlamydia pneumoniae*	Database	atpD (V)
687	*Halobacterium salinarum*	Database	atpD (V)
688	*Homo sapiens*	Database	atpD (V)
689	*Plasmodium falciparum*	Database	atpD (V)
690	*Saccharomyces cerevisiae*	Database	atpD (V)
691	*Schizosaccharomyces pombe*	Database	atpD (V)
692	*Trypanosoma congolense*	Database	atpD (V)
693	*Thermus thermophilus*	Database	atpD (V)
698	*Escherichia coli*	WO98/20157	tuf
709	*Borrelia burgdorferi*	Database	atpD (V)
710	*Treponema pallidum*	Database	atpD (V)
711	*Chlamydia trachomatis*	Genome project	atpD (V)
712	*Enterococcus faecalis*	Genome project	atpD (V)
713	*Methanosarcina barkeri*	Database	atpD (V)
714	*Methanococcus jannaschii*	Database	atpD (V)
715	*Porphyromonas gingivalis*	Genome project	atpD (V)
716	*Streptococcus pneumoniae*	Genome project	atpD (V)
717	*Burkholderia mallei*	This patent	tuf
718	*Burkholderia pseudomallei*	This patent	tuf
719	*Clostridium beijerinckii*	This patent	tuf
720	*Clostridium innocuum*	This patent	tuf
721	*Clostridium novyi*	This patent	tuf
722	*Clostridium septicum*	This patent	tuf
723	*Clostridium tertium*	This patent	tuf
724	*Clostridium tetani*	This patent	tuf
725	*Enterococcus malodoratus*	This patent	tuf
726	*Enterococcus sulfureus*	This patent	tuf
727	*Lactococcus garvieae*	This patent	tuf
728	*Mycoplasma pirum*	This patent	tuf
729	*Mycoplasma salivarium*	This patent	tuf
730	*Neisseria polysaccharea*	This patent	tuf
731	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Enteritidis	This patent	tuf
732	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Gallinarum	This patent	tuf
733	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi B	This patent	tuf
734	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Virchow	This patent	tuf
735	*Serratia grimesii*	This patent	tuf
736	*Clostridium difficile*	This patent	tuf
737	*Burkholderia pseudomallei*	This patent	atpD
738	*Clostridium bifermentans*	This patent	atpD
739	*Clostridium beijerinckii*	This patent	atpD
740	*Clostridium difficile*	This patent	atpD
741	*Clostridium ramosum*	This patent	atpD
742	*Clostridium septicum*	This patent	atpD
743	*Clostridium tertium*	This patent	atpD
744	*Comamonas acidovorans*	This patent	atpD
745	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	atpD
746	*Neisseria canis*	This patent	atpD
747	*Neisseria cinerea*	This patent	atpD
748	*Neisseria cuniculi*	This patent	atpD
749	*Neisseria elongata* subsp. *elongata*	This patent	atpD
750	*Neisseria flavescens*	This patent	atpD
751	*Neisseria gonorrhoeae*	This patent	atpD
752	*Neisseria gonorrhoeae*	This patent	atpD
753	*Neisseria lactamica*	This patent	atpD
754	*Neisseria meningitidis*	This patent	atpD
755	*Neisseria mucosa*	This patent	atpD
756	*Neisseria subflava*	This patent	atpD
757	*Neisseria weaveri*	This patent	atpD
758	*Neisseria animalis*	This patent	atpD
759	*Proteus penneri*	This patent	atpD
760	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Enteritidis	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
761	*Yersinia pestis*	This patent	atpD
762	*Burkholderia mallei*	This patent	atpD
763	*Clostridium sordellii*	This patent	atpD
764	*Clostridium novyi*	This patent	atpD
765	*Clostridium botulinum*	This patent	atpD
766	*Clostridium histolyticum*	This patent	atpD
767	*Peptostreptococcus prevotii*	This patent	atpD
768	*Absidia corymbifera*	This patent	atpD
769	*Alternaria alternata*	This patent	atpD
770	*Aspergillus flavus*	This patent	atpD
771	*Mucor circinelloides*	This patent	atpD
772	*Piedraia hortai*	This patent	atpD
773	*Pseudallescheria boydii*	This patent	atpD
774	*Rhizopus oryzae*	This patent	atpD
775	*Scopulariopsis koningii*	This patent	atpD
776	*Trichophyton mentagrophytes*	This patent	atpD
777	*Trichophyton tonsurans*	This patent	atpD
778	*Trichosporon cutaneum*	This patent	atpD
779	*Cladophialophora carrionii*	This patent	tuf (EF-1)
780	*Cunninghamella bertholletiae*	This patent	tuf (EF-1)
781	*Curvularia lunata*	This patent	tuf (EF-1)
782	*Fonsecaea pedrosoi*	This patent	tuf (EF-1)
783	*Microsporum audouinii*	This patent	tuf (EF-1)
784	*Mucor circinelloides*	This patent	tuf (EF-1)
785	*Phialophora verrucosa*	This patent	tuf (EF-1)
786	*Saksenaea vasiformis*	This patent	tuf (EF-1)
787	*Syncephalastrum racemosum*	This patent	tuf (EF-1)
788	*Trichophyton tonsurans*	This patent	tuf (EF-1)
789	*Trichophyton mentagrophytes*	This patent	tuf (EF-1)
790	*Bipolaris hawaiiensis*	This patent	tuf (EF-1)
791	*Aspergillus fumigatus*	This patent	tuf (M)
792	*Trichophyton mentagrophytes*	This patent	tuf (M)
827	*Clostridium novyi*	This patent	atpD (V)
828	*Clostridium difficile*	This patent	atpD (V)
829	*Clostridium septicum*	This patent	atpD (V)
830	*Clostridium botulinum*	This patent	atpD (V)
831	*Clostridium perfringens*	This patent	atpD (V)
832	*Clostridium tetani*	This patent	atpD (V)
833	*Streptococcus pyogenes*	Database	atpD (V)
834	*Babesia bovis*	This patent	atpD (V)
835	*Cryptosporidium parvum*	This patent	atpD (V)
836	*Leishmania infantum*	This patent	atpD (V)
837	*Leishmania major*	This patent	atpD (V)
838	*Leishmania tarentolae*	This patent	atpD (V)
839	*Trypanosoma brucei*	This patent	atpD (V)
840	*Trypanosoma cruzi*	This patent	tuf (EF-1)
841	*Trypanosoma cruzi*	This patent	tuf (EF-1)
842	*Trypanosoma cruzi*	This patent	tuf (EF-1)
843	*Babesia bovis*	This patent	tuf (M)
844	*Leishmania aethiopica*	This patent	tuf (M)
845	*Leishmania amazonensis*	This patent	tuf (M)
846	*Leishmania donovani*	This patent	tuf (M)
847	*Leishmania infantum*	This patent	tuf (M)
848	*Leishmania enriettii*	This patent	tuf (M)
849	*Leishmania gerbilli*	This patent	tuf (M)
850	*Leishmania major*	This patent	tuf (M)
851	*Leishmania mexicana*	This patent	tuf (M)
852	*Leishmania tarentolae*	This patent	tuf (M)
853	*Trypanosoma cruzi*	This patent	tuf (M)
854	*Trypanosoma cruzi*	This patent	tuf (M)
855	*Trypanosoma cruzi*	This patent	tuf (M)
856	*Babesia bigemina*	This patent	atpD
857	*Babesia bovis*	This patent	atpD
858	*Babesia microti*	This patent	atpD
859	*Leishmania guyanensis*	This patent	atpD
860	*Leishmania mexicana*	This patent	atpD
861	*Leishmania tropica*	This patent	atpD
862	*Leishmania tropica*	This patent	atpD
863	*Bordetella pertussis*	Database	tuf
864	*Trypanosoma brucei brucei*	Database	tuf (EF-1)
865	*Cryptosporidium parvum*	This patent	tuf (EF-1)
866	*Staphylococcus saprophyticus*	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
867	*Zoogloea ramigera*	This patent	atpD
868	*Staphylococcus saprophyticus*	This patent	tuf
869	*Enterococcus casseliflavus*	This patent	tuf
870	*Enterococcus casseliflavus*	This patent	tuf
871	*Enterococcus flavescens*	This patent	tuf
872	*Enterococcus gallinarum*	This patent	tuf
873	*Enterococcus gallinarum*	This patent	tuf
874	*Staphylococcus haemolyticus*	This patent	tuf
875	*Staphylococcus epidermidis*	This patent	tuf
876	*Staphylococcus epidermidis*	This patent	tuf
877	*Staphylococcus epidermidis*	This patent	tuf
878	*Staphylococcus epidermidis*	This patent	tuf
879	*Enterococcus gallinarum*	This patent	tuf
880	*Pseudomonas aeruginosa*	This patent	tuf
881	*Enterococcus casseliflavus*	This patent	tuf
882	*Enterococcus casseliflavus*	This patent	tuf
883	*Enterococcus faecalis*	This patent	tuf
884	*Enterococcus faecalis*	This patent	tuf
885	*Enterococcus faecium*	This patent	tuf
886	*Enterococcus faecium*	This patent	tuf
887	*Zoogloea ramigera*	This patent	tuf
888	*Enterococcus faecalis*	This patent	tuf
889	*Aspergillus fumigatus*	This patent	atpD
890	*Penicillium mameffei*	This patent	atpD
891	*Paecilomyces lilacinus*	This patent	atpD
892	*Penicillium mameffei*	This patent	atpD
893	*Sporothrix schenckii*	This patent	atpD
894	*Malbranchea filamentosa*	This patent	atpD
895	*Paecilomyces lilacinus*	This patent	atpD
896	*Aspergillus niger*	This patent	atpD
897	*Aspergillus fumigatus*	This patent	tuf (EF-1)
898	*Penicillium mameffei*	This patent	tuf (EF-1)
899	*Piedraia hortai*	This patent	tuf (EF-1)
900	*Paecilomyces lilacinus*	This patent	tuf (EF-1)
901	*Paracoccidioides brasiliensis*	This patent	tuf (EF-1)
902	*Sporothrix schenckii*	This patent	tuf (EF-1)
903	*Penicillium mameffei*	This patent	tuf (EF-1)
904	*Curvularia lunata*	This patent	tuf (M)
905	*Aspergillus niger*	This patent	tuf (M)
906	*Bipolaris hawaiiensis*	This patent	tuf (M)
907	*Aspergillus flavus*	This patent	tuf (M)
908	*Alternaria alternata*	This patent	tuf (M)
909	*Penicillium mameffei*	This patent	tuf (M)
910	*Penicillium mameffei*	This patent	tuf (M)
918	*Escherichia coli*	Database	recA
929	*Bacteroides fragilis*	This patent	atpD (V)
930	*Bacteroides distasonis*	This patent	atpD (V)
931	*Porphyromonas asaccharolytica*	This patent	atpD (V)
932	*Listeria monocytogenes*	This patent	tuf
939	*Saccharomyces cerevisiae*	Database	recA (Rad51)
940	*Saccharomyces cerevisiae*	Database	recA (Dmc1)
941	*Cryptococcus humicolus*	This patent	atpD
942	*Escherichia coli*	This patent	atpD
943	*Escherichia coli*	This patent	atpD
944	*Escherichia coli*	This patent	atpD
945	*Escherichia coli*	This patent	atpD
946	*Neisseria polysaccharea*	This patent	atpD
947	*Neisseria sicca*	This patent	atpD
948	*Streptococcus mitis*	This patent	atpD
949	*Streptococcus mitis*	This patent	atpD
950	*Streptococcus mitis*	This patent	atpD
951	*Streptococcus oralis*	This patent	atpD
952	*Streptococcus pneumoniae*	This patent	atpD
953	*Streptococcus pneumoniae*	This patent	atpD
954	*Streptococcus pneumoniae*	This patent	atpD
955	*Streptococcus pneumoniae*	This patent	atpD
956	*Babesia microti*	This patent	atpD (V)
957	*Entamoeba histolytica*	This patent	atpD (V)
958	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	atpD (V)
959	*Leishmania aethiopica*	This patent	atpD (V)
960	*Leishmania tropica*	This patent	atpD (V)
961	*Leishmania guyanensis*	This patent	atpD (V)

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
962	*Leishmania donovani*	This patent	atpD (V)
963	*Leishmania hertigi*	This patent	atpD (V)
964	*Leishmania mexicana*	This patent	atpD (V)
965	*Leishmania tropica*	This patent	atpD (V)
966	*Peptostreptococcus anaerobius*	This patent	atpD (V)
967	*Bordetella pertussis*	This patent	tuf
968	*Bordetella pertussis*	This patent	tuf
969	*Enterococcus columbae*	This patent	tuf
970	*Enterococcus flavescens*	This patent	tuf
971	*Streptococcus pneumoniae*	This patent	tuf
972	*Escherichia coli*	This patent	tuf
973	*Escherichia coli*	This patent	tuf
974	*Escherichia coli*	This patent	tuf
975	*Escherichia coli*	This patent	tuf
976	*Mycobacterium avium*	This patent	tuf
977	*Streptococcus pneumoniae*	This patent	tuf
978	*Mycobacterium gordonae*	This patent	tuf
979	*Streptococcus pneumoniae*	This patent	tuf
980	*Mycobacterium tuberculosis*	This patent	tuf
981	*Staphylococcus warneri*	This patent	tuf
982	*Streptococcus mitis*	This patent	tuf
983	*Streptococcus mitis*	This patent	tuf
984	*Streptococcus mitis*	This patent	tuf
985	*Streptococcus oralis*	This patent	tuf
986	*Streptococcus pneumoniae*	This patent	tuf
987	*Enterococcus hirae*	This patent	tuf (C)
988	*Enterococcus mundtii*	This patent	tuf (C)
989	*Enterococcus raffinosus*	This patent	tuf (C)
990	*Bacillus anthracis*	This patent	recA
991	*Prevotella melaninogenica*	This patent	recA
992	*Enterococcus casseliflavus*	This patent	tuf
993	*Streptococcus pyogenes*	Database	speA
1002	*Streptococcus pyogenes*	WO98/20157	tuf
1003	*Bacillus cereus*	This patent	recA
1004	*Streptococcus pneumoniae*	This patent	pbp1a
1005	*Streptococcus pneumoniae*	This patent	pbp1a
1006	*Streptococcus pneumoniae*	This patent	pbp1a
1007	*Streptococcus pneumoniae*	This patent	pbp1a
1008	*Streptococcus pneumoniae*	This patent	pbp1a
1009	*Streptococcus pneumoniae*	This patent	pbp1a
1010	*Streptococcus pneumoniae*	This patent	pbp1a
1011	*Streptococcus pneumoniae*	This patent	pbp1a
1012	*Streptococcus pneumoniae*	This patent	pbp1a
1013	*Streptococcus pneumoniae*	This patent	pbp1a
1014	*Streptococcus pneumoniae*	This patent	pbp1a
1015	*Streptococcus pneumoniae*	This patent	pbp1a
1016	*Streptococcus pneumoniae*	This patent	pbp1a
1017	*Streptococcus pneumoniae*	This patent	pbp1a
1018	*Streptococcus pneumoniae*	This patent	pbp1a
1019	*Streptococcus pneumoniae*	This patent	pbp2b
1020	*Streptococcus pneumoniae*	This patent	pbp2b
1021	*Streptococcus pneumoniae*	This patent	pbp2b
1022	*Streptococcus pneumoniae*	This patent	pbp2b
1023	*Streptococcus pneumoniae*	This patent	pbp2b
1024	*Streptococcus pneumoniae*	This patent	pbp2b
1025	*Streptococcus pneumoniae*	This patent	pbp2b
1026	*Streptococcus pneumoniae*	This patent	pbp2b
1027	*Streptococcus pneumoniae*	This patent	pbp2b
1028	*Streptococcus pneumoniae*	This patent	pbp2b
1029	*Streptococcus pneumoniae*	This patent	pbp2b
1030	*Streptococcus pneumoniae*	This patent	pbp2b
1031	*Streptococcus pneumoniae*	This patent	pbp2b
1032	*Streptococcus pneumoniae*	This patent	pbp2b
1033	*Streptococcus pneumoniae*	This patent	pbp2b
1034	*Streptococcus pneumoniae*	This patent	pbp2x
1035	*Streptococcus pneumoniae*	This patent	pbp2x
1036	*Streptococcus pneumoniae*	This patent	pbp2x
1037	*Streptococcus pneumoniae*	This patent	pbp2x
1038	*Streptococcus pneumoniae*	This patent	pbp2x
1039	*Streptococcus pneumoniae*	This patent	pbp2x
1040	*Streptococcus pneumoniae*	This patent	pbp2x
1041	*Streptococcus pneumoniae*	This patent	pbp2x

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1042	*Streptococcus pneumoniae*	This patent	pbp2x
1043	*Streptococcus pneumoniae*	This patent	pbp2x
1044	*Streptococcus pneumoniae*	This patent	pbp2x
1045	*Streptococcus pneumoniae*	This patent	pbp2x
1046	*Streptococcus pneumoniae*	This patent	pbp2x
1047	*Streptococcus pneumoniae*	This patent	pbp2x
1048	*Streptococcus pneumoniae*	This patent	pbp2x
1049	*Enterococcus faecium*	This patent	vanA
1050	*Enterococcus gallinarum*	This patent	vanA
1051	*Enterococcus faecium*	This patent	vanA
1052	*Enterococcus faecium*	This patent	vanA
1053	*Enterococcus faecium*	This patent	vanA
1054	*Enterococcus faecalis*	This patent	vanA
1055	*Enterococcus gallinarum*	This patent	vanA
1056	*Enterococcus faecium*	This patent	vanA
1057	*Enterococcus flavescens*	This patent	vanA
1058	*Enterococcus gallinarum*	This patent	vanC1
1059	*Enterococcus gallinarum*	This patent	vanC1
1060	*Enterococcus casseliflavus*	This patent	vanC2
1061	*Enterococcus casseliflavus*	This patent	vanC2
1062	*Enterococcus casseliflavus*	This patent	vanC2
1063	*Enterococcus casseliflavus*	This patent	vanC2
1064	*Enterococcus flavescens*	This patent	vanC3
1065	*Enterococcus flavescens*	This patent	vanC3
1066	*Enterococcus flavescens*	This patent	vanC3
1067	*Enterococcus faecium*	This patent	vanXY
1068	*Enterococcus faecium*	This patent	vanXY
1069	*Enterococcus faecium*	This patent	vanXY
1070	*Enterococcus faecalis*	This patent	vanXY
1071	*Enterococcus gallinarum*	This patent	vanXY
1072	*Enterococcus faecium*	This patent	vanXY
1073	*Enterococcus flavescens*	This patent	vanXY
1074	*Enterococcus faecium*	This patent	vanXY
1075	*Enterococcus gallinarum*	This patent	vanXY
1076	*Escherichia coli*	Database	stx_1
1077	*Escherichia coli*	Database	stx_2
1093	*Staphylococcus saprophyticus*	This patent	unknown
1117	*Enterococcus faecium*	Database	vanB
1138	*Enterococcus gallinarum*	Database	vanC1
1139	*Enterococcus faecium*	Database	vanA
1140	*Enterococcus casseliflavus*	Database	vanC2
1141	*Enterococcus faecium*	Database	vanHAXY
1169	*Streptococcus pneumoniae*	Database	pbp1a
1172	*Streptococcus pneumoniae*	Database	pbp2b
1173	*Streptococcus pneumoniae*	Database	pbp2x
1178	*Staphylococcus aureus*	Database	mecA
1183	*Streptococcus pneumoniae*	Database	hexA
1184	*Streptococcus pneumoniae*	This patent	hexA
1185	*Streptococcus pneumoniae*	This patent	hexA
1186	*Streptococcus pneumoniae*	This patent	hexA
1187	*Streptococcus pneumoniae*	This patent	hexA
1188	*Streptococcus oralis*	This patent	hexA
1189	*Streptococcus mitis*	This patent	hexA
1190	*Streptococcus mitis*	This patent	hexA
1191	*Streptococcus mitis*	This patent	hexA
1198	*Staphylococcus saprophyticus*	This patent	unknown
1215	*Streptococcus pyogenes*	Database	pcp
1230	*Escherichia coli*	Database	tuf (EF-G)
1242	*Enterococcus faecium*	Database	ddl
1243	*Enterococcus faecalis*	Database	mtlF, mtlD
1244	*Staphylococcus aureus* subsp. *aureus*	This patent	unknown
1245	*Bacillus anthracis*	This patent	atpD
1246	*Bacillus mycoides*	This patent	atpD
1247	*Bacillus thuringiensis*	This patent	atpD
1248	*Bacillus thuringiensis*	This patent	atpD
1249	*Bacillus thuringiensis*	This patent	atpD
1250	*Bacillus weihenstephanensis*	This patent	atpD
1251	*Bacillus thuringiensis*	This patent	atpD
1252	*Bacillus thuringiensis*	This patent	atpD
1253	*Bacillus cereus*	This patent	atpD
1254	*Bacillus cereus*	This patent	atpD
1255	*Staphylococcus aureus*	This patent	gyrA

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.			
SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1256	*Bacillus weihenstephanensis*	This patent	atpD
1257	*Bacillus anthracis*	This patent	atpD
1258	*Bacillus thuringiensis*	This patent	atpD
1259	*Bacillus cereus*	This patent	atpD
1260	*Bacillus cereus*	This patent	atpD
1261	*Bacillus thuringiensis*	This patent	atpD
1262	*Bacillus thuringiensis*	This patent	atpD
1263	*Bacillus thuringiensis*	This patent	atpD
1264	*Bacillus thuringiensis*	This patent	atpD
1265	*Bacillus anthracis*	This patent	atpD
1266	*Paracoccidioides brasiliensis*	This patent	tuf (EF-1)
1267	*Blastomyces dermatitidis*	This patent	tuf (EF-1)
1268	*Histoplasma capsulatum*	This patent	tuf (EF-1)
1269	*Trichophyton rubrum*	This patent	tuf (EF-1)
1270	*Microsporum canis*	This patent	tuf (EF-1)
1271	*Aspergillus versicolor*	This patent	tuf (EF-1)
1272	*Exophiala moniliae*	This patent	tuf (EF-1)
1273	*Hortaea wemeckii*	This patent	tuf (EF-1)
1274	*Fusarium solani*	This patent	tuf (EF-1)
1275	*Aureobasidium pullulans*	This patent	tuf (EF-1)
1276	*Blastomyces dermatitidis*	This patent	tuf (EF-1)
1277	*Exophiala dermatitidis*	This patent	tuf (EF-1)
1278	*Fusarium moniliforme*	This patent	tuf (EF-1)
1279	*Aspergillus terreus*	This patent	tuf (EF-1)
1280	*Aspergillus fumigatus*	This patent	tuf (EF-1)
1281	*Cryptococcus laurentii*	This patent	tuf (EF-1)
1282	*Emmonsia parva*	This patent	tuf (EF-1)
1283	*Fusarium solani*	This patent	tuf (EF-1)
1284	*Sporothrix schenckii*	This patent	tuf (EF-1)
1285	*Aspergillus nidulans*	This patent	tuf (EF-1)
1286	*Cladophialophora carrionii*	This patent	tuf (EF-1)
1287	*Exserohilum rostratum*	This patent	tuf (EF-1)
1288	*Bacillus thuringiensis*	This patent	recA
1289	*Bacillus thuringiensis*	This patent	recA
1299	*Staphylococcus aureus*	Database	gyrA
1300	*Escherichia coli*	Database	gyrA
1307	*Staphylococcus aureus*	Database	gyrB
1320	*Escherichia coli*	Database	parC (grlA)
1321	*Staphylococcus aureus*	Database	parC (grlA)
1328	*Staphylococcus aureus*	Database	parE (grlB)
1348	unidentified bacterium	Database	aac2Ia
1351	*Pseudomonas aeruginosa*	Database	aac3Ib
1356	*Serratia marcescens*	Database	aac3IIb
1361	*Escherichia coli*	Database	aac3IVa
1366	*Enterobacter cloacae*	Database	aac3VIa
1371	*Citrobacter koseri*	Database	aac6Ia
1376	*Serratia marcescens*	Database	aac6Ic
1381	*Escherichia coli*	Database	ant3Ia
1386	*Staphylococcus aureus*	Database	ant4Ia
1391	*Escherichia coli*	Database	aph3Ia
1396	*Escherichia coli*	Database	aph3IIa
1401	*Enterococcus faecalis*	Database	aph3IIIa
1406	*Acinetobacter baumannii*	Database	aph3VIa
1411	*Pseudomonas aeruginosa*	Database	blaCARB
1416	*Klebsiella pneumoniae*	Database	blaCMY-2
1423	*Escherichia coli*	Database	blaCTX-M-1
1428	*Salmonella choleraesuis* subsp. *choleraesuis serotype* Typhimurium	Database	blaCTX-M-2
1433	*Pseudomonas aeruginosa*	Database	blaIMP
1438	*Escherichia coli*	Database	blaOXA2
1439	*Pseudomonas aeruginosa*	Database	blaOXA10
1442	*Pseudomonas aeruginosa*	Database	blaPER1
1445	*Salmonella choleraesuis* subsp. *choleraesuis serotype* Typhimurium	Database	blaPER2
1452	*Staphylococcus epidermidis*	Database	dfrA
1461	*Escherichia coli*	Database	dhfrIa
1470	*Escherichia coli*	Database	dhfrIb
1475	*Escherichia coli*	Database	dhfrV
1480	*Proteus mirabilis*	Database	dhfrVI
1489	*Escherichia coli*	Database	dhfrVII
1494	*Escherichia coli*	Database	dhfrVIII
1499	*Escherichia coli*	Database	dhfrIX

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1504	*Escherichia coli*	Database	dhfrXII
1507	*Escherichia coli*	Database	dhfrXIII
1512	*Escherichia coli*	Database	dhfrXV
1517	*Escherichia coli*	Database	dhfrXVII
1518	*Acinetobacter lwoffii*	This patent	fusA
1519	*Acinetobacter lwoffii*	This patent	fusA-tuf spacer
1520	*Acinetobacter lwoffii*	This patent	tuf
1521	*Haemophilus influenzae*	This patent	fusA
1522	*Haemophilus influenzae*	This patent	fusA-tuf spacer
1523	*Haemophilus influenzae*	This patent	tuf
1524	*Proteus mirabilis*	This patent	fusA
1525	*Proteus mirabilis*	This patent	fusA-tuf spacer
1526	*Proteus mirabilis*	This patent	fuf
1527	*Campylobacter curvus*	This patent	atpD
1530	*Escherichia coli*	Database	ereA
1535	*Escherichia coli*	Database	ereB
1540	*Staphylococcus haemolyticus*	Database	linA
1545	*Enterococcus faecium*	Database	linB
1548	*Streptococcus pyogenes*	Database	mefA
1551	*Streptococcus pneumoniae*	Database	mefE
1560	*Escherichia coli*	Database	mphA
1561	*Candida albicans*	This patent	tuf (EF-1)
1562	*Candida dubliniensis*	This patent	tuf (EF-1)
1563	*Candida famata*	This patent	tuf (EF-1)
1564	*Candida glabrata*	This patent	tuf (EF-1)
1565	*Candida guilliermondii*	This patent	tuf (EF-1)
1566	*Candida haemulonii*	This patent	tuf (EF-1)
1567	*Candida kefyr*	This patent	tuf (EF-1)
1568	*Candida lusitaniae*	This patent	tuf (EF-1)
1569	*Candida sphaerica*	This patent	tuf (EF-1)
1570	*Candida tropicalis*	This patent	tuf (EF-1)
1571	*Candida viswanathii*	This patent	tuf (EF-1)
1572	*Alcaligenes faecalis* subsp. *faecalis*	This patent	tuf
1573	*Prevotella buccalis*	This patent	tuf
1574	*Succinivibrio dextrinosolvens*	This patent	tuf
1575	*Tetragenococcus halophilus*	This patent	tuf
1576	*Campylobacter jejuni* subsp. *jejuni*	This patent	atpD
1577	*Campylobacter rectus*	This patent	atpD
1578	*Enterococcus casseliflavus*	This patent	fusA
1579	*Enterococcus gallinarum*	This patent	fusA
1580	*Streptococcus mitis*	This patent	fusA
1585	*Enterococcus faecium*	Database	satG
1590	Cloning vector pFW16	Database	tetM
1594	*Enterococcus faecium*	Database	vanD
1599	*Enterococcus faecalis*	Database	vanE
1600	*Campylobacter jejuni* subsp. *doylei*	This patent	atpD
1601	*Enterococcus sulfurous*	This patent	atpD
1602	*Enterococcus solitarius*	This patent	atpD
1603	*Campylobacter sputorum* subsp. *sputorum*	This patent	atpD
1604	*Enterococcus pseudoavium*	This patent	atpD
1607	*Klebsiella omithinolytica*	This patent	gyrA
1608	*Klebsiella oxytoca*	This patent	gyrA
1613	*Staphylococcus aureus*	Database	vatB
1618	*Staphylococcus cohnii*	Database	vatC
1623	*Staphylococcus aureus*	Database	vga
1628	*Staphylococcus aureus*	Database	vgaB
1633	*Staphylococcus aureus*	Database	vgb
1638	*Aspergillus fumigatus*	This patent	atpD
1639	*Aspergillus fumigatus*	This patent	atpD
1640	*Bacillus mycoides*	This patent	atpD
1641	*Bacillus mycoides*	This patent	atpD
1642	*Bacillus mycoides*	This patent	atpD
1643	*Bacillus pseudomycoides*	This patent	atpD
1644	*Bacillus pseudomycoides*	This patent	atpD
1645	*Budvicia aquatica*	This patent	atpD
1646	*Buttiauxella agrestis*	This patent	atpD
1647	*Candida norvegica*	This patent	atpD
1648	*Streptococcus pneumoniae*	This patent	pbp1a
1649	*Campylobacter lari*	This patent	atpD
1650	*Coccidioides immitis*	This patent	atpD
1651	*Emmonsia parva*	This patent	atpD
1652	*Erwinia amylovora*	This patent	atpD

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1653	*Fonsecaea pedrosoi*	This patent	atpD
1654	*Fusarium moniliforme*	This patent	atpD
1655	*Klebsiella oxytoca*	This patent	atpD
1656	*Microsporum audouinii*	This patent	atpD
1657	*Obesumbacterium proteus*	This patent	atpD
1658	*Paracoccidioides brasiliensis*	This patent	atpD
1659	*Plesiomonas shigelloides*	This patent	atpD
1660	*Shewanella putrefaciens*	This patent	atpD
1662	*Campylobacter curvus*	This patent	tuf
1663	*Campylobacter rectus*	This patent	tuf
1664	*Fonsecaea pedrosoi*	This patent	tuf
1666	*Microsporum audouinii*	This patent	tuf
1667	*Piedraia hortai*	This patent	tuf
1668	*Escherichia coli*	Database	tuf
1669	*Saksenaea vasiformis*	This patent	tuf
1670	*Trichophyton tonsurans*	This patent	tuf
1671	*Enterobacter aerogenes*	This patent	atpD
1672	*Bordetella pertussis*	Database	atpD
1673	*Arcanobacterium haemolyticum*	This patent	tuf
1674	*Butyrivibrio fibrisolvens*	This patent	tuf
1675	*Campylobacter jejuni* subsp. *doylei*	This patent	tuf
1676	*Campylobacter lari*	This patent	tuf
1677	*Campylobacter sputorum* subsp. *sputorum*	This patent	tuf
1678	*Campylobacter upsaliensis*	This patent	tuf
1679	*Globicatella sanguis*	This patent	tuf
1680	*Lactobacillus acidophilus*	This patent	tuf
1681	*Leuconostoc mesenteroides* subsp. *dextranicum*	This patent	tuf
1682	*Prevotella buccalis*	This patent	tuf
1683	*Ruminococcus bromii*	This patent	tuf
1684	*Paracoccidioides brasiliensis*	This patent	atpD
1685	*Candida norvegica*	This patent	tuf (EF-1)
1686	*Aspergillus nidulans*	This patent	tuf
1687	*Aspergillus terreus*	This patent	tuf
1688	*Candida norvegica*	This patent	tuf
1689	*Candida parapsilosis*	This patent	tuf
1702	*Streptococcus gordonii*	WO98/20157	recA
1703	*Streptococcus mutans*	WO98/20157	recA
1704	*Streptococcus pneumoniae*	WO98/20157	recA
1705	*Streptococcus pyogenes*	WO98/20157	recA
1706	*Streptococcus salivarius* subsp. *thermophilus*	WO98/20157	recA
1707	*Escherichia coli*	WO98/20157	oxa
1708	*Enterococcus faecalis*	WO98/20157	blaZ
1709	*Pseudomonas aeruginosa*	WO98/20157	aac6'-IIa
1710	*Staphylococcus aureus*	WO98/20157	ermA
1711	*Escherichia coli*	WO98/20157	ermB
1712	*Staphylococcus aureus*	WO98/20157	ermC
1713	*Enterococcus faecalis*	WO98/20157	vanB
1714	*Campylobacter jejuni* subsp. *jejuni*	This patent	recA
1715	*Abiotrophia adiacens*	WO98/20157	tuf
1716	*Abiotrophia defectiva*	WO98/20157	tuf
1717	*Corynebacterium accolens*	WO98/20157	tuf
1718	*Corynebacterium genitalium*	WO98/20157	tuf
1719	*Corynebacterium jeikeium*	WO98/20157	tuf
1720	*Corynebacterium pseudodiphtheriticum*	WO98/20157	tuf
1721	*Corynebacterium striatum*	WO98/20157	tuf
1722	*Enterococcus avium*	WO98/20157	tuf
1723	*Gardnerella vaginalis*	WO98/20157	tuf
1724	*Listeria innocua*	WO98/20157	tuf
1725	*Listeria ivanovii*	WO98/20157	tuf
1726	*Listeria monocytogenes*	WO98/20157	tuf
1727	*Listeria seeligeri*	WO98/20157	tuf
1728	*Staphylococcus aureus*	WO98/20157	tuf
1729	*Staphylococcus saprophyticus*	WO98/20157	tuf
1730	*Staphylococcus simulans*	WO98/20157	tuf
1731	*Streptococcus agalactiae*	WO98/20157	tuf
1732	*Streptococcus pneumoniae*	WO98/20157	tuf
1733	*Streptococcus salivarius*	WO98/20157	tuf
1734	*Agrobacterium radiobacter*	WO98/20157	tuf
1735	*Bacillus subtilis*	WO98/20157	tuf
1736	*Bacteroides fragilis*	WO98/20157	tuf
1737	*Borrelia burgdorferi*	WO98/20157	tuf
1738	*Brevibacterium linens*	WO98/20157	tuf

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1739	*Chlamydia trachomatis*	WO98/20157	tuf
1740	*Fibrobacter succinogenes*	WO98/20157	tuf
1741	*Flavobacterium ferrugineum*	WO98/20157	tuf
1742	*Helicobacter pylori*	WO98/20157	tuf
1743	*Micrococcus luteus*	WO98/20157	tuf
1744	*Mycobacterium tuberculosis*	WO98/20157	tuf
1745	*Mycoplasma genitalium*	WO98/20157	tuf
1746	*Neisseria gonorrhoeae*	WO98/20157	tuf
1747	*Rickettsia prowazekii*	WO98/20157	tuf
1748	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	WO98/20157	tuf
1749	*Shewanella putrefaciens*	WO98/20157	tuf
1750	*Stigmatella aurantiaca*	WO98/20157	tuf
1751	*Thiomonas cuprina*	WO98/20157	tuf
1752	*Treponema pallidum*	WO98/20157	tuf
1753	*Ureaplasma urealyticum*	WO98/20157	tuf
1754	*Wolinella succinogenes*	WO98/20157	tuf
1755	*Burkholderia cepacia*	WO98/20157	tuf
1756	*Bacillus anthracis*	This patent	recA
1757	*Bacillus anthracis*	This patent	recA
1758	*Bacillus cereus*	This patent	recA
1759	*Bacillus cereus*	This patent	recA
1760	*Bacillus mycoides*	This patent	recA
1761	*Bacillus pseudomycoides*	This patent	recA
1762	*Bacillus thuringiensis*	This patent	recA
1763	*Bacillus thuringiensis*	This patent	recA
1764	*Klebsiella oxytoca*	This patent	gyrA
1765	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	gyrA
1766	*Klebsiella planticola*	This patent	gyrA
1767	*Klebsiella pneumoniae*	This patent	gyrA
1768	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	gyrA
1769	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	gyrA
1770	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	gyrA
1771	*Klebsiella terrigena*	This patent	gyrA
1772	*Legionella pneumophila* subsp. *pneumophila*	This patent	gyrA
1773	*Proteus mirabilis*	This patent	gyrA
1774	*Providencia rettgeri*	This patent	gyrA
1775	*Proteus vulgaris*	This patent	gyrA
1776	*Yersinia enterocolitica*	This patent	gyrA
1777	*Klebsiella oxytoca*	This patent	parC (grlA)
1778	*Klebsiella oxytoca*	This patent	parC (grlA)
1779	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	parC (grlA)
1780	*Klebsiella planticola*	This patent	parC (grlA)
1781	*Klebsiella pneumoniae*	This patent	parC (grlA)
1782	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	parC (grlA)
1783	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	parC (grlA)
1784	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	parC (grlA)
1785	*Klebsiella terrigena*	This patent	parC (grlA)
1786	*Bacillus cereus*	This patent	fusA
1787	*Bacillus cereus*	This patent	fusA
1788	*Bacillus anthracis*	This patent	fusA
1789	*Bacillus cereus*	This patent	fusA
1790	*Bacillus anthracis*	This patent	fusA
1791	*Bacillus pseudomycoides*	This patent	fusA
1792	*Bacillus cereus*	This patent	fusA
1793	*Bacillus anthracis*	This patent	fusA
1794	*Bacillus cereus*	This patent	fusA
1795	*Bacillus weihenstephanensis*	This patent	fusA
1796	*Bacillus mycoides*	This patent	fusA
1797	*Bacillus thuringiensis*	This patent	fusA
1798	*Bacillus weihenstephanensis*	This patent	fusA-tuf spacer
1799	*Bacillus thuringiensis*	This patent	fusA-tuf spacer
1800	*Bacillus anthracis*	This patent	fusA-tuf spacer
1801	*Bacillus pseudomycoides*	This patent	fusA-tuf spacer
1802	*Bacillus anthracis*	This patent	fusA-tuf spacer
1803	*Bacillus cereus*	This patent	fusA-tuf spacer
1804	*Bacillus cereus*	This patent	fusA-tuf spacer
1805	*Bacillus mycoides*	This patent	fusA-tuf spacer
1806	*Bacillus cereus*	This patent	fusA-tuf spacer
1807	*Bacillus cereus*	This patent	fusA-tuf spacer
1808	*Bacillus cereus*	This patent	fusA-tuf spacer
1809	*Bacillus anthracis*	This patent	fusA-tuf spacer

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1810	*Bacillus mycoides*	This patent	tuf
1811	*Bacillus thuringiensis*	This patent	tuf
1812	*Bacillus cereus*	This patent	tuf
1813	*Bacillus weihenstephanensis*	This patent	tuf
1814	*Bacillus anthracis*	This patent	tuf
1815	*Bacillus cereus*	This patent	tuf
1816	*Bacillus cereus*	This patent	tuf
1817	*Bacillus anthracis*	This patent	tuf
1818	*Bacillus cereus*	This patent	tuf
1819	*Bacillus anthracis*	This patent	tuf
1820	*Bacillus pseudomycoides*	This patent	tuf
1821	*Bacillus cereus*	This patent	tuf
1822	*Streptococcus oralis*	This patent	fusA
1823	*Budvicia aquatica*	This patent	fusA
1824	*Buttiauxella agrestis*	This patent	fusA
1825	*Klebsiella oxytoca*	This patent	fusA
1826	*Plesiomonas shigelloides*	This patent	fusA
1827	*Shewanella putrefaciens*	This patent	fusA
1828	*Obesumbacterium proteus*	This patent	fusA
1829	*Klebsiella oxytoca*	This patent	fusA-tuf spacer
1830	*Budvicia aquatica*	This patent	fusA-tuf spacer
1831	*Plesiomonas shigelloides*	This patent	fusA-tuf spacer
1832	*Obesumbacterium proteus*	This patent	fusA-tuf spacer
1833	*Shewanella putrefaciens*	This patent	fusA-tuf spacer
1834	*Buttiauxella agrestis*	This patent	fusA-tuf spacer
1835	*Campylobacter coli*	This patent	tuf
1836	*Campylobacter fetus* subsp. *fetus*	This patent	tuf
1837	*Campylobacter fetus* subsp. *venerealis*	This patent	tuf
1838	*Buttiauxella agrestis*	This patent	tuf
1839	*Klebsiella oxytoca*	This patent	tuf
1840	*Plesiomonas shigelloides*	This patent	tuf
1841	*Shewanella putrefaciens*	This patent	tuf
1842	*Obesumbacterium proteus*	This patent	tuf
1843	*Budvicia aquatica*	This patent	tuf
1844	*Abiotrophia adiacens*	This patent	atpD
1845	*Arcanobacterium haemolyticum*	This patent	atpD
1846	*Basidiobolus ranarum*	This patent	atpD
1847	*Blastomyces dermatitidis*	This patent	atpD
1848	*Blastomyces dermatitidis*	This patent	atpD
1849	*Campylobacter coli*	This patent	atpD
1850	*Campylobacter fetus* subsp. *fetus*	This patent	atpD
1851	*Campylobacter fetus* subsp. *venerealis*	This patent	atpD
1852	*Campylobacter gracilis*	This patent	atpD
1853	*Campylobacter jejuni* subsp. *jejuni*	This patent	atpD
1854	*Enterococcus cecorum*	This patent	atpD
1855	*Enterococcus columbae*	This patent	atpD
1856	*Enterococcus dispar*	This patent	atpD
1857	*Enterococcus malodoratus*	This patent	atpD
1858	*Enterococcus mundtii*	This patent	atpD
1859	*Enterococcus raffinosus*	This patent	atpD
1860	*Globicatella sanguis*	This patent	atpD
1861	*Lactococcus garvieae*	This patent	atpD
1862	*Lactococcus lactis*	This patent	atpD
1863	*Listeria ivanovii*	This patent	atpD
1864	*Succinivibrio dextrinosolvens*	This patent	atpD
1865	*Tetragenococcus halophilus*	This patent	atpD
1866	*Campylobacter fetus* subsp. *fetus*	This patent	recA
1867	*Campylobacter fetus* subsp. *venerealis*	This patent	recA
1868	*Campylobacter jejuni* subsp. *jejuni*	This patent	recA
1869	*Enterococcus avium*	This patent	recA
1870	*Enterococcus faecium*	This patent	recA
1871	*Listeria monocytogenes*	This patent	recA
1872	*Streptococcus mitis*	This patent	recA
1873	*Streptococcus oralis*	This patent	recA
1874	*Aspergillus fumigatus*	This patent	tuf (M)
1875	*Aspergillus versicolor*	This patent	tuf (M)
1876	*Basidiobolus ranarum*	This patent	tuf (M)
1877	*Campylobacter gracilis*	This patent	tuf
1878	*Campylobacter jejuni* subsp. *jejuni*	This patent	tuf
1879	*Coccidioides immitis*	This patent	tuf (M)
1880	*Erwinia amylovora*	This patent	tuf

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
1881	*Salmonella choleraesuis* subsp. *choleraesuis serotype* Typhimurium	This patent	tuf
1899	*Klebsiella pneumoniae*	Database	blaSHV
1900	*Klebsiella pneumoniae*	Database	blaSHV
1901	*Escherichia coli*	Database	blaSHV
1902	*Klebsiella pneumoniae*	Database	blaSHV
1903	*Klebsiella pneumoniae*	Database	blaSHV
1904	*Escherichia coli*	Database	blaSHV
1905	*Pseudomonas aeruginosa*	Database	blaSHV
1927	*Neisseria meningitidis*	Database	blaTEM
1928	*Escherichia coli*	Database	blaTEM
1929	*Klebsiella oxytoca*	Database	blaTEM
1930	*Escherichia coli*	Database	blaTEM
1931	*Escherichia coli*	Database	blaTEM
1932	*Escherichia coli*	Database	blaTEM
1933	*Escherichia coli*	Database	blaTEM
1954	*Klebsiella pneumoniae* subsp. *pneumoniae*	Database	gyrA
1956	*Candida inconspicua*	This patent	tuf (M)
1957	*Candida utilis*	This patent	tuf (M)
1958	*Candida zeylanoides*	This patent	tuf (M)
1959	*Candida catenulata*	This patent	tuf (M)
1960	*Candida krusei*	This patent	tuf (M)
1965	Plasmid pGS05	Database	sulII
1970	Transposon Tn10	Database	tetB
1985	*Cryptococcus neoformans*	Database	tuf (EF-1)
1986	*Cryptococcus neoformans*	Database	tuf (EF-1)
1987	*Saccharomyces cerevisiae*	Database	tuf (EF-1)
1988	*Saccharomyces cerevisiae*	Database	tuf (EF-1)
1989	*Eremothecium gossypii*	Database	tuf (EF-1)
1990	*Eremothecium gossypii*	Database	tuf (EF-1)
1991	*Aspergillus oryzae*	Database	tuf (EF-1)
1992	*Aureobasidium pullulans*	Database	tuf (EF-1)
1993	*Histoplasma capsulatum*	Database	tuf (EF-1)
1994	*Neurospora crassa*	Database	tuf (EF-1)
1995	*Podospora anserina*	Database	tuf (EF-1)
1996	*Podospora curvicolla*	Database	tuf (EF-1)
1997	*Sordaria macrospora*	Database	tuf (EF-1)
1998	*Trichoderma reesei*	Database	tuf (EF-1)
2004	*Candida albicans*	Database	tuf (M)
2005	*Schizosaccharomyces pombe*	Database	tuf (M)
2010	*Klebsiella pneumoniae*	Database	blaTEM
2011	*Klebsiella pneumoniae*	Database	blaTEM
2013	*Kluyvera ascorbata*	This patent	gyrA
2014	*Kluyvera georgiana*	This patent	gyrA
2047	*Streptococcus pneumoniae*	Database	pbp1A
2048	*Streptococcus pneumoniae*	Database	pbp1A
2049	*Streptococcus pneumoniae*	Database	pbp1A
2050	*Streptococcus pneumoniae*	Database	pbp1A
2051	*Streptococcus pneumoniae*	Database	pbp1A
2052	*Streptococcus pneumoniae*	Database	pbp1A
2053	*Streptococcus pneumoniae*	Database	pbp1A
2054	*Streptococcus pneumoniae*	Database	gyrA
2055	*Streptococcus pneumoniae*	Database	parC
2056	*Streptococcus pneumoniae*	This patent	pbp1A
2057	*Streptococcus pneumoniae*	This patent	pbp1A
2058	*Streptococcus pneumoniae*	This patent	pbp1A
2059	*Streptococcus pneumoniae*	This patent	pbp1A
2060	*Streptococcus pneumoniae*	This patent	pbp1A
2061	*Streptococcus pneumoniae*	This patent	pbp1A
2062	*Streptococcus pneumoniae*	This patent	pbp1A
2063	*Streptococcus pneumoniae*	This patent	pbp1A
2064	*Streptococcus pneumoniae*	This patent	pbp1A
2072	*Mycobacterium tuberculosis*	Database	rpoB
2097	*Mycoplasma pneumoniae*	Database	tuf
2101	*Mycobacterium tuberculosis*	Database	inhA
2105	*Mycobacterium tuberculosis*	Database	embB
2129	*Clostridium difficile*	Database	cdtA
2130	*Clostridium difficile*	Database	cdtB
2137	*Pseudomonas putida*	Genome project	tuf
2138	*Pseudomonas aeruginosa*	Genome project	tuf
2139	*Campylobacter jejuni*	Database	atpD
2140	*Streptococcus pneumoniae*	Database	pbp1a

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
2144	*Staphylococcus aureus*	Database	mupA
2147	*Escherichia coli*	Database	catI
2150	*Escherichia coli*	Database	catII
2153	*Shigella flexneri*	Database	catIII
2156	*Clostridium perfringens*	Database	catP
2159	*Staphylococcus aureus*	Database	cat
2162	*Staphylococcus aureus*	Database	cat
2165	*Salmonella typhimurium*	Database	ppflo-like
2183	*Alcaligenes faecalis* subsp. *faecalis*	This patent	tuf
2184	*Campylobacter coli*	This patent	fusA
2185	*Succinivibrio dextrinosolvens*	This patent	tuf
2186	*Tetragenococcus halophilus*	This patent	tuf
2187	*Campylobacter jejuni* subsp. *jejuni*	This patent	fusA
2188	*Campylobacter jejuni* subsp. *jejuni*	This patent	fusA
2189	*Leishmania guyanensis*	This patent	atpD
2190	*Trypanosoma brucei brucei*	This patent	atpD
2191	*Aspergillus nidulans*	This patent	atpD
2192	*Leishmania panamensis*	This patent	atpD
2193	*Aspergillus nidulans*	This patent	tuf (M)
2194	*Aureobasidium pullulans*	This patent	tuf (M)
2195	*Emmonsia parva*	This patent	tuf (M)
2196	*Exserohilum rostratum*	This patent	tuf (M)
2197	*Fusarium moniliforme*	This patent	tuf (M)
2198	*Fusarium solani*	This patent	tuf (M)
2199	*Histoplasma capsulatum*	This patent	tuf (M)
2200	*Kocuria kristinae*	This patent	tuf
2201	*Vibrio mimicus*	This patent	tuf
2202	*Citrobacter freundii*	This patent	recA
2203	*Clostridium botulinum*	This patent	recA
2204	*Francisella tularensis*	This patent	recA
2205	*Peptostreptococcus anaerobius*	This patent	recA
2206	*Peptostreptococcus asaccharolyticus*	This patent	recA
2207	*Providencia stuartii*	This patent	recA
2208	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Paratyphi A	This patent	recA
2209	*Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	This patent	recA
2210	*Staphylococcus saprophyticus*	This patent	recA
2211	*Yersinia pseudotuberculosis*	This patent	recA
2212	*Zoogloea ramigera*	This patent	recA
2214	*Abiotrophia adiacens*	This patent	fusA
2215	*Acinetobacter baumannii*	This patent	fusA
2216	*Actinomyces meyeri*	This patent	fusA
2217	*Clostridium difficile*	This patent	fusA
2218	*Corynebacterium diphtheriae*	This patent	fusA
2219	*Enterobacter cloacae*	This patent	fusA
2220	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	fusA
2221	*Listeria monocytogenes*	This patent	fusA
2222	*Mycobacterium avium*	This patent	fusA
2223	*Mycobacterium gordonae*	This patent	fusA
2224	*Mycobacterium kansasii*	This patent	fusA
2225	*Mycobacterium terrae*	This patent	fusA
2226	*Neisseria polysaccharea*	This patent	fusA
2227	*Staphylococcus epidermidis*	This patent	fusA
2228	*Staphylococcus haemolyticus*	This patent	fusA
2229	*Succinivibrio dextrinosolvens*	This patent	fusA
2230	*Tetragenococcus halophilus*	This patent	fusA
2231	*Veillonella parvula*	This patent	fusA
2232	*Yersinia pseudotuberculosis*	This patent	fusA
2233	*Zoogloea ramigera*	This patent	fusA
2234	*Aeromonas hydrophila*	This patent	fusA
2235	*Abiotrophia adiacens*	This patent	fusA-tuf spacer
2236	*Acinetobacter baumannii*	This patent	fusA-tuf spacer
2237	*Actinomyces meyeri*	This patent	fusA-tuf spacer
2238	*Clostridium difficile*	This patent	fusA-tuf spacer
2239	*Corynebacterium diphtheriae*	This patent	fusA-tuf spacer
2240	*Enterobacter cloacae*	This patent	fusA-tuf spacer
2241	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	fusA-tuf spacer
2242	*Listeria monocytogenes*	This patent	fusA-tuf spacer
2243	*Mycobacterium avium*	This patent	fusA-tuf spacer
2244	*Mycobacterium gordonae*	This patent	fusA-tuf spacer
2245	*Mycobacterium kansasii*	This patent	fusA-tuf spacer

TABLE 7-continued

Origin of the nucleic acids and/or sequences in the sequence listing.

SEQ ID NO.	Archaeal, bacterial, fungal or parasitical species	Source	Gene*
2246	*Mycobacterium terrae*	This patent	fusA-tuf spacer
2247	*Neisseria polysaccharea*	This patent	fusA-tuf spacer
2248	*Staphylococcus epidermidis*	This patent	fusA-tuf spacer
2249	*Staphylococcus haemolyticus*	This patent	fusA-tuf spacer
2255	*Abiotrophia adiacens*	This patent	tuf
2256	*Acinetobacter baumannii*	This patent	tuf
2257	*Actinomyces meyeri*	This patent	tuf
2258	*Clostridium difficile*	This patent	tuf
2259	*Corynebacterium diphtheriae*	This patent	tuf
2260	*Enterobacter cloacae*	This patent	tuf
2261	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	tuf
2262	*Listeria monocytogenes*	This patent	tuf
2263	*Mycobacterium avium*	This patent	tuf
2264	*Mycobacterium gordonae*	This patent	tuf
2265	*Mycobacterium kansasii*	This patent	tuf
2266	*Mycobacterium terrae*	This patent	tuf
2267	*Neisseria polysaccharea*	This patent	tuf
2268	*Staphylococcus epidermidis*	This patent	tuf
2269	*Staphylococcus haemolyticus*	This patent	tuf
2270	*Aeromonas hydrophila*	This patent	tuf
2271	*Bilophila wadsworthia*	This patent	tuf
2272	*Brevundimonas diminuta*	This patent	tuf
2273	*Streptococcus mitis*	This patent	pbp1a
2274	*Streptococcus mitis*	This patent	pbp1a
2275	*Streptococcus mitis*	This patent	pbp1a
2276	*Streptococcus oralis*	This patent	pbp1a
2277	*Escherichia coli*	This patent	gyrA
2278	*Escherichia coli*	This patent	gyrA
2279	*Escherichia coli*	This patent	gyrA
2280	*Escherichia coli*	This patent	gyrA
2288	*Enterococcus faecium*	Database	ddl
2293	*Enterococcus faecium*	Database	vanA
2296	*Enterococcus faecalis*	Database	vanB

*tuf indicates tuf sequences, tuf (C) indicates tuf sequences divergent from main (usually A and B) copies of the elongation factor-Tu, tuf (EF-1) indicates tuf sequences of the eukaryotic type (elongation factor 1α), tuf (M) indicates tuf sequences from organellar (mostly mitochondrial) origin.
fusA indicates fusA sequences; fusA-tuf spacer indicates the intergenic region between fusA and tuf.
atpD indicates atpD sequences of the F-type, atpD (V) indicates atpD sequences of the V-type.
recA indicates recA sequences, recA(Rad51) indicates rad51 sequences or homologs and recA(Dmc1) indicates dmc1 sequences or homologs.

TABLE 8

Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers derived from tuf sequences.

Strain	Reference number	Strain	Reference number
Streptococcus acidominimus	ATCC 51726	*Bacteroides caccae*	ATCC 43185
Streptococcus agalactiae	ATCC 12403	*Bacteroides vulgatus*	ATCC 8482
Streptococcus agalactiae	ATCC 12973	*Bacteroides fragilis*	ATCC 25285
Streptococcus agalactiae	ATCC 13813	*Candida albicans*	ATCC 11006
Streptococcus agalactiae	ATCC 27591	*Clostridium innoculum*	ATCC 14501
Streptococcus agalactiae	CDCs 1073	*Clostridium ramosum*	ATCC 25582
Streptococcus anginosus	ATCC 27335	*Lactobacillus casei* subsp. *casei*	ATCC 393
Streptococcus anginosus	ATCC 33397	*Clostridium septicum*	ATCC 12464
Streptococcus bovis	ATCC 33317	*Corynebacterium cervicis*	NCTC 10604
Streptococcus anginosus	ATCC 27823	*Corynebacterium genitalium*	ATCC 33031
Streptococcus cricetus	ATCC 19642	*Corynebacterium urealyticum*	ATCC 43042
Streptococcus cristatus	ATCC 51100	*Enterococcus faecalis*	ATCC 29212
Streptococcus downei	ATCC 33748	*Enterococcus faecium*	ATCC 19434
Streptococcus dysgalactiae	ATCC 43078	*Eubacterium lentum*	ATCC 43055
Streptococcus equi subsp. *equi*	ATCC 9528	*Eubacterium nodutum*	ATCC 33099
Streptococcus ferus	ATCC 33477	*Gardnerella vaginalis*	ATCC 14018
Streptococcus gordonii	ATCC 10558	*Lactobacillus acidophilus*	ATCC 4356
Streptococcus macacae	ATCC 35911	*Lactobacillus crispatus*	ATCC 33820
Streptococcus mitis	ATCC 49456	*Lactobacillus gasseri*	ATCC 33323
Streptococcus mutans	ATCC 25175	*Lactobacillus johnsonii*	ATCC 33200
Streptococcus oralis	ATCC 35037	*Lactococcus lactis* subsp. *lactis*	ATCC 19435
Streptococcus parasanguinis	ATCC 15912	*Lactococcus lactis* subsp. *lactis*	ATCC 11454
Streptococcus parauberis	DSM 6631	*Listeria innocua*	ATCC 33090

TABLE 8-continued

Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers derived from tuf sequences.

Strain	Reference number	Strain	Reference number
Streptococcus pneumoniae	ATCC 27336	*Micrococcus luteus*	ATCC 9341
Streptococcus pyogenes	ATCC 19615	*Escherichia coli*	ATCC 25922
Streptococcus ratti	ATCC 19645	*Micrococcus lylae*	ATCC 27566
Streptococcus salivarius	ATCC 7073	*Porphyromonas asaccharolytica*	ATCC 25260
Streptococcus sanguinis	ATCC 10556	*Prevotella corporis*	ATCC 33547
Streptococcus sobrinus	ATCC 27352	*Prevotella melanogenica*	ATCC 25845
Streptococcus suis	ATCC 43765	*Staphylococcus aureus*	ATCC 13301
Streptococcus uberis	ATCC 19436	*Staphylococcus epidermidis*	ATCC 14990
Streptococcus vestubularis	ATCC 49124	*Staphylococcus saprophyticus*	ATCC 15305

TABLE 9

Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers derived from atpD sequences.

Strain	Reference number
Streptococcus acidominimus	ATCC 51726
Streptococcus agalactiae	ATCC 12400
Streptococcus agalactiae	ATCC 12403
Streptococcus agalactiae	ATCC 12973
Streptococcus agalactiae	ATCC 13813
Streptococcus agalactiae	ATCC 27591
Streptococcus agalactiae	CDCs-1073
Streptococcus anginosus	ATCC 27335
Streptococcus anginosus	ATCC 27823
Streptococcus bovis	ATCC 33317
Streptococcus cricetus	ATCC 19642
Streptococcus cristatus	ATCC 51100
Streptococcus downei	ATCC 33748
Streptococcus dysgalactiae	ATCC 43078
Streptococcus equi subsp. *equi*	ATCC 9528
Streptococcus ferus	ATCC 33477
Streptococcus gordonii	ATCC 10558
Streptococcus macacae	ATCC 35911
Streptococcus mitis	ATCC 49456
Streptococcus mutans	ATCC 25175
Streptococcus oralis	ATCC 35037
Streptococcus parasanguinis	ATCC 15912
Streptococcus parauberis	DSM 6631
Streptococcus pneumoniae	ATCC 27336
Streptococcus pyogenes	ATCC 19615
Streptococcus ratti	ATCC 19645
Streptococcus salivarius	ATCC 7073
Streptococcus sanguinis	ATCC 10556
Streptococcus sobrinus	ATCC 27352
Streptococcus suis	ATCC 43765
Streptococcus uberis	ATCC 19436
Streptococcus vestibularis	ATCC 49124

TABLE 10

Bacterial species used to test the specificity of the *Enterococcusspecific* amplification primers derived from tuf sequences.

Strain	Reference number
Gram-positive species (n = 74)	
Abiotrophia adiacens	ATCC 49176
Abiotrophia defectiva	ATCC 49175
Bacillus cereus	ATCC 14579
Bacillus subtilis	ATCC 27370
Bifidobacterium adolescentis	ATCC 27534

TABLE 10-continued

Bacterial species used to test the specificity of the *Enterococcusspecific* amplification primers derived from tuf sequences.

Strain	Reference number
Bifidobacterium breve	ATCC 15700
Bifidobacterium dentium	ATCC 27534
Bifidobacterium longum	ATCC 15707
Clostridium perfringens	ATCC 3124
Clostridium septicum	ATCC 12464
Corynebacterium aquaticus	ATCC 14665
Corynebacterium pseudodiphtheriticum	ATCC 10700
Enterococcus avium	ATCC 14025
Enterococcus casseliflavus	ATCC 25788
Enterococcus cecorum	ATCC 43199
Enterococcus columbae	ATCC 51263
Enterococcus dispar	ATCC 51266
Enterococcus durans	ATCC 19432
Enterococcus faecalis	ATCC 29212
Enterococcus faecium	ATCC 19434
Enterococcus flavescens	ATCC 49996
Enterococcus gallinarum	ATCC 49573
Enterococcus hirae	ATCC 8044
Enterococcus malodoratus	ATCC 43197
Enterococcus mundtii	ATCC 43186
Enterococcus pseudoavium	ATCC 49372
Enterococcus raffinosus	ATCC 49427
Enterococcus saccharolyticus	ATCC 43076
Enterococcus solitarius	ATCC 49428
Enterococcus sulfureus	ATCC 49903
Eubacterium lentum	ATCC 49903
Gemella haemolysans	ATCC 10379
Gemella morbillorum	ATCC 27842
Lactobacillus acidophilus	ATCC 4356
Leuconostoc mesenteroides	ATCC 19225
Listeria grayi	ATCC 19120
Listeria grayi	ATCC 19123
Listeria innocua	ATCC 33090
Listeria ivanovii	ATCC 19119
Listeria monocytogenes	ATCC 15313
Listeria seeligeri	ATCC 35967
Micrococcus luteus	ATCC 9341
Pediococcus acidilacti	ATCC 33314
Pediococcus pentosaceus	ATCC 33316
Peptococcus niger	ATCC 27731
Peptostreptococcus anaerobius	ATCC 27337
Peptostreptococcus indolicus	ATCC 29247
Peptostreptococcus micros	ATCC 33270
Propionibacterium acnes	ATCC 6919
Staphylococcus aureus	ATCC 43300
Staphylococcus capitis	ATCC 27840
Staphylococcus epidermidis	ATCC 14990
Staphylococcus haemolyticus	ATCC 29970
Staphylococcus hominis	ATCC 27844

TABLE 10-continued

Bacterial species used to test the specificity of the *Enterococcusspecific* amplification primers derived from tuf sequences.

Strain	Reference number
Staphylococcus lugdunensis	ATCC 43809
Staphylococcus saprophyticus	ATCC 15305
Staphylococcus simulans	ATCC 27848
Staphylococcus warneri	ATCC 27836
Streptococcus agalactiae	ATCC 13813
Streptococcus anginosus	ATCC 33397
Streptococcus bovis	ATCC 33317
Streptococcus constellatus	ATCC 27823
Streptococcus cristatus	ATCC 51100
Streptococcus intermedius	ATCC 27335
Streptococcus mitis	ATCC 49456
Streptococcus mitis	ATCC 3639
Streptococcus mutans	ATCC 27175
Streptococcus parasanguinis	ATCC 15912
Streptococcus pneumoniae	ATCC 27736
Streptococcus pneumoniae	ATCC 6303
Streptococcus pyogenes	ATCC 19615
Streptococcus salivarius	ATCC 7073
Streptococcus sanguinis	ATCC 10556
Streptococcus suis	ATCC 43765
Gram-negative species (n = 39)	
Acidominococcus fermentans	ATCC 2508
Acinetobacter baumannii	ATCC 19606
Alcaligenes faecalis	ATCC 8750
Anaerobiospirillum succiniproducens	ATCC 29305
Anaerorhabdus furcosus	ATCC 25662
Bacteroides distasonis	ATCC 8503
Bacteroides thetaiotaomicron	ATCC 29741
Bacteroides vulgatus	ATCC 8482
Bordetella pertussis	LSPQ 3702

TABLE 10-continued

Bacterial species used to test the specificity of the *Enterococcusspecific* amplification primers derived from tuf sequences.

Strain	Reference number
Bulkholderia cepacia	LSPQ 2217
Butyvibrio fibrinosolvens	ATCC 19171
Cardiobacterium hominis	ATCC 15826
Citrobacter freundii	ATCC 8090
Desulfovibrio vulgaris	ATCC 29579
Edwardsiellae tarda	ATCC 15947
Enterobacter cloacae	ATCC 13047
Escherichia coli	ATCC 25922
Fusobacterium russii	ATCC 25533
Haemophilus influenzae	ATCC 9007
Hafnia alvei	ATCC 13337
Klebsiella oxytoca	ATCC 13182
Meganomonas hypermegas	ATCC 25560
Mitsukoella multiacidus	ATCC 27723
Moraxella catarrhalis	ATCC 43628
Morganella morganii	ATCC 25830
Neisseria meningitidis	ATCC 13077
Pasteurella aerogenes	ATCC 27883
Proteus vulgaris	ATCC 13315
Providencia alcalifaciens	ATCC 9886
Providencia rettgeri	ATCC 9250
Pseudomonas aeruginosa	ATCC 27853
Salmonella typhimurium	ATCC 14028
Serratia marcescens	ATCC 13880
Shigella flexneri	ATCC 12022
Shigella sonnei	ATCC 29930
Succinivibrio dextrinosolvens	ATCC 19716
Tissierella praeacuta	ATCC 25539
Veillonella parvuala	ATCC 10790
Yersinia enterocolitica	ATCC 9610

TABLE 11

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
tuf sequences			
Bacteria			
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	tuf
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	tuf (EF-G)
Agrobacterium tumefaciens		X99673	tuf
Agrobacterium tumefaciens		X99673	tuf (EF-G)
Agrobacterium tumefaciens		X99674	tuf
Anacystis nidulans	PCC 6301	X17442	tuf
Aquifex aeolicus	VF5	AE000669	tuf
Aquifex aeolicus	VF5	AE000669	tuf (EF-G)
Aquifex pyrophilus		Genome project[2]	tuf (EF-G)
Aquifex pyrophilus		Y15787	tuf
Bacillus anthracis	Ames	Genome project[2]	tuf
Bacillus anthracis	Ames	Genome project[2]	tuf (EF-G)
Bacillus halodurans	C-125	AB017508	tuf
Bacillus halodurans	C-125	AB017508	tuf (EF-G)
Bacillus stearothermophilus	CCM 2184	AJ000260	tuf
Bacillus subtilis	168	D64127	tuf
Bacillus subtilis	168	D64127	tuf (EF-G)
Bacillus subtilis	DSM 10	Z99104	tuf
Bacillus subtilis	DSM 10	Z99104	tuf (EF-G)
Bacteroides forsythus	ATCC 43037	AB035466	tuf
Bacteroides fragilis	DSM 1151	—[1]	tuf
Bordetella bronchiseptica	RB50	Genome project[2]	tuf
Bordetella pertussis	Tohama 1	Genome project[2]	tuf
Bordetella pertussis	Tohama 1	Genome project[2]	tuf (EF-G)

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Borrelia burdorgferi	B31	U78193	tuf
Borrelia burgdorferi		AE001155	tuf (EF-G)
Brevibacterium linens	DSM 20425	X76863	tuf
Buchnera aphidicola	Ap	Y12307	tuf
Burkholderia pseudomallei	K96243	Genome project[2]	tuf (EF-G)
Campylobacter jejuni	NCTC 11168	Y17167	tuf
Campylobacter jejuni	NCTC 11168	CJ11168X2	tuf (EF-G)
Chlamydia pneumoniae	CWL029	AE001592	tuf
Chlamydia pneumoniae	CWL029	AE001639	tuf (EF-G)
Chlamydia trachomatis		M74221	tuf
Chlamydia trachomatis	D/UW-3/CX	AE001317	tuf (EF-G)
Chlamydia trachomatis	D/UW-3/CX	AE001305	tuf
Chlamydia trachomatis	F/IC-Cal-13	L22216	tuf
Chlorobium vibrioforme	DSM 263	X77033	tuf
Chloroflexus aurantiacus	DSM 636	X76865	tuf
Clostridium acetobutylicum	ATCC 824	Genome project[2]	tuf
Clostridium difficile	630	Genome project[2]	tuf
Clostridium difficile	630	Genome project[2]	tuf (EF-G)
Corynebacterium diphtheriae	NCTC 13129	Genome project[2]	tuf
Corynebacterium diphtheriae	NCTC 13129	Genome project[2]	tuf (EF-G)
Corynebacterium glutamicum	ASO 19	X77034	tuf
Corynebacterium glutamicum	MJ-233	E09634	tuf
Coxiella burnetii	Nine Mile phase I	AF136604	tuf
Cytophaga lytica	DSM 2039	X77035	tuf
Deinococcus radiodurans	R1	AE001891	tuf (EF-G)
Deinococcus radiodurans	R1	AE180092	tuf
Deinococcus radiodurans	R1	AE002041	tuf
Deinonema sp.		—[1]	tuf
Eikenella corrodens	ATCC 23834	Z12610	tuf
Eikenella corrodens	ATCC 23834	Z12610	tuf (EF-G)
Enterococcus faecalis		Genome project[2]	tuf (EF-G)
Escherichia coli		J01690	tuf
Escherichia coli		J01717	tuf
Escherichia coli		X00415	tuf (EF-G)
Escherichia coli		X57091	tuf
Escherichia coli	K-12 MG1655	U00006	tuf
Escherichia coli	K-12 MG1655	U00096	tuf
Escherichia coli	K-12 MG1655	AE000410	tuf (EF-G)
Fervidobacterium islandicum	DSM 5733	Y15788	tuf
Fibrobacter succinogenes	S85	X76866	tuf
Flavobacterium ferrigeneum	DSM 13524	X76867	tuf
Flexistipes sinusarabici		X59461	tuf
Gloeobacter violaceus	PCC 7421	U09433	tuf
Gloeothece sp.	PCC 6501	U09434	tuf
Haemophilus actinomycetemcomitans	HK1651	Genome project[2]	tuf
Haemophilus ducreyi	35000	AF087414	tuf (EF-G)
Haemophilus influenzae	Rd	U32739	tuf
Haemophilus influenzae	Rd	U32746	tuf
Haemophilus influenzae	Rd	U32739	tuf (EF-G)
Helicobacter pylori	26695	AE000511	tuf
Helicobacter pylori	J99	AE001539	tuf (EF-G)
Helicobacter pylori	J99	AE001541	tuf
Herpetosiphon aurantiacus	Hpga1	X76868	tuf
Klebsiella pneumoniae	M6H 78578	Genome project[2]	tuf
Klebsiella pneumoniae	M6H 78578	Genome project[2]	tuf (EF-G)
Lactobacillus paracasei		E13922	tuf
Legionella pneumophila	Philadelphia-1	Genome project[2]	tuf
Leptospira interrogans		AF115283	tuf
Leptospira interrogans		AF115283	tuf (EF-G)
Micrococcus luteus	IFO 3333	M17788	tuf (EF-G)
Micrococcus luteus	IFO 3333	M17788	tuf
Moraxella sp.	TAC II 25	AJ249258	tuf
Mycobacterium avium	104	Genome project[2]	tuf
Mycobacterium avium	104	Genome project[2]	tuf (EF-G)
Mycobacterium bovis	AF2122/97	Genome project[2]	tuf
Mycobacterium bovis	AF2122/97	Genome project[2]	tuf (EF-G)
Mycobacterium leprae		L13276	tuf
Mycobacterium leprae		Z14314	tuf
Mycobacterium leprae		Z14314	tuf (EF-G)
Mycobacterium leprae	Thai 53	D13869	tuf
Mycobacterium tuberculosis	Erdmann	S40925	tuf

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Mycobacterium tuberculosis	H37Rv	AL021943	tuf (EF-G)
Mycobacterium tuberculosis	H37Rv	Z84395	tuf
Mycobacterium tuberculosis	y42	AD000005	tuf
Mycobacterium tuberculosis	CSU#93	Genome project[2]	tuf
Mycobacterium tuberculosis	CSU#93	Genome project[2]	tuf (EF-G)
Mycoplasma capricolum	PG-31	X16462	tuf
Mycoplasma genitalium	G37	U39732	tuf
Mycoplasma genitalium	G37	U39689	tuf (EF-G)
Mycoplasma hominis		X57136	tuf
Mycoplasma hominis	PG21	M57675	tuf
Mycoplasma pneumoniae	M129	AE000019	tuf
Mycoplasma pneumoniae	M129	AE000058	tuf (EF-G)
Neisseria gonorrhoeae	MS11	L36380	tuf
Neisseria gonorrhoeae	MS11	L36380	tuf (EF-G)
Neisseria meningitidis	Z2491	Genome project[2]	tuf (EF-G)
Neisseria meningitidis	Z2491	Genome project[2]	tuf
Pasteurella multocida	Pm70	Genome project[2]	tuf
Peptococcus niger	DSM 20745	X76869	tuf
Phormidium ectocarpi	PCC 7375	U09443	tuf
Planobispora rosea	ATCC 53773	U67308	tuf
Planobispora rosea	ATCC 53733	X98830	tuf
Planobispora rosea	ATCC 53733	X98830	tuf (EF-G)
Plectonema boryanum	PCC 73110	U09444	tuf
Porphyromonas gingivalis	W83	Genome project[2]	tuf
Porphyromonas gingivalis	W83	Genome project[2]	tuf (EF-G)
Porphyromonas gingivalis	FDC 381	AB035461	tuf
Porphyromonas gingivalis	W83	AB035462	tuf
Porphyromonas gingivalis	SUNY 1021	AB035463	tuf
Porphyromonas gingivalis	A7A1-28	AB035464	tuf
Porphyromonas gingivalis	ATCC 33277	AB035465	tuf
Porphyromonas gingivalis	ATCC 33277	AB035471	tuf (EF-G)
Prochlorothrix hollandica		U09445	tuf
Pseudomonas aeruginosa	PAO-1	Genome project[2]	tuf
Pseudomonas putida		Genome project[2]	tuf
Rickettsia prowazekii	Madrid E	AJ235272	tuf
Rickettsia prowazekii	Madrid E	AJ235270	tuf (EF-G)
Rickettsia prowazekii	Madrid E	Z54171	tuf (EF-G)
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*		X64591	tuf (EF-G)
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*	LT2 trpE91	X55116	tuf
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*	LT2 trpE91	X55117	tuf
Serpulina hyodysenteriae	B204	U51635	tuf
Serratia marcescens		AF058451	tuf
Shewanella putrefaciens	DSM 50426	—[1]	tuf
Shewanella putrefaciens	MR-1	Genome project[2]	tuf
Spirochaeta aurantia	DSM 1902	X76874	tuf
Staphylococcus aureus		AJ237696	tuf (EF-G)
Staphylococcus aureus	EMRSA-16	Genome project[2]	tuf
Staphylococcus aureus	NCTC 8325	Genome project[2]	tuf
Staphylococcus aureus	COL	Genome project[2]	tuf
Staphylococcus aureus	EMRSA-16	Genome project[2]	tuf (EF-G)
Stigmatella aurantiaca	DW4	X82820	tuf
Stigmatella aurantiaca	Sg a1	X76870	tuf
Streptococcus mutans	GS-5 Kuramitsu	U75481	tuf
Streptococcus mutans	UAB159	Genome project[2]	tuf
Streptococcus oralis	NTCC 11427	P331701	tuf
Streptococcus pyogenes		Genome project[2]	tuf (EF-G)
Streptococcus pyogenes	M1-GAS	Genome project[2]	tuf
Streptomyces aureofaciens	ATCC 10762	AF007125	tuf
Streptomyces cinnamoneus	Tue89	X98831	tuf
Streptomyces coelicolor	A3(2)	AL031013	tuf (EF-G)
Streptomyces coelicolor	A3(2)	X77039	tuf (EF-G)
Streptomyces coelicolor	M145	X77039	tuf
Streptomyces collinus	BSM 40733	S79408	tuf
Streptomyces netropsis	Tu1063	AF153618	tuf
Streptomyces ramocissimus		X67057	tuf
Streptomyces ramocissimus		X67058	tuf
Streptomyces ramocissimus		X67057	tuf (EF-G)
Synechococcus sp.	PCC 6301	X17442	tuf (EF-G)

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Synechococcus sp.	PCC 6301	X17442	tuf
Synechocystis sp.	PCC 6803	D90913	tuf (EF-G)
Synechocystis sp.	PCC 6803	D90913	tuf
Synechocystis sp.	PCC 6803	X65159	tuf (EF-G)
Taxeobacter occealus	Myx 2105	X77036	tuf
Thermotoga maritima		Genome project[2]	tuf (EF-G)
Thermotoga maritima		M27479	tuf
Thermus aquaticus	EP 00276	X66322	tuf
Thermus thermophilus	HB8	X16278	tuf (EF-G)
Thermus thermophilus	HB8	X05977	tuf
Thermus thermophilus	HB8	X06657	tuf
Thiomonas cuprina	DSM 5495	U78300	tuf
Thiomonas cuprina	DSM 5495	U78300	tuf (EF-G)
Thiomonas cuprina	Hoe5	X76871	tuf
Treponema denticola		Genome project[2]	tuf
Treponema denticola		Genome project[2]	tuf (EF-G)
Treponema pallidum		AE001202	tuf
Treponema pallidum		AE001222	tuf (EF-G)
Treponema pallidum		AE001248	tuf (EF-G)
Ureaplasma urealyticum	ATCC 33697	Z34275	tuf
Ureaplasma urealyticum	serovar 3 biovar 1	AE002151	tuf
Ureaplasma urealyticum	serovar 3 biovar 1	AE002151	tuf (EF-G)
Vibrio cholerae	N16961	Genome project[2]	tuf
Wolinella succinogenes	DSM 1740	X76872	tuf
Yersinia pestis	CO-92	Genome project[2]	tuf
Yersinia pestis	CO-92	Genome project[2]	tuf (EF-G)
Archaebacteria			
Archaeoglobus fulgidus		Genome project[2]	tuf (EF-G)
Halobacterium marismortui		X16677	tuf
Methanobacterium thermoautrophicum	delta H	AE000877	tuf
Methanococcus jannaschii	ATCC 43067	U67486	tuf
Methanococcus vannielii		X05698	tuf
Pyrococcus abyssi	Orsay	AJ248285	tuf
Thermoplasma acidophilum	DSM 1728	X53866	tuf
Fungi			
Absidia glauca	CBS 101.48	X54730	tuf (EF-1)
Arxula adeninivorans	Ls3	Z47379	tuf (EF-1)
Aspergillus oryzae	KBN616	AB007770	tuf (EF-1)
Aureobasidium pullulans	R106	U19723	tuf (EF-1)
Candida albicans	SC5314	Genome project[2]	tuf (M)
Candida albicans	SC5314	M29934	tuf (EF-1)
Candida albicans	SC5314	M29935	tuf (EF-1)
Cryptococcus neoformans	B3501	U81803	tuf (EF-1)
Cryptococcus neoformans	M1-106	U81804	tuf (EF-1)
Eremothecium gossypii	ATCC 10895	X73978	tuf (EF-1)
Eremothecium gossypii		A29820	tuf (EF-1)
Fusarium oxysporum	NRRL 26037	AF008498	tuf (EF-1)
Histoplasma capsulatum	186AS	U14100	tuf (EF-1)
Podospora anserina		X74799	tuf (EF-1)
Podospora curvicolla	VLV	X96614	tuf (EF-1)
Prototheca wickerhamii	263-11	AJ245645	tuf (EF-1)
Puccinia graminis	race 32	X73529	tuf (EF-1)
Reclinomonas americana	ATCC 50394	AF007261	tuf (M)
Rhizomucor racemosus	ATCC 1216B	X17475	tuf (EF-1)
Rhizomucor racemosus	ATCC 1216B	J02605	tuf (EF-1)
Rhizomucor racemosus	ATCC 1216B	X17476	tuf (EF-1)
Rhodotorula mucilaginosa		AF016239	tuf (EF-1)
Saccharomyces cerevisiae		K00428	tuf (M)
Saccharomyces cerevisiae		M59369	tuf (EF-G)
Saccharomyces cerevisiae		X00779	tuf (EF-1)
Saccharomyces cerevisiae		X01638	tuf (EF-1)
Saccharomyces cerevisiae		M10992	tuf (EF-1)
Saccharomyces cerevisiae	Alpha S288	X78993	tuf (EF-1)
Saccharomyces cerevisiae		M15666	tuf (EF-1)
Saccharomyces cerevisiae		Z35987	tuf (EF-1)
Saccharomyces cerevisiae	S288C (AB972)	U51033	tuf (EF-1)
Schizophyllum commune	1-40	X94913	tuf (EF-1)
Schizosaccharomyces pombe	972h-	AL021816	tuf (EF-1)
Schizosaccharomyces pombe	972h-	AL021813	tuf (EF-1)

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Schizosaccharomyces pombe	972h-	D82571	tuf (EF-1)
Schizosaccharomyces pombe		U42189	tuf (EF-1)
Schizosaccharomyces pombe	PR745	D89112	tuf (EF-1)
Sordaria macrospora	OOO	X96615	tuf (EF-1)
Trichoderma reesei	QM9414	Z23012	tuf (EF-1)
Yarrowia lipolytica		AF054510	tuf (EF-1)
Parasites			
Blastocystis hominis	HE87-1	D64080	tuf (EF-1)
Cryptosporidium parvum		U69697	tuf (EF-1)
Eimeria tenella	LS18	AI755521	tuf (EF-1)
Entamoeba histolytica	HM1:IMSS	X83565	tuf (EF-1)
Entamoeba histolytica	NIH 200	M92073	tuf (EF-1)
Giardia lamblia		D14342	tuf (EF-1)
Kentrophoros sp.		AF056101	tuf (EF-1)
Leishmania amazonensis	IFLA/BR/67/PH8	M92653	tuf (EF-1)
Leishmania braziliensis		U72244	tuf (EF-1)
Onchocerca volvulus		M64333	tuf (EF-1)
Porphyra purpurea	Avonport	U08844	tuf (EF-1)
Plasmodium berghei	ANKA	AJ224150	tuf (EF-1)
Plasmodium falciparum	K1	X60488	tuf (EF-1)
Plasmodium knowlesi	line H	AJ224153	tuf (EF-1)
Toxoplasma gondii	RH	Y11431	tuf (EF-1)
Trichomonas tenax	ATCC 30207	D78479	tuf (EF-1)
Trypanosoma brucei	LVH/75/	U10562	tuf (EF-1)
Trypanosoma cruzi	USAMRU-K/18Y	L76077	tuf (EF-1)
Human and plants			
Arabidopsis thaliana	Columbia	X89227	tuf (EF-1)
Glycine max	Ceresia	X89058	tuf (EF-1)
Glycine max	Ceresia	Y15107	tuf (EF-1)
Glycine max	Ceresia	Y15108	tuf (EF-1)
Glycine max	Maple Arrow	X66062	tuf (EF-1)
Homo sapiens		X03558	tuf (EF-1)
Pyramimonas disomata		AB008010	tuf
atpD sequences			
Bacteria			
Acetobacterium woodi	DSM 1030	U10505	atpD
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	atpD
Bacillus anthracis	Ames	Genome project[2]	atpD
Bacillus firmus	OF4	M60117	atpD
Bacillus megaterium	QM B1551	M20255	atpD
Bacillus stearothermophilus		D38058	atpD
Bacillus stearothermophilus	IFO1035	D38060	atpD
Bacillus subtilis	168	Z28592	atpD
Bacteroides fragilis	DSM 2151	M22247	atpD
Bordetella bronchiseptica	RB50	Genome project[2]	atpD
Bordetella pertussis	Tohama 1	Genome project[2]	atpD
Borrelia burgdorferi	B31	AE001122	atpD (V)
Burkholderia cepacia	DSM50181	X76877	atpD
Burkholderia pseudomallei	K96243	Genome project[2]	atpD
Campylobacter jejuni	NCTC 11168	CJ11168X1	atpD
Chlamydia pneumoniae		Genome project[2]	atpD (V)
Chlamydia trachomatis	MoPn	Genome project[2]	atpD (V)
Chlorobium vibrioforme	DSM 263	X76873	atpD
Citrobacter freundii	JEO503	AF037156	atpD
Clostridium acetobutylicum	ATCC 824	Genome project[2]	atpD
Clostridium acetobutylicum	DSM 792	AF101055	atpD
Clostridium difficile	630	Genome project[2]	atpD
Corynebacterium diphtheriae	NCTC13129	Genome project[2]	atpD
Corynebacterium glutamicum	ASO 19	X76875	atpD
Corynebacterium glutamicum	MJ-233	E09634	atpD
Cytophaga lytica	DSM 2039	M22535	atpD
Enterobacter aerogenes	DSM 30053	—[3]	atpD
Enterococcus faecalis	V583	Genome project[2]	atpD (V)
Enterococcus hirae		M90060	atpD
Enterococcus hirae	ATCC 9790	D17462	atpD (V)
Escherichia coli		J01594	atpD
Escherichia coli		M25464	atpD

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Escherichia coli		V00267	atpD
Escherichia coli		V00311	atpD
Escherichia coli	K12 MG1655	L10328	atpD
Flavobacterium ferrugineum	DSM 13524	—[3]	atpD
Haemophilus actinomycetemcomitans		Genome project[2]	atpD
Haemophilus influenzae	Rd	U32730	atpD
Helicobacter pylori	NCTC 11638	AF004014	atpD
Helicobacter pylori	26695	Genome project[2]	atpD
Helicobacter pylori	J99	Genome project[2]	atpD
Klebsiella pneumoniae	M6H 78578	Genome project[2]	atpD
Lactobacillus casei	DSM 20021	X64542	atpD
Legionella pneumophila	Philadelphia-1	Genome project[2]	atpD
Moorella thermoacetica	ATCC 39073	U64318	atpD
Mycobacterium avium	104	Genome project[2]	atpD
Mycobacterium bovis	AF2122/97	Genome project[2]	atpD
Mycobacterium leprae		U15186	atpD
Mycobacterium leprae		Genome project[2]	atpD
Mycobacterium tuberculosis	H37Rv	Z73419	atpD
Mycobacterium tuberculosis	CSU#93	Genome project[2]	atpD
Mycoplasma gallisepticum		X64256	atpD
Mycoplasma genitalium	G37	U39725	atpD
Mycoplasma pneumoniae	M129	U43738	atpD
Neisseria gonorrhoeae	FA 1090	Genome project[2]	atpD
Neisseria meningitidis	Z2491	Genome project[2]	atpD
Pasteurella multocida	Pm70	Genome project[2]	atpD
Pectinatus frisingensis	DSM 20465	X64543	atpD
Peptococcus niger	DSM 20475	X76878	atpD
Pirellula marina	IFAM 1313	X57204	atpD
Porphyromonas gingivalis	W83	Genome project[2]	atpD (V)
Propionigenium modestum	DSM 2376	X58461	atpD
Pseudomonas aeruginosa	PAO1	Genome project[2]	atpD
Pseudomonas putida		Genome project[2]	atpD
Rhodobacter capsulatus	B100	X99599	atpD
Rhodospirillum rubrum		X02499	atpD
Rickettsia prowazekii	F-12	AF036246	atpD
Rickettsia prowazekii	Madrid	Genome project[2]	atpD
Ruminococcus albus	7ATCC	AB006151	atpD
Salmonella bongori	JEO4162	AF037155	atpD
Salmonella bongori	BR1859	AF037154	atpD
Salmonella choleraesuis subsp. *arizonae*	S83769	AF037146	atpD
Salmonella choleraesuis subsp. *arizonae*	u24	AF037147	atpD
Salmonella choleraesuis subsp. *choleraesuis* serotype Dublin	K228	AF037140	atpD
Salmonella choleraesuis subsp. *choleraesuis* serotype Dublin	K771	AF037139	atpD
Salmonella choleraesuis subsp. *choleraesuis* serotype Infantis	Div36-86	AF037142	atpD
Salmonella choleraesuis subsp. *choleraesuis* serotype Tennessee	Div95-86	AF037143	atpD
Salmonella choleraesuis subsp. *choleraesuis* serotype *Typhimurium*	LT2	AF037141	atpD
Salmonella choleraesuis subsp. *diarizonae*	DS210/89	AF037149	atpD
Salmonella choleraesuis subsp. *diarizonae*	JEO307	AF037148	atpD
Salmonella choleraesuis subsp. *diarizonae*	S109671	AF037150	atpD
Salmonella choleraesuis subsp. *houtenae*	S84366	AF037151	atpD
Salmonella choleraesuis subsp. *houtenae*	S84098	AF037152	atpD
Salmonella choleraesuis subsp. *indica*	BR2047	AF037153	atpD
Salmonella choleraesuis subsp. *salamae*	NSC72	AF037144	atpD
Salmonella choleraesuis subsp. *salamae*	S114655	AF037145	atpD
Shewanella putrefaciens	MR-1	Genome project[2]	atpD
Staphylococcus aureus	COL	Genome project[2]	atpD

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Stigmatella aurantiaca	Sga1	X76879	atpD
Streptococcus bovis	JB-1	AB009314	atpD
Streptococcus mutans	GS-5	U31170	atpD
Streptococcus mutans	UAB159	Genome project[2]	atpD
Streptococcus pneumoniae	Type 4	Genome project[2]	atpD (V)
Streptococcus pneumoniae	Type 4	Genome project[2]	atpD
Streptococcus pyogenes	M1-GAS	Genome project[2]	atpD (V)
Streptococcus pyogenes	M1-GAS	Genome project[2]	atpD
Streptococcus sanguinis	10904	AF001955	atpD
Streptomyces lividans	1326	Z22606	atpD
Thermus thermophilus	HB8	D63799	atpD (V)
Thiobacillus ferrooxidans	ATCC 33020	M81087	atpD
Treponema pallidum	Nichols	AE001228	atpD (V)
Vibrio alginolyticus		X16050	atpD
Vibrio cholerae	N16961	Genome project[2]	atpD
Wolinella succinogenes	DSM 1470	X76880	atpD
Yersinia enterocolitica	NCTC 10460	AF037157	atpD
Yersinia pestis	CO-92	Genome project[2]	atpD
Archaebacteria			
Archaeoglobus fulgidus	DSM 4304	AE001023	atpD (V)
Halobacterium salinarum		S56356	atpD (V)
Haloferax volcanii	WR 340	X79516	atpD
Methanococcus jannaschii	DSM 2661	U67477	atpD (V)
Methanosarcina barkeri	DSM 800	J04836	atpD (V)
Fungi			
Candida albicans	SC5314	Genome project[2]	atpD
Candida tropicalis		M64984	atpD (V)
Kluyveromyces lactis	2359/152	U37764	atpD
Neurospora crassa		X53720	atpD
Saccharomyces cerevisiae		M12082	atpD
Saccharomyces cerevisiae	X2180-1A	J05409	atpD (V)
Schizosaccharomyces pombe	972 h-	S47814	atpD (V)
Schizosaccharomyces pombe	972 h-	M57956	atpD
Parasites			
Giardia lamblia	WB	U18938	atpD
Plasmodium falciparum	3D7	L08200	atpD (V)
Trypanosoma congolense	IL3000	Z25814	atpD (V)
Human and plants			
Homo sapiens		L09234	atpD (V)
Homo sapiens		M27132	atpD
	recA sequences		
Bacteria			
Acetobacter aceti	no. 1023	S60630	recA
Acetobacter altoacetigenes	MH-24	E05290	recA
Acetobacter polyoxogenes	NBI 1028	D13183	recA
Acholeplasma laidlawii	8195	M81465	recA
Acidiphilium facilis	ATCC 35904	D16538	recA
Acidothermus cellulolyticus	ATCC 43068	AJ006705	recA
Acinetobacter calcoaceticus	BD413/ADP1	L26100	recA
Actinobacillus actinomycetemcomitans	HK1651	Genome project[2]	recA
Aeromonas salmonicida	A449	U83688	recA
Agrobacterium tumefaciens	C58	L07902	recA
Allochromatium vinosum		AJ000677	recA
Aquifex aeolicus	VF5	AE000775	recA
Aquifex pyrophilus	Kol5a	L23135	recA
Azotobacter vinelandii		S96898	recA
Bacillus stearothermophilus	10	Genome project[2]	recA
Bacillus subtilis	PB1831	U87792	recA
Bacillus subtilis	168	Z99112	recA
Bacteroides fragilis		M63029	recA
Bifidobacterium breve	NCFB 2258	AF094756	recA
Blastochloris viridis	DSM 133	AF022175	recA
Bordetella pertussis	165	X53457	recA
Bordetella pertussis	Tohama I	Genome project[2]	recA
Borrelia burgdorferi	Sh-2-82	U23457	recA

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Borrelia burgdorferi	B31	AE001124	recA
Brevibacterium flavum	MJ-233	E10390	recA
Brucella abortus	2308	L00679	recA
Burkholderia cepacia	ATCC 17616	U70431	recA
Burkholderia cepacia		D90120	recA
Burkholderia pseudomallei	K96243	Genome project[2]	recA
Campylobacter fetus subsp. *fetus*	23D	AF020677	recA
Campylobacter jejuni	81-176	U03121	recA
Campylobacter jejuni	NCTC 11168	AL139079	recA
Chlamydia trachomatis	L2	U16739	recA
Chlamydia trachomatis	D/UW-3/CX	AE001335	recA
Chlamydophila pneumoniae	CWL029	AE001658	recA
Chloroflexus aurantiacus	J-10-fl	AF037259	recA
Clostridium acetobutylicum		M94057	recA
Clostridium perfringens	13	U61497	recA
Corynebacterium diphtheriae	NCTC13129	Genome project[2]	recA
Corynebacterium glutamicum	AS019	U14965	recA
Corynebacterium pseudotuberculosis	C231	U30387	recA
Deinococcus radiodurans	KD8301	AB005471	recA
Deinococcus radiodurans	R1	U01876	recA
Enterobacter agglomerans	339	L03291	recA
Enterococcus faecalis	OGIX	M81466	recA
Erwinia carotovora		X55554	recA
Escherichia coli		J01672	recA
Escherichia coli		X55552	recA
Escherichia coli	K-12	AE000354	recA
Frankia alni	Arl3	AJ006707	recA
Gluconobacter oxydans		U21001	recA
Haemophilus influenzae	Rd	U32687	recA
Haemophilus influenzae	Rd	U32741	recA
Haemophilus influenzae	Rd	L07529	recA
Helicobacter pylori	69A	Z35478	recA
Helicobacter pylori	26695	AE000536	recA
Helicobacter pylori	J99	AE001453	recA
Klebsiella pneumoniae	M6H 78578	Genome project[2]	recA
Lactococcus lactis	ML3	M88106	recA
Legionella pneumophila		X55453	recA
Leptospira biflexa	serovar patoc	U32625	recA
Leptospira interrogans	serovar pomona	U29169	recA
Magnetospirillum magnetotacticum	MS-1	X17371	recA
Methylobacillus flagellatus	MFK1	M35325	recA
Methylomonas clara	ATCC 31226	X59514	recA
Mycobacterium avium	104	Genome project[2]	recA
Mycobacterium bovis	AF122/97	Genome project[2]	recA
Mycobacterium leprae		X73822	recA
Mycobacterium tuberculosis	H37Rv	X58485	recA
Mycobacterium tuberculosis	CSU#93	Genome project[2]	recA
Mycoplasma genitalium	G37	U39717	recA
Mycoplasma mycoides	GM9	L22073	recA
Mycoplasma pneumoniae	ATCC 29342	MPAE000033	recA
Mycoplasma pulmonis	KD735	L22074	recA
Myxococcus xanthus		L40368	recA
Myxococcus xanthus		L40367	recA
Neisseria animalis	NCTC 10212	U57910	recA
Neisseria cinerea	LCDC 81-176	AJ223869	recA
Neisseria cinerea	LNP 1646	U57906	recA
Neisseria cinerea	NCTC 10294	AJ223871	recA
Neisseria cinerea	Vedros M601	AJ223870	recA
Neisseria elongata	CCUG 2131	AJ223882	recA
Neisseria elongata	CCUG 4165A	AJ223880	recA
Neisseria elongata	NCTC 10660	AJ223881	recA
Neisseria elongata	NCTC 11050	AJ223878	recA
Neisseria elongata	NHITCC 2376	AJ223877	recA
Neisseria elongata subsp. *intermedia*	CCUG 4557	AJ223879	recA
Neisseria flava	Bangor 9	AJ223873	recA
Neisseria flavescens	LNP 444	U57907	recA
Neisseria gonorrhoeae	CH95	U57902	recA
Neisseria gonorrhoeae	FA19	X64842	recA
Neisseria gonorrhoeae	MS11	X17374	recA
Neisseria gonorrhoeae		Genome project[2]	recA

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Neisseria lactamica	CCUC 7757	AJ223866	recA
Neisseria lactamica	CCUG 7852	Y11819	recA
Neisseria lactamica	LCDC 77-143	Y11818	recA
Neisseria lactamica	LCDC 80-111	AJ223864	recA
Neisseria lactamica	LCDC 845	AJ223865	recA
Neisseria lactamica	NCTC 10617	U57905	recA
Neisseria lactamica	NCTC 10618	AJ223863	recA
Neisseria meningitidis	44/46	X64849	recA
Neisseria meningitidis	Bangor 13	AJ223868	recA
Neisseria meningitidis	HF116	X64848	recA
Neisseria meningitidis	HF130	X64844	recA
Neisseria meningitidis	HF46	X64847	recA
Neisseria meningitidis	M470	X64850	recA
Neisseria meningitidis	N94II	X64846	recA
Neisseria meningitidis	NCTC 8249	AJ223867	recA
Neisseria meningitidis	P63	X64845	recA
Neisseria meningitidis	S3446	U57903	recA
Neisseria meningitidis	FAM18	Genome project[2]	recA
Neisseria mucosa	LNP 405	U57908	recA
Neisseria mucosa	Vedros M1801	AJ223875	recA
Neisseria perflava	CCUG 17915	AJ223876	recA
Neisseria perflava	LCDC 85402	AJ223862	recA
Neisseria pharyngis var. *flava*	NCTC 4590	U57909	recA
Neisseria polysaccharea	CCUG 18031	Y11815	recA
Neisseria polysaccharea	CCUG 24845	Y11816	recA
Neisseria polysaccharea	CCUG 24846	Y11814	recA
Neisseria polysaccharea	INS MA 3008	Y11817	recA
Neisseria polysaccharea	NCTC 11858	U57904	recA
Neisseria sicca	NRL 30016	AJ223872	recA
Neisseria subflava	NRL 30017	AJ223874	recA
Paracoccus denitrificans	DSM 413	U59631	recA
Pasteurella multocida		X99324	recA
Porphyromonas gingivalis	W83	U70054	recA
Prevotella ruminicola	JCM 8958	U61227	recA
Proteus mirabilis	pG1300	X14870	recA
Proteus vulgaris		X55555	recA
Pseudomonas aeruginosa		X05691	recA
Pseudomonas aeruginosa	PAM 7	X52261	recA
Pseudomonas aeruginosa	PAO12	D13090	recA
Pseudomonas fluorescens	OE 28.3	M96558	recA
Pseudomonas putida		L12684	recA
Pseudomonas putida	PpS145	U70864	recA
Rhizobium leguminosarum biovar viciae	VF39	X59956	recA
Rhizobium phaseoli	CNPAF512	X62479	recA
Rhodobacter capsulatus	J50	X82183	recA
Rhodobacter sphaeroides	2.4.1	X72705	recA
Rhodopseudomonas palustris	N 7	D84467	recA
Rickettsia prowazekii	Madrid E	AJ235273	recA
Rickettsia prowazekii	Madrid E	U01959	recA
Serratia marcescens		M22935	recA
Shigella flexneri		X55553	recA
Shigella sonnei	KNIH104S	AF101227	recA
Sinorhizobium meliloti	2011	X59957	recA
Staphylococcus aureus		L25893	recA
Streptococcus gordonii	Challis V288	L20574	recA
Streptococcus mutans	UA96	M81468	recA
Streptococcus mutans	GS-5	M61897	recA
Streptococcus pneumoniae		Z17307	recA
Streptococcus pneumoniae	R800	Z34303	recA
Streptococcus pyogenes	NZ131	U21934	recA
Streptococcus pyogenes	D471	M81469	recA
Streptococcus salivarius subsp. *thermophilus*		M94062	recA
Streptomyces ambofaciens	DSM 40697	Z30324	recA
Streptomyces coelicolor	A3(2)	AL020958	recA
Streptomyces lividans	TK24	X76076	recA
Streptomyces rimosus	R6	X94233	recA
Streptomyces venezuelae	ATCC10712	U04837	recA
Synechococcus sp.	PR6	M29495	recA
Synechocystis sp.	PCC6803	D90917	recA

TABLE 11-continued

Microbial species for which tuf and/or atpD and/or recA sequences are available in public databases.

Species	Strain	Accession number	Coding gene*
Thermotoga maritima		L23425	recA
Thermotoga maritima		AE001823	recA
Thermus aquaticus		L20095	recA
Thermus thermophilus	HB8	D17392	recA
Thiobacillus ferrooxidans		M26933	recA
Treponema denticola		Genome project[2]	recA
Treponema pallidum	Nichols	AE001243	recA
Vibrio anguillarum		M80525	recA
Vibrio cholerae	017	X71969	recA
Vibrio cholerae	2740-80	U10162	recA
Vibrio cholerae	569B	L42384	recA
Vibrio cholerae	M549	AF117881	recA
Vibrio cholerae	M553	AF117882	recA
Vibrio cholerae	M645	AF117883	recA
Vibrio cholerae	M793	AF117878	recA
Vibrio cholerae	M794	AF117880	recA
Vibrio cholerae	M967	AF117879	recA
Xanthomonas citri	XW47	AF006590	recA
Xanthomonas oryzae		AF013600	recA
Xenorhabdus bovienii	T228/1	U87924	recA
Xenorhabdus nematophilus	AN6	AF127333	recA
Yersinia pestis	231	X75336	recA
Yersinia pestis	CO-92	Genome project[2]	recA
Fungi, parasites, human and plants			
Anabaena variabilis	ATCC 29413	M29680	recA
Arabidopsis thaliana		U43652	recA (Rad51)
Candida albicans		U39808	recA (Dmc1)
Coprinus cinereus	Okayama-7	U21905	recA (Rad51)
Emericella nidulans		Z80341	recA (Rad51)
Gallus gallus		L09655	recA (Rad51)
Homo sapiens		D13804	recA (Rad51)
Homo sapiens		D63882	recA (Dmc1)
Leishmania major	Friedlin	AF062379	recA (Rad51)
Leishmania major	Friedlin	AF062380	recA (Dmc1)
Mus musculus		D58419	recA (Dmc1)
Neurospora crassa	74-OR23-1A	D29638	recA (Rad51)
Saccharomyces cerevisiae		D10023	recA (Rad51)
Schizosaccharomyces pombe		Z22691	recA (Rad51)
Schizosaccharomyces pombe	972h-	AL021817	recA (Dmc1)
Tetrahymena thermophila	PB9R	AF064516	recA (Rad51)
Trypanosoma brucei	stock 427	Y13144	recA (Rad51)
Ustilago maydis		U62484	recA (Rad51)
Xenopus laevis		D38488	recA (Rad51)
Xenopus laevis		D38489	recA (Rad51)

*tuf indicates tuf sequences, including tuf genes, fusA genes and fusA-tuf intergenic spacers. tuf (C) indicates tuf sequences divergent from main (usually A and B) copies of the elongation factor-Tu tuf (EF-1) indicates tuf sequences of the eukaryotic type (elongation factor 1α) tuf (M) indicates tuf sequences from organellar (mostly mitochondrial) origin atpD indicates atpD sequences of the F-type atpD (V) indicates atpD sequences of the V-Type recA indicates recA sequences recA (Rad51) indicates rad51 sequences or homologs recA (Dmc1) indicates dmc1 sequences or homologs

[1]Nucleotides sequences published in Arch. Microbiol. 1990 153: 241-247

[2]These sequences are from theTIGR database (http://www.tigr.org/tdb/tdb.html)

[3]Nucleotides sequences published in FEMS Microbiology Letters 1988 50: 101-106

TABLE 12

Bacterial species used to test the specificity of the *Staphylococcus*specific amplification primers derived from tuf sequences.

Strain	Reference number
Staphylococcal species (n = 27)	
Staphylococcus arlettae	ATCC 43957
Staphylococcus aureus subsp. *anaerobius*	ATCC 35844
Staphylococcus aureus subsp. *aureus*	ATCC 43300

TABLE 12-continued

Bacterial species used to test the specificity of the *Staphylococcus*specific amplification primers derived from tuf sequences.

Strain	Reference number
Staphylococcus auricularis	ATCC 33753
Staphylococcus capitis subsp. *capitis*	ATCC 27840
Staphylococcus caprae	ATCC 35538
Staphylococcus carnosus	ATCC 51365
Staphylococcus chromogenes	ATCC 43764

TABLE 12-continued

Bacterial species used to test the specificity of the *Staphylococcus*specific amplification primers derived from tuf sequences.

Strain	Reference number
Staphylococcus cohnii subsp. *urealyticum*	DSM 20260
Staphylococcus delphini	ATCC 49171
Staphylococcus epidermidis	ATCC 14990
Staphylococcus equorum	ATCC 43958
Staphylococcus felis	ATCC 49168
Staphylococcus gallinarum	ATCC 35539
Staphylococcus haemolyticus	ATCC 29970
Staphylococcus hominis	ATCC 27844
Staphylococcus hyicus	ATCC 11249
Staphylococcus intermedius	ATCC 29663
Staphylococcus kloosis	ATCC 43959
Staphylococcus lentus	ATCC 29070
Staphylococcus lugdunensis	ATCC 43809
Staphylococcus saprophyticus	ATCC 15305
Staphylococcus schleiferi subsp. *coagulans*	ATCC 49545
Staphylococcus sciuri subsp. *sciuri*	ATCC 29060
Staphylococcus simulans	ATCC 27848
Staphylococcus warneri	ATCC 27836
Staphylococcus xylosus	ATCC 29971
Gram-negative bacteria (n = 33)	
Acinetobacter baumannii	ATCC 19606
Bacteroides distasonis	ATCC 8503
Bacteroides fragilis	ATCC 25285
Bulkholderia cepacia	ATCC 25416
Bordetella pertussis	ATCC 9797
Citrobacter freundii	ATCC 8090
Enterobacter aerogenes	ATCC 13048
Enterobacter cloacae	ATCC 13047
Escherichia coli	ATCC 25922
Haemophilus influenzae	ATCC 8907
Haemophilus parahaemolyticus	ATCC 10014
Haemophilus parainfluenzae	ATCC 7901
Hafnia alvei	ATCC 13337
Kingella indologenes	ATCC 25869
Klebsiella oxytoca	ATCC 13182
Klebsiella pneumoniae	ATCC 13883
Moraxella catarrhalis	ATCC 25240
Morganella morganii	ATCC 25830
Neisseria gonorrhoeae	ATCC 35201
Neisseria meningitidis	ATCC 13077
Proteus mirabilis	ATCC 25933
Proteus vulgaris	ATCC 13315
Providencia rettgeri	ATCC 9250
Providencia stuartii	ATCC 29914
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas fluorencens	ATCC 13525
Salmonella choleraesuis	ATCC 7001
Salmonella typhimurium	ATCC 14028
Serratia marcescens	ATCC 8100
Shigella flexneri	ATCC 12022
Shigella sonnei	ATCC 29930
Stenotrophomonas maltophilia	ATCC 13843
Yersinia enterocolitica	ATCC 9610
Other Gram-positive bacteria (n = 20)	
Bacillus subtilis	ATCC 27370
Enterococcus avium	ATCC 14025
Enterococcus durans	ATCC 19432
Enterococcus faecalis	ATCC 19433
Enterococcus faecium	ATCC 19434
Enterococcus flavescens	ATCC 49996
Enterococcus gallinarum	ATCC 49573
Lactobacillus acidophilus	ATCC 4356
Lactococcus lactis	ATCC 11454
Listeria innocua	ATCC 33090
Listeria ivanovii	ATCC 19119
Listeria monocytogenes	ATCC 15313

TABLE 12-continued

Bacterial species used to test the specificity of the *Staphylococcus*specific amplification primers derived from tuf sequences.

Strain	Reference number
Macrococcus caseolyticus	ATCC 13548
Streptococcus agalactiae	ATCC 13813
Streptococcus anginosus	ATCC 33397
Streptococcus bovis	ATCC 33317
Streptococcus mutans	ATCC 25175
Streptococcus pneumoniae	ATCC 6303
Streptococcus pyogenes	ATCC 19615
Streptococcus salivarius	ATCC 7073

TABLE 13

Bacterial species used to test the specificity of the penicillin-resistant *Streptococcus pneumoniae* assay.

Strain	Reference number
Gram-positive species (n = 67)	
Abiotrophia adiacens	ATCC 49175
Abiotrophia defectiva	ATCC 49176
Actinomyces pyogenes	ATCC 19411
Bacillus anthracis	ATCC 4229
Bacillus cereus	ATCC 14579
Bifidobacterium breve	ATCC 15700
Clostridium difficile	ATCC 9689
Enterococcus avium	ATCC 14025
Enterococcus casseliflavus	ATCC 25788
Enterococcus dispar	ATCC 51266
Enterococcus durans	ATCC 19432
Enterococcus faecalis	ATCC 29212
Enterococcus faecium	ATCC 19434
Enterococcus flavescens	ATCC 49996
Enterococcus gallinarum	ATCC 49573
Enterococcus hirae	ATCC 8043
Enterococcus mundtii	ATCC 43186
Enterococcus raffinosus	ATCC 49427
Lactobacillus lactis	ATCC 19435
Lactobacillus monocytogenes	ATCC 15313
Mobiluncus curtisii	ATCC 35242
Peptococcus niger	ATCC 27731
Peptostreptococcus acones	ATCC 6919
Peptostreptococcus anaerobius	ATCC 27337
Peptostreptococcus asaccharolyticus	ATCC 2639
Peptostreptococcus lactolyticus	ATCC 51172
Peptostreptococcus magnus	ATCC 15794
Peptostreptococcus prevotii	ATCC 9321
Peptostreptococcus tetradius	ATCC 35098
Staphylococcus aureus	ATCC 25923
Staphylococcus capitis	ATCC 27840
Staphylococcus epidermidis	ATCC 14990
Staphylococcus haemolyticus	ATCC 29970
Staphylococcus hominis	ATCC 27844
Staphylococcus lugdunensis	ATCC 43809
Staphylococcus saprophyticus	ATCC 15305
Staphylococcus simulans	ATCC 27848
Staphylococcus. warneri	ATCC 27836
Streptococcus acidominimus	ATCC 51726
Streptococcus agalactiae	ATCC 12403
Streptococcus anginosus	ATCC 33397
Streptococcus bovis	ATCC 33317
Streptococcus constellatus	ATCC 27823
Streptococcus cricetus	ATCC 19624
Streptococcus cristatus	ATCC 51100
Streptococcus downei	ATCC 33748
Streptococcus dysgalactiae	ATCC 43078
Streptococcus equi	ATCC 9528
Streptococcus ferus	ATCC 33477

TABLE 13-continued

Bacterial species used to test the specificity of the penicillin-resistant *Streptococcus pneumoniae* assay.

Strain	Reference number
Streptococcus gordonii	ATCC 10558
Streptococcus intermedius	ATCC 27335
Streptococcus mitis	ATCC 903
Streptococcus mitis	LSPQ 2583
Streptococcus mitis	ATCC 49456
Streptococcus mutans	ATCC 27175
Streptococcus oralis	ATCC 10557
Streptococcus oralis	ATCC 9811
Streptococcus oralis	ATCC 35037
Streptococcus parasanguinis	ATCC 15912
Streptococcus parauberis	ATCC 6631
Streptococcus rattus	ATCC 15912
Streptococcus salivarius	ATCC 7073
Streptococcus sanguinis	ATCC 10556
Streptococcus suis	ATCC 43765
Streptococcus uberis	ATCC 19436
Streptococcus vestibularis	ATCC 49124
Gram-negative species (n = 33)	
Actinetobacter baumannii	ATCC 19606
Bordetella pertussis	ATCC 9797
Citrobacter diversus	ATCC 27028
Citrobacter freundii	ATCC 8090
Enterobacter aerogenes	ATCC 13048
Enterobacter agglomerans	ATCC 27155
Enterobacter cloacae	ATCC 13047
Escherichia coli	ATCC 25922
Haemophilus ducreyi	ATCC 33940
Haemophilus haemolyticus	ATCC 33390
Haemophilus influenzae	ATCC 9007
Haemophilus parainfluenzae	ATCC 7901
Hafnia alvei	ATCC 13337
Klebsiella oxytoca	ATCC 13182
Klebsiella pneumoniae	ATCC 13883
Moraxella atlantae	ATCC 29525
Moraxella catarrhalis	ATCC 43628
Moraxella morganii	ATCC 13077
Neisseria gonorrhoeae	ATCC 35201
Neisseria meningitidis	ATCC 13077
Proteus mirabilis	ATCC 25933
Proteus vulgaris	ATCC 13315
Providencia alcalifaciens	ATCC 9886
Providencia rettgeri	ATCC 9250
Providencia rustigianii	ATCC 33673
Providencia stuartii	ATCC 33672
Pseudomonas aeruginosa	ATCC 35554
Pseudomonas fluorescens	ATCC 13525
Pseudomonas stutzeri	ATCC 17588
Salmonella typhimurium	ATCC 14028
Serratia marcescens	ATCC 13880
Shigella flexneri	ATCC 12022
Yersina enterocolitica	ATCC 9610

TABLE 14

Bacterial species (n = 104) detected by the platelet contaminants assay.

Abiotrophia adiacens
Abiotrophia defective
Acinetobacter baumannii
Acinetobacter lwoffi
Aerococcus viridans
Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Brucella abortus
Burkholderia cepacia
Citrobacter diversus

TABLE 14-continued

Bacterial species (n = 104) detected by the platelet contaminants assay.

Citrobacter freundii
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter cloacae
Enterococcus avium
Enterococcus casseliflavus
Enterococcus dispar
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Enterococcus gallinarum
Enterococcus mundtii
Enterococcus raffinosus
Enterococcus solitarius
Escherichia coli
Gemella morbillorum
Haemophilus ducreyi
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Hafnia alvei
Kingella kingae
Klebsiella oxytoca
Klebsiella pneumoniae
Legionella pneumophila
Megamonas hypermegale
Moraxella atlantae
Moraxella catarrhalis
Morganella morganii
Neisseria gonorrheae
Neisseria meningitidis
Pasteurella aerogenes
Pasteurella multocida
Peptostreptococcus magnus
Proteus mirabilis
Providencia alcalifaciens
Providencia rettgeri
Providencia rustigianii
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas stutzeri
Salmonella bangori
Salmonella choleraesuis
Salmonella enteritidis
Salmonella gallinarum
Salmonella typhimurium
Serratia liquefaciens
Serratia marcescens
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus capitis
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus simulans
Staphylococcus warneri
Stenotrophomonas maltophilia
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus constellatus
Streptococcus cricetus
Streptococcus cristatus
Streptococcus dysgalactiae
Streptococcus equi
Streptococcus ferus

TABLE 14-continued

Bacterial species (n = 104) detected by the platelet contaminants assay.
Streptococcus gordonii
Streptococcus intermedius
Streptococcus macacae
Streptococcus mitis
Streptococcus mutans
Streptococcus oralis
Streptococcus parasanguinis
Streptococcus parauberis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivarius
Streptococcus sanguinis
Streptococcus sobrinus
Streptococcus uberis
Streptococcus vestibularis
Vibrio cholerae
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculos

Bold characters indicate the major bacterial contaminants found in platelet concentrates.

TABLE 15

Microorganisms identified by commercial systems[1].
Abiotrophia adiacens (*Streptococcus adjacens*)
Abiotrophia defectiva (*Streptococcus defectivus*)
Achromobacter species
Acidaminococcus fermentans
Acinetobacter alcaligenes
Acinetobacter anitratus
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter calcoaceticus biovar *anitratus*
Acinetobacter calcoaceticus biovar *lwoffi*
Acinetobacter genomospecies
Acinetobacter haemolyticus
Acinetobacter johnsonii
Acinetobacter junii
Acinetobacter lwoffii
Acinetobacter radioresistens
Acinetobacter species
Actinobacillus actinomycetemcomitans
Actinobacillus capsulatus
Actinobacillus equuli
Actinobacillus hominis
Actinobacillus lignieresii
Actinobacillus pleuropneumoniae
Actinobacillus species
Actinobacillus suis
Actinobacillus ureae
Actinomyces bovis
Actinomyces israelii
Actinomyces meyeri
Actinomyces naeslundii
Actinomyces neuii subsp. *anitratus*
Actinomyces neuii subsp. *neuii*
Actinomyces odontolyticus
Actinomyces pyogenes
Actinomyces radingae
Actinomyces species
Actinomyces turicensis
Actinomyces viscosus
Aerococcus species
Aerococcus viridans

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Aeromonas caviae
Aeromonas hydrophila
Aeromonas hydrophila group
Aeromonas jandaei
Aeromonas salmonicida
Aeromonas salmonicida subsp. *achromogenes*
Aeromonas salmonicida subsp. *masoucida*
Aeromonas salmonicida subsp. *salmonicida*
Aeromonas schubertii
Aeromonas sobria
Aeromonas species
Aeromonas trota
Aeromonas veronii
Aeromonas veronii biovar *sobria*
Aeromonas veronii biovar *veronii*
Agrobacterium radiobacter
Agrobacterium species
Agrobacterium tumefaciens
Alcaligenes denitrificans
Alcaligenes faecalis
Alcaligenes odorans
Alcaligenes odorans (*Alcaligenes faecalis*)
Alcaligenes species
Alcaligenes xylosoxidans
Alcaligenes xylosoxidans subsp. *denitrificans*
Alcaligenes xylosoxidans subsp. *xylosoxidans*
Alloiococcus otitis
Anaerobiospirillum succiniciproducens
Anaerovibrio lipolytica
Arachnia propionica
Arcanobacterium (*Actinomyces*) *bemardiae*
Arcanobacterium (*Actinomyces*) *pyogenes*
Arcanobacterium haemolyticum
Arcobacter cryaerophilus (*Campylobacter cryaerophila*)
Arthrobacter globiformis
Arthrobacter species
Arxiozyma telluris (*Torulopsis pintolopesii*)
Atopobium minutum (*Lactobacillus minutus*)
Aureobacterium species
Bacillus amyloliquefaciens
Bacillus anthracis
Bacillus badius
Bacillus cereus
Bacillus circulans
Bacillus coagulans
Bacillus firmus
Bacillus lentus
Bacillus licheniformis
Bacillus megaterium
Bacillus mycoides
Bacillus pantothenticus
Bacillus pumilus
Bacillus species
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacteroides caccae
Bacteroides capillosus
Bacteroides distasonis
Bacteroides eggerthii
Bacteroides fragilis
Bacteroides merdae

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Bacteroides ovatus
Bacteroides species
Bacteroides splanchnicus
Bacteroides stercoris
Bacteroides thetaiotaomicron
Bacteroides uniformis
Bacteroides ureolyticus (*B. corrodens*)
Bacteroides vulgatus
Bergeyella (*Weeksella*) *zoohelcum*
Bifidobacterium adolescentis
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium dentium
Bifidobacterium infantis
Bifidobacterium species
Blastoschizomyces (*Dipodascus*) *capitatus*
Bordetella avium
Bordetella bronchiseptica
Bordetella parapertussis
Bordetella pertussis
Bordetella species
Borrelia species
Branhamella (*Moraxella*) *catarrhalis*
Branhamella species
Brevibacillus brevis
Brevibacillus laterosporus
Brevibacterium casei
Brevibacterium epidermidis
Brevibacterium linens
Brevibacterium species
Brevundimonas (*Pseudomonas*) *diminuta*
Brevundimonas (*Pseudomonas*) *vesicularis*
Brevundimonas species
Brochothrix thermosphacta
Brucella abortus
Brucella canis
Brucella melitensis
Brucella ovis
Brucella species
Brucella suis
Budvicia aquatica
Burkholderia (*Pseudomonas*) *cepacia*
Burkholderia (*Pseudomonas*) *gladioli*
Burkholderia (*Pseudomonas*) *mallei*
Burkholderia (*Pseudomonas*) *pseudomallei*
Burkholderia species
Buttiauxella agrestis
Campylobacter coli
Campylobacter concisus
Campylobacter fetus
Campylobacter fetus subsp. *fetus*
Campylobacter fetus subsp. *venerealis*
Campylobacter hyointestinalis
Campylobacter jejuni subsp. *doylei*
Campylobacter jejuni subsp. *jejuni*
Campylobacter lari
Campylobacter lari subsp. UPTC
Campylobacter mucosalis
Campylobacter species
Campylobacter sputorum
Campylobacter sputorum subsp. *bubulus*
Campylobacter sputorum subsp. *fecalis*
Campylobacter sputorum subsp. *sputorum*
Campylobacter upsaliensis
Candida (*Clavispora*) *lusitaniae*
Candida (*Pichia*) *guilliermondii*

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Candida (*Torulopsis*) *glabrata*
Candida albicans
Candida boidinii
Candida catenulata
Candida ciferrii
Candida colliculosa
Candida conglobata
Candida curvata (*Cryptococcus curvatus*)
Candida dattila
Candida dubliniensis
Candida famata
Candida globosa
Candida hellenica
Candida holmii
Candida humicola
Candida inconspicua
Candida intermedia
Candida kefyr
Candida krusei
Candida lambica
Candida magnoliae
Candida maris
Candida melibiosica
Candida membranaefaciens
Candida norvegensis
Candida norvegica
Candida parapsilosis
Candida paratropicalis
Candida pelliculosa
Candida pseudotropicalis
Candida pulcherrima
Candida ravautii
Candida rugosa
Candida sake
Candida silvicola
Candida species
Candida sphaerica
Candida stellatoidea
Candida tenuis
Candida tropicalis
Candida utilis
Candida valida
Candida vini
Candida viswanathii
Candida zeylanoides
Capnocytophaga gingivalis
Capnocytophaga ochracea
Capnocytophaga species
Capnocytophaga sputigena
Cardiobacterium hominis
Camobacterium divergens
Camobacterium piscicola
CDC group ED-2
CDC group EF4 (*Pasteurella* sp.)
CDC group EF-4A
CDC group EF-4B
CDC group EQ-Z
CDC group HB-5
CDC group II K-2
CDC group IV C-2 (*Bordetella*-like)
CDC group M5
CDC group M6
Cedecea davisae
Cedecea lapagei
Cedecea neteri
Cedecea species
Cellulomonas (*Oerskovia*) *turbata*
Cellulomonas species
Chlamydia species
Chromobacterium violaceum
Chryseobacterium (*Flavobacterium*) *indologenes*

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Chryseobacterium (*Flavobacterium*) *meningosepticum*
Chryseobacterium gleum
Chryseobacterium species
Chryseomonas indologenes
Citeromyces matritensis
Citrobacter amalonaticus
Citrobacter braakii
Citrobacter diversus
Citrobacter farmeri
Citrobacter freundii
Citrobacter freundii complex
Citrobacter koseri
Citrobacter sedlakii
Citrobacter species
Citrobacter werkmanii
Citrobacter youngae
Clostridium acetobutylicum
Clostridium barati
Clostridium beijerinckii
Clostridium bifermentans
Clostridium botulinum
Clostridium botulinum (NP) B&F
Clostridium botulinum (NP) E
Clostridium botulinum (P) A&H
Clostridium botulinum (P) F
Clostridium botulinum G1
Clostridium botulinum G2
Clostridium butyricum
Clostridium cadaveris
Clostridium chauvoei
Clostridium clostridiiforme
Clostridium difficile
Clostridium fallax
Clostridium glycolicum
Clostridium hastiforme
Clostridium histolyticum
Clostridium innocuum
Clostridium limosum
Clostridium novyi
Clostridium novyi A
Clostridium paraputrificum
Clostridium perfringens
Clostridium putrificum
Clostridium ramosum
Clostridium septicum
Clostridium sordellii
Clostridium species
Clostridium sphenoides
Clostridium sporogenes
Clostridium subterminale
Clostridium tertium
Clostridium tetani
Clostridium tyrobutyricum
Comamonas (*Pseudomonas*) *acidovorans*
Comamonas (*Pseudomonas*) *testosteroni*
Comamonas species
Corynebacterium accolens
Corynebacterium afermentans
Corynebacterium amycolatum
Corynebacterium aquaticum
Corynebacterium argentoratense
Corynebacterium auris
Corynebacterium bovis
Corynebacterium coyleae
Corynebacterium cystitidis
Corynebacterium diphtheriae
Corynebacterium diphtheriae biotype *belfanti*
Corynebacterium diphtheriae biotype *gravis*

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Corynebacterium diphtheriae biotype *intermedius*
Corynebacterium diphtheriae biotype *mitis*
Corynebacterium flavescens
Corynebacterium glucuronolyticum
Corynebacterium glucuronolyticum-seminale
Corynebacterium group A
Corynebacterium group A-4
Corynebacterium group A-5
Corynebacterium group ANF
Corynebacterium group B
Corynebacterium group B-3
Corynebacterium group F
Corynebacterium group F-1
Corynebacterium group F-2
Corynebacterium group G
Corynebacterium group G-1
Corynebacterium group G-2
Corynebacterium group I
Corynebacterium group I-2
Corynebacterium jeikeium (group JK)
Corynebacterium kutscheri (*C. murium*)
Corynebacterium macginleyi
Corynebacterium minutissimum
Corynebacterium pilosum
Corynebacterium propinquum
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudotuberculosis
Corynebacterium pyogenes
Corynebacterium renale
Corynebacterium renale group
Corynebacterium seminale
Corynebacterium species
Corynebacterium striatum (*C. flavidum*)
Corynebacterium ulcerans
Corynebacterium urealyticum (group D2)
Corynebacterium xerosis
Cryptococcus albidus
Cryptococcus ater
Cryptococcus cereanus
Cryptococcus gastricus
Cryptococcus humicolus
Cryptococcus lactativorus
Cryptococcus laurentii
Cryptococcus luteolus
Cryptococcus melibiosum
Cryptococcus neoformans
Cryptococcus species
Cryptococcus terreus
Cryptococcus uniguttulatus
Debaryomyces hansenii
Debaryomyces marama
Debaryomyces polymorphus
Debaryomyces species
Dermabacter hominis
Dermacoccus (*Micrococcus*) *nishinomiyaensis*
Dietzia species
Edwardsiella hoshinae
Edwardsiella ictaluri
Edwardsiella species
Edwardsiella tarda
Eikenella corrodens
Empedobacter brevis (*Flavobacterium breve*)
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter amnigenus
Enterobacter amnigenus asburiae (CDC enteric group 17)

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Enterobacter amnigenus biogroup 1
Enterobacter amnigenus biogroup 2
Enterobacter asburiae
Enterobacter cancerogenus
Enterobacter cloacae
Enterobacter gergoviae
Enterobacter hormaechei
Enterobacter intermedius
Enterobacter sakazakii
Enterobacter species
Enterobacter taylorae
Enterobacter taylorae (CDC enteric group 19)
Enterococcus (*Streptococcus*) *cecorum*
Enterococcus (*Streptococcus*) *faecalis* (Group D)
Enterococcus (*Streptococcus*) *faecium*(Group D)
Enterococcus (*Streptococcus*) *saccharolyticus*
Enterococcus avium (Group D)
Enterococcus casseliflavus (*Steptococcus faecium* subsp. *casseliflavus*)
Enterococcus durans (*Streptococcus faecium* subsp. *durans*) (Group D)
Enterococcus gallinarum
Enterococcus hirae
Enterococcus malodoratus
Enterococcus mundtii
Enterococcus raffinosus
Enterococcus species
Erwinia amylovora
Erwinia carotovora
Erwinia carotovora subsp. *atroseptica*
Erwinia carotovora subsp. *betavasculorum*
Erwinia carotovora subsp. *carotovora*
Erwinia chrysanthemi
Erwinia cypripedii
Erwinia mallotivora
Erwinia nigrifluens
Erwinia quercina
Erwinia rhapontici
Erwinia rubrifaciens
Erwinia salicis
Erwinia species
Erysipelothrix rhusiopathiae
Erysipelothrix species
Escherichia blattae
Escherichia coli
Escherichia coli A-D
Escherichia coli O157:H7
Escherichia fergusonii
Escherichia hermannii
Escherichia species
Escherichia vulneris
Eubacterium aerofaciens
Eubacterium alactolyticum
Eubacterium lentum
Eubacterium limosum
Eubacterium species
Ewingella americana
Filobasidiella neoformans
Filobasidium floriforme
Filobasidium uniguttulatum
Flavimonas oryzihabitans
Flavobacterium gleum
Flavobacterium indologenes
Flavobacterium odoratum
Flavobacterium species
Francisella novicida
Francisella philomiragia

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Francisella species
Francisella tularensis
Fusobacterium mortiferum
Fusobacterium necrogenes
Fusobacterium necrophorum
Fusobacterium nucleatum
Fusobacterium species
Fusobacterium varium
Gaffkya species
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Gemella species
Geotrichum candidum
Geotrichum fermentans
Geotrichum penicillarum
Geotrichum penicillatum
Geotrichum species
Gordona species
Haemophilus aegyptius
Haemophilus aphrophilus
Haemophilus ducreyi
Haemophilus haemoglobinophilus
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus influenzae biotype I
Haemophilus influenzae biotype II
Haemophilus influenzae biotype III
Haemophilus influenzae biotype IV
Haemophilus influenzae biotype V
Haemophilus influenzae biotype VI
Haemophilus influenzae biotype VII
Haemophilus influenzae biotype VIII
Haemophilus paragallinarum
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus parainfluenzae biotype I
Haemophilus parainfluenzae biotype II
Haemophilus parainfluenzae biotype III
Haemophilus parainfluenzae biotype IV
Haemophilus parainfluenzae biotype V
Haemophilus parainfluenzae biotype VI
Haemophilus parainfluenzae biotype VII
Haemophilus parainfluenzae biotype VIII
Haemophilus paraphrohaemolyticus
Haemophilus paraphrophilus
Haemophilus segnis
Haemophilus somnus
Haemophilus species
Hafnia alvei
Hanseniaspora guilliermondii
Hanseniaspora uvarum
Hanseniaspora valbyensis
Hansenula anomala
Hansenula holstii
Hansenula polymorpha
Helicobacter (*Campylobacter*) *cinaedi*
Helicobacter (*Campylobacter*) *fennelliae*
Helicobacter (*Campylobacter*) *pylori*
Issatchenkia orientalis
Kingella denitrificans
Kingella indologenes
Kingella kingae
Kingella species
Klebsiella ornithinolytica
Klebsiella oxytoca
Klebsiella planticola

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Klebsiella pneumoniae subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*
Klebsiella pneumoniae subsp. *rhinoscleromatis*
Klebsiella species
Klebsiella terrigena
Kloeckera apiculata
Kloeckera apis
Kloeckera japonica
Kloeckera species
Kluyvera ascorbata
Kluyvera cryocrescens
Kluyvera species
Kluyveromyces lactis
Kluyveromyces marxianus
Kluyveromyces thermotolerans
Kocuria (*Micrococcus*) *kristinae*
Kocuria (*Micrococcus*) *rosea*
Kocuria(*Micrococcus*) *varians*
Koserella trabulsii
Kytococcus (*Micrococcus*) *sedentarius*
Lactobacillus (*Weissella*) *viridescens*
Lactobacillus A
Lactobacillus acidophilus
Lactobacillus B
Lactobacillus brevis
Lactobacillus buchneri
Lactobacillus casei
Lactobacillus casei subsp. *casei*
Lactobacillus casei subsp. *lactosus*
Lactobacillus casei subsp. *rhamnosus*
Lactobacillus catenaformis
Lactobacillus cellobiosus
Lactobacillus collinoides
Lactobacillus coprophilus
Lactobacillus crispatus
Lactobacillus curvatus
Lactobacillus delbrueckii subsp. *bulgaricus*
Lactobacillus delbrueckii subsp. *delbrueckii*
Lactobacillus delbrueckii subsp. *lactis*
Lactobacillus fermentum
Lactobacillus fructivorans
Lactobacillus helveticus
Lactobacillus helveticus subsp. *jugurti*
Lactobacillus jensenii
Lactobacillus lindneri
Lactobacillus minutus
Lactobacillus paracasei subsp. *paracasei*
Lactobacillus pentosus
Lactobacillus plantarum
Lactobacillus salivarius
Lactobacillus salivarius var. *salicinius*
Lactobacillus species
Lactococcus diacitilactis
Lactococcus garvieae
Lactococcus lactis subsp. *cremoris*
Lactococcus lactis subsp. *diacitilactis*
Lactococcus lactis subsp. *hordniae*
Lactococcus lactis subsp. *lactis*
Lactococcus plantarum
Lactococcus raffinolactis
Leclercia adecarboxylata
Legionella species
Leminorella species
Leptospira species
Leptotrichia buccalis
Leuconostoc (*Weissella*) *paramesenteroides*

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Leuconostoc carnosum
Leuconostoc citreum
Leuconostoc gelidum
Leuconostoc lactis
Leuconostoc mesenteroides
Leuconostoc mesenteroides subsp. *cremoris*
Leuconostoc mesenteroides subsp. *dextranicum*
Leuconostoc mesenteroides subsp. *mesenteroides*
Leuconostoc species
Listeria grayi
Listeria innocua
Listeria ivanovii
Listeria monocytogenes
Listeria murrayi
Listeria seeligeri
Listeria species
Listeria welshimeri
Megasphaera elsdenii
Methylobacterium mesophilicum
Metschnikowia pulcherrima
Microbacterium species
Micrococcus luteus
Micrococcus lylae
Micrococcus species
Mobiluncus curtisii
Mobiluncus mulieris
Mobiluncus species
Moellerella wisconsensis
Moraxella (*Branhamella*) *catarrhalis*
Moraxella atlantae
Moraxella bovis
Moraxella lacunata
Moraxella nonliquefaciens
Moraxella osloensis
Moraxella phenylpyruvica
Moraxella species
Morganella morganii
Morganella morganii subsp. *morganii*
Morganella morganii subsp. *sibonii*
Mycobacterium africanum
Mycobacterium asiaticum
Mycobacterium avium
Mycobacterium bovis
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium malmoense
Mycobacterium marinum
Mycobacterium phlei
Mycobacterium scrofulaceum
Mycobacterium smegmatis
Mycobacterium species
Mycobacterium tuberculosis
Mycobacterium ulcerans
Mycobacterium xenopi
Mycoplasma fermentans
Mycoplasma hominis
Mycoplasma orale
Mycoplasma pneumoniae
Mycoplasma species
Myroides species
Neisseria cinerea
Neisseria elongata subsp. *elongata*
Neisseria flava
Neisseria flavescens
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Neisseria mucosa
Neisseria perflava

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Neisseria polysaccharea
Neisseria saprophytes
Neisseria sicca
Neisseria subflava
Neisseria weaveri
Neisseria weaveri (CDC group M5)
Nocardia species
Ochrobactrum anthropi
Oerskovia species
Oerskovia xanthineolytica
Oligella (*Moraxella*) *urethralis*
Oligella species
Oligella ureolytica
Paenibacillus alvei
Paenibacillus macerans
Paenibacillus polymyxa
Pantoea agglomerans
Pantoea ananas (*Erwinia uredovora*)
Pantoea dispersa
Pantoea species
Pantoea stewartii
Pasteurella (*Haemophilus*) *avium*
Pasteurella aerogenes
Pasteurella gallinarum
Pasteurella haemolytica
Pasteurella haemolyticus
Pasteurella multocida
Pasteurella multocida SF
Pasteurella multocida subsp. *multocida*
Pasteurella multocida subsp. *septica*
Pasteurella pneumotropica
Pasteurella species
Pasteurella ureae
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Pediococcus species
Peptococcus niger
Peptococcus species
Peptostreptococcus anaerobius
Peptostreptococcus asaccharolyticus
Peptostreptococcus indolicus
Peptostreptococcus magnus
Peptostreptococcus micros
Peptostreptococcus parvulus
Peptostreptococcus prevotii
Peptostreptococcus productus
Peptostreptococcus species
Peptostreptococcus tetradius
Phaecoccomyces exophialiae
Photobacterium damselae
Pichia (*Hansenula*) *anomala*
Pichia (*Hansenula*) *jadinii*
Pichia (*Hansenula*) *petersonii*
Pichia angusta (*Hansenula polymorpha*)
Pichia carsonii (*P. vini*)
Pichia etchellsii
Pichia farinosa
Pichia fermentans
Pichia membranaefaciens
Pichia norvegensis
Pichia ohmeri
Pichia spartinae
Pichia species
Plesiomonas shigelloides
Porphyromonas asaccharolytica
Porphyromonas endodontalis
Porphyromonas gingivalis
Porphyromonas levii
Prevotella (*Bacteroides*) *buccae*
Prevotella (*Bacteroides*) *buccalis*
Prevotella (*Bacteroides*) *corporis*

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Prevotella (*Bacteroides*) *denticola*
Prevotella (*Bacteroides*) *loescheii*
Prevotella (*Bacteroides*) *oralis*
Prevotella (*Bacteroides*) *disiens*
Prevotella (*Bacteroides*) *oris*
Prevotella bivia (*Bacteroides bivius*)
Prevotella intermedia (*Bacteroides intermedius*)
Prevotella melaninogenica (*Bacteroides melaninogenicus*)
Prevotella ruminicola
Propionibacterium acnes
Propionibacterium avidum
Propionibacterium granulosum
Propionibacterium propionicum
Propionibacterium species
Proteus mirabilis
Proteus penneri
Proteus species
Proteus vulgaris
Prototheca species
Prototheca wickerhamii
Prototheca zopfii
Providencia alcalifaciens
Providencia heimbachae
Providencia rettgeri
Providencia rustigianii
Providencia species
Providencia stuartii
Providencia stuartii urea +
Pseudomonas (*Chryseomonas*) *luteola*
Pseudomonas acidovorans
Pseudomonas aeruginosa
Pseudomonas alcaligenes
Pseudomonas cepacia
Pseudomonas chlororaphis (*P. aureofaciens*)
Pseudomonas fluorescens
Pseudomonas fluorescens group
Pseudomonas mendocina
Pseudomonas pseudoalcaligenes
Pseudomonas putida
Pseudomonas species
Pseudomonas stutzeri
Pseudomonas testosteroni
Pseudomonas vesicularis
Pseudoramibacter (*Eubacterium*) *alactolyticus*
Psychrobacter (*Moraxella*) *phenylpyruvicus*
Rahnella aquatilis
Ralstonia (*Pseudomonas, Burkholderia*) *pickettii*
Rhodococcus (*Corynebacterium*) *equi*
Rhodococcus species
Rhodosporidium toruloides
Rhodotorula glutinis
Rhodotorula minuta
Rhodotorula mucilaginosa (*R. rubra*)
Rhodotorula species
Rickettsia species
Rothia dentocariosa
Saccharomyces cerevisiae
Saccharomyces exiguus
Saccharomyces kluyverii
Saccharomyces species
Sakaguchia dacryoides (*Rhodosporidium dacryoidum*)
Salmonella arizonae
Salmonella choleraesuis
Salmonella enteritidis
Salmonella gallinarum
Salmonella paratyphi A
Salmonella paratyphi B

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Salmonella pullorum
Salmonella species
Salmonella typhi
Salmonella typhimurium
Salmonella typhisuis
Salmonella/Arizona
Serratia ficaria
Serratia fonticola
Serratia grimesii
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Serratia odorifera type 1
Serratia odorifera type 2
Serratia plymuthica
Serratia proteamaculans
Serratia proteamaculans subsp. *proteamaculans*
Serratia proteamaculans subsp. *quinovora*
Serratia rubidaea
Serratia species
Shewanella (*Pseudomonas*, *Alteromonas*) *putrefaciens*
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonneri
Shigella species
Sphingobacterium multivorum
Sphingobacterium species
Sphingobacterium spiritivorum
Sphingobacterium thalpophilum
Sphingomonas (*Pseudomonas*) *paucimobilis*
Sporidiobolus salmonicolor
Sporobolomyces roseus
Sporobolomyces salmonicolor
Sporobolomyces species
Staphylococcus (*Peptococcus*) *saccharolyticus*
Staphylococcus arlettae
Staphylococcus aureus
Staphylococcus aureus (*Coagulase-negative*)
Staphylococcus auricularis
Staphylococcus capitis
Staphylococcus capitis subsp. *capitis*
Staphylococcus capitis subsp. *ureolyticus*
Staphylococcus caprae
Staphylococcus carnosus
Staphylococcus caseolyticus
Staphylococcus chromogenes
Staphylococcus cohnii
Staphylococcus cohnii subsp. *cohnii*
Staphylococcus cohnii subsp. *urealyticum*
Staphylococcus epidermidis
Staphylococcus equorum
Staphylococcus gallinarum
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus hominis subsp. *hominis*
Staphylococcus hominis subsp. *novobiosepticus*
Staphylococcus hyicus
Staphylococcus intermedius
Staphylococcus kloosii
Staphylococcus lentus
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus schleiferi
Staphylococcus sciuri
Staphylococcus simulans
Staphylococcus species
Staphylococcus warneri
Staphylococcus xylosus
Stenotrophomonas (*Xanthomonas*) *maltophilia*
Stephanoascus ciferrii
Stomatococcus mucilaginosus
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus agalactiae (Group B)
Streptococcus agalactiae hemolytic
Streptococcus agalactiae non-hemolytic
Streptococcus alactolyticus
Streptococcus anginosus
Streptococcus anginosus (Group D, nonenterococci)
Streptococcus beta-hemolytic group A
Streptococcus beta-hemolytic non-group A or B
Streptococcus beta-hemolytic non-group A
Streptococcus beta-hemolytic
Streptococcus bovis (Group D, nonenterococci)
Streptococcus bovis I
Streptococcus bovis II
Streptococcus canis
Streptococcus constellatus
Streptococcus constellatus (*Streptococcus milleri* I)
Streptococcus constellatus (*viridans Streptococcus*)
Streptococcus downei
Streptococcus dysgalactiae subsp. *dysgalactiae*
Streptococcus dysgalactiae subsp. *equisimilis*
Streptococcus equi (Group C/Group G *Streptococcus*)
Streptococcus equi subsp. *equi*
Streptococcus equi subsp. *zooepidemicus*
Streptococcus equinus
Streptococcus equinus (Group D, nonenterococci)
Streptococcus equisimilis
Streptococcus equisimulis (Group C/Group G *Streptococcus*)
Streptococcus Gamma (non)-hemolytic
Streptococcus gordonii
Streptococcus Group B
Streptococcus Group C
Streptococcus Group D
Streptococcus Group E
Streptococcus Group F
Streptococcus Group G
Streptococcus Group L
Streptococcus Group P
Streptococcus Group U
Streptococcus intermedius
Streptococcus intermedius (*Streptococcus milleri* II)
Streptococcus intermedius (*viridans Streptococcus*)
Streptococcus milleri group
Streptococcus mitis
Streptococcus mitis (*viridans Streptococcus*)
Streptococcus mitis group
Streptococcus mutans

TABLE 15-continued

Microorganisms identified by commercial systems[1].
Streptococcus mutans (*viridans Streptococcus*)
Streptococcus oralis
Streptococcus parasanguis
Streptococcus pneumoniae
Streptococcus porcinus
Streptococcus pyogenes
Streptococcus pyogenes (Group A)
Streptococcus salivarius
Streptococcus salivarius (*viridans Streptococcus*)
Streptococcus salivarius subsp. *salivarius*
Streptococcus salivarius subsp. *thermophilus*
Streptococcus sanguis
Streptococcus sanguis I (*viridans Streptococcus*)
Streptococcus sanguis II
Streptococcus sanguis II (*viridans Streptococcus*)
Streptococcus sobrinus
Streptococcus species
Streptococcus suis I
Streptococcus suis II
Streptococcus uberis
Streptococcus uberis (*viridans Streptococcus*)
Streptococcus vestibularis
Streptococcus zooepidemicus
Streptococcus zooepidemicus (Group C)
Streptomyces somaliensis
Streptomyces species
Suttonella (*Kingella*) *indologenes*
Tatumella ptyseos
Tetragenococcus (*Pediococcus*) *halophilus*
Torulaspora delbrueckii (*Saccharomyces rosei*)
Torulopsis candida
Torulopsis haemulonii
Torulopsis inconspicua
Treponema species
Trichosporon asahii
Trichosporon asteroides
Trichosporon beigelii
Trichosporon cutaneum
Trichosporon inkin
Trichosporon mucoides
Trichosporon ovoides
Trichosporon pullulans
Trichosporon species
Turicella otitidis
Ureaplasma species
Ureaplasma urealyticum
Veillonella parvula (*V. alcalescens*)
Veillonella species
Vibrio alginolyticus
Vibrio cholerae
Vibrio damsela
Vibrio fluvialis
Vibrio furnissii
Vibrio harveyi
Vibrio hollisae
Vibrio metschnikovii
Vibrio mimicus
Vibrio parahaemolyticus
Vibrio species
Vibrio species SF
Vibrio vulnificus
Weeksella (*Bergeylla*) *virosa*
Weeksella species
Weeksella virosa
Williopsis (*Hansenula*) *satumus*
Xanthomonas campestris
Xanthomonas species
Yarrowia (*Candida*) *lipolytica*
Yersinia aldovae
Yersinia enterocolitica
Yersinia enterocolitica group
Yersinia frederiksenii
Yersinia intermedia
Yersinia intermedius
Yersinia kristensenii
Yersinia pestis
Yersinia pseudotuberculosis
Yersinia pseudotuberculosis SF
Yersinia ruckeri
Yersinia species
Yokenella regensburgei
Yokenella regensburgei (*Koserella trabulsii*)
Zygoascus hellenicus
Zygosaccharomyces species

[1]The list includes microorganisms that may be identified by API identification test systems and VITEK ® automated identification system from bioMerieux Inc., or by the MicroScan ®-WalkAway ® automated systems from Dade Behring. Identification relies on classical identification methods using batteries of biochemical and other phenotypical tests.

TABLE 16

tuf gene sequences obtained in our laboratory (Example 42).

Species	Strain no.	Gene	GenBank Accession no.*
Abiotrophia adiacens	ATCC49175	tuf	AF124224
Enterococcus avium	ATCC14025	tufA	AF124220
		tufB	AF274715
Enterococcus casseliflavus	ATCC25788	tufA	AF274716
		tufB	AF274717
Enterococcus cecorum	ATCC43198	tuf	AF274718
Enterococcus columbae	ATCC51263	tuf	AF274719
Enterococcus dispar	ATCC51266	tufA	AF274720
		tufB	AF274721
Enterococcus durans	ATCC19432	tufA	AF274722
		tufB	AF274723
Enterococcus faecalis	ATCC29212	tuf	AF124221
Enterococcus faecium	ATCC 19434	tufA	AF124222
		tufB	AF274724
Enterococcus gallinarum	ATCC49573	tufA	AF124223
		tufB	AF274725
Enterococcus hirae	ATCC8043	tufA	AF274726
		tufB	AF274727
Enterococcus malodoratus	ATCC43197	tufA	AF274728
		tufB	AF274729
Enterococcus mundtii	ATCC43186	tufA	AF274730
		tufB	AF274731
Enterococcus pseudoavium	ATCC49372	tufA	AF274732
		tufB	AF274733
Enterococcus raffinosus	ATCC49427	tufA	AF274734
		tufB	AF274735
Enterococcus saccharolyticus	ATCC43076	tuf	AF274736
Enterococcus solitarius	ATCC49428	tuf	AF274737
Enterococcus sulfureus	ATCC49903	tuf	AF274738
Lactococcus lactis	ATCC11154	tuf	AF274745
Listeria monocytogenes	ATCC15313	tuf	AF274746
Listeria seeligeri	ATCC35967	tuf	AF274747
Staphylococcus aureus	ATCC25923	tuf	AF274739
Staphylococcus epidermidis	ATCC14990	tuf	AF274740
Streptococcus mutans	ATCC25175	tuf	AF274741
Streptococcus pneumoniae	ATCC6303	tuf	AF274742

TABLE 16-continued

tuf gene sequences obtained in our laboratory (Example 42).

Species	Strain no.	Gene	GenBank Accession no.*
Streptococcus pyogenes	ATCC19615	tuf	AF274743
Streptococcus suis	ATCC43765	tuf	AF274744

*Corresponding sequence ID NO. for the above ATCC strains are given in table 7.

TABLE 17

tuf gene sequences selected from databases for Example 42.

Species	Gene	Accession no.*
Agrobacterium tumefaciens	tufA	X99673
	tufB	X99674
Anacystis nidulans	tuf	X17442
Aquifex aeolicus	tufA	AE000657
	tufB	AE000657
Bacillus stearothermophilus	tuf	AJ000260
Bacillus subtilis	tuf	AL009126
Bacteroides fragilis	tuf	P33165
Borrelia burgdorferi	tuf	AE000783
Brevibacterium linens	tuf	X76863
Bulkholderia cepacia	tuf	P33167
Campylobacter jejuni	tufB	Y17167
Chlamydia pneumoniae	tuf	AE001363
Chlamydia trachomatis	tuf	M74221
Corynebacterium glutamicum	tuf	X77034
Cytophaga lytica	tuf	X77035
Deinococcus radiodurans	tuf	AE000513
Escherichia coli	tufA	J01690
	tufB	J01717
Fervidobacterium islandicum	tuf	Y15788
Haemophilus influenzae	tufA	L42023
	tufB	L42023
Helicobacter pylori	tuf	AE000511

TABLE 17-continued

tuf gene sequences selected from databases for Example 42.

Species	Gene	Accession no.*
Homo sapiens (Human)	EF-1α	X03558
Methanococcus jannaschii	EF-1α	U67486
Mycobacterium leprae	tuf	D13869
Mycobacterium tuberculosis	tuf	X63539
Mycoplasma genitalium	tuf	L43967
Mycoplasma pneumoniae	tuf	U00089
Neisseria gonorrhoeae	tufA	L36380
Nicotiana tabacum (Tobacco)	EF-1α	U04632
Peptococcus niger	tuf	X76869
Planobispora rosea	tuf1	U67308
Saccharomyces cerevisiae (Yeast)	EF-1α	X00779
Salmonella typhimurium	tufA	X55116
	tufB	X55117
Shewanella putrefaciens	tuf	P33169
Spirochaeta aurantia	tuf	X76874
Spirulina platensis	tufA	X15646
Streptomyces aureofaciens	tuf1	AF007125
Streptomyces cinnamoneus	tuf1	X98831
Streptomyces coelicolor	tuf1	X77039
	tuf3	X77040
Streptomyces collinus	tuf1	S79408
Streptomyces ramocissimus	tuf1	X67057
	tuf2	X67058
	tuf3	X67059
Synechocystis sp.	tuf	AB001339
Taxeobacter ocellatus	tuf	X77036
Thermotoga maritima	tuf	AE000512
Thermus aquaticus	tuf	X66322
Thermus thermophilus	tuf	X06657
Thiobacillus cuprinus	tuf	U78300
Treponema pallidum	tuf	AE000520
Wolinella succinogenes	tuf	X76872

*Sequence data were obtained from GenBank, EMBL, and SWISSPROT databases. Genes were designated as appeared in the references.

TABLE 18

Nucleotide and amino acid sequence identities of EF-Tu between different *enterococci* and other low G + C gram-positive bacteria.

Bacterial tuf gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1. *E. avium* tufA		96	98	96	96	96	96	97	95	98	99	95	95	96	94	96	93	86	87	85	86	86	85	86	85	86	87	86	92	91	90	90	90	92	84	85	84	82	83
2. *E.* ? tufA	90		97	96	96	99	96	95	96	96	96	95	95	96	96	94	93	87	88	86	87	87	86	87	87	87	88	88	94	91	90	91	91	92	86	87	85	85	85
3. *E.* ? tufA	93	90		95	95	96	95	96	95	97	97	91	90	95	95	95	93	86	87	85	87	87	86	87	86	87	87	87	93	90	89	90	90	92	85	86	84	85	84
4. *E. durans* tufA	90	89	90		98	96	99	93	99	95	96	90	91	94	95	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	90	90	90	91	85	86	84	84	84
5. *E.* ? tufA	89	90	89	96		96	98	93	98	95	96	89	91	88	94	93	92	87	88	86	86	87	87	86	87	87	88	87	94	92	91	91	91	93	85	86	84	84	84
6. *E.* ? tufA	90	97	89	89	89		96	93	95	96	96	88	89	89	96	93	92	87	87	86	87	87	87	86	87	87	88	87	93	92	90	90	90	93	85	86	84	83	84
7. *E.* ? tufA	90	90	89	99	96	89		93	99	95	96	91	91	89	95	94	92	86	87	86	86	86	85	86	86	87	87	87	94	90	90	90	90	91	85	86	84	84	84
8. *E.* ? tufA	96	91	94	90	89	90	89		92	97	97	89	89	90	93	96	92	86	85	82	85	85	85	85	83	85	88	86	92	90	88	88	89	91	83	84	83	83	82
9. *E.* ? tufA	89	89	88	96	93	89	96	88		94	95	88	90	88	94	94	92	87	87	86	86	86	85	86	87	87	88	87	94	90	89	90	89	91	85	86	84	84	84
10. *E.* ? tufA	97	92	93	90	89	91	89	97	89		98	90	90	91	95	96	94	87	87	86	87	87	86	87	86	87	88	88	93	90	89	90	90	91	85	86	85	85	84
11. *E.* ? tufA	97	91	93	90	89	89	89	97	88	97		91	90	90	94	96	93	86	87	85	86	86	85	86	85	87	87	87	93	89	89	90	89	91	84	85	84	84	83
12. *E.* ? tufA	90	90	95	96	96	95	96	92	95	95	95		98	95	93	93	93	88	88	87	87	87	86	85	89	87	89	89	93	90	90	91	91	93	86	86	84	85	84
13. *E.* ? tufA	90	90	95	96	97	96	96	93	95	95	95	97		95	94	92	92	89	88	86	87	88	88	87	87	87	89	89	94	92	91	91	92	93	86	86	85	86	85
14. *E.* ? tufA	91	91	90	89	96	97	94	94	94	95	96	90	89		94	94	93	87	87	86	87	87	86	86	87	87	88	87	93	91	89	90	91	93	86	86	86	85	85
15. *E.* ? tufA	91	91	91	90	87	90	89	91	89	92	91	89	89	92		94	92	86	87	85	87	86	84	86	85	87	87	87	92	90	89	89	88	90	84	85	84	84	84
16. *E.* ? tufA	91	89	90	91	88	88	90	91	89	92	91	88	89	91	94		91	85	84	81	84	85	84	84	81	84	85	85	91	90	87	88	89	91	82	83	83	82	82
17. *E.* ? tufA	83	84	83	83	84	83	82	84	83	84	84	84	83	84	83	83		88	87	86	87	87	86	87	88	88	88	89	92	91	89	90	90	91	86	85	85	85	84
18. *E. avium* tufB	77	77	78	78	76	77	78	78	77	78	77	78	78	78	77	76	77		93	93	94	94	94	92	98	93	99	97	87	86	87	86	85	86	89	88	87	85	86
19. *E.* ? tufB	71	72	72	72	70	72	72	70	71	72	72	72	70	72	72	68	72	79		93	95	95	96	95	93	95	94	94	87	86	88	88	84	85	90	90	89	88	88
20. *E. dispar* tufB	76	78	77	77	77	77	77	76	77	76	77	77	77	77	78	75	78	82	79		91	91	92	91	94	92	93	93	86	83	85	85	82	84	89	89	87	87	86
21. *E. durans* tufB	77	78	78	78	76	77	78	77	78	77	78	77	77	78	78	75	75	83	80	82		98	95	97	94	97	95	94	87	85	88	88	84	85	90	91	89	88	89
22. *E.* ? tufB	76	75	76	76	75	77	76	76	76	75	76	77	77	77	78	74	74	80	78	79	85		96	97	95	97	95	94	87	87	88	88	84	86	90	90	89	87	87
23. *E.* ? tufB	72	73	72	73	72	74	72	71	72	72	72	72	72	73	73	72	72	78	81	77	81	82		94	94	95	95	94	85	87	89	89	84	86	90	90	89	87	88
24. *E.* ? tufB	75	74	75	75	75	75	75	75	76	75	75	74	74	74	75	72	74	80	79	79	84	83	79		93	97	93	94	87	85	86	88	83	85	89	90	88	88	87
25. *E.* ? tufB	76	76	76	77	77	77	77	74	77	76	76	77	75	77	77	73	78	90	79	83	81	80	77	79		93	98	97	87	86	87	87	85	85	88	89	87	85	86

TABLE 18-continued

Nucleotide and amino acid sequence identities of EF-Tu between different *enterococci* and other low G + C gram-positive bacteria.

Bacterial tuf gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
26. *E.* (?) tufB	74	74	74	75	73	74	74	74	74	74	74	74	74	75	74	71	73	80	80	78	85	85	80	84	80		94	94	87	86	88	88	84	86	90	90	89	88	89
27. *E.* (?) tufB	77	77	78	77	76	78	77	77	76	78	78	77	77	78	78	77	78	91	80	85	84	81	79	80	91	80		98	88	87	88	87	85	87	90	89	88	86	87
28. *E.* (?) tufB	78	79	79	78	77	77	78	78	77	79	79	78	78	78	79	77	79	90	79	84	84	81	77	80	90	81	92		87	85	87	88	84	86	90	89	88	88	87
29. *A.* (?) tuf	88	87	87	86	88	86	86	89	88	88	88	87	88	88	88	90	82	77	70	76	77	76	71	73	77	73	78	78		90	88	89	90	91	85	86	84	85	83
30. *B. subtilis* tuf	81	80	79	79	80	80	79	79	79	80	81	80	81	81	80	78	78	73	69	73	73	71	70	71	72	71	74	74	78		91	92	90	90	82	82	83	82	84
31. *L. monocytogenes* tuf	82	81	82	82	82	82	82	81	81	81	82	81	81	81	81	79	79	76	71	75	75	75	73	74	75	73	78	76	79	82		99	88	90	84	84	84	84	84
32. *L.* (?) tuf	82	81	82	82	82	81	82	81	82	81	82	81	82	80	81	79	79	76	71	76	75	74	73	75	75	73	77	76	79	82	99		88	91	84	85	85	84	85
33. *S. aureus* tuf	84	84	83	83	83	84	84	82	84	83	84	86	86	84	82	81	79	75	69	75	75	73	69	72	74	72	74	74	83	79	81	81		96	81	82	82	80	82
34. *S. epidermidis* tuf	83	85	83	84	83	84	84	82	84	83	83	88	87	85	83	82	79	75	69	75	75	73	68	72	74	72	74	75	81	79	82	81	94		83	83	83	83	83
35. *S. mutans* tuf	76	77	76	76	76	77	76	75	76	76	76	77	76	76	76	74	78	79	72	77	78	77	74	75	78	75	78	81	77	75	76	77	74	73		97	96	94	88
36. *S. pneumonise* tuf	76	77	76	77	77	77	77	75	78	76	76	77	76	77	75	74	75	76	72	75	78	76	73	74	77	75	75	78	75	76	77	76	74	74	87		96	96	89
37. *S. pyogeness* tuf	76	77	76	77	76	75	77	74	77	76	75	76	75	77	75	73	75	74	71	75	78	75	73	74	75	75	75	77	76	77	76	76	73	72	87	93		94	89
38. *S. suis* tuf	74	78	76	76	74	75	76	74	78	76	77	77	75	78	76	73	75	74	71	75	78	74	70	74	75	73	73	77	77	77	77	77	73	73	88	93	91		88
39. *L. lactis* tuf	75	76	75	76	75	75	76	75	76	76	76	77	76	76	75	72	74	75	72	75	77	76	71	75	74	75	75	75	75	75	77	76	74	74	80	83	82	81	

The upper right triangle represents the deduced amino acid sequence identities of gram-positive bacterial EF-Tu; while the lower left triangle represents the DNA sequence identities of the corresponding tuf genes. The sequence identities between different *enterococcal*tufA genes are boxed while those between *enterococcal*tufB genes are shaded.

(?) indicates text missing or illegible when filed

TABLE 19

Strains analyzed in Example 43.

Taxon	Strain*	Strain†	16S rDNA sequence accession number
Cedecea davisae	ATCC 33431^T		
Cedecea lapagei	ATCC 33432^T		
Cedecea neteri	ATCC 33855^T		
Citrobacter amalonaticus	ATCC 25405^T	CDC 9020-77^T	AF025370
Citrobacter braakii	ATCC 43162	CDC 080-58^T	AF025368
Citrobacter farmeri	ATCC 51112^T	CDC 2991-81^T	AF025371
Citrobacter freundii	ATCC 8090^T	DSM 30039^T	AJ233408
Citrobacter koseri	ATCC 27156^T		
Citrobacter sedlakii	ATCC 51115^T	CDC 4696-86^T	AF025364
Citrobacter werkmanii	ATCC 51114^T	CDC 0876-58^T	AF025373
Citrobacter youngae	ATCC 29935^T		
Edwardsiella hoshinae	ATCC 33379^T		
Edwardsiella tarda	ATCC 15947^T	CDC 4411-68	AF015259
Enterobacter aerogenes	ATCC 13048^T	JCM 1235^T	AB004750
Enterobacter agglomerans	ATCC 27989		
Enterobacter amnigenus	ATCC 33072^T	JCM 1237^T	AB004749
Enterobacter asburiae	ATCC 35953^T	JCM 6051^T	AB004744
Enterobacter cancerogenus	ATCC 35317^T		
Enterobacter cloacae	ATCC 13047^T		
Enterobacter gergoviae	ATCC 33028^T	JCM 1234^T	AB004748
Enterobacter hormaechei	ATCC 49162^T		
Enterobacter sakazakii	ATCC 29544^T	JCM 1233^T	AB004746
Escherichia coli	ATCC 11775^T	ATCC 11775^T	X80725
Escherichia coli	ATCC 25922	ATCC 25922	X80724
Escherichia coli (ETEC)	ATCC 35401		
Escherichia coli (O157:H7)	ATCC 43895	ATCC 43895	Z83205
Escherichia fergusonii	ATCC 35469^T		
Escherichia hermanii	ATCC 33650^T		
Escherichia vulneris	ATCC 33821^T	ATCC 33821^T	X80734
Ewingella americana	ATCC 33852^T	NCPPB 3905	X88848
Hafnia alvei	ATCC 13337^T	ATCC 13337^T	M59155
Klebsiella ornithinolytica	ATCC 31898	CIP 103.364	U78182
Klebsiella oxytoca	ATCC 33496	ATCC 13182^T	U78183
Klebsiella planticola	ATCC 33531^T	JCM 7251^T	AB004755
Klebsiella pneumoniae			
subsp. *pneumoniae*	ATCC 13883^T	DSM 30104^T	AJ233420
subsp. *ozaenae*	ATCC 11296^T	ATCC 11296^T	Y17654
subsp. *rhinoscleromatis*	ATCC 13884^T		
Kluyvera ascorbata	ATCC 33433^T	ATCC 14236	Y07650
Kluyvera cryocrescens	ATCC 33435^T		
Kluyvera georgiana	ATCC 51603^T		
Leclercia adecarboxylata	ATCC 23216^T		
Leminorella grimontii	ATCC 33999^T	DSM 5078^T	AJ233421
Moellerella wisconsensis	ATCC 35017^T		
Morganella morganii	ATCC 25830^T		
Pantoea agglomerans	ATCC 27155^T	DSM 3493^T	AJ233423
Pantoea dispersa	ATCC 14589^T		
Plesiomonas shigelloides	ATCC 14029^T		
Pragia fontium	ATCC 49100^T	DSM 5563^T	AJ233424
Proteus mirabilis	ATCC 25933		
Proteus penneri	ATCC 33519^T		
Proteus vulgaris	ATCC 13315^T	DSM 30118^T	AJ233425
Providencia alcalifaciens	ATCC 9886^T		
Providencia rettgeri	ATCC 9250		
Providencia rustigianii	ATCC 33673^T		
Providencia stuartii	ATCC 33672		
Rahnella aquatilis	ATCC 33071^T	DSM 4594^T	AJ233426
Salmonella choleraesuis			
subsp. *arizonae*	ATCC 13314^T		
subsp. *choleraesuis*			
serotype Choleraesuis	ATCC 7001		
serotype Enteritidis‡	ATCC 13076^T	SE22	SE22
serotype Gallinarum	ATCC 9184		
serotype Heidelberg	ATCC 8326		
serotype Paratyphi A	ATCC 9150		
serotype Paratyphi B	ATCC 8759		
serotype Typhi‡	ATCC 10749	St111	U88545
serotype Typhimurium‡	ATCC 14028		
serotype Virchow	ATCC 51955		
subsp. *diarizonae*	ATCC 43973^T		
subsp. *houtenae*	ATCC 43974^T		
subsp. *indica*	ATCC 43976^T		
subsp. *salamae*	ATCC 43972^T		
Serratia fonticola	DSM 4576^T	DSM 4576^T	AJ233429
Serratia grimesii	ATCC 14460^T	DSM 30063^T	AJ233430
Serratia liquefaciens	ATCC 27592^T		
Serratia marcescens	ATCC 13880^T	DSM 30121^T	AJ233431
Serratia odorifera	ATCC 33077^T	DSM 4582^T	AJ233432
Serratia plymuthica	DSM 4540^T	DSM 4540^T	AJ233433
Serratia rubidaea	DSM 4480^T	DSM 4480^T	AJ233436
Shigella boydii	ATCC 9207	ATCC 9207	X96965
Shigella dysenteriae	ATCC 11835		
		ATCC 13313^T	X96966
		ATCC 25931	X96964
Shigella flexneri	ATCC 12022	ATCC 12022	X96963
Shigella sonnei	ATCC 29930^T		
Tatumella ptyseos	ATCC 33301^T	DSM 5000^T	AJ233437
Trabulsiella guamensis	ATCC 49490^T		
Yersinia enterocolitica	ATCC 9610^T	ATCC 9610^T	M59292
Yersinia frederiksenii	ATCC 33641^T		
Yersinia intermedia	ATCC 29909^T		
Yersinia pestis	RRB KIMD27	ATCC 19428^T	X75274
Yersinia pseudotuberculosis	ATCC 29833^T		
Yersinia rohdei	ATCC 43380^T	ER-2935^T	X75276
Shewanella putrefaciens	ATCC 8071^T		
Vibrio cholerae	ATCC 25870	ATCC 14035^T	X74695

T Type strain

*Strains used in this study for sequencing of partial tuf and atpD genes. SEQ ID NOs. for tuf and atpD sequences corresponding to the above reference strains are given in table 7.

†Strains used in other studies for sequencing of 16S rDNA gene. When both strain numbers are on the same row, both strains are considered to be the same although strain numbers may be different.

‡Phylogenetic serotypes considered species by the Bacteriological Code (1990 Revision).

TABLE 20

PCR primer pairs used in this study

Primer SEQ ID NO.	Sequence	Nucleotide positions*	Amplicon length (bp)
Tuf			
664	5'-AAYATGATIACIGGIGCIGCICARATGGA-3'	271-299	884
697	5'-CCIACIGTICKICCRCCYTCRCG-3'	1132-1156	

TABLE 20-continued

PCR primer pairs used in this study			
Primer SEQ ID NO.	Sequence	Nucleotide positions*	Amplicon length (bp)
atpD			
568	5'-RTIATIGGIGCIGTIRTIGAYGT-3'	25-47	884
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	
700	5'-TIRTIGAYGTCGARTTCCCTCARG-3'	38-61	871
567	5'-TCRTCIGCIGGIACRTAIAYIGCYTG-3'	883-908	

*The nucleotide positions given are for *E. coli* tuf and atpD sequences (GenBank accession no. AE000410 and V00267, respectively). Numbering starts from the first base of the initiation codon.

TABLE 21

Selection of *M. catarrhalis*-specific primer pairs from SEQ ID NO: 29[1] (466 pb DNA fragment) other than those previously tested[2].

Primer	Sequence	Amplicon size (bp)	*Moraxella catarrhalis* ATCC 43628	*Moraxella catarrhalis* ATCC 53879	*Moraxella nonliquefaciens*
SEQ ID NO: 118 SEQ ID NO: 119	CGCTGACGGCTTGTTTGTACCA TGTTTTGAGCTTTTTATTTTTTGA	118	+[3]	+	–
VBmcat1 VBmcat2	TGCTTAAGATTCACTCTGCCATTTT TAAGTCGCTGACGGCTTGTTT	93	+	+	–
VBmcat3 VBmcat4	CCTGCACCACAAGTCATCAT AATTCACCAACAATGTCAAAGC	140	+	+	–
VBmcat5 VBmcat6	AATGATAACCAGTCAAGCAAGC GGTGCATGGTGATTTGTAAAA	219	+	+	–
VBmcat7 VBmcat8	GTGTGCGTTCACTTTTACAAAT GGTGTTAAGCTGATGATGAGAG	160	+	+	–
VBmcat9 VBmcat10	TGACCATGCACACCCTTATT TCATTGGGATGAAAGTATCGTT	167	+	+	–

Primer	*Moraxella lacunata*	*Moraxella osloensis*	*Moraxella atlantae*	*Moraxella phenylpyruvica*	*Kingella indologenes*	*Kingella kingea*
SEQ ID NO: 118 SEQ ID NO: 119	–	–	–	–	–	–
VBmcat1 VBmcat2	–	–	–	–	–	–
VBmcat3 VBmcat4	–	–	–	–	–	–
VBmcat5 VBmcat6	–	–	–	–	–	–
VBmcat7 VBmcat8	–	–	–	–	–	–
VBmcat9 VBmcat10	–	–	–	–	–	–

Primer	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Escherichia coli*	*Staphylococcus aureus*
SEQ ID NO: 118 SEQ ID NO: 119	–	–	–	–
VBmcat1 VBmcat2	–	–	–	–

TABLE 21-continued

Selection of *M. catarrhalis*-specific primer pairs from SEQ ID NO: 29[1] (466 pb DNA fragment) other than those previously tested[2].

VBmcat3 VBmcat4	–	–	–	–
VBmcat5 VBmcat6	–	–	–	–
VBmcat7 VBmcat8	–	–	–	–
VBmcat9 VBmcat10	–	–	–	–

[1]SEQ ID NO. from U.S. Pat. No. 6,001,564.
[2]All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55° C. and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.
[3]All position results showed a strong amplification signal with genomic DNA from the target species *M. catarrhalis*.

TABLE 22

Selection of *S. epidermidis*-specific primer pairs from SEQ ID NO: 36[1] (705 pb DNA fragment) other than those previously tested.

Primer	Sequence (all 25 nucleotides)	Amplicon size (bp)	*Staphylococcus epidermidis*, ATCC 14990	*Staphylococcus epidermidis*, ATCC 12228	*Staphylo coccus capitis*	*Staphylo coccus cohnii*
SEQ ID NO: 145	ATCAAAAAGTTGGCGAACCTTTTCA	125	+[3]	+	–	–
SEQ ID NO: 146	CAAAAGAGCGTGGAGAAAAGTATCA					
VBsep3	CATAGTCTGATTGCTCAAAGTCTTG	208	+	+	–	–
VBsep4	GCGAATAGTGAACTACATTCTGTTG		+	+	–	–
VBsep5	CACGCTCTTTTGCAATTTCCATTGA	208	+	+	+	+
VBsep6	GAAGCAAATATTCAAAATGCACCAG		+	+	+	+
VBsep7	AAAGTCTTTTGCTTCTTCAGATTCA	177	+	+	–	–
VBsep8	GTGTTCACAGGTATGGATGCTCTTA		+	+	NT	NT
			+	+	NT	NT
VBsep9	GAGCATCCATACCTGTGAACACAGA	153	+	+	–	–
VBsep10	TTTTCCAATTACAAGAGACATCAGT		+	+	NT	NT
			+	+	NT	NT
VBsep11	TTTGAATTCGCATGTACTTTGTTTG	135	+	+	–	–
VBsep12	CCCCGGGTTCGAAATCGATAAAAAG					

Primer	*Staphylo coccus aureus*	*Staphylo coccus auricularis*	*Staphylo coccus*	*Staphylo coccus hominis*	*Staphylo coccus*	*Staphylo coccus*	*Staphylo coccus simulans*	*Staphylo coccus warneri*	*Bacillus subtilis*	*Entero coccus faecalis*
SEQ ID NO: 145	–	–	–	–	–	–	–	–	–	–
SEQ ID NO: 146										
VBsep3	–	–	+	–	–	–	–	–	–	–
VBsep4	–	–	–	–	–	–	–	–	–	–
VBsep5	+	–	+	+	–	–	–	–	–	–
VBsep6	+	–	+	+	–	–	–	NT	NT	NT
VBsep7	–	–	+	–	–	–	+	–	–	–
VBsep8	–	NT	–	NT	–	–	+	NT	NT	NT
	–	NT	–	NT	–	–	–	NT	NT	NT
VBsep9	–	–	+	–	+	+	–	–	–	–
VBsep10	–	NT	+	NT	+	–	–	NT	NT	NT
	–	NT	–	NT	–	–	–	NT	NT	NT
VBsep11	–	–	–	–	–	–	–	–	–	–
VBsep12										

TABLE 22-continued

Selection of *S. epidermidis*-specific primer pairs from SEQ ID NO: 36[1] (705 pb DNA fragment) other than those previously tested.

Primer	*Entero coccus faecium*	*Entero coccus gallinarum*	*Listeria monocytogenes*	*Strepto coccus agalactiae*	*Strepto coccus pneumoniae*	*Strepto coccus pyogenes*	Annealing temperature[2] (° C.)
SEQ ID NO: 145	–	–	–	–	–	–	55
SEQ ID NO: 146							
VBsep3	–	–	–	–	–	–	55
VBsep4	–	–	–	–	–	–	60
VBsep5	–	–	–	–	–	–	55
VBsep6	NT	NT	NT	NT	NT	NT	65
VBsep7	–	–	–	–	–	–	55
VBsep8	NT	NT	NT	NT	NT	NT	60
	NT	NT	NT	NT	NT	NT	65
VBsep9	–	–	–	–	–	–	55
VBsep10	NT	NT	NT	NT	NT	NT	60
	NT	NT	NT	NT	NT	NT	65
VBsep11	–	–	–	–	–	–	55
VBsep12							

[1]SEQ ID NO. from U.S. Pat. No. 6,001,564.
[2]All PCR assays were performed with 1 ng of purified genomic DNA by using an annealing temperature of 55 to 65° C. and 30 cycles of amplification. The genomic DNA from the various bacterial species above was always isolated from reference strains obtained from ATCC.
[3]All positive results showed a strong amplification signal with genomic DNA from the target species *S. epidermidis*. The instensity of the positive amplification signal with species other than *S. epidermidis* was variable.
NT = NOT TESTED.

TABLE 23

Influence of nucleotide variation(s) on the efficiency of the PCR amplification: Example with SEQ ID NO: 146 from *S. epidermidis*.

			Staphyloccus epidermidis[2] ATCC 14990				*Staphylococcus aureus*[3]
	Sequence	Number of	50° C.	55° C.			50° C.
Primer[1]	(all 25 nucleotides)	mutation	1	1	0.1	0.01	1
SEQ ID NO: 145	ATCAAAAAGTTGGCGAACCTTTTCA	0					
SEQ ID NO: 146	CAAAAGAGCGTGGAGAAAAGTATCA	0	3+[4]	3+	2+	+	–
VBmut1	CAAAAGAGCGTGGAGAAAAGTA$\boxed{C}$CA	1	3+	3+	2+	+	–
VBmut2	CAAAAGAGCGTGGAGAAAA$\boxed{A}$TATCA	1	3+	3+	2+	+	–
VBmut3	CAAAAGAGCGTGGAGA$\boxed{G}$AAGTATCA	1	3+	3+	2+	+	–
VBmut4	CAAAAGAGCGTGG$\boxed{T}$GAAAAGTATCA	1	3+	3+	2+	+	–
VBmut5	CAAAAGAGCG$\boxed{C}$GGAGAAAAGTATCA	1	3+	3+	2+	+	–
VBmut6	CAAAAGA$\boxed{A}$CGTGGAGAAAAGTATCA	1	3+	3+	2+	+	–
VBmut7	CAAA$\boxed{G}$GAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+	–
VBmut8	C$\boxed{T}$AAAGAGCGTGGAGAAAAGTATCA	1	3+	3+	2+	+	–
VBmut9	CAAAAGAGCGTGGAGA$\boxed{G}$AAGTA$\boxed{C}$CA	2	3+	3+	2+	+	–
VBmut10	CAAAAGAGCG$\boxed{C}$GGAGA$\boxed{G}$AAGTATCA	2	3+	3+	2+	+	–
VBmut11	CAAA$\boxed{G}$GAGCG$\boxed{C}$GGAGAAAAGTATCA	2	3+	3+	2+	+	–

TABLE 23-continued

Influence of nucleotide variation(s) on the efficiency of the PCR amplification: Example with SEQ ID NO: 146 from *S. epidermidis*.

			Staphyloccus epidermidis[2] ATCC 14990				*Staphylococcus aureus*[3]
	Sequence	Number of	50° C.	55° C.			50° C.
Primer[1]	(all 25 nucleotides)	mutation	1	1	0.1	0.01	1
VBmut12	CAAA$\boxed{G}$GAGCGTGG$\boxed{T}$GAAAAGTA$\boxed{C}$CA	3	3+	3+	2+	+	−
VBmut13	CAAA$\boxed{G}$GAGCG$\boxed{C}$GGAGA$\boxed{G}$AAGTA$\boxed{C}$CA	4	3+	2+	+	−	−

[1]All PCR tests were performed with SEQ ID NO: 145 without modification combined with SEQ ID NO: 146 or 13 modified versions of SEQ ID NO: 146. Boxed nucleotides indicate changes in SEQ ID NO: 146. All SEQ ID NOs. are from U.S. Pat No. 6,001,564.
[2]The tests with *S. epidermidis* were performed by using an annealing temperature of 55° C. with 1, 0.1 and 0.01 ng of purified genomic DNA or at 50° C. with 1 ng of purified genomic DNA.
[3]The tests with *S. aureus* were performed only at 50° C. with 1 ng of genomic DNA.
[4]The intensity of the positive amplification signal was quantified as follows: 3+ = strong signal, 2+ = intermediate signal and + = weak signal.

TABLE 24

Effect of the primer length on the efficiency of the PCR amplification[1]: Example with the AT-rich SEQ ID NO: 145[2] and SEQ ID NO: 146[2] from *S. epidermidis*.

			Staphylococcus epidermidis[3] ATCC 14990						*Staphylo coccus aureus*[4]		*Staphylo coccus haemo lyticus*		*Staphylo coccus capitis*		*Staphylo coccus warneri*	
		Length	45° C.			55° C.										
Primer	Sequence	(nt)	1	0.1	0.01	1	0.1	0.01	45	55	45	55	45	55	45	55
VBsep301	ATATCATCAAAAAGTTGGCGAACCTTTTCA	30	NT	NT	NT	4+	3+	2+	NT	−	NT	−	NT	−	NT	−
VBsep302	AATTGCAAAAGAGCGTGGAGAAAAGTATCA	30														
SEQ ID NO: 145	ATCAAAAAGTTGGCGAACCTTTTCA	25	4+[5]	3+	2+	4+	3+	2+	−	−	−	−	+	−	−	−
SEQ ID NO: 146	CAAAAGAGCGTGGAGAAAAGTATCA	25														
VBsep201	AAAGTTGGCGAACCTTTTCA	20	NT	NT	NT	4+	3+	2+	NT	−	NT	−	NT	−	NT	−
VBsep202	GAGCGTGGAGAAAAGTATCA	20														
VBsep171	GTTGGCGAACCTTTTCA	17	4+	3+	2+	3+	2+	+	−	−	−	−	−	−	−	−
VBsep172	CGTGGAGAAAAGTATCA	17														
VBsep151	TGGCGAACCTTTTCA	15	3+	2+	+	−	−	−	−	−	−	−	−	−	−	−
VBsep152	TGGAGAAAAGTATCA	15														

[1]All PCR tests were performed using an annealing temperature of 45 or 55° C. and 30 cycles of amplification.
[2]All SEQ ID NOs. in this Table are from U.S. pat. No. 6,001,546.
[3]The tests with S. epidermidis were made with 1, 0.1 and 0.01 ng of purified genomic DNA.
[4]The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.
[5]The intensity of the positive amplification signal was quantified as follows: 4+ = very strong signal, 3+ = strong signal, 2+ = intermediate signal and + = weak signal.
NT = not tested.

TABLE 25

Effect of the primer length on the efficiency of the PCR amplification[1]: Example with the GC-rich SEQ ID NO: 83[2] and SEQ ID NO: 84[2] from *P. aeruginosa*.

			Pseudomonas aeruginosa[3] ATCC 35554				
		Length				*Pseudomonas*	*Burkholderia*
Primer	Sequence	(nt)	1	0.1	0.01	*fluorescens*[4]	*cepacia*
SEQ ID NO 83	CGAGCGGG TGGTGTTCATC	19	2+[5]	+	−	−	−

TABLE 25-continued

Effect of the primer length on the efficiency of the PCR amplification[1]: Example with the GC-rich SEQ ID NO: 83[2] and SEQ ID NO: 84[2] from *P. aeruginosa*.

SEQ ID NO 84	CAAGTCGT CGTCGGAGGGA	19					
Pse554-16a	CGAGCGGG TGGTGTTC	16	2+	+	–	–	–
Pse674-16a	GTCGTCGT CGGAGGGA	16					
Pse554-13b	GCGGGTGG TGTTC	13	2+	+	–	–	–
Pse674-13a	GTCGTCGG AGGGA	13					

Primer	*Shewanella putida*	*Stenotrophomonas maltophilia*	*Neisseria meningitidis*	*Haemophilus parahaemolyticus*
SEQ ID NO 83	–	–	–	–
SEQ ID NO 84				
Pse554-16a	–	–	–	–
Pse674-16a				
Pse554-13b	–	–	–	–
Pse674-13a				

[1]All PCR tests were performed using an annealing temperature of 55° C. and 30 cycles of amplification.
[2]All SEQ ID NOs. in this Table are from U.S. Pat. No. 6,001,546.
[3]The tests with *P. aeruginosa* were made with 1, 0.1 and 0.01 ng of purified genomic DNA.
[4]The tests with all other bacterial species were made only with 1 ng of purified genomic DNA.
[5]The intensity of the positive amplification signal was quantified as follows: 2+ = strong signal and + = moderately strong signal.

TABLE 26

Sequences used for the consensus of the vanA gene

Organism	GenBank access number	Source
E. faecium	M97297	
E. faecium	AX111560	Sequence 2293 of patent WO0123604
E. faecium	AX085668	Sequence 21 of patent WO0112803
E. faecium	AX085648	Sequence 1 of patent WO0112803
E. faecium	AX110408	Sequence 1141 of Patent WO0123604
E. faecium	AX110406	Sequence 1139 of patent WO0123604
E. faecium	AX110320	Sequence 1053 of patent WO0123604
E. faecium	AX110319	Sequence 1052 of patent WO0123604
E. faecium	AX110318	Sequence 1051 of patent WO0123604

TABLE 26-continued

Sequences used for the consensus of the vanA gene

Organism	GenBank access number	Source
E. faecium	AX110316	Sequence 1049 of patent WO0123604
E. faecalis	AX110321	Sequence 1054 of patent WO0123604
E. gallinarum	AX110317	Sequence 1050 of patent WO0123604
E. gallinarum	AX110322	Sequence 1055 of patent WO0123604
E. flavescens	AX110324	Sequence 1057 of patent WO0123604
S. aureus (VRSA)	AE017171.1	Direct deposit to NCBI

TABLE 27

Sequences used for the consensus of vanB gene

Organism	GenBank access number	Source
E. faecium SLH475	AF310954.1	Tn5382 transposon ligase VanB2 (vanB2) gene
E. faecium UI709	AY145441.1	D-alanine:D-lactate ligase (vanB2)
E. faecium VRE-1	AF310953.1	Tn5382 ligase VanB2 (vanB2) gene
E. faecium	U94526.1	vancomycin resistance protein B (vanB) gene,
E. faecium	AJ306727.1	plasmid pVREM3123/00 partial vanHB gene and partial vanB2 gene

TABLE 27-continued

Sequences used for the consensus of vanB gene

Organism	GenBank access number	Source
E. faecium	AJ306726.1	plasmid pVREM2497/00 partial vanHB gene and partial vanB2 gene
E. faecium	Z83305.1	vanB2, vanHB2 and vanXB2 genes
E. faecium	Z83305.1	vanB2, vanHB2 and vanXB2 genes
E. faecium CG4248	AF310957.1	transposon Tn5382 ligase VanB2 (vanB2) gene,
E. faecalis	L15304.1	vancomycin resistance vanB2 gene
E. faecalis	U35369.1	vancomycin resistance genes, response regulator (vanRB), protein histidine kinase (vanSB), D,D-carboxypeptidase (vanYB), putative D-2-hydroxyacid dehydrogenase (vanHB), D-Ala:D-Lac ligase (vanB), and putative D,D-dipeptidase (vanXB) genes
E. faecalis	U00456.1	vanB gene
E. faecalis	L06138.1	VANB gene
E. faecalis	U72704.1	vancomycin resistance protein (vanB) gene
E. faecalis T4059	AF310955.1	transposon Tn5382 ligase VanB2 (vanB2) gene
E. faecalis	L15304.1	vancomycin resistance vanB2 gene
E. faecalis T4059	AF310955.1	transposon Tn5382 ligase VanB2 (vanB2) gene
E. faecalis	AF192329.1	transposon Tn1549, complete sequence
S. bovis biotype II	Z70527.1	partial vanB2 gene
S. lutetiensis strain 5-F9	AY035703.1	Tn5382-like transposon ligase VanB2 (vanB2) gene

*Deposited directly to NCBI

TABLE 28

Number of positive assay results out of five in VanR Assay.

DNA copies number	*E. faecium*	*E. faecalis*
0.5	2	2
2.5	5	3
5	5	4
10	5	5
20	5	5

TABLE 29

First list of exemplary strains tested with VanR assay.

No	Strains	ATCC or Ref. number
1	*Enterococcus casseliflavus*	25788
2	*Enterococcus cecorum*	43198
3	*Enterococcus columbae*	51263
4	*Enterococcus malodoratus*	43197
5	*Enterococcus mundtii*	43186
6	*Enterococcus pseudoavium*	49372
7	*Enterococcus raffinosus*	49427
8	*Enterococcus dispar*	51266
9	*Enterococcus durans*	19432
10	*Enterococcus faecalis*	19433
11	*Enterococcus faecalis*	29212
12	*Enterococcus faecalis*	R830
13	*Enterococcus faecium*	19434
14	*Enterococcus faecium*	N97-330
15	*Enterococcus flavescens*	49996
16	*Enterococcus gallinarum*	49573
17	*Enterococcus saccharolyticus*	43076
18	*Enterococcus sulfureus*	49903
19	*Enterococcus faecium* (vanD)	CCRI-14889
20	*Enterococcus faecium* (vanD)	CCRI-14889
21	*Enterococcus faecalis* (vanE)	CCRI-1908
22	*Enterococcus faecalis* (vanE)	CCRI-1908
23	*Enterococcus faecalis* (vanG)	CCRI-12848
24	*Enterococcus faecalis* (vanG)	CCRI-12848

TABLE 30

Second list of exemplary strains tested with VanR assay

No	Strains	ATCC or Ref. number
1	*Acinetobacter twoffii*	CDCF 3697
2	*Aeromonas hydrophila*	7966
3	*Bacteroides fragilis*	25285
4	*Bacillus cereus*	13472
5	*Bifidobacterium breve*	15700
6	*Candida albicans*	10231
7	*Clostridium difficile*	9689
8	*Clostridium perfringens*	13124
9	*Corynebacterium bovis*	7715
10	*Escherichia coli*	25922
11	*Escherichia coli*	23511
12	*Fusobacterium nucleatum*	10953
13	*Gardnerella vaginalis*	14019
14	*Klebsiella oxytoca*	33496
15	*Klebsiella pneumoniae*	13883
16	*Listeria monocytogenes*	L 374
17	*Morganella morganii* subsp. *morganii*	25830
18	*Peptostreptococcus anaerobius*	27337
19	*Peptostreptococcus asaccharolyticus*	LSPQ 2639
20	*Porphyromonas asaccharolytica*	25260
21	*Prevotella melaninogenica*	25845
22	*Propionibacterium acnes*	6919
23	*Staphylococcus epidermidis*	14990
24	*Stenotrophomonas maltophilia*	13637

TABLE 31

Third list of exemplary strains tested with VanR assay.

No	Strains	ATCC or Ref. number
1	*Abiotrophia defectiva*	49176
2	*Acinetobacter baumannii*	19606

TABLE 31-continued

Third list of exemplary strains tested with VanR assay.

No	Strains	ATCC or Ref. number
3	*Actinomyces pyogenes*	19411
4	*Citrobacter braakii*	43162
5	*Citrobacter koseri*	27028
6	*Corynebacterium genitalium*	LSPQ 3583
7	*Enterobacter cloacae*	13047
8	*Hafnia alvei*	13337
9	*Homo sapiens*	2.16
10	*Lactobacillus acidophilus*	4356
11	*Lactobacillus gasseri*	33323
12	*Mobiluncus curtisii* subsp. *holmesii*	35242
13	*Neisseria gonorrhoeae*	35201
14	*Pantoea agglomerans*	27155
15	*Pseudomonas aeruginosa*	35554
16	*Salmonella enterica* subsp. *Arizonae*	13314
17	*Salmonella enterica* subsp. *Enterica*	7001
18	*Salmonella typhimurium*	14028
19	*Shigella flexneri*	12022
20	*Shigella sonnei*	29930
21	*Staphylococcus aureus*	43300
22	*Streptococcus anginosus*	33397
23	*Streptococcus bovis*	33317
24	*Vibrio cholerae*	25870
25	*Yersinia enterocolitica* subs. *enterolitica*	23715

TABLE 32

Vancomycin resistant strains tested with VanR assay at 10^5 CFU/rx.

No	Strains	Resistance gene	ATCC or Ref. number
1-2	*Enterococcus casseliflavus* (10^4 CFU/rx)	vanC2	25788
3-4	*Enterococcus casseliflavus*	vanC2	25788
5-6	*Enterococcus faecalis*	vanG	CCRI-12849
7-8	*Enterococcus faecium*	vanD	R832

TABLE 32-continued

Vancomycin resistant strains tested with VanR assay at 10^5 CFU/rx.

No	Strains	Resistance gene	ATCC or Ref. number
9-10	*Enterococcus faecium*	vanD2	CCRI-15140
11-12	*Enterococcus faecium*	vanD	CCRI-2062
13-14	*Enterococcus faecium*	vanD	CCRI-8824
15-16	*Enterococcus gallinarum*	vanC	CCRI-9133
17-18	*Enterococcus gallinarum*	vanC	CCRI-9131
19-20	*Enterococcus gallinarum*	vanA and vanC1	CCRI-1561

TABLE 33

Composition of premix (PM)

COMPONENT	CONCENTRATION IN FRESHLY PREPARED MIX (28.8 μL FINAL VOLUME)
FastStart Taq DNA Polymerase	0.060 U/μL
$MgCl_2$	3.010 mM
Tris	100.333 mM
KCl	10.033 mM
$(NH_4)_2SO_4$	5.017 mM
dNTPs	200.667 μM
Primer vanA649 (SEQ ID NO: 1090)	0.120 μM
Primer vanA754 (SEQ ID NO: 1091)	0.101 μM
Primer vanB626 (SEQ ID NO: 2298)	0.702 μM
Primer vanB774 (SEQ ID NO: 1096)	0.702 μM
Probe VanA-B5c-A0 (SEQ ID NO: 2299)	0.120 μM
Probe Van8-850-F0 (SEW ID NO: 2300)	0.351 μM
Probe Sign-B4-B0 (SEQ ID NO: 2301)	0.201 μM
pERVd Internal control (SEQ ID NO: 2302)	3.582 copies/μL (usual)
S. epidermidis DNA	895.836 copies/μL
BSA	0.301 mg/mL
Trehalose	4.014%

*These components were added following Table 21

TABLE 34

Composition of final mix (MM)

	n	Sign-B4 (SEQ ID NO: 2301)	VanA-B5c (SEQ ID NO: 2299)	VanB-B50 (SEQ ID NO: 2300)	IC (SEQ ID NO: 2302)	Specimen
MM1-a	10	x				DNA[3]
MM1-b	5	x			x[1]	TE 1X
MM2-a	10		X			DNA[3]
MM2-b	5		X		x[1]	TE 1X
MM3-a	10			x		DNA[3]
MM3-b	5			X	x[1]	TE 1X
MM4-std	15	x	X	X	x[2]	DNA[4]/TE 1X

[1]At 100 times the usual concentration

[2]At usual concentration

[3]At 25/50 VanA/VanB DNA copies

[4]At 1 ng/rx VanA/VanB DNA copies

TABLE 35

Positive results obtained in indicated beacon channel				
	Sign-B4 (SEQ ID NO: 2301) TET	VanA-B5 (SEQ ID NO: 2299)c FAM	VanB-B50 (SEQ ID NO: 2300) Texas Red	Target
MM1-a	0/5	—	—	Efm
	0/5	—	—	Efs
MM1-b	5/5	—	—	IC
MM2-a	—	5/5	—	Efm
	—	0/5	—	Efs
MM2-b	—	0/5	—	IC
MM3-a	—	—	0/5	Efm
	—	—	5/5	Efs
MM3-b	—	—	0/5	IC
MM4-std	5/5	5/5	0/5	Efm
	5/5	0/5	5/5	Efs
	5/5	0/5	0/5	IC

TABLE 36

List of *enterococcal* strains from different locations around the world tested with VanR assay (dilutions 10^{-3}).

No	Strains	Reference number	Location	vanA CT	vanB CT
1	*Enterococcus faecalis*	CCRI-1471	Texas, USA	29.91	—
2	*Enterococcus faecalis*	CCRI-1528	Quebec, CAN	27.05	—
3	*Enterococcus faecalis*	CCRI-9741	USA	—	31.05
4	*Enterococcus gallinarum*	CCRI-1568	Quebec, CAN	28.60	—
5	*Enterococcus faecium*	CCRI-9911	Taiwan, China	—	31.96
6	*Enterococcus faecalis*	725	Israel	—	31.55
7	*Enterococcus faecalis*	1435	Brazil	28.28	—
8	*Enterococcus faecium*	6169	Italy	31.63	—
9	*Enterococcus gallinarum*	CCRI-9737	Norway	—	29.50
10	*Enterococcus faecalis*	CCRI-9738	Germany	—	31.35
11	*Enterococcus faecium*	CCRI-9740	Germany	—	29.41
12	*Enterococcus faecalis*	13024	Germany	27.49	—
13	*Enterococcus faecalis*	CCRI-9739	Germany	—	31.00
14	*Enterococcus faecium*	CCRI-9733	USA	—	31.93
15	*Enterococcus faecium*	1585	Argentina	29.00	—
16	*Enterococcus faecium*	715	Sweden	—	30.87
17	*Enterococcus faecalis*	CCRI-9954	Netherlands	—	31.83
18	*Enterococcus faecium*	CCRI-1482	Toronto, CAN	29.40	—

*All strains gave vanA or vanB positive PCR results.

TABLE 37

List of resistant non enterococcal strains (vanB genotype) tested with VanR assay.

No	Strains	Reference number	Texas Red CT (cycles)
1	*Clostridium innocuum*	CCRI-9927	29.6
2	*Clostridium* sp.	IDI-1987	31.9
3	*Clostridium* sp.	CCRI-9929	32.5
4	*Clostridium symbosium*	CCRI-9928	31.6
5	*Eggerthella lenta*	CCRI-9926	32.4

*All strains gave vanB positive PCR results.

TABLE 38

VanR Assay Formulation

Raw Material	Concentration of components at final volume (28 μL) Lyophilized		Concentration of components at final volume (28.8 μL) Fresh	
FastStart Taq DNA Polymerase	0..060	U/μL[1]	0.060	U/μL[1]
$MgCl_2$	3.000	mM[2]	3.010	mM[2]
Tris	100.000	mM (pH 8.3)[2]	100.333	mM (pH 8.3)[2]
KCl	10.000	mM[2]	10.033	mM[2]
$(NH_4)_2S0_4$	5.000	mM[2]	5.017	mM[2]
dNTP	200.000	μM	200.667	μM
Primer VanA 649 (SEQ ID NO: 1090)	0.120	μM	0.120	μM
Primer Van A 754 (SEQ ID NO: 1091)	0.100	μM	0.101	μM
Primer Van B 626 (SEQ ID NO: 2298)	0.700	μM	0.702	μM
Primer van B 774 (SEQ ID NO: 1096)	0.700	μM	0.702	μM
Probe VanA-B5c-A0 (SEQ ID NO: 2299)	0.120	μM	0.120	μM
Probe VanB-B50-F0 (SEQ ID NO: 2300)	0.350	μM	0.351	μM
Probe Sign-B4-B0 (SEQ ID NO: 2301)	0.200	μM	0.201	μM
Internal control pERVd (SEQ ID NO: 2302)	3.570	copies/μL	3.582	copies/μL

TABLE 38-continued

VanR Assay Formulation		
Raw Material	Concentration of components at final volume (28 μL) Lyophilized	Concentration of components at final volume (28.8 μL) Fresh
S. epidermidis DNA	892.857 copies/μL^3	895.836 copies/μL^3
BSA	0.300 mg/mL	0.301 mg/mL
Trehalose	4.000%	4.014%

Annex I: Specific and ubiquitous primers for nucleic acid amplification (tuf sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: *Acinetobacter baumannii*			
1692	5'-GGT GAG AAC TGT GGT ATC TTA CTT	1	478-501
1693[a]	5'-CAT TTC AAC GCC TTC TTT CAA CTG	1	691-714
Bacterial species: *Chlamydia pneumoniae*			
630	5'-CGG AGC TAT CCT AGT CGT TTC A	20	2-23
629[a]	5'-AAG TTC CAT CTC AAC AAG GTC AAT A	20	146-170
2085	5'-CAA ACT AAA GAA CAT ATC TTG CTA	20	45-68
2086[a]	5'-ATA TAA TTT GCA TCA CCT TCA AG	20	237-259
2087	5'-TCA GCT CGT GGG ATT AGG AGA G	20	431-452
2088[a]	5'-AGG CTT CAC GCT GTT AGG CTG A	20	584-605
Bacterial species: *Chlamydia trachomatis*			
554	5'-GTT CCT TAC ATC GTT GTT TTT CTC	22	82-105
555[a]	5'-TCT CGA ACT TTC TCT ATG TAT GCA	22	249-272
Parasitical species: *Cryptosporidium parvum*			
798	5'-TGG TTG TCC CAG CCG ATC GTT T	865	158-179
804[a]	5'-CCT GGG ACG GCC TCT GGC AT	865	664-683
799	5'-ACC TGT GAA TAC AAG CAA TCT	865	280-300
805[a]	5'-CTC TTG TCC ATC TTA GCA GT	865	895-914
800	5'-GAT GAA ATC TTC AAC GAA GTT GAT	865	307-330
806[a]	5'-AGC ATC ACC AGA CTT GAT AAG	865	946-966
801	5'-ACA ACA CCG AGA AGA TCC CA	865	353-372
803[a]	5'-ACT TCA GTG GTA ACA CCA GC	865	616-635
802	5'-TTG CCA TTT CTG GTT TCG TT	865	377-396
807[a]	5'-AAA GTG GCT TCA AAG GTT GC	865	981-1000
Bacterial species: *Enterococcus faecium*			
1696	5'-ATG TTC CTG TAG TTG CTG GA	64	189-208
1697[a]	5'-TTT CTT CAG CAA TAC CAA CAA C	64	422-443

-continued

Bacterial species: *Klebsiella pneumoniae*			
1329	5'-TGT AGA GCG CGG TAT CAT CAA AGT A	103	352-377
1330[a]	5'-AGA TTC GAA CTT GGT GTG CGG G	103	559-571

[a]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Bacterial species: *Mycoplasma pneumoniae*			
2093	5'-TGT TGG CAA TCG AAG ACA CC	2097[a]	635-654
2094[b]	5'-TTC AAT TTC TTG ACC TAC TTT CAA	2097[a]	709-732
Bacterial species: *Neisseria gonorrhoeae*			
551	5'-GAA GAA AAA ATC TTC GAA CTG GCT A	126	256-280
552[b]	5'-TAC ACG GCC GGT GAC TAC G	126	378-396
2173	5'-AAG AAA AAA TCT TCG AAC TGG CTA	126	257-280
2174[b]	5'-TCT ACA CGG CCG GTG	126	384-398
2175	5'-CCG CCA TAC CCC GTT T	126	654-669
2176[b]	5'-CGG CAT TAC CAT TTC CAC ACC TTT	126	736-759
Bacterial species: *Pseudomonas aeruginosa*			
1694	5'-AAG GCA AGG ATG ACA ACG GC	153	231-250
1695[b]	5'-ACG ATT TCC ACT TCT TCC TGG	153	418-438
Bacterial species: *Streptococcus agalactiae*			
549	5'-GAA CGT GAT ACT GAC AAA CCT TTA	207-210[c]	308-331[d]
550[b]	5'-GAA GAA GAA CAC CAA CGT TG	207-210[c]	520-539[d]
Bacterial species: *Streptococcus pyogenes*			
999	5'-TTG ACC TTG TTG ATG ACG AAG AG	1002	143-165
1000[b]	5'-TTA GTG TGT GGG TTG ATT GAA CT	1002	622-644
1001	5'-AAG AGT TGC TTG AAT TAG TTG AG	1002	161-183
1000[b]	5'-TTA GTG TGT GGG TTG ATT GAA CT	1002	622-644
Parasitical species: *Trypanosoma brucei*			
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
821[b]	5'-GGC GCA AAC GTC ACC ACA TCA	864	789-809
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
822[b]	5'-CGG CGG ATG TCC TTA ACA GAA	864	909-929

[a]Sequence from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the *S. agalactiae* tuf sequence fragment (SEQ ID NO. 209).

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SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Parasitical species: *Trypanosoma cruzi*		
794	5'-GAC GAC AAG TCG GTG AAC TT	840-842[a]	281-300[c]
795[b]	5'-ACT TGC ACG CGA TGT GGC AG	840-842[a]	874-893[c]
	Bacterial genus: *Clostridium* sp.		
796	5'-GGT CCA ATG CCW CAA ACW AGA	32,719-724, 736[a]	32-52[d]
797[b]	5'-CAT TAA GAA TGG YTT ATC TGT SKC TCT	32, 719-724, 736[a]	320-346[d]
808	5'-GCI TTA IWR GCA TTA GAA RAY CCA	32, 719-724, 736[a]	224-247[d]
809[b]	5'-TCT TCC TGT WGC AAC TGT TCC TCT	32, 719-724, 736[a]	337-360[d]
810	5'-AGA GMW ACA GAT AAR SCA TTC TTA	32, 719-724, 736[a]	320-343[d]
811[b]	5'-TRA ART AGA ATT GTG GTC TRT ATC C	32, 719-724, 736[a]	686-710[d]
	Bacterial genus: *Corynebacterium* sp.		
545	5'-TAC ATC CTB GTY GCI CTI AAC AAG TG	34-44, 662[a]	89-114[e]
546[b]	5'-CCR CGI CCG GTR ATG GTG AAG AT	34-44, 662[a]	350-372[e]
	Bacterial genus: *Enterococcus* sp.		
656	5'-AAT TAA TGG CTG CAG TTG AYG A	58-72[a]	273-294[f]
657[b]	5'-TTG TCC ACG TTC GAT RTC TTC A	58-72[a]	556-577[f]
656	5'-AAT TAA TGG CTG CAG TTG AYG A	58-72[a]	273-294[f]
271[b]	5'-TTG TCC ACG TTG GAT RTC TTC A	58-72[a]	556-577[f]
1137	5'-AAT TAA TGG CTG CWG TTG AYG AA	58-72[a]	273-295[f]
1136[b]	5'-ACT TGT CCA CGT TSG ATR TCT	58-72[a]	559-579[f]

[a]These sequences were aligned to derive the corresponding primer.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c] The nucleotide positions refer to the *T. cruzi* tuf sequence fragment (SEQ ID NO. 842).
[d] The nucleotide positions refer to the *C. perfringens* tuf sequence fragment (SEQ ID NO. 32).
[e] The nucleotide positions refer to the *C. diphtheriae* tuf sequence fragment (SEQ ID NO. 662).
[f] The nucleotide positions refer to the *E. durans* tuf sequence fragment (SEQ ID NO. 61).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Bacterial genus: *Legionella* sp.		
2081	5'-GRA TYR TYA AAG TTG GTG AGG AAG	111-112[a]	411-434[b]
2082[c]	5'-CMA CTT CAT CYC GCT TCG TAC C	111-112[a]	548-569[b]

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	Bacterial genus: *Staphylococcus* sp.		
553	5'-GGC CGT GTT GAA CGT GGT CAA ATC A	176-203[a]	313-337[d]
575[c]	5'-TIA CCA TTT CAG TAC CTT CTG GTA A	176-203[a]	653-677[d]
553	5'-GGC CGT GTT GAA CGT GGT CAA ATC A	176-203[a]	313-337[d]
707[c]	5'-TWA CCA TTT CAG TAC CTT CTG GTA A	176-203[a]	653-677[d]
	Bacterial genus: *Streptococcus* sp.		
547	5'-GTA CAG TTG CTT CAG GAC GTA TC	206-231[a]	372-394[e]
548[c]	5'-ACG TTC GAT TTC ATC ACG TTG	206-231[a]	548-568[e]
	Fungal genus: *Candida* sp.		
576	5'-AAC TTC RTC AAG AAG GTY GGT TAC AA	407-426, 428-432[a]	332-357[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]
631	5'-CAG ACC AAC YGA IAA RCC ATT RAG AT	407-426, 428-432[a]	523-548[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]
633	5'-CAG ACC AAC YGA IAA RCC ITT RAG AT	407-426, 428-432[a]	523-548[f]
632[c]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[a]	791-813[f]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *L. pneumophila* tuf sequence fragment (SEQ ID NO. 112).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *S. aureus* tuf sequence fragment (SEQ ID NO. 179).
[e]The nucleotide positions refer to the *S. agalactiae* tuf sequence fragment (SEQ ID NO. 209).
[f]The nucleotide positions refer to the *C. albicans* tuf(EF-1) sequence fragment (SEQ ID NO. 408).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Fungal genus: *Cryptococcus* sp.		
1971	5'-CYG ACT GYG CCA TCC TYA TCA	434, 623, 1281, 1985, 1986[a]	150-170[b]
1973[c]	5'-RAC ACC RGI YTT GGW ITC CTT	434, 623, 1281, 1985, 1986[a]	464-484[b]
1972	5'-MGI CAG CTC ATY ITT GCW KSC	434, 623, 1281, 1985, 1986[a]	260-280[b]
1973[c]	5'-RAC ACC RGI YTT GGW ITC CTT	434, 623, 1281, 1985, 1986[a]	464-484[b]
	Parasitical genus: *Entamoeba* sp.		
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57

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705[c]	5'-GTA CAG TTC CAA TAC CTG AA	512	534-553
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
706[c]	5'-TGA AAT CTT CAC ATC CAA CA	512	768-787
793	5'-TTA TTG TTG CTG CTG GTA CT	512	149-168
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461
	Parasitical genus: *Giardia* sp.		
816	5'-GCT ACG ACG AGA TCA AGG GC	513	305-324
819[c]	5'-TCG AGC TTC TGG AGG AAG AG	513	895-914
817	5'-TGG AAG AAG GCC GAG GAG TT	513	355-374
818[c]	5'-AGC CGG GCT GGA TCT TCT TC	513	825-844
	Parasitical genus: *Leishmania* sp.		
701	5'-GTG TTC ACG ATC ATC GAT GCG	514-526[a]	94-114[d]
702[c]	5'-CTC TCG ATA TCC GCG AAG CG	514-526[a]	913-932[d]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *C. neoformans* tuf (EF-1) sequence fragment (SEQ ID NO. 623).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *L. tropica* tuf(EF-1) sequence fragment (SEQ ID NO. 526).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Parasitical genus: *Trypanosoma* sp.		
823	5'-GAG CGG TAT GAY GAG ATT GT	529, 840-842, 864[a]	493-512[b]
824[c]	5'-GGC TTC TGC GGC ACC ATG CG	529, 840-842, 864[a]	1171-1190[b]
	Bacterial family: Enterobacteriaceae		
933	5'-CAT CAT CGT ITT CMT GAA CAA RTG	78, 103, 146, 168, 238, 698[a]	390-413[d]
934[c]	5'-TCA CGY TTR RTA CCA CGC AGI AGA	78, 103, 146, 168, 238, 698[a]	831-854[d]
	Bacterial family: Mycobacteriaceae		
539	5'-CCI TAC ATC CTB GTY GCI CTI AAC AAG	122	85-111
540[c]	5'-GGD GCI TCY TCR TCG WAI TCC TG	122	181-203
	Bacterial group: *Escherichia coli* and *Shigella*		
1661	5'-TGG GAA GCG AAA ATC CTG	1668[e]	283-300
1665[c]	5'-CAG TAC AGG TAG ACT TCT G	1668[e]	484-502
	Bacterial group: Pseudomonads group		
541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[f]
542[c]	5'-CGG AAR TAG AAC TGS GGA CGG TAG	153-155[a]	679-702[f]

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541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[f]
544[c]	5'-AYG TTG TCG CCM GGC ATT MCC AT	153-155[a]	749-771[f]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *T. brucei* tuf (EF-1) sequence fragment (SEQ ID NO. 864).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *E. coli* tuf sequence fragment (SEQ ID NO. 698).
[e]Sequence from databases.
[f]The nucleotide positions refer to the *P. aeruginosa* tuf sequence fragment (SEQ ID NO. 153).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Parasitical group: Trypanosomatidae family		
923	5'-GAC GCI GCC ATC CTG ATG ATC	511, 514-526, 529, 840-842, 864[a]	166-188[b]
924[c]	5'-ACC TCA GTC GTC ACG TTG GCG	511, 514-526, 529, 840-842, 864[a]	648-668[b]
925	5'-AAG CAG ATG GTT GTG TGC TG	511, 514-526, 529, 840-842, 864[a]	274-293[b]
926[c]	5'-CAG CTG CTC GTG GTG CAT CTC GAT	511, 514-526, 529, 840-842, 864[a]	676-699[b]
927	5'-ACG CGG AGA AGG TGC GCT T	511, 514-526, 529, 840-842, 864[a]	389-407[b]
928[c]	5'-GGT CGT TCT TCG AGT CAC CGC A	511, 514-526, 529, 840-842, 864[a]	778-799[b]
	Universal primers (bacteria)		
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	7, 54, 78, 100, 103, 159, 209, 224, 227[b]	470-492[d]
637[c]	5'-ACG TCA GTI GTA CGG AAR TAG AA	7, 54, 78, 100, 103, 159, 209, 224, 227[b]	692-714[d]
638	5'-CCA ATG CCA CAA ACI CGT GAR CAC AT	7, 54, 78, 100, 103, 159, 209, 224, 227[b]	35-60[e]
639[c]	5'-TTT ACG GAA CAT TTC WAC ACC WGT IAC A	7, 54, 78, 100, 103, 159, 209, 224, 227[b]	469-496[e]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *L. tropica* tuf (EF-1) sequence fragment (SEQ ID NO. 526).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *E. coli* tuf sequence fragment (SEQ ID NO. 78).
[e]The nucleotide positions refer to the *B. cereus* tuf sequence fragment (SEQ ID NO. 7).

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SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Universal primer (bacteria) (continued)		
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1, 3, 4, 7, 12, 13, 16, 49, 54, 72, 78, 85, 88, 91, 94, 98, 103, 108, 112, 115, 116, 120, 121, 126, 128, 134, 136, 146, 154, 159, 179, 186, 205, 209, 212, 224, 238[a]	470-492[b]
644[c]	5'-ACG TCI GTI GTI CKG AAR TAG AA	same as SEQ ID NO. 643	692-714[b]
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1, 3, 4, 7, 12, 13, 16, 49, 54, 72, 78, 85, 88, 91, 94, 98, 103, 108, 112, 115, 116, 120, 121, 126, 128, 134, 136, 146, 154, 159, 179, 186, 205, 209, 212, 224, 238[a]	470-492[b]
645[c]	5'-ACG TCI GTI GTI CKG AAR TAR AA	same as SEQ ID NO. 643	692-714[b]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2, 13, 82 122, 145[a]	317-339[d]
647[c]	5'-ACG TCC GTS GTR CGG AAG TAG AAC TG	2, 13, 82 122, 145[a]	686-711[d]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2, 13, 82 122, 145[a]	317-339[d]
648[c]	5'-ACG TCS GTS GTR CGG AAG TAG AAC TG	2, 13, 82 122, 145[a]	686-711[d]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *E. coli* tuf sequence fragment (SEQ ID NO. 78).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *A. meyeri* tuf sequence fragment (SEQ ID NO. 2)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Universal primer (bacteria) (continued)		
649	5'-GTC CTA TGC CTC ARA CWC GIG AGC AC	8, 86, 141, 143[a]	33-58[b]
650[c]	5'-TTA CGG AAC ATY TCA ACA CCI GT	8, 86, 141, 143[a]	473-495[b]
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	8, 86, 141, 143[a]	473-495[b]
651[c]	5'-TGA CGA CCA CCI TCY TCY TTY TTC A	8, 86, 141, 143[a]	639-663[b]

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Universal primers (fungi)			
1974	5'-ACA AGG GIT GGR MSA AGG AGA C	404, 405, 433, 445, 898, 1268, 1276, 1986[a]	443-464[d]
1975[c]	5'-TGR CCR GGG TGG TTR AGG ACG	404, 405, 433, 445, 898, 1268, 1276, 1986[a]	846-866[d]
1976	5'-GAT GGA YTC YGT YAA ITG GGA	407-412, 414-426, 428-431, 439, 443, 447, 448, 622, 624, 665, 1685, 1987-1990[a]	286-306[e]
1978[c]	5'-CAT CIT GYA ATG GYA ATC TYA AT	same as SEQ ID NO. 1976	553-575[e]
1977	5'-GAT GGA YTC YGT YAA RTG GGA	same as SEQ ID NO. 1976	286-306[e]
1979[c]	5'-CAT CYT GYA ATG GYA ASC TYA AT	same as SEQ ID NO. 1976	553-575[e]
1981	5'-TGG ACA CCI SCA AGI GGK CYG	401-405, 433, 435, 436, 438, 444, 445, 449, 453, 455, 457, 779, 781-783, 785, 786, 788-790, 897-903, 1267-1272, 1274-1280, 1282-1287, 1991-1998[a]	281-301[d]
1980[c]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[d]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *B. distasonis* tuf sequence fragment (SEQ ID NO. 8).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *A. fumigatus* tuf (EF-1) sequence fragment (SEQ ID NO. 404).
[e]The nucleotide positions refer to the *C. albicans* tuf (EF-1) sequence fragment (SEQ ID NO. 407).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Universal primers (fungi) (continued)			
1982	5'-TGG ACA CYI SCA AGI GGK CYG	same as SEQ ID NO. 1981	281-301[a]
1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
1983	5'-CYG AYT GCG CYA TIC TCA TCA	same as SEQ ID NO. 1981	143-163[a]
1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
1984	5'-CYG AYT GYG CYA TYC TSA TCA	same as SEQ ID NO. 1981	143-163[a]

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1980[b]	5'-TCR ATG GCI TCI AIR AGR GTY T	same as SEQ ID NO. 1981	488-509[a]
	Sequencing primers		
556	5'-CGG CGC NAT CYT SGT TGT TGC	668[c]	306-326
557[b]	5'-CCM AGG CAT RAC CAT CTC GGT G	668[c]	1047-1068
694	5'-CGG CGC IAT CYT SGT TGT TGC	668[c]	306-326
557[b]	5'-CCM AGG CAT RAC CAT CTC GGT G	668[c]	1047-1068
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[c]	604-632
652[b]	5'-CCW AYA GTI YKI CCI CCY TCY CTI ATA	619[c]	1482-1508
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[c]	604-632
561[b]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[c]	1483-1505
543	5'-ATC TTA GTA GTT TCT GCT GCT GA	607	8-30
660[b]	5'-GTA GAA TTG AGG ACG GTA GTT AG	607	678-700
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
659[b]	5'-GCT TTT TGI GTT TCW GGT TTR AT	621	443-465
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
661[b]	5'-GTA GAA YTG TGG WCG ATA RTT RT	621	678-700
558	5'-TCI TTY AAR TAY GCI TGG GT	665[c]	157-176
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[c]	1279-1301
813	5'-AAT CYG TYG AAA TGC AYC ACG A	665[c]	687-708
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[c]	1279-1301

[a]The nucleotide positions refer to the *A. fumigatus* tuf (EF-1) sequence fragment (SEQ ID NO. 404).
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]Sequences from databases.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Sequencing primers (continued)		
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
815[b]	5'-TGG TGC ATY TCK ACR GAC TT	665[a]	686-705
560	5'-GAY TTC ATY AAR AAY ATG ATY AC	665[a]	289-311
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
653	5'-GAY TTC ATI AAR AAY ATG AT	665[a]	289-308
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
655[b]	5'-CCR ATA CCI CMR ATY TTG TA	665[a]	754-773
654	5'-TAC AAR ATY KGI GGT ATY GG	665[a]	754-773
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
696	5'-ATI GGI CAY RTI GAY CAY GGI AAR AC	698[a]	52-77

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697[b]	5'-CCI ACI GTI CKI CCR CCY TCR CG	698[a]	1132-1154
911	5'-GAC GGM KKC ATG CCG CAR AC	853	22-41
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	853	700-720
912	5'-GAC GGC GKC ATG CCG CAR AC	846	20-39
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	846	692-712
913	5'-GAC GGY SYC ATG CCK CAG AC	843	251-270
915[b]	5'-AAA CGC CTG AGG RCG GTA GTT	843	905-925
916	5'-GCC GAG CTG GCC GGC TTC AG	846	422-441
561[b]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[a]	1483-1505
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[a]	604-632
917[b]	5'-TCG TGC TAC CCG TYG CCG CCA T	846	593-614

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Sequencing primers (continued)		
1221	5'-GAY ACI CCI GGI CAY GTI GAY TT	1230[a]	292-314
1226[b]	5'-GTI RMR TAI CCR AAC ATY TC	1230[a]	2014-2033
1222	5'-ATY GAY ACI CCI GGI CAY GTI GAY TT	1230[a]	289-314
1223[b]	5'-AYI TCI ARR TGI ARY TCR CCC ATI CC	1230[a]	1408-1433
1224	5'-CCI GYI HTI YTI GAR CCI ATI ATG	1230[a]	1858-1881
1225[b]	5'-TAI CCR AAC ATY TCI SMI ARI GGI AC	1230[a]	2002-2027
1227	5'-GTI CCI YTI KCI GAR ATG TTY GGI TA	1230[a]	2002-2027
1229[b]	5'-TCC ATY TGI GCI GCI CCI GTI ATC AT	698[a]	4-29
1228	5'-GTI CCI YTI KCI GAR ATG TTY GGI TAY GC	1230[a]	2002-2030
1229[b]	5'-TCC ATY TGI GCI GCI CCI GTI ATC AT	698[a]	4-29
1999	5'-CAT GTC AAY ATT GGT ACT ATT GGT CAT GT	498-500, 502, 505, 506, 508, 619, 2004, 2005[c]	25-53[d]
2000[b]	5'-CCA CCY TCI CTC AMG TTG AAR CGT T	same as SEQ ID NO. 1999	1133-1157[d]
2001	5'-ACY ACI TTR ACI GCY GCY ATY AC	same as SEQ ID NO. 1999	67-89[d]
2003[b]	5'-CAT YTC RAI RTT GTC ACC TGG	same as SEQ ID NO. 1999	1072-1092[d]
2002	5'-CCI GAR GAR AGA GCI MGW GGT	same as SEQ ID NO. 1999	151-171[d]

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2003[b]	5'-CAT YTC RAI RTT GTC ACC TGG	same as SEQ ID NO. 1999	1072-1092[d]

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the *C. albicans* tuf sequence fragment (SEQ ID NO. 2004).

Annex II: Specific and ubiquitous primers for nucleic acid amplification (atpD sequences).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Bacterial species: *Acinetobacter baumannii*		
1690	5'-CAG GTC CTG TTG CGA CTG AAG AA	243	186-208
1691[b]	5'-CAC AGA TAA ACC TGA GTG TGC TTT C	243	394-418
	Bacterial species: *Bacteroides fragilis*		
2134	5'-CGC GTG AAG CTT CTG TG	929	184-200
2135[b]	5'-TCT CGC CGT TAT TCA GTT TC	929	395-414
	Bacterial species: *Bordetella pertussis*		
2180	5'-TTC GCC GGC GTG GGC	1672[c]	544-558
2181[b]	5'-AGC GCC ACG CGC AGG	1672[c]	666-680
	Bacterial species: *Enterococcus faecium*		
1698	5'-GGA ATC AAC AGA TGG TTT ACA AA	292	131-153
1699[b]	5'-GCA TCT TCT GGG AAA GGT GT	292	258-277
1700	5'-AAG ATG CGG AAA GAA GCG AA	292	271-290
1701[b]	5'-ATT ATG GAT CAG TTC TTG GAT CA	292	439-461
	Bacterial species: *Klebsiella pneumoniae*		
1331	5'-GCC CTT GAG GTA CAG AAT GGT AAT GAA GTT	317	88-118
1332[b]	5'-GAC CGC GGC GCA GAC CAT CA	317	183-203

[a]These sequences were aligned to derive the corresponding primer.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]Sequence from databases.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Bacterial species: *Streptococcus agalactiae*		
627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]

-continued

627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
	Bacterial group: *Campylobacter jejuni* and *C. coli*		
2131	5'-AAG CMA TTG TTG TAA ATT TTG AAA G	1576, 1600, 1849, 1863, 2139[d,a]	7-31[e]
2132[c]	5'-TCA TAT CCA TAG CAA TAG TTC TA	1576, 1600, 1849, 1863, 2139[d,a]	92-114[e]
	Bacterial genus: *Bordetella* sp.		
825	5'-ATG AGC ARC GSA ACC ATC GTT CAG TG	1672[d]	1-26
826[c]	5'-TCG ATC GTG CCG ACC ATG TAG AAC GC	1672[d]	1342-1367
	Fungal genus: *Candida* sp.		
634	5'-AAC ACY GTC AGR RCI ATT GCY ATG GA	460-472, 474-478[a]	101-126[f]
635[c]	5'-AAA CCR GTI ARR GCR ACT CTI GCT CT	460-472, 474-478[a]	617-642[f]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *S. agalactiae* atpD sequence fragment (SEQ ID NO. 380).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]Sequence from databases.
[e]The nucleotide positions refer to the *C. jejuni* atpD sequence fragment (SEQ ID NO. 1576).
[f]The nucleotide positions refer to the *C. albicans* atpD sequence fragment (SEQ ID NO. 460).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Universal primers		
562	5'-CAR ATG RAY GAR CCI CCI GGI GYI MGI ATG	243, 244, 262, 264, 280, 284, 291, 297, 309, 311, 315, 317, 324, 329, 332, 334-336, 339, 342, 343, 351, 356, 357, 364-366, 370, 375, 379, 393[a]	528-557[b]
563[c]	5'-GGY TGR TAI CCI ACI GCI GAI GGC AT	243, 244, 262, 264, 280, 284, 291, 297, 309, 311, 315, 317, 324, 329, 332, 334-336, 339, 342, 343, 351, 356, 357, 364-366, 370, 375, 379, 393[a]	687-712[b]
564	5'-TAY GGI CAR ATG AAY GAR CCI CCI GGI AA	243, 244, 262, 264, 280, 284, 291, 297, 309, 311, 315, 317, 324, 329, 332, 334-336, 339,	522-550[b]

-continued

		342, 343, 351, 356, 357, 364-366, 370, 375, 379, 393[a]	
565[c]	5'-GGY TGR TAI CCI ACI GCI GAT GGD AT	243, 244, 262, 264, 280, 284, 291, 297, 309, 311, 315, 317, 324, 329, 332, 334-336, 339, 342, 343, 351, 356, 357, 364-366, 370, 375, 379, 393[a]	687-712[b]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *K. pneumoniae* atpD sequence fragment (SEQ ID NO. 317).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Universal primers (continued)		
640	5'-TCC ATG GTI TWY GGI CAR ATG AA	248, 284, 315, 317, 343, 357, 366, 370, 379, 393[a]	513-535[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248, 284, 315, 317, 343, 357, 366, 370, 379, 393[a]	684-709[b]
642	5'-GGC GTI GGI GAR CGI ACI CGT GA	248, 284, 315, 317, 343, 357, 366, 370, 379, 393[a]	438-460[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248, 284, 315, 317, 343, 357, 366, 370, 379, 393[a]	684-709[b]
	Sequencing primers		
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
814	5'-GCI GGC ACG TAC ACI GCC TG	666[d]	901-920
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[d]	25-47
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
570	5'-RTI RYI GGI CCI GTI RTI GAY GT	672[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
572	5'-RTI RTI GGI SCI GTI RTI GA	669[d]	25-44
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
569	5'-RTI RTI GGI SCI GTI RTI GAT AT	671[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908

-continued

571	5'-RTI RTI GGI CCI GTI RTI GAT GT	670[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908

[a] These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *K. pneumoniae* atpD sequence fragment (SEQ ID NO. 317).
[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d] Sequences from databases.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Sequencing primers (continued)			
700	5'-TIR TIG AYG TCG ART TCC CTC ARG	669[a]	38-61
567[b]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[a]	883-908
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[a]	25-47
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
708[b]	5'-TCR TCC ATI CCI ARI ATI GCI ATI AT	666[a]	1258-1283
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
682[b]	5'-GTI ACI GGY TCY TCR AAR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
683[b]	5'-GTI ACI GGI TCI SWI AWR TCI CCI CC	685	1180-1205
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
699	5'-GTI ACI GGY TCY TYR ARR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
812[b]	5'-GTI ACI GGI TCY TYR ARR TTI CCI CC	685	1180-1205
1213	5'-AAR GGI GGI ACI GCI GCI ATH CCI GG	714[a]	697-722
1212[b]	5'-CCI CCI RGI GGI GAI ACI GCW CC	714[a]	1189-1211
1203	5'-GGI GAR MGI GGI AAY GAR ATG	709[a]	724-744
1207[b]	5'-CCI TCI TCW CCI GGC ATY TC	709[a]	985-1004
1204	5'-GCI AAY AAC ITC IWM YAT GCC	709[a]	822-842
1206[b]	5'-CKI SRI GTI GAR TCI GCC A	709[a]	926-944
1205	5'-AAY ACI TCI AWY ATG CCI GT	709[a]	826-845
1207[b]	5'-CCI TCI TCW CCI GGC ATY TC	709[a]	985-1004
2282	5'-AGR RGC IMA RAT GTA TGA	714[a]	84-101
2284[b]	5'-TCT GWG TRA CIG GYT CKG AGA	714[a]	1217-1237

-continued

2283	5'-ATI TAT GAY GGK ITT CAG AGG C	714[a]	271-292
2285[b]	5'-CMC CIC CWG GTG GWG AWA C	714[a]	1195-1213

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex III: Internal hybridization probes for specific detection of tuf sequences.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Bacterial species: *Abiotrophia adiacens*		
2170	5'-ACG TGA CGT TGA CAA ACC A	1715	313-331
	Bacterial species: *Chlamydia pneumoniae*		
2089	5'-ATG CTG AAC TTA TTG ACC TT	20	136-155
2090	5'-CGT TAC TGG AGT CGA AAT G	20	467-485
	Bacterial species: *Enterococcus faecalis*		
580	5'-GCT AAA CCA GCT ACA ATC ACT CCA C	62-63, 607[a]	584-608[b]
603	5'-GGT ATT AAA GAC GAA ACA TC	62-63, 607[a]	440-459[b]
1174	5'-GAA CGT GGT GAA GTT CGC	62-63, 607[a]	398-415[b]
	Bacterial species: *Enterococcus faecium*		
602	5'-AAG TTG AAG TTG TTG GTA TT	64, 608[a]	426-445[c]
	Bacterial species: *Enterococcus gallinarum*		
604	5'-GGT GAT GAA GTA GAA ATC GT	66, 609[a]	419-438[d]
	Bacterial species: *Escherichia coli*		
579	5'-GAA GGC CGT GCT GGT GAG AA	78	503-522
2168	5'-CAT CAA AGT TGG TGA AGA AGT TG	78	409-431
	Bacterial species: *Neisseria gonorrhoeae*		
2166	5'-GAC AAA CCA TTC CTG CTG	126	322-339[e]
	Fungal species: *Candida albicans*		
577	5'-CAT GAT TGA ACC ATC CAC CA	407-411[a]	406-425[f]
	Fungal species: *Candida dubliniensis*		
578	5'-CAT GAT TGA AGC TTC CAC CA	412, 414-415[a]	418-437[g]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *E. faecalis* tuf sequence fragment (SEQ ID NO. 607).
[c]The nucleotide positions refer to the *E. faecium* tuf sequence fragment (SEQ ID NO. 608).
[d]The nucleotide positions refer to the *E. gallinarum* tuf sequence fragment (SEQ ID NO. 609).
[e]The nucleotide positions refer to the *N. gonorrhoeae* tuf sequence fragment (SEQ ID NO. 126).
[f]The nucleotide positions refer to the *C. albicans* tuf(EF-1) sequence fragment (SEQ ID NO. 408).
[g]The nucleotide positions refer to the *C. dubliniensis* tuf(EF-1) sequence fragment (SEQ ID NO. 414).

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SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Haemophilus influenzae*		
581	5'-ACA TCG GTG CAT TAT TAC GTG G	610[a]	551-572
	Bacterial species: *Mycoplasma pneumoniae*		
2095	5'-CGG TCG GGT TGA ACG TGG	2097[a]	687-704
	Bacterial species: *Staphylococcus aureus*		
584	5'-ACA TGA CAC ATC TAA AAC AA	176-180[b]	369-388[c]
585	5'-ACC ACA TAC TGA ATT CAA AG	176-180[b]	525-544[c]
586	5'-CAG AAG TAT ACG TAT TAT CA	176-180[b]	545-564[c]
587	5'-CGT ATT ATC AAA AGA CGA AG	176-180[b]	555-574[c]
588	5'-TCT TCT CAA ACT ATC GTC CA	176-180[b]	593-612[c]
	Bacterial species: *Staphylococcus epidermidis*		
589	5'-GCA CGA AAC TTC TAA AAC AA	185, 611[b]	445-464[d]
590	5'-TAT ACG TAT TAT CTA AAG AT	185, 611[b]	627-646[d]
591	5'-TCC TGG TTC TAT TAC ACC AC	185, 611[b]	586-605[d]
592	5'-CAA AGC TGA AGT ATA CGT AT	185, 611[b]	616-635[d]
593	5'-TTC ACT AAC TAT CGC CCA CA	185, 611[b]	671-690[d]
	Bacterial species: *Staphylococcus haemolyticus*		
594	5'-ATT GGT ATC CAT GAC ACT TC	186, 188-190[b]	437-456[e]
595	5'-TTA AAG CAG ACG TAT ACG TT	186, 188-190[b]	615-634[e]
	Bacterial species: *Staphylococcus hominis*		
596	5'-GAA ATT ATT GGT ATC AAA GA	191, 193-196[b]	431-450[f]
597	5'-ATT GGT ATC AAA GAA ACT TC	191, 193-196[b]	437-456[f]
598	5'-AAT TAC ACC TCA CAC AAA AT	191, 193-196[b]	595-614[f]

[a]Sequences from databases.
[b]These sequences were aligned to derive the corresponding probe.
[c]The nucleotide positions refer to the *S. aureus* tuf sequence fragment (SEQ ID NO. 179).
[d]The nucleotide positions refer to the *S. epidermidis* tuf sequence fragment (SEQ ID NO. 611).
[e]The nucleotide positions refer to the *S. haemolyticus* tuf sequence fragment (SEQ ID NO. 186).
[f]The nucleotide positions refer to the *S. hominis* tuf sequence fragment (SEQ ID NO. 191).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Staphylococcus saprophyticus*		
599	5'-CGG TGA AGA AAT CGA AAT CA	198-200[a]	406-425[b]
600	5'-ATG CAA GAA GAA TCA AGC AA	198-200[a]	431-450[b]
601	5'-GTT TCA CGT GAT GAT GTA CA	198-200[a]	536-555[b]
695	5'-GTT TCA CGT GAT GAC GTA CA	198-200[a]	563-582[b]

-continued

	Bacterial species: *Streptococcus agalactiae*		
582[c]	5'-TTT CAA CTT CGT CGT TGA CAC GAA CAG T	207-210[a]	404-431[d]
583[c]	5'-CAA CTG CTT TTT GGA TAT CTT CTT TAA TAC CAA CG	207-210[a]	433-467[d]
1199	5'-GTA TTA AAG AAG ATA TCC AAA AAG C	207-210[a]	438-462[d]
	Bacterial species: *Streptococcus pneumoniae*		
1201	5'-TCA AAG AAG AAA CTA AAA AAG CTG T	971, 977, 979, 986[a]	513-537[e]
	Bacterial species: *Streptococcus pyogenes*		
1200	5'-TCA AAG AAG AAA CTA AAA AAG CTG T	1002	473-497
	Bacterial group: *Enterococcus casseliflavus-flavescens-gallinarum* group		
620	5'-ATT GGT GCA TTG CTA CGT	58, 65, 66[a]	527-544[f]
1122	5'-TGG TGC ATT GCT ACG TGG	58, 65, 66[a]	529-546[f]
	Bacterial group: *Enterococcus* sp., *Gemella* sp., *A. adiacens*		
2172	5'-GTG TTG AAA TGT TCC GTA AA	58-62, 67-71, 87-88, 607-609, 727, 871 1715, 1722[a]	477-496[g]

[a]These sequences were aligned to derive the corresponding primer.
[b] The nucleotide positions refer to the *S. saprophyticus* tuf sequence fragment (SEQ ID NO. 198).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the *S. agalactiae* tuf sequence fragment (SEQ ID NO. 209).
[e]The nucleotide positions refer to the *S. pneumoniae* tuf sequence fragment (SEQ ID NO. 986).
[f]The nucleotide positions refer to the *E. flavescens* tuf sequence fragment (SEQ ID NO. 65).
[g]The nucleotide positions refer to the *E. faecium* tuf sequence fragment (SEQ ID NO. 608).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Bacterial genus: *Gemella*		
2171	5'-TCG TTG GAT TAA CTG AAG AA	87, 88[a]	430-449[b]
	Bacterial genus: *Staphylococcus* sp.		
605	5'-GAA ATG TTC CGT AAA TTA TT	176-203[a]	403-422[c]
606	5'-ATT AGA CTA CGC TGA AGC TG	176-203[a]	420-439[c]
1175	5'-GTT ACT GGT GTA GAA ATG TTC	176-203[a]	391-411[c]
1176	5'-TAC TGG TGT AGA AAT GTT C	176-203[a]	393-411[c]
	Bacterial genus: *Streptococcus* sp.		
1202	5'-GTG TTG AAA TGT TCC GTA AAC A	206-231, 971, 977, 979, 982-986[a]	466-487[d]
	Fungal species: *Candida albicans*		
1156	5'-GTT GAA ATG CAT CAC GAA CAA TT	407-412, 624[a]	680-702[e]

-continued

	Fungal group: *Candida albicans* and *C. tropicalis*		
1160	5'-CGT TTC TGT TAA AGA AAT TAG AAG	407-412, 429, 624[a]	748-771[e]
	Fungal species: *Candida dubliniensis*		
1166	5'-ACG TTA AGA ATG TTT CTG TCA A	414-415[a]	750-771[f]
1168	5'-GAA CAA TTG GTT GAA GGT GT	414-415[a]	707-726[f]
	Fungal species: *Candida glabrata*		
1158	5'-AAG AGG TAA TGT CTG TGG T	417	781-799
1159	5'-TGA AGG TTT GCC AGG TGA	417	718-735
	Fungal species: *Candida krusei*		
1161	5'-TCC AGG TGA TAA CGT TGG	422	720-737

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *G. haemolysans* tuf sequence fragment (SEQ ID NO. 87).
[c]The nucleotide positions refer to the *S. aureus* tuf sequence fragment (SEQ ID NO. 179).
[d]The nucleotide positions refer to the *S. pneumoniae* tuf sequence fragment (SEQ ID NO. 986).
[e]The nucleotide positions refer to the *C. albicans* tuf(EF-1) sequence fragment (SEQ ID NO. 408).
[f]The nucleotide positions refer to the *C. dubliniensis* tuf(EF-1) sequence fragment (SEQ ID NO. 414).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Fungal group: *Candida lusitaniae* and *C. guillermondii*		
1162	5'-CAA GTC CGT GGA AAT GCA	418, 424[a]	682-699[b]
	Fungal species: *Candida parapsilosis*		
1157	5'-AAG AAC GTT TCA GTT AAG GAA AT	426	749-771
	Fungal species: *Candida zeylanoides*		
1165	5'-GGT TTC AAC GTG AAG AAC	432	713-730
	Fungal genus: *Candida* sp.		
1163	5'-GTT GGT TTC AAC GTT AAG AAC	407-412, 414-415, 417, 418, 422, 429[a]	728-748[c]
1164	5'-GGT TTC AAC GTC AAG AAC	413, 416, 420, 421, 424, 425, 426, 428, 431[a]	740-757[b]
1167	5'-GTT GGT TTC AAC GT	406-426, 428-432, 624[a]	728-741[c]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the *C. lusitaniae* tuf(EF-1) sequence fragment (SEQ ID NO. 424).
[c]The nucleotide positions refer to the *C. albicans* tuf(EF-1) sequence fragment (SEQ ID NO. 408).

Annex IV: Strategy for the selection of amplification/sequencing primers from atpD (F-type) sequences.

	23 49 443 472 881 910	SEQ ID NO.:	Accession #:
B. cepacia	AGTgCAT CGGCGCCGTT ATCGACGTGG...TGTTCG GCGGTGCTGG CGTGGGCAAG ACCG...TCCA GGCCGTGT ACGTCCCTGC GGACGACT	—	X76877
B. pertussis	AGTgCAT CGGCGCCGTG GTGGATATTC...TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...TCCA GGCCGTGT ACGTGCCTGC CGACGACT	—	Genome project
P. aeruginosa	AAATCAT CGGCGCCGTG ATCGACGTGG...TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...TCCA GGCCGTAT ACGTTCCCGC GGACGACC	—	Genome project
E. coli	AGGTAAT CGGCGCCGTA GTTGACGTCG...TGTTCG GTGGTGCGGG TGTAGGTAAA ACCG...TACA GGCAGTAT ACGTACCTGC GGATGACT	—	J01594
N. gonorrhoeae	AAATTAT CGGTGCGGTT GTTGACGTGG...TGTTCG GCGGTGCCGG TGTGGGTAAA ACCG...TCCA AGCCGTAT ATGTACCTGC GGATGACT	—	Genome project
M. thermoacetica	AGGTTAT TGGCCCGGTG GTTGACGTCG...TCTTCG GCGGCGCCGG GGTCGGCAAG ACGG...TGCA AGCTATCT ATGTGCCGGC CGACGACC	—	U64318
S. aurantiaca	AGGTTcT CGGTCCCGTG ATTGACGTGG...TGTTCG GCGGCGCCGG CGTGGGCAAG ACGG...TGCA GGCCATCT ACGTGCCCGC CGACGACC	—	X76879
M. tuberculosis	GGGTCAC TGGGCCCGTC GTCGACGTCG...TGTTCG GCGGTGCCGG GGTGGGCAAG ACGG...TGCA AGCCGTCT ACGTGCCCGC CGACGACT	—	Z73419
B. fragilis	AGGTAAT TGGCCCTGTG GTCGATGTGT...TGTTTG GCGGGGCCGG AGTGGGTAAA ACTG...TGCA GGCTGTTT ACGTACCGGC TGATGACT	—	M22247
C. lytica	AAATTAT TGGCCCAGTT ATAGATGTGG...TATTTG GAGGTGCCGG AGTAGGTAAA ACAG...TACA GGCGGTTT ACGTACCTGC GGATGATT	672	M22535
A. woodii	AGGTTAT TGGACCAGTA GTCGATGTTA...TTTTCG GTGGTGCCGG AGTTGGTAAA ACCG...TTCA GGCCGTTT ACGaTCCAGC CGATGACT	—	U10505
C. acetobutylicum	AGGTAAT AGGACCTGTT GTGGATATTA...TGTTCG GTGGTGCCGG TGTTGGTAAA ACAG...TTCA GGCTGTAT ATGTTCCTGC TGATGACC	671	AF101055
M. pneumoniae	AAGTGAT TGGCCCGGTA GTTGATGTCA...TATTTG GTGGTGCTGG TGTTGGTAAA ACGG...TGCA AGCGATCT ATGTGCCAGC TGATGACT	—	U43738
H. pylori	AGGTTtT AGGCCCGGTG GTAGATGTGG...TGTTTG GTGGGGCTGG CGTAGGCAAA ACGG...TTCA AGCGGTGT ATGTGCCAGC AGACGACT	670	AF004014
Selected sequences for universal primers	RTIAT IGGIGCIGTI RTIGAYGT	568	
	RTIRY IGGICCIGTI RTIGAYGT	570	
	RTIRT IGGISCIGTI RTIGA	572	
	RTIRT IGGISCIGTI RTIGATAT	569	
	RTIRT IGGICCIGTI RTIGATGT	571	
	TTYG GIGGIGCIGG IGTIGGIAAR AC	566	
Selected sequence for universal primer[a]	CA RGCIRTIT AYGTICCIGC IGAYGA	567	

The sequence numbering refers to the *Escherichia coli* atpD gene fragment (SEQ ID NO. 669). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]This sequence is the reverse-complement of the selected primer.

Annex V: Strategy for the selection of universal amplification/sequencing primers from atpD (V-type) sequences.

	691 719 1177 1208	SEQ ID NO.:
E. hirae	CC **AGGTCCGTTT** GGTGCAGGG**A** **AGA**CAGT...TCT**GGTGGAg** **ATaTCtctGA** **ACCAGTGA**CT CA	685
H. salinarum	CC **GGGGCCGTTC** GGGTCCGGG**A** **AGA**CGGT...CCC**GGCGGGg** **ACTTCtccGA** **GCCGGTCA**CC CA	687
T. thermophilus	CC **TGGGCCCTTC** GGCAGCGGC**A** **AGA**CCGT...CCG**GGCGGCg** **ACaTgtccGA** **GCCCGTGA**CC CA	693
Human	CC **TGGGGCCTTC** GGATGTGGC**A** **AGA**CTGT...CCC**GGTGGAg** **ACTTCtcAGA** **tCCCGTGA**CG AC	688
T. congolense	CC **TGGCGCGTTT** GGATGCGG**AA** **AGA**CGGT...CCT**GGAGGTg** **ACTTTtctGA** **cCCAGTGA**CG TC	692
P. falciparum	CC **TGGTGCATTT** GGTTGTGG**AA** **AAA**CTTG...CCA**GGTGGTg** **ATTTCtctGA** **cCCTGTAA**CT AC	689
C. pneumoniae	CC **AGGACCTTTT** GGTGCAGGG**A** **AAA**CAGT...GCA**GGAGGAA** **ACTTTGAAGA** **ACCAGTCA**CT CA	686
Selected sequences for universal primers	**GGISSITTY GGIISIGGIA ARAC**	681
Selected sequences for universal primers[a]	**GGIGGIA AYTTYGARGA RCCIGTIAC**	682
	GGIGGIG AYWTIWSIGA ICCIGTIAC	683

The sequence numbering refers to the *Enterococcus hirae* atpD gene fragment (SEQ ID NO. 685). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NOs. 681 and 682 are indicated by lower-case letters. Mismatches for SEQ ID NO. 683 are indicated by underlined nucleotides. Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]These sequences are the reverse-complement of the selected primers.

Annex VI: Strategy for the selection of universal amplification/sequencing primers from tuf (M) sequences (organelle origin).

	601 635
C. neoformans[a]	AAG**AA** **CATGATCACC** **GGTaCCtCCC** **AGgctGA**CTG...
S. cerevisiae[a]	AAG**AA** **CATGATTACT** **GGTaCTtCTC** **AAgctGA**CTG...
O. volvulus[a]	AAG**AA** **TATGATCACA** **GGTaCTtCTC** **AGgctGA**CTG...
Human[a]	AAA**AA** **CATGATTACA** **GGGaCAtCTC** **AGgctGA**CTG...
*G. max*B1[b]	AAG**AA** **CATGATCACC** **GGCGCTGCCC** **AGATGGA**CGG...
*G. max*B2[b]	AAA**AA** **CATGATCACC** **GGCGCCGCCC** **AGATGGA**CGG...
E. coli[c]	AAA**AA** **CATGATCACC** **GGTGCTGCTC** **AGATGGA**CGG...
S. aureofaciens[c]	AAG**AA** **CATGATCACC** **GGTGCCGCCC** **AGATGGA**CGG...
E. tenella[b]	AAA**AA** **TATGATTACA** **GGAGCAGCAC** **AAATGGA**TGG...
T. gondii[b]	AAG**AA** **TATGATTACT** **GGAGCCGCAC** **AAATGGA**TGG...
S. cerevisiae[b]	AAG**AA** **TATGATTACC** **GGTGCTGCTC** **AAATGGA**TGG...
A. thaliana[b]	AAA**AA** **TATGATTACT** **GGAGCTGCGC** **AAATGGA**TGG...
Selected sequence for universal primer	**AA YATGATIACI GGIGCIGCIC ARATGGA**
Selected sequences for universal primers	

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Annex VI: Strategy for the selection of universal amplification/sequencing primers from tuf (M) sequences (organelle origin).

	1479 1511	SEQ ID NO.:	Accession #:
C. neoformans[a]	CGC**cgTCcGA GAcatGcGAC AGACcGTTGc** CGT	—	U81803
S. cerevisiae[a]	CGC**TgTCAGA GAcatGaGAC AAACTGTcGc** TGT	665	X00779
O. volvulus[a]	TGC**TgTGcGt GAtatGaGAC AAACaGTTGc** GGT	—	M64333
Human[a]	TGC**TgTTcGt GAtatGaGAC AGACaGTTGc** TGT	—	X03558
*G. max*B1[b]	TGC**TATTAGA GAAGGAGGCA AAACTGTTGG** AGC	—	Y15107
*G. max*B2[b]	TGC**TATTAGA GAAGGAGGCA AAACTGTTGG** AGC	—	Y15108
E. coli[c]	CGC**aATCcGt GAAGGGGCC GTACcGTTGG** CGC	78	—
S. aureofaciens[c]	CGC**cATCcGt GAGGGTGGTC GTACcGTgGG** CGC	—	AF007125
E. tenella[b]	TGC**TATAAGAGAAGGAGGAA AAACTATAGG** AGC	—	AI755521
T. gondii[b]	TGC**TATTAGA GAAGGAGGTC GTACTATAGG** AGC	—	Y11431
S. cerevisiae[b]	CAA**TATCAGA GAGGGTGGAA GAACTGTTGG** TAC	619	K00428
A. thaliana[b]	TGC**ctTAAGG GAAGGAGGTA GAACaGTTGG** AGC	—	X89227
Selected sequence for universal primer		664	
Selected sequences for universal primers	**TATIAGR GARGGIGGIM RIACTRTWGG**[d] **ATCCGT GAGGGYGGCC GITCIGT**[d]	652 561	

The sequence numbering refers to the Saccharomyces cerevisiae tuf (M) gene (SEQ ID NO. 619).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches for SEQ ID NOs. 652 and 664 are indicated by lower-case letters.
Mismatches for SEQ ID NO. 561 are indicated by underlined nucleotides.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G; "Y" stands for C or T;
"M" stands for A or C; "K" stands for G or T;
"W" stands for A or T; "S" stands for C or G.
"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]This sequence refers to tuf(EF-1) gene.
[b]This sequence refers to tuf (M) or organelle gene.
[c]This sequence refers to tuf gene from bacteria.
[d]These sequences are the reverse-complement of the selected primers.

Annex VII: Strategy for the selection of eukaryotic sequencing primers from tuf (EF-1) sequences.

	154	179 286	314	SEQ ID NO.:	Accession #:
S. cerevisiae	GG TTCTTTCAAG TACGCTTGGG TTTT...AGAGA	TTTCATCAAG AACATGATTA	CTGG...	665	X00779
B. hominis	GG CTCCTTCAAG TACGCGTGGG TGCT...CGTGA	CTTCATaAAG AACATGATCA	CGGG...	—	D64080
C. albicans	GG TTCTTTCAAA TACGCTTGGG TCTT...AGAGA	TTTCATCAAG AATATGATCA	CTGG...	—	M29934
C. neoformans	TC TTCTTTCAAG TACGCTTGGG TTCT...CGAGA	CTTCATCAAG AACATGATCA	CCGG...	—	U81803
E. histolytica	GG ATCATTCAAA TATGCTTGGG TCTT...AGAGA	TTTCATTAAG AACATGATTA	CTGG...	—	M92073
G. lamblia	GG CTCCTTCAAG TACGCGTGGG TCCT...CGCGA	CTTCATCAAG AACATGATCA	CGGG...	—	D14342
H. capsulatum	AA ATCCTTCAAA TATGCGTGGG TCCT...CGTGA	CTTCATCAAG AACATGATCA	CTGG...	—	U14100
Human	GG CTCCTTCAAG TATGCCTGGG TCTT...AGAGA	CTTtATCAAA AACATGATTA	CAGG...	—	X03558
L. braziliensis	GC GTCCTTCAAG TACGCGTGGG TGCT...CGCGA	CTTCATCAAG AACATGATCA	CCGG...	—	U72244
O. volvulus	GG CTCATTTAAA TATGCTTGGG TATT...CGTGA	TTTCATTAAG AATATGATCA	CAGG...	—	M64333
P. berghei	GG TagTTTCAAA TATGCATGGG TTTT...AAAcA	TTTtATTAAA AATATGATTA	CTGG...	—	AJ224150
P. knowlesi	GG AagTTTTAAG TACGCATGGG TGTT...AAGGA	TTTCATTAAA AATATGATTA	CCGG...	—	AJ224153
S. pombe	GG TTCCTTCAAG TACGCCTGGG TTTT...CGTGA	TTTCATCAAG AACATGATTA	CCGG...	—	U42189
T. cruzi	TC TTCTTTCAAG TACGCGTGGG TCTT...CGCGA	CTTCATCAAG AACATGATCA	CGGG...	—	L76077
Y. lipolytica	GG TTCTTTCAAG TACGCTTGGG TTCT...CGAGA	TTTCATCAAG AACATGATCA	CCGG...	—	AF054510
Selected sequences for amplification primers	TCITTYAAR TAYGCITGGG T			558	
		GA YTTCATYAAR AAYATGATYA C		560	
		GA YTTCATIAAR AAYATGAT		653	

The sequence numbering refers to the *Saccharomyces cerevisiae* tuf (EF-1) gene fragment (SEQ ID NO. 665).
Nucleotides in capitals are identical to the selected sequences SEQ ID NOs. 558, 560 or 653, or match those sequences.
Mismatches for SEQ ID no. 558 and 560 are indicated by lower-case letters.
Mismatches for SEQ ID NO. 653 are indicated by underlined nucleotides.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G;
"Y" stands for C. or T;
"M" stands for A or C;
"K" stands for G or T;
"W" stands for A or T;
"S" stands for C or G.
"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

	751	776 1276	1304	SEQ ID NO.:	Accession #:
S. cerevisiae	...GTTTACAA GATCGGTGGT ATTGGTAC...GACATG	AGACAAACTG TCGCTGTCGG	TGT	665	X00779
B. hominis	...GTGTACAA GATTGGCGGT ATTGGTAC...GATATG	AGACAGACTG TCGCTGTCGG	TAT	—	D64080
C. albicans	...GTTTACAA GATCGGTGGT ATTGGTAC...GATATG	AGACAAACCG TTGCTGTtGG	TGT	—	M29934
C. neoformans	...GTCTACAA GATCGGTGGT ATCGGCAC...GACATG	CGACAGACCG TTGCCGTtGG	TGT	—	U81803
E. histolytica	...GTTTACAA GATTTcAGGT ATTGGAAC...GATATG	AaACAAACCG TTGCTGTtGG	AGT	—	M92073
G. lamblia	...GTCTACAA GATCTcGGGc gTCGGGAC...~~~~~~	~~~~~~~~~~ ~~~~~~~~~~	~~~	—	D14342

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H. capsulatum	...GTGTACAA AATCTcTGGT ATTGGCAC...GACATG AGACAAACCG TCGCTGTCGG TGT	—	U14100
Human	...GTCTACAA AATTGGTGGT ATTGGTAC...GATATG AGACAGACAG TTGCgGTgGG TGT	—	X03558
L. braziliensis	...GTGTACAA GATCGGCGGT ATCGGCAC...GACATG CGCagAACGG TCGCCGTCGG CAT	—	U72244
O. volvulus	...GTTTACAA AATTGGAGGT ATTGGAAC...GATATG AGACAAACAG TTGCTGTtGG CGT	—	M64333
P. berghei	...GTATACAA AATTGGTGGT ATTGGTAC...GATATG AGACAAACAA TTGCTGTCGG TAT	—	AJ224150
P. knowlesi	...GTATACAA AATCGGTGGT ATTGGTAC...GATATG AGACAAACCA TTGCTGTCGG TAT	—	AJ224153
S. pombe	...GTTTACAA GATCGGTGGT ATTGGTAC...GACATG CGTCAAACCG TCGCTGTCGG TGT	—	U42189
T. cruzi	...GTGTACAA GATCGGCGGT ATCGGCAC...GACATG CGCCAGACGG TCGCCGTCGG CAT	—	L76077
Y. lipolytica	...GTCTACAA GATCGGTGGT ATCGGCAC...GACATG CGACAGACCG TTGCTGTCGG TGT	—	AF054510
Selected sequence for amplification primer	TACAA RATYKGIGGT ATYGG	654	
Selected sequences for amplification primers[a]	TACAA RATYKGIGGT ATYGG	655	
	ATG MGICARACIR TYGCYGTCGG	559	

The sequence numbering refers to the *Saccharomyces cerevisiae* tuf (EF-1) gene fragment (SEQ ID NO. 665).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
"~" indicate incomplete sequence data.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G;
"Y" stands for C or T;
"M" stands for A or C;
"K" stands for G or T;
"W" stands for A or T;
"S" stands for C or G.
"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]This sequences are the reverse-complement of the selected primers.

Annex VIII: Strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from tuf sequences.

	305 334 517 542	SEQ ID NO.:	Accession #:
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	207	—
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	208	—
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	209	—
S. agalactiae	CCAGAA CGTGATACTG ACAAACCTTT ACTT...GGAC AACGTTGGTG TTCTTCTTCG TG	210	—
S. anginosus	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AACGTaGGgG TTCTTCTTCG TG	211	—
S. anginosus	CCAGAA CGTGATACTG ACAAACCaTT gCTT...AGAt AACGTaGGTG TTCTTCTTCG TG	221	—
S. bovis	CCAaAA CGTGATACTG ACAAACCaTT gCTT...GGAt AACGTTGGTG TTCTTCTTCG TG	212	—
S. gordonii	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AAtGTaGGTG TcCTTCTTCG TG	223	—
S. mutans	CCAGAA CGTGATACTG ACAAgCCgcT cCTT...GGAt AAtGTTGGTG TTCTcCTTCG TG	224	—
S. pneumoniae	CCAGAA CGTGAcACTG ACAAACCaTT gCTT...AGAt AACGTaGGTG TcCTTCTTCG TG	145[a]	
S. sanguinis	CCAGAA CGcGATACTG ACAAgCCaTT gCTT...GGAC AACGTaGGTG TgCTTCTcCG TG	227	—
S. sobrinus	CCAaAA CGcGATACTG AtAAgCCaTT gCTT...AGAt AACGTTGGTG TgCTTCTTCG TG	228	—

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Annex VIII: Strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from tuf sequences.

	305 334 517 542	SEQ ID NO.:	Accession #:
B. cepacia	CCGGAg CGTGcagtTG ACggcgCgTT cCTG...CGAC AACGTTGGTa TcCTgCTgCG cG	16	—
B. fragilis	CCTccg CGcGATgtTG AtAAACCTTT ctTG...TGAC AACGTaGGTc TgtTgCTTCG TG	—	P33165
B. subtilis	CCAGAA CGcGAcACTG AaAAACCaTT caTG...TGAC AACaTTGGTG ccCTTCTTCG cG	—	Z99104
C. diphtheriae	CCAGAg CGTGAgACcG ACAAgCCaTT cCTC...CGAC AACtgTGGTc TgCTTCTcCG TG	662	—
C. trachomatis	CCAGAA aGaGAaAtTG ACAAgCCTTT cTTA...AGAg AAtGTTGGat TgCTcCTcaG aG	22	—
E. coli	CCAGAg CGTGcgAtTG ACAAgCCgTT cCTg...TGAg AACGTaGGTG TTCTgCTgCG TG	78	—
G. vaginalis	CCAact CacGATctTG ACAAgCCaTT cTTg...CGAt RACacTGGTc TTCTTCTcCG cG	135[a]	
S. aureus	CCAGAA CGTGATtCTG ACAAACCaTT cATg...TGAC AACaTTGGTG catTatTaCG TG	179	—
Selected sequence for species-specific primer	GAA CGTGATACTG ACAAACCTTT A	549	
Selected sequence for species-specific primer[b]	C AACGTTGGTG TTCTTCTTC	550	

The sequence numbering refers to the *Streptococcus agalactiae* tuf gene fragment (SEQ ID NO. 209).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G;
"Y" stands for C or T;
"M" stands for A or C;
"K" stands for G or T;
"W" stands for A or T;
"S" stands for C or G.
"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.
[b]This sequence is the reverse-complement of the selected primer.

Annex IX: Strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from tuf sequences.

	401 431 433 470	SEQ ID NO.:	Accession #:
S. acidominimus	GGTACTGT TaaaGTtAAt GACGAAGTTG AAATCGTTGG TATcAAAGAc GAaATCtctA AAGCAGTTGT TA	206	
S. agalactiae	GGTACTGT TCGTGTCAAC GACGAAGTTG AAATCGTTGG TATTAAAGAA GATATCCAAA AAGCAGTTGT TA	209	
S. agalactiae	GGTACTGT TCGTGTCAAC GACGAAGTTG AAATCGTTGG TATTAAAGAA GATATCCAAA AAGCAGTTGT TA	144[a]	
S. agalactiae	GGTACTGT TCGTGTCAAC GACGAAGTTG AAATCGTTGG TATTAAAGAA GATATCCAAA AAGCAGTTGT TA	207	
S. agalactiae	GGTACTGT TCGTGTCAAC GACGAAGTTG AAATCGTTGG TATTAAAGAA GATATCCAAA AAGCAGTTGT TA	210	
S. agalactiae	GGTACTGT TCGTGTCAAC GACGAAGTTG AAATCGTTGG TATTAAAGAA GATATCCAAA AAGCAGTTGT TA	208	
S. anginosus	GGTACTGT TaaaGTCAAC GACGAAGTTG AAATCGTTGG TATccgtGAt GAaATCCAAA AAGCAGTTGT TA	211	
S. anginosus	GGTACTGT TaaaGTCAAC GAtGAAGTTG AAATCGTTGG TATccgcGAg GAaATCCAAA AAGCAGTTGT TA	221	
S. bovis	GGTACTGT TaaaGTCAAC GACGAAGTTG AAATCGTTGG TATccgtGAc GAcATCCAAA AAGCtGTTGT TA	212	

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Annex IX: Strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from tuf sequences.

	401 431 433 470	SEQ ID NO.:	Accession #:
S. anginosus	GGTACTGT TaaaGTCAAt GAtGAAGTTG AAATCGTTGG TATTcgtGAc GAaATCCAAA AAGCAGTTGT TA	213	
S. cricetus	GGTACTGT TaagGTCAAt GACGAAGTTG AAATCGTTGG TATcAAgGAc GAaATCCAAA AAGCgGTTGT TA	214	
S. cristatus	GGTACTGT TCGTGTCAAC GAtGAAaTcG AAATCGTTGG TATcAAAGAA GAaATCCAAA AAGCAGTTGT TA	215	
S. downei	GGTACTGT TaagGTCAAC GACGAAGTTG AAATCGTTGG TATcAAgGAc GAaATCCAAA AAGCAGTTGT TA	216	
S. dysgalactiae	GGTACTGT TCGTGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaActaAAA AAGCtGTTGT TA	217	
S. equi equi	GGTACTGT TCGTGTCAAC GACGAAaTcG AAATCGTTGG TATcAgAGAc GAgATCaAAA AAGCAGTTGT TA	218	
S. ferus	GGTACTGT aaGaGTCAAC GAtGAAGTTG AAATCGTTGG TATcAAAGAc GAaATCactA AAGCAGTTGT TA	219	
S. gordonii	GGTAtcGT TaaaGTCAAt GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaATCCAAA AAGCAGTTGT TA	220	
S. macacae	GGTACTGT TaagGTtAAt GAtGAAGTTG AAATCGTTGG TATTcgtGAc GATATtCAAA AAGCAGTTGT TA	222	
S. gordonii	GGTAtcGT TaaaGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaActCAAA AAGCAGTTGT TA	223	
S. mutans	GGTACTGT TaaaGTtAAC GAtGAAGTTG AAATCGTTGG TATccgtGAt GAcATtCAAA AAGCtGTTGT TA	224	
S. oralis	GGTACTGT TCGTGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaActCAAA AAGCAGTTGT TA	—	P33170
S. parasanguinis	GGTgtTGT TCGTGTCAAt GAtGAAaTcG AAATCGTTGG TATcAAAGAA GAaATCCAAA AAGCAGTTGT TA	225	
S. pneumoniae	GGTAtcGT TaaaGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaActCAAA AAGCAGTTGT TA	145[a]	
S. pyogenes	GGTACTGT TCGTGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaActaAAA AAGCtGTTGT TA	—	Genome project
S. ratti	GGTACTGT TaaaGTCAAt GACGAAGTTG AAATCGTTGG TATccgtGAt GAcATCCAAA AAGCtGTTGT TA	226	
S. salivarius	GGTgtTGT TCGTGTCAAt GACGAAGTTG AAATCGTTGG TcTTAAAGAA GAcATCCAAA AAGCAGTTGT TA	146[a]	
S. sanguinis	GGTAtcGT TaaaGTCAAC GACGAAaTcG AAATCGTTGG TATcAAAGAA GAaATCCAAA AAGCAGTTGT TA	227	
S. sobrinus	GGTACTGT TaagGTCAAC GACGAAGTTG AAATCGTTGG TATccgtGAc GATATCCAAA AAGCAGTTGT TA	228	
S. suis	GGTACTGT TCGTGTCAAC GACGAAaTcG AAATCGTTGG TcTTcAAGAA GAaAaatcta AAGCAGTTGT TA	229	
S. uberis	GGTACTGT TCGTGTCAAC GACGAAaTTG AAATCGTTGG TATcAAAGAA GAaActaAAA AAGCAGTTGT TA	230	
S. vestibularis	GGTgtTGT TCGTGTtAAt GACGAAGTTG AAATCGTTGG TcTTAAAGAA GAaATCCAAA AAGCAGTTGT TA	231	
Selected sequences for species-specific hybridization probes[b]	ACTGT TCGTGTCAAC GACGAAGTTG AAA	582	
	CGTTGG TATTAAAGAA GATATCCAAA AAAGTTG	583	

The sequence numbering refers to the *Streptococcus agalactiae* tuf gene fragment (SEQ ID NO. 209).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
Dots indicate gaps in the sequences displayed.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.
[b]These sequences are the reverse-complement of the selected probes.

Annex X: Strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from atpD sequences.

	39 80 203 234 368 399	SEQ ID NO.:
S. agalactiae	TT GATTGTCTAT AAAAATGGCG ATAAGTCACA AAAAGTAGTA...TAAGGATA CTTTGGGTCG TGTCTTCAAC GTTC...CTT ATTAGCACCT TACTTAAAAG GTGGTAAAG	380
S. agalactiae	TT GATTGTCTAT AAAAATGGCG ATAAGTCACA AAAAGTAGTA...TAAGGATA CTTTGGGTCG TGTCTTCAAC GTTC...CTT ATTAGCACCT TACTTAAAAG GTGGTAAAG	379
S. agalactiae	TT GATTGTCTAT AAAAATGGCG ATAAGTCACA AAAAGTAGTA...TAAGGATA CTTTGGGTCG TGTCTTCAAC GTTC...CTT ATTAGCACCT TACTTAAAAG GTGGTAAAG	381
S. agalactiae	TT GATTGTCTAT AAAAATGGCG ATAAGTCACA AAAAGTAGTA...TAAGGATA CTTTGGGTCG TGTCTTCAAC GTTC...CTT ATTAGCACCT TACTTAAAAG GTGGTAAAG	382
S. agalactiae	TT GATTGTCTAT AAAAATGGCG ATAAGTCACA AAAAGTAGTA...TAAGGATA CTTTGGGTCG TGTCTTCAAC GTTC...CTT ATTAGCACCT TACTTAAAAG GTGGTAAAG	383
S. bovis	TT GATTGTtTAT AAAgATGGCG ATAAGTCtCA AAAAaTcGTg...TAAaGAaA CTTTGGGTCG TGTgTTtAAt GTTC...CcT tcTtGCcCCT TACcTAAAAG GTGGTAAAG	—[a]
S. salivarius	TT GgTcGTtTAT ActgATGaac AaAAGTCtaA AcgtaTcGTg...TAAaGATA CccTtGGaCG TGTCTTtAAC GTTC...CTT gcTAGCcCCT TACcTtAAgG GTGGTAAAG	387
S. pneumoniae	cT tgTcGTCTAc AAAAATGaCG AaAgaaaAac AAAAaTcGTc...TAAaGAaA CTTTGGGaCG TGTCTTCAAC GTTt...CcT tcTtGCcCCT TACcTtAAAG GTGGTAAAG	—[b]
S. pyogenes	TT GATTGTtTAT AAAgATaGtG ATAAaaagCA AAAAaTcGTc...TAAaGAaA CTTTGGGaCG cGTCTTtAAt GTaC...CcT tcTtGCcCCT TACcTtAAAG GTGGTAAAG	—[c]
S. anginosus	cT tgTaGTCTAT AAAAATGaCG AaAAtaaAtc AAAAaTcGTc...gAAaGAaA CacTtGGTCG cGTCTTtAAC GTTt...CcT tTTAGCcCCc TACcTcAAAG GTGGgAAAG	386
S. sanguinis	cT tgTaGTCTAT AAAAATGatG AgAAaaaAtc AAAAaTcGTc...aAAGGAaA CTcTaGGcCG gGT gTTCAAt GTTt...CcT gcTAGCACCT TAtcTgAAAG GTGGgAAAG	—[d]
S. mutans	TT GgTcGTtTAT AAAgATGGCG AcAAGTCtCA AAgAaTtGTt...aAAaGAaA CacTaGGTCG TGTCTTtAAt GTTC...CcT tcTtGCcCCT TAtcTtAAAG GTGGTAAAG	—[e]
B. anthracis	gT aAaacagagc AAcgAaaaCG gaAcaagcat tAActTAacA...TgAtGcaA CacTtGGTCG TGTaTTtAAC GTat...CTT AcTtGCtCCT TACaTtAAgG GTGGTAAga	247
B. cereus	gT aAaacaaagc AAcgAaaaCG g...aagcat gAActTAacA...TgAtGcaA CacTtGGaCG TGTaTTCAAC GTat...CTT AcTtGCtCCT TACaTtAAgG GTGGTAAga	248
E. faecium	TT agTTGTtTAT AAAAATGaCG AaAAtaaAtc AAAAGTtGTt...TAAaGAaA CaTTaGGTCG cGTaTTCAAC GTaC...tTT gcTtGCcCCa TAtTTAAAAG GTGGgAAAG	292
E. gallinarum	TT GATcGTtTAc AAAAAaGaCG AgAAaaaAac AAAAGTAGTA...aAcaGATA CTcTaGGcCG aGTaTTtAAt GTaC...tTT ATTAGCtCCT TACTTAAAAG GTGGTAAAG	293

-continued

Annex X: Strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from atpD sequences.

	39 80 203 234 368 399	SEQ ID NO.:
E. faecalis	TT agTcGTtTAT AAAAATGGCG AagcaaaACA AAAAGTAGTA...TAAaGATA CaTTaGGTCG TGTgTTtAAC GTTt...CTT ATTAGCACCT TAtcTAAAAG GTGGTAAAG	291
E. coli	Ta cgaTGctctT gAggtgcaaa ATggtaatgA gcgtcTgGTg...TAAaGcgA CTcTGGGcCG TaTCaTgAAC GTaC...CcT gaTgtgtCCg TtCgctAAgG GcGGTAAAG	f
L. monocytogenes	Ta tAaatctgAT gcAgAaGaaG caccaaCtag ccAAcTtact...TAcaGtaA CTcTtGGTCG TGTaTTtAAt GTat...CTT gcTAGCtCCT TACTTAAAAG GTGGTAAAa	324
S. aureus	gT tATTGatgtg cctAAaGaaG AaggtaCAat AcAAcTAacA...TgAtGAaA CaTTaGGTCG TGTaTTtAAt GTaC...tTT AcTAGCACCT TAtaTtAAAG GTGGTAAAa	366
S. epidermidis	ca cATcGaagtT cctAAaGaaG ATggagCgCt tcAAtTAacA...TgAcGtaA CTcTaGGaaG aGTgTTtAAC GTaC...CTT ATTAGCACCT TACaTAAAAG GTGGTAAAa	370
Selected sequences for species-specific primer	ATTGTCTAT AAAAATGGCG ATAAGTC	627
	AAAATGGCG ATAAGTCACA AAAAGTA	628
Selected sequences for species-specific primers[g]	GGATA CTTTGGGTCG TGTCTTCAAC G	625
	ATTAGCACCT TACTTAAAAG GTGGTA	626

The sequence numbering refers to the *Streptococcus agalactiae* tuf gene fragment (SEQ ID NO. 380). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a,d,e,f]These sequences were obtained from Genbank and have accession #: a = AB009314, d = AF001955, e = U31170, and f = V00311.

[b,c]These sequences were obtained from genome sequencing projects,

[g]These sequences are the reverse-complement of the selected primers.

Annex XI: Strategy for the selection of *Candida albicans*/*dubliniensis*-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe from tuf sequences.

	337 368 403 428 460 491	SEQ ID NO.:	Accession #:
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	624	—
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	409	—
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	410	—
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	407	—
C. albicans	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAACCATC CACCAACT...C AAATCCGGTA AAGTTACTGG TAAGACCTTG T	408	—
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	412	—
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	414	—
C. dubliniensis	CGTC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtTC CACCAACT...C AAATCCGGTA AgGTTACTGG TAAGACCTTG T	415	—
C. glabrata	CATC AAGAAGGTcG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCcaC CACCAACG...C AAggCtGGTg tcGTcAagGG TAAGACCTTG T	417	—
C. guilliermondii	CGTC AAGAAGGTTG GTTACAACCC tAAGACTG...CAACATGA TTGAggCtTC tACCAACT...C AAggCtGGTA AgtccACcGG TAAGACtTTG T	418	—
C. kefyr	CATC AAGAAGGTcG GTTACAACCC AAAGAATG...CAACATGA TTGAAgCcaC CACCAACG...C AAggCtGGTA ccGTcAagGG TAAGACCTTG T	421	—
C. krusei	CATC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCATC CACCAACT...C AAggCaGGTg ttGTTAagGG TAAGACCTTA T	422	—
C. lusitaniae	CGTC AAGAAGGTTG GTTACAACCC tAAGACTG...CAACATGA TTGAgCCATC YACCAACT...C AAgTCYGGTA AgtccACcGG TAAGACCTTG T	424	—
C. neoformans	CATC AAGAAGGTTG GTTACAACCC cAAGgCTG...CAACATGt TgGAggagaC CACCAAGT...C AAgTCtGGTg tttccAagGG TAAGACCcTC C	623	—
C. parapsilosis	CGTC AAGAAGGTTG GTTACAACCC tAAagCTG...CAAtATGA TTGAACCATC aACCAACT...T AAAgCtGGTA AgGTTACcGG TAAGACCTTG T	426	—
C. tropicalis	CGTC AAGAAGGTTG GTTACAACCC tAAGgCTG...CAACATGA TTGAAgCtTC tACCAACT...C AAggCtGGTA AgGTTACcGG TAAGACtTTG T	429	—
A. fumigatus	CATC AAGAAGGTcG GcTACAACCC cAAGgCCG...CAACATGc TTGAgCCcTC CtCCAACT...C AAggCCGGcA AgGTcACTGG TAAGACCcTC A	404	—
Human	CATt AAGAAaaTTG GcTACAACCC cgAcACAG...CAACATGc TgGAgCCAag tgCtAACA...T AAggatGGcA AtGccAgTGG aAccACgcTG C	—	X03558
P. anomala	TATC AAGAAaGTTG GTTACAACCC AAAaACTG...TAACATGA TTGAACCATC aWCtAACT...C AAAgCtGGTg AAGcTAaaGG TAAaACtTTA T	447	—
S. cerevisiae	TATC AAGAAGGTTG GTTACAACCC AAAGACTG...CAACATGA TTGAAgCtaC CACCAACG...C AAggCCGGTg tcGTcAagGG TAAGACtTTG T	622	—
S. pombe	CATC AAGAAGGTcG GTTtCAACCC cAAGACCG...TAACATGA TTGAgCCcaC CACCAACA...C AAggCtGGTg tcGTcAagGG TAAGACtcTT T	—	U42189

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Annex XI: Strategy for the selection of *Candida albicans*/*dubliniensis*-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe from tuf sequences.

	337 368	403 428	460 491	SEQ ID NO.:	Accession #:
Selected sequence for species-specific amplification primer[a]	**C AAGAAGGTTG GTTACAACCC AAAGA**				
Selected sequence for species-specific amplification primer[a,b]			**ATCCGGTA AAGTTACTGG TAAGACCT**		
Selected sequences for species-specific hybridization probes		**CATGA TTGAACCATC CACCA** (*C. albicans*)		577	
		CATGA TTGAAGCTTC CACCA (*C. dubliniensis*)		578	

The sequence numbering refers to the *Candida albicans* tuf gene fragment (SEQ ID NO. 408). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches for SEQ ID NO. 577 are indicated by lower-case letters. Mismatches for SEQ ID NO. 578 are indicated by underlined nucleotides.
Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.

"R" stands for A or G;

"Y" stands for C or T;

"M" stands for A or C;

"K" stands for G or T;

"W" stands for A or T;

"S" stands for C or G.

"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a] *C. albicans* primers have been described in a previous patent (publication WO98/20157, SEQ ID NOs. 11-12)

[b] This sequence is the reverse-complement of the selected primer.

Annex XII: Strategy for the selection of *Staphylococcus*-specific amplification primers from tuf sequences.

	310 340	652 682	SEQ ID NO.:	Accession #:
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTACTG AAATGGTAAT GC	179	—
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACC~ ~~~~~~~~~~ ~~~~~~~~~~ GC	176	—
S. aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTMCTG AAATGGTAAT GC	177	—
S. aureus aureus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CACTTACCA GAAGGTACTG AAATGGTAAT GC	180	—
S. auricularis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	ActTTACCA GAAGGTACaG AAATGGTAAT GC	181	—
S. capitis capitis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	182	—
M. caseolyticus	A CTGGaCGTGT TGAgCGTGGa CAAgTtAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	183	—
S. cohnii	A CAGGgCGTGT TGAACGTGGT CAAATCAAAG...	ActTTACCA GAAGGTACTG AAATGGTTAT GC	184	
S. epidermidis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	185	—
S. epidermidis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACaG AAATGGTTAT GC	141[a]	—
S. haemolyticus	A CAGGCCGTGT TGAACGTGGg CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	186	—
S. haemolyticus	A CAGGtCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAG~~~~~~ ~~~~~~~~~~ ~~	188	—
S. haemolyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGG~~~~ ~~	189	—
S. hominis hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	191	—
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	193	—
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGG~~~~~ ~~~~~~~~~~ ~~	194	—
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTMCTG AAATGGTAAT GC	195	—
S. hominis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTAAT GC	196	—
S. lugdunensis	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACaG AAATGGTTAT GC	197	—
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	198	—
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	199	—
S. saprophyticus	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	200	—
S. sciuri sciuri	A CAGGCCGTGT TGAACGTGGT CAAATCACTG...	AACTTACCA GAAGGTACTG AAATGGTTAT GC	201	—
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CAaTTACCA GAAGGTACTG ~~~~~~~~~~ ~~	187	—
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~	192	—
S. warneri	A CAGGCCGTGT TGAACGTGGT CAAATCAAAG...	CAaTTACCA GAAGGTACTG AAATGGTTAT GC	202	—
B. subtilis	A CTGGCCGTGT aGAACGcGGa CAAgTtAAAG...	CAtcTtCCA GAAGGcgtaG AAATGGTTAT GC	—	Z99104
E. coli	A CCGGtCGTGT aGAACGcGGT atcATCAAAG...	GAacTgCCg GAAGGcgtaG AgATGGTAAT GC	78	—
L. monocytogenes	A CTGGaCGTGT TGAACGTGGa CAAgTtAAAG...	AcacTtCCA GAAGGTACTG AAATGGTAAY GC	138[a]	—
Selected sequence for genus-specific primer	GGCCGTGT TGAACGTGGT CAAATCA		553	

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Annex XII: Strategy for the selection of *Staphylococcus*-specific amplification primers from tuf sequences.						
	310	340	652	682	SEQ ID NO.:	Accession #:
Selected sequences for genus-specific primers[b]			**TTACCA GAAGGTACTG AAATGGTIA**		575	
			TTACCA GAAGGTACTG AAATGCTWA		707	

The sequence numbering refers to the *Staphylococcus aureus* tuf gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "~" indicate incomplete sequence data.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G;
"Y" stands for C or T;
"M" stands for A or C;
"K" stands for G or T;
"W" stands for A or T;
"S" stands for C or G.
"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.
[b]These sequences are the reverse-complement of the selected primers.

Annex XIII: Strategy for the selection of the *Staphylococcus*-specific hybridization probe from tuf sequences.				
	400	425	SEQ ID NO.:	Accession #:
S. aureus	G **TT**GAAATGTT CCGTAAATTA **TT**AGA		179	—
S. aureus	G **TT**GAAATGTT CCGTAAATTA **TT**AGA		176	—
S. aureus	G **TT**GAAATGTT CCGTAAATTA **TT**AGA		177	—
S. aureus	G **TT**GAAATGTT CCGTAAATTA **TT**AGA		178	—
S. aureus aureus	G **TT**GAAATGTT CCGTAAATTA **TT**AGA		180	—
S. auricularis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		181	—
S. capitis capitis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		182	—
M. caseolyticus	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		183	—
S. cohnii	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		184	—
S. epidermidis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		185	—
S. haemolyticus	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		186	—
S. haemolyticus	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		189	—
S. haemolyticus	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		190	—
S. haemolyticus	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		188	—
S. hominis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		196	—
S. hominis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		194	—
S. hominis hominis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		191	—
S. hominis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		193	—
S. hominis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		195	—
S. lugdunensis	G **TA**GAAATGTT CCGTAAATTA **TT**AGA		197	—

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Annex XIII: Strategy for the selection of the *Staphylococcus*-specific hybridization probe from tuf sequences.

	400 425	SEQ ID NO.:	Accession #:
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	198	—
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	200	—
S. saprophyticus	G TAGAAATGTT CCGTAAATTA TTAGA	199	—
S. sciuri sciuri	G TTGAAATGTT CCGTAAATTA TTAGA	201	—
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	187	—
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	192	—
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	202	—
S. warneri	G TAGAAATGTT CCGTAAgTTA TTAGA	203	—
B. subtilis	G TTGAAATGTT CCGTAAgcTt cTTGA	—	Z99104
E. coli	G TTGAAATGTT CCGcAAAcTg cTGGA	78	—
L. monocytogenes	G TAGAAATGTT CCGTAAATTA cTAGA	138[a]	—
Selected sequence for genus-specific hybridization probe	GAAATGTT CCGTAAATTA TT	605	

The sequence numbering refers to the *Staphylococcus aureus* tuf gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequence or match that sequence. Mismatches are indicated by lower-case letters.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XIV: Strategy for the selection of *Staphylococcus saprophyticus*-specific and of *Staphylococcus haemolyticus*-specific hybridization probes from tuf sequences.

	339 383	SEQ ID NO.:
S. aureus	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACaTC TAA	179
S. aureus	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACaTC TAA	176
S. aureus	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACaTC TAA	177
S. aureus	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACaTC TAA	178
S. aureus aureus	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACaTC TAA	180
S. auricularis	AG TCGGTGAAGA AgTtGAAATC ATcGGTATga AaGACggTTC AAA	181
S. capitis capitis	AG TtGGTGAAGA AgTtGAAATC ATcGGTATCC AcGAaACTTC TAA	182
M. caseolyticus	AG TtGGTGAAGA AgTtGAAATC ATTGGTtTaa cTGAagaacC AAA	183
S. cohnii	AG TCGGTGAAGA AgTtGAAATC ATcGGTATgC AaGAagaTTC CAA	184
S. epidermidis	AG TtGGTGAAGA AgTtGAAATC ATcGGTATgC AcGAaACTTC TAA	185
S. haemolyticus	AG TtGGTGAAGA AgTtGAAATC ATTGGTATCC ATGACACTTC TAA	186
S. haemolyticus	AG TtGGTGAAGA AgTtGAAATC ATTGGTATCC ATGACACTTC TAA	189
S. haemolyticus	AG TtGGTGAAGA AgTtGAAATC ATTGGTATCC ATGACACTTC TAA	190
S. haemolyticus	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAaACTTC TAA	188

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Annex XIV: Strategy for the selection of *Staphylococcus saprophyticus*-specific and of *Staphylococcus haemolyticus*-specific hybridization probes from tuf sequences.

	339 383	SEQ ID NO.:
S. hominis	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAaACTTC TAA	194
S. hominis hominis	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAaACTTC TAA	191
S. hominis	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAaACTTC TAA	193
S. hominis	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAaACTTC TAA	195
S. hominis	AG TtGGTGAAGA AgTtGAAATt ATTGGTATCa AaGAtACTTC TAA	196
S. lugdunensis	AG TCGGTGAAGA AgTtGAAATt ATTGGTATCC AcGAtACTaC TAA	197
S. saprophyticus	AG TCGGTGAAGA AATCGAAATC ATcGGTATgC AaGAagaaTC CAA	198
S. saprophyticus	AG TCGGTGAAGA AATCGAAATC ATcGGTATgC AaGAagaaTC CAA	200
S. saprophyticus	AG TCGGTGAAGA AATCGAAATC ATcGGTATgC AaGAagaaTC CAA	199
S. sciuri sciuri	TG TtGGTGAAGA AgTtGAAATC ATcGGTtTaa cTGAagaaTC TAA	201
S. warneri	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACTTC TAA	187
S. warneri	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACTTC TAA	192
S. warneri	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACTTC TAA	202
S. warneri	AG TtGGTGAAGA AgTtGAAATC ATcGGTtTaC ATGACACTTC TAA	203
B. subtilis	AG TCGGTGAcGA AgTtGAAATC ATcGGTcTtC AaGAagagag AAA	—[a]
E. coli	AG TtGGTGAAGA AgTtGAAATC gTTGGTATCa AaGAgACTca GAA	78
L. monocytogenes	AG TtGGTGAcGA AgTaGAAgTt ATcGGTATCg AaGAagaaag AAA	138[b]
Selected sequences for species-specific hybridization probes	CGGTGAAGA AATCGAAATC A (*S. saprophyticus*) (*S. haemolyticus*) ATTGGTATCC ATGACACTTC	599 594

The sequence numbering refers to the *Staphylococcus aureus* tuf gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters.
[a]This sequence was obtained from Genbank accession #Z99104.
[b]The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XV: Strategy for the selection of *Staphylococcus aureus*-specific and of *Staphylococcus epidermidis*-specific hybridization probes from tuf sequences.

	521 547 592 617	SEQ ID NO.:
S. aureus	TACACCACA TACTGAATTC AAAGCAG...TTCTTCtCa AACTA TCGtC CACAAAT	179
S. aureus	TACACCACA TACTGAATTC AAAGCAG...TTCTTCtC~ ~~~~~~~~~~ ~~~~~~~	178
S. aureus	TACACCACA TACTGAATTC AAAGCAG...TTCTTCtCa AACTA TCGtC CACAAAT	176
S. aureus	TACACCACA TACTGAATTC AAAGCAG...TTCTTCtCa AACTA TCGtC CACAAAT	177
S. aureus aureus	TACACCACA TACTGAATTC AAAGCAG...TTCTTCtCa AACTA TCGtC CACAAAT	180
S. auricularis	TACACCACA cACTaAATTC ActGCAG...TTCTTCtCT AACTA cCGtC CACAAAT	181

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Annex XV: Strategy for the selection of *Staphylococcus aureus*-specific and of *Staphylococcus epidermidis*-specific hybridization probes from tuf sequences.

	521 547	592 617	SEQ ID NO.:
S. capitis capitis	CACACCACA cACTaAATTC AAAGCGG...	TTCTTCAgT AACTA cCGCC CACAAAT	182
M. caseolyticus	TACtCCACA TACTaAATTC AAAGCTG...	TTCTTCACT AACTA cCGCC CtCAGTT	183
S. cohnii	TACACCACA cACaaAcTTt AAAGCGG...	TTCTTCAgT AACTA TCGCC CACAAAT	184
S. epidermidis	TACACCACA cACaaAATTC AAAGCTG...	TTCTTCACT AACTA TCGCC CACAAAT	185
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	TTCTTCACa AACTA TCGtC CACAAAT	186
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	TTCTTCACa AACTA TCGtC CACAAAT	189
S. haemolyticus	CACACCtCA cACaaAATTt AAAGCAG...	TTCTTCACa AACTA TCGtC CACAAAT	190
S. haemolyticus	TACACCtCA cACaaAATTC AAAGCAG...	TTCTTCACT AACTA TCGtC CACAAAT	188
S. hominis	CACACCtCA cACaaAATTC AAAGCAG...	TTCTTCACT AACTA TCGtC CACAAAT	195
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	TTCTTCACT AACTA TCGtC CACAAAT	196
S. hominis hominis	TACACCtCA cACaaAATTC AAAGCAG...	TTCTTCtCT AACTA TCGtC CACAAAT	191
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	TTCTTCtCT AACTA TCGtC CACAAAT	193
S. hominis	TACACCtCA cACaaAATTC AAAGCAG...	TTCTTCtCT AACTA TCGtC CACAAAT	194
S. lugdunensis	TACACCtCA cACTaAATTt AAAGCTG...	TTCTTCtCa AACTA cCGCC CACAAAT	197
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCACT AACTA cCGCC CACAAAT	198
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCACT AACTA cCGCC CACAAAT	199
S. saprophyticus	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCACT AACTA cCGCC CACAAAT	200
S. sciuri sciuri	CACACCtCA cACTaAATTC AAAGCTG...	TTCTTCACa AACTA cCGCC CACAAAT	201
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~	192
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCAgT AACTA cCGCC CACAAAT	187
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCAgT AACTA cCGCC CACAAAT	202
S. warneri	TACACCACA TACaaAATTC AAAGCGG...	TTCTTCAgT AACTA cCGCC CACAAAT	203
B. subtilis	CACtCCACA cAgcaAATTC AAAGCTG...	TTCTTCtCT AACTA cCGtC CtCAGTT	—[a]
E. coli	CAAgCCgCA cACcaAgTTC gAAtCTG...	TTCTTCAaa ggCTA cCGtC CgCAGTT	78
L. monocytogenes	TACtCCACA cACTaAcTTC AAAGCTG...	TTCTTCAac AACTA cCGCC CACAAAT	138[b]
Selected sequences for species-specific hybridization probes	ACCACA TACTGAATTC AAAG (*S. aureus*)		585
	(*S. epidermidis*)	TTCACT AACTATCGCC CACA	593

The sequence numbering refers to the *Staphylococcus aureus* tut gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. "~" indicate incomplete sequence data. Dots indicate gaps in the sequences displayed.

[a]This sequence was obtained from Genbank accession #Z99104.

[b]The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XVI: Strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from tuf sequences.

	358 383	SEQ ID NO.:
S. aureus	ATC ATcGGTtTac AtGAcACaTC TAA	179
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	176
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	177
S. aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	178
S. aureus aureus	ATC **ATcGGTtTac AtGAcACaTC** TAA	180
S. auricularis	ATC **ATcGGTATgA AAGAcggTTC** AAA	181
S. capitis capitis	ATC **ATcGGTATCc AcGAAACTTC** TAA	182
M. caseolyticus	ATC **ATTGGTtTaA ctGAAgaacC** AAA	183
S. cohnii	ATC **ATcGGTATgc AAGAAgaTTC** CAA	184
S. epidermidis	ATC **ATcGGTATgc AcGAAACTTC** TAA	185
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	186
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	189
S. haemolyticus	ATC **ATTGGTATCc AtGAcACTTC** TAA	190
S. haemolyticus	ATT **ATTGGTATCA AAGAAACTTC** TAA	188
S. hominis	ATT **ATTGGTATCA AAGAtACTTC** TAA	196
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	194
S. hominis hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	191
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	193
S. hominis	ATT **ATTGGTATCA AAGAAACTTC** TAA	195
S. lugdunensis	ATT **ATTGGTATCc AcGAtACTaC** TAA	197
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	198
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	200
S. saprophyticus	ATC **ATcGGTATgc AAGAAgaaTC** CAA	199
S. sciuri sciuri	ATC **ATcGGTtTaA ctGAAgaaTC** TAA	201
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	187
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	192
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	202
S. warneri	ATC **ATcGGTtTac AtGAcACTTC** TAA	203
B. subtilis	ATC **ATcGGTcTtc AAGAAgagag** AAA	—[a]
E. coli	ATC **gTTGGTATCA AAGAgACTca** GAA	78
L. monocytogenes	GTT **ATcGGTATCg AAGAAgaaag** AAA	138[b]
Selected sequence for species-specific hybridization probe	**ATTGGTATCA AAGAAACTTC**	597

The sequence numbering refers to the *Staphylococcus aureus* tuf gene fragment (SEQ ID NO. 179). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
[a]This sequence was obtained from Genbank accession #Z99104.
[b]The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XVII: Strategy for the selection of the *Enterococcus*-specific amplification primers from tuf sequences.

	270 … 298 556 … 582	SEQ ID NO.:	Accession #:
E. avium	TAGAATTAAT GGCTGCTGTT GACGAATAT...TGAA GATATCCAAC GTGGACAAGTATT	131[a]	—
E. casseliflavus	TGGAATTAAT GGCTGCAGTT GACGAATAC...TGAA GACATCCAAC GTGGACAAGT ATT	58	—
E. cecorum	TAGAATTAAT GGCTGCAGTT GACGAATAC...TGAA GATATCCAAC GTGGtCAAGT ATT	59	—
E. dispar	TAGAATTAAT GGCTGCAGTT GACGAATAT...TGAA GATATCCAAC GTGGtCAAGT ATT	60	—
E. durans	TTGAATTAAT GGCTGCAGTT GACGAATAT...TGAA GACATCCAAC GTGGACAAGT TTT	61	—
E. flavescens	TGGAATTAAT GGCTGCAGTT GACGAATAC...TGAA GACATCCAAC GTGGACAAGT ATT	65	—
E. faecium	TTGAATTAAT GGCTGCAGTT GACGAATAC...TGAA GACATCCAAC GTGGACAAGT TTT	608	—
E. faecalis	TAGAATTAAT GGCTGCAGTT GACGAATAT...TGAA GATATCGAAC GTGGACAAGT ATT	607	—
E. gallinarum	TGGAATTgAT GGCTGCAGTT GACGAATAC...TGAA GACATCCAAC GTGGACAAGT ATT	609	—
E. hirae	TTGAATTgAT GGCTGCAGTT GACGAATAT...TGAA GACATCCAAC GTGGACAAGT TTT	67	—
E. mundtii	TTGAATTgAT GGCTGCAGTT GACGAATAT...TGAA GACATCCAAC GTGGtCAAGT TTT	68	—
E. pseudoavium	TAGAATTAAT GSCTGCTGTT GACGAATAC...TGAA GACATCCAAC GTGGACAAGT ATT	69	—
E. raffinosus	TAGAATTAAT GGCTGCTGTT GATGAATAC...TGAA GACATCCAAC GTGGACAAGT ATT	70	—
E. saccharolyticus	TCGAATTAAT GGCTGCAGTT GACGAATAT...TGAA GACATCCAAC GTGGACAAGT ATT	71	—
E. solitarius	TGGAcTTAAT GGaTGCAGTT GATGAcTAC...TGAt GATATCGAAC GTGGtCAAGT ATT	72	—
E. coli	TGGAAcTggc tGgcttccTg GATtctTAY...TGAA GAaATCGAAC GTGGtCAgGT ACT	78	—
B. cepacia	TGAgccTggc cGacGCgcTg GACacgTAC...TGAA GACgTgGAgC GTGGcCAgGT TCT	16	—
B. fragilis	TGGAAcTgAT GGaaGCTGTT GATactTGG...GAAc GAaATCaAAC GTGGtatgGT TCT	—	M22247
B. subtilis	TCGAAcTtAT GGaTGCgGTT GATGAgTAC...TGAA GAaATCCAAC GTGGtCAAGT ACT	—	Z99104
C. diphtheriae	TCGAccTcAT GcagGCTtgc KATGAtTCC...CGAA GACgTtGAgC GTGGcCAgGT TGT	662	—
C. trachomatis	GAGAgcTAAT GcaaGCcGTc GATGAtAAT...GAAc GATgTgGAAa GaGGAatgGT TGT	22	—
G. vaginalis	AGGAAcTcAT GaagGCTGTT GACGAgTAC...TACc GACgTtGAgC GTGGtCAgGT TGT	135[a]	—
S. aureus	TAGAATTART GGaaGCTGTa GATactTAC...TGAA GACgTaCAAC GTGGtCAAGT ATT	179	—
S. pneumoniae	TGGAATTgAT GaacaCAGTT GATGAgTAT...TGAt GAaATCGAAC GTGGACAAGT TAT	145[a]	—
A. adiacens	TAGAATTAAT GGCTGCTGTT GACGAATAC...TGAA aACATCGAAC GTGGACAAGT TCT	118[a]	—
G. haemolysans	TCGAATTAAT GGaaaCAGTT GACGAATAC...TGAA GACATCGAAC GTGGACAAGT TTT	87	—
G. morbillorum	TCGAATTAAT GGaaaCAGTT GACGAgTAC...TGAA GATATCGAAC GTGGACAAGT TTT	88	—
Selected sequence for amplification primer	AATTAAT GGCTGCWGTT GAYGAA	1137	
Selected sequence for amplification primer[b]	A GAYATCSAAC GTGGACAAGT	1136	

The sequence numbering refers to the *Enterococcus durans* tuf gene fragment (SEQ ID NO. 61). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed. "Y" "W" and "S" designate nucleotide positions which are degenerated. "Y" stands for C or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a]The SEQ ID NO. refers to previous patent publication WO98/20157.

[b]This sequence is the reverse-complement of the selected primer.

Annex XVIII: Strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from tuf sequences.

	395	448...526	549	SEQ ID NO.:	Accession #:
E. avium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa	GGTATcGCT...CATc	GGTGCtTTGt TACGTGGTGT	131[a]	—
E. casseliflavus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT	GGTATTGCT...CATT	GGTGCATTGC TACGTGGTGT	58	—
E. cecorum	GTTGA ACGTGGacAA GTaCGtGTTG GTGACGAAGT TGAAaTaGTT	GGTATcCAT...CATc	GGTGCATTat TACGTGGTGT	59	—
E. dispar	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa	GGTATcGCT...CATT	GGTGCATTat TACGTGGTGT	60	—
E. durans	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAtaTcGTT	GGTATcGCA...CATT	GGTGCtTTaC TACGTGGTGT	61	—
E. faecalis	GTTGA ACGTGGTGAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT	GGTATTAAA...CTTc	GGTGCtTTat TACGTGGTGT	62	—
E. faecium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAGTTGTT	GGTATTGCT...CATT	GGTGCtTTaC TACGTGGTGT	608	—
E. flavescens	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTT	GGTATTGCT...CATT	GGTGCATTGC TACGTGGGGT	65	—
E. gallinarum	GTTGA ACGTGGacAA GTTCGCGTTG GTGATGAAGT aGAAaTcGTT	GGTATTGCT...CATT	GGTGCATTGC TACGTGGGGT	609	—
E. hirae	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAtaTcGTT	GGTATcGCA...CATT	GGTGCtTTaC TACGTGGTGT	67	—
E. mundtii	GTTGA ACGTGGacAA GYTCGtGTTG GTGACGttaT cGAtaTcGTT	GGTATcGCA...CATT	GGTGCgTTaC TACGTGGTGT	68	—
E. pseudoavium	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa	GGTATCGCT...CATc	GGTGCATTat TACGTGGTGT	69	—
E. raffinosus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGAAGT TGAAaTcGTa	GGTATTGCT...CATT	GGTGCATTat TACGTGGTGT	70	—
E. saccharolyticus	GTTGA ACGTGGacAA GTTCGCGTTG GTGACGttGT aGAAaTcGTT	GGTATcGAC...CATc	GGTGCtTTat TACGTGGGGT	71	—
E. solitarius	GTTGA ACGcGGgact aTcaaaGTCG GCGATGAAGT TGAcaTTaTT	GGTATTCAT...CATT	GGTaCtTTGt TACGTGGTGT	72	—
C. diphtheriae	GTTGA gCGTGGctcc cTgaagGTCA ACGAGGAcGT cGAgaTcaTc	GGTATcCGC...CTGT	GGTctgcTtC TcCGTGGCGT	662	—
G. vaginalis	GTTGA gCGTGGTaAg cTcCcaATCA ACACCCcAGT TGAgaTcGTT	GGTtTgCGC...CACT	GGTcttcTtC TcCGcGGTAT	135[a]	—

-continued

Annex XVIII: Strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum* group-specific hybridization probe from tuf sequences.

	395	448...526	549	SEQ ID NO.:	Accession #:
B. cepacia	GTCG**A** gCGcGGc**a**tc GTg**aag**GTCG GCGAAG**AAaT cGAAaTcGTc** GGTATcAAG...CGTT GGTatccTGC TgCGcGGCAC			16	—
S. aureus	GTTG**A** ACGTGGTc**AA** aTc**aaa**GTTG GTGAAG**AAGT TGAAaTcaTc** GGTtTaCAT...CATT GGTGCATTat TACGTGGTGT			179	—
B. subtilis	GTAG**A** ACGcGGac**AA** GTT**aaa**GTCG GTGACG**AAGT TGAAaTcaTc** GGTcTTCAA...CATT GGTGCccTtC TtCGcGGTGT			—	Z99104
S. pneumoniae	ATCG**A** cCGTGGTatc GTT**aaa**GTCA ACGACG**AAaT cGAAaTcGTT** GGTATcAAA...CGTa GGTGtccTtC TtCGTGGTGT			145[a]	—
E. coli	GTAG**A** **A**CGcGGTatc aTc**aaa**GTTG GTGAAG**AAGT TGAAaTcGTT** GGTATcAAA...CGTa GGTGttcTGC TgCGTGGTAT			78	—
B. fragilis	ATCG**A** **Aa**cTGGTGtt aTcCatGTAG GTGATG**AAaT cGAAaTccTc** GGTtTgGGT...CGTa GGTctgTTGC TtCGTGGTGT			—	M22247
C. trachomatis	ATTG**A** gCGTGGaatt GTT**aaa**GTTT CCGATA**AAGT TcAgtTgGTc** GGTcTTAGA...CGTT GGattgcTcC TcaGaGGTAT			22	—
Selected sequences for species-specific or group-specific hybridization probes	GA ACGTGGTGAA GTTCGC (*E. faecalis*) AAGT TGAAGTTGTT GGTATT (*E. faecium*) T GGTGCATTGC TACGTGG			1174 602 1122	

The sequence numbering refers to the *Enterococcus faecium* tuf gene fragments (SEQ ID NO. 608).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
Dots indicate gaps in the sequences displayed.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.

Annex XIX: Strategy for the selection of primers for the identification of platelets contaminants from tuf sequences.

	467 495	689 717	SEQ ID NO.:	Accession #:
B. cereus	GTA ACTGGTGTaG AGATGTTCCG TAAACT...	C AGTTCTACTT CCGTACAACT GACGTAAC	7	—
B. subtilis	GTT ACaGGTGTTG AAATGTTCCG TAAGCT...	C AGTTCTACTT CCGTACAACT GACGTAAC	—	Z99104
E. cloacae	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GACGTGAC	54	—
E. coli	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	78	—
K. oxytoca	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GACGTGAC	100	—
K. pneumoniae	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	103	—
P. aeruginosa	TGC ACcGGCGTTG AAATGTTCCG CAAGCT...	C AGTTCTACTT CCGTACCACK GACGTGAC	153	—
S. agalactiae	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	209	—
S. aureus	GTT ACaGGTGTTG AAATGTTCCG TAAATT...	C AATTCTATTT CCGTACTACT GACGTAAC	140[a]	—
S. choleraesuis	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACTACT GACGTGAC	159	—
S. epidermidis	GTT ACTGGTGTaG AAATGTTCCG TAAATT...	C AATTCTATTT CCGTACTACT GACGTAAC	611	—
S. marcescens	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACCACT GACGTGAC	168	—
S. mutans	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	224	—
S. pyogenes	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AATTCTACTT CCGTACAACT GACGTAAC	—	U40453
S. salivarius	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AGTTCTACTT CCGTACAACT GACGTAAC	146[a]	—
S. sanguinis	GTT ACTGGTGTTG AAATGTTCCG TAAACA...	C AGTTCTACTT CCGTACAACT GACGTTAC	227	—
Y. enterocolitica	TGT ACTGGCGTTG AAATGTTCCG CAAACT...	C AGTTCTACTT CCGTACAACT GAtGTAAC	235	—
Selected sequence for amplification primer	**ACTGGYGTTG AIATGTTCCG YAA**		636	
Selected sequence for amplification primer[b]		**TTCTAYTT CCGTACIACT GACGT**	637	

The sequence numbering refers to the *E. coli* tuf gene fragment (SEQ ID NO. 78).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.
[b]This sequence is the reverse-complement of the selected primer.

Annex XX: Strategy for the selection of the universal amplification primers from atpD sequences.

	616	657 781	812	SEQ ID NO.	ACCESSION #:
C. glutamicum	GTGTTCGGTC AGATGGATGA GCCACCAGGA GTCCGTATG	CGC...CGTATg	CCTTCCGCCG TGGGTTACCA GCCAAC	—	X76875
M. tuberculosis	GTATTCGGAC AGATGGACGA GCCGCCGGGC aCCCGTATG	CGT...CGGATg	CCGTCGGCCG TGGGATACCA GCCCAC	—	Z73419
E. faecalis	GTGTTCGGAC AAATGAACGA ACCACCAGGT GCTCGGATG	CGG...CGTATg	CCTTCTGCCG TTGGTTACCA ACCAAC	291	—
S. agalactiae	GTCTTTGGTC AAATGAATGA ACCACCAGGA GCACGTATG	CGT...CGTATg	CCTTCAGCCG TTGGTTATCA ACCAAC	380	—
B. subtilis	GTATTCGGAC AAATGAACGA GCCGCCGGGC GCACGTATG	CGT...CGTATg	CCTTCAGCGG TTGGTTATCA GCCGAC	—	Z28592
L. monocytogenes	GTATTCGGTC AAATGAACGA GCCACCAGGT GCGCGTATG	CGT...CGTATg	CCATCTGCGG TAGGTTACCA ACCAAC	324	—
S. aureus	GTATTCGGGC AAATGAATGA GCCACCTGGT GCACGTATG	CGT...CGTATg	CCTTCTGCAG TAGGTTACCA ACCAAC	366	—
A. baumannii	GTCTACGGTC AGATGAACGA GCCACCAGGT aaCCGTtTa	CGC...CGTATg	CCATCTGCGG TAGGTTACCA ACCTAC	243	—
N. gonorrhoeae	GTGTATGGCC AAATGAACGA ACCTCCAGGC aaCCGTcTG	CGC...CGTATg	CCTTCTGCAG TGGGTTACCA ACCGAC	—	Genome project
C. freundii	GTATATGGCC AGATGAACGA GCCGCCTGGA aaCCGTcTG	CGT...CGTATg	CCATCAGCGG TAGGCTACCA GCCGAC	264	—
E. cloacae	GTTTACGGCC AGATGAACGA GCCACCAGGA aaCCGTcTG	CGC...CGTATg	CCTTCAGCGG TAGGTTATCA GCCTAC	284	—
E. coli	GTGTATGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG	CGC...CGTATg	CCTTCAGCGG TAGGTTATCA GCCGAC	669	V00267
S. typhimurium	GTGTATGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG	CGC...CGTATg	CCTTCCGCAG TAGGTTACCA GCCGAC	351	—
K. pneumoniae	GTGTACGGCC AGATGAACGA GCCGCCGGGA aaCCGTcTG	CGC...CGTATg	CCTTCAGCGG TAGGTTATCA GCCGAC	317	—
S. marcescens	GTTTACGGCC AGATGAACGA GCCACCAGGT aaCCGTcTG	CGC...CGTATg	CCATCCGCGG TAGGTTATCA GCCAAC	357	—
Y. enterocolitica	GTTTATGGCC AAATGAATGA GCCACCAGGT aaCCGTcTG	CGC...CGTATg	CCATCTGCCG TAGGTTACCA GCCAAC	393	—

-continued

Annex XX: Strategy for the selection of the universal amplification primers from atpD sequences.

	616 ... 657	781 ... 812	SEQ ID NO.	ACCESSION #:
B. cepacia	GTGT**AC**GGCC **AGATGAACGA** GCCGCCGGGC aaCCGTcTG CGC...	...CGT**ATg** CCGTCGGCAG **TGGGCTATCA** GCCGAC	—	X76877
H. influenzae	GTTT**ATG**GTC **AAATGAACGA** GCCACCAGGT aaCCGTtTa CGT...	...CGT**ATg** CCATCCGCGG **TAGGTTACCA** ACCGAC	—	U32730
M. pneumoniae	GTGTTTGGTC **AGATGAACGA** ACCCCCAGGA GCACGGATG CGG...	...CGG**ATg** CCATCAGCCG **TGGGTTACCA** ACCAAC	—	U43738
H. pylori	TGC**TAT**GGGC **AAATGAATGA** GCCACCAGGT GCAAGGAat CGC...	...CGT**ATC** CCTTCAGCGG **TGGGGTATCA** GCCCAC	670	V00267
B. fragilis	GTGTTCGGAC **AGATGAACGA** ACCTCCTGGA GCACGTgct TCA...	...CGT**ATg** CCTTCTGCGG **TAGGTTATCA** ACCTAC	—	M22247
Selected sequences for universal primers	C **ARATGRAYGA** RCCICCIGGI GYIMGIATG TAYGGIC **ARATGAAYGA**RCCICCIGGI **AA**		562 564	
Selected sequences for universal primers[a]		**ATH CCITCIGCIG TIGGITAYCA RCC** **ATG CCITCIGCIG TIGGITAYCA RCC**	565 563	

The sequence numbering refers to the *Escherichia coli* atpD gene fragment (SEQ ID NO. 669).

Nucleotides in capitals are identical to the selected sequences or match those sequences.

Mismatches for SEQ ID NOs. 562 and 565 are indicated by lower-case letters.

Mismatches for SEQ ID NOs. 564 and 563 are indicated by underlined nucleotides.

Dots indicate gaps in the sequences displayed.

"R" "Y" "M" "K" "W" and "S" letters designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "H" stands for A, C or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a]These sequences are the reverse-complement of the selected primers.

Annex XXI: Specific and ubiquitous primers for nucleic acid amplification (recA sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Universal primers (recA)		
919	5'-GGI CCI GAR TCI TMI GGI AAR AC	918[a]	437-459
920[b]	5'-TCI CCV ATI TCI CCI TCI AIY TC	918[a]	701-723
921	5'-TIY RTI GAY GCI GAR CAI GC	918[a]	515-534
922[b]	5'-TAR AAY TTI ARI GCI YKI CCI CC	918[a]	872-894
	Sequencing primers (recA)		
1605	5'-ATY ATY GAA RTI TAY GCI CC	1704[a]	220-239
1606	5'-CCR AAC ATI AYI CCI ACT TTT TC	1704[a]	628-650
	Universal primers (rad51)		
935	5'-GGI AAR WSI CAR YTI TGY CAY AC	939[a]	568-590
936[b]	5'-TCI SIY TCI GGI ARR CAI GG	939[a]	1126-1145
	Universal primers (dmc1)		
937	5'-ATI ACI GAR GYI TTY GGI GAR TT	940[a]	1038-1060
938[b]	5'-CYI GTI GYI SWI GCR TGI GC	940[a]	1554-1573

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXII: Specific and ubiquitous primers for nucleic acid amplification (speA sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Streptococcus pyogenes*		
994	5'-TGG ACT AAC AAT CTC GCA AGA GG	993[a]	60-82
995[b]	5'-ACA TTC TCG TGA GTA ACA GGG T	993[a]	173-194
996	5'-ACA AAT CAT GAA GGG AAT CAT TTA G	993[a]	400-424
997[b]	5'-CTA ATT CTT GAG CAG TTA CCA TT	993[a]	504-526
998	5'-GGA GGG GTA ACA AAT CAT GAA GG	993[a]	391-413
997[b]	5'-CTA ATT CTT GAG CAG TTA CCA TT	993[a]	504-526

[a]Sequence from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXIII: First strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from speA sequences.

ACCESSION #	57 85 170 197	SEQ ID NO.:
speA X61573	CCTT GGgCTAACAA cCTCaCAAGA aGTAT...GTGAtCCT.GT cgtTCAtGAG AATGTAAA	—
speA AF029051	~~~~ GGgCTAACAA cCTCaCAAGA aGTAT...GTGAtCCT.GT cgtTCAtGAG AATGTAAA	—
speA X61571	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61570	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61568	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61569	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61572	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61560	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA U40453	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	993
speA X61554	TCTT GGACTAACAA TCTtGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61557	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61559	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61558	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61556	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61555	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61560	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61561	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61566	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61567	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61562	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61563	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61564	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X61565	TCTT GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA AF055698	~~~~ GGACTAACAA TCTCGCAAGA GGTAT...GTGACCCT.GT TACTCACGAG AATGTGAA	—
speA X03929[a]	TCTT GGACTAACAA TCTtGCcAaA aGGTA...GTGACCCTGGT TACTCACGAG AATGTGAA	—
Selected sequence for species-specific primer	T GGACTAACAA TCTCGCAAGA GG	994
Selected sequence for species-specific primer[b]	ACCCT.GT TACTCACGAG AATGT	995

The sequence numbering refers to the *Streptococcus pyogenes* speA gene fragment (SEQ ID NO. 993).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
"~" indicate incomplete sequence data.
Dots indicate gaps in the sequences displayed.
[a]The extra G nucleotide introducing a gap in the sequence is probably a sequencing error.
[b]This sequence is the reverse-complement of the selected primer.

Annex XXIV: Second strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from speA sequences.

	Accession #	388 427 501 529	SEQ ID NO.:
speA	X61573	TA TGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	AF029051	TA TGGAGGGG**TA ACAAATCATG** AAGGGAATCA TTTAGAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61571	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AAAAATGGT AACTGCTCAA GAATTAGACT	–
speA	X61570	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61568	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61569	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61572	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61560	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	U40453	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	993
speA	X61554	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61557	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61559	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61558	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61556	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61555	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61560	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61561	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61566	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61567	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61562	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61563	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61564	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X61565	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	AF055698	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**GACT	–
speA	X03929	TA CGGAGGGG**TA ACAAATCATG AAGGGAATCA TTTA**GAAA...AA**AAAT**GGT **AACTGCTCAA GAATTA**G.CT	–
Selected sequences for species-specific primers		GGAGGGG**TA ACAAATCATG AAGG**	998
		ACAAATCATG AAGGGAATCA TTTAG	996
Selected sequence for species-specific primer[a]		**AAT**GGT **AACTGCTCAA GAATTA**G	997

The sequence numbering refers to the *Streptococcus pyogenes* speA gene fragment (SEQ ID NO. 993).
Dots indicate gaps in the sequences displayed.
[a]This sequence is the reverse-complement of the selected primer.

Annex XXV: Strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from tuf sequences.

	140 186 619 647	SEQ ID NO.:
S. anginosus	A AGTTGACtTg GTTGACGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cAtCCACACA CTAAATT	211
S. bovis	A AGTTGACCTT GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC cACCCACACA CTAAATT	212
S. dysgalactiae	A AATTGACCTT GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	217
S. pyogenes	A AGTTGACCTT GTTGATGACG AAGAGTTGCT TGAATTAGTT GAGATG...CC AAGTTCAATC AACCCACABCA CTAAATT	1002
S. agalactiae	A AGTTGACCTT GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	144[a]
S. oralis	A AATTGACtTg GTAGAcGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	985
S. pneumoniae	A AGTTGACtTg GTTGAcGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	145[a]
S. cristatus	A GATCGACtTg GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	215
S. mitis	A GATCGACtTg GTTGATGACG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	982
S. gordonii	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAGTTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	200
S. sanguinis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	227
S. parasanguinis	A AGTTGACtTg GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATC AACCCACABCA CTAAATT	225
S. salivarius	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC TgGTTCAATC AACCCACABCA CTAAATT	146[a]
S. vestibularis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCTTGAATTgGTT GAaATG.. CC TgGTTCAATC AACCCACABCA CTAAATT	231
S. suis	A AGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAgTTgGTT GAaATG...CC AgGTTCtATC AACCCACABCA CTAAATT	229
S. mutans	A AGTTGAttTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cACCCACACA CTAAATT	224
S. ratti	A GGTTGACtTg GTTGATGAtG AAGAa TTGCT TGAATTgGTT GAaATG...CC AgGTTCAATt cAtCCgCAcA CTAAATT	226

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Annex XXV: Strategy for the selection of *Streptococcus pyogenes*-specific amplification primers from tuf sequences.

	140 186 619 647	SEQ ID NO.:
S. macacae	A AGTTGACtTa GTTGATGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC AgGATCAATt cAtCCACAcA CTAAATT	222
S. cricetus	A GGTTGACtTg GTTGAcGAtG AAGAaTTGCT TGAATTgGTT GAaATG...CC TgGTTCAATC cAtCCACACA CTAAATT	214
E. faecalis	A AATgGAtaTg GTTGATGACG AAGAaTTatT aGAATTAGTa GAaATG...CC AgcTaCAATC ActCCACACA CaAAATT	607
S. aureus	A AGTTGACaTg GTTGAcGAtG AAGAaTTatT aGAATTAGTa GAaATG...CC TgGTTCAATt AcaCCACACA CTgAATT	176
B. cereus	A ATgcGACaTg GTaGATGACG AAGAaTTatT aGAATTAGTa GAaATG...AG CgGTTCtgTa AAagCtCACg CTAAATT	7
E. coli	A ATgcGACaTg GTTGATGACG AAGAGcTGCT gGAAcTgGTT GAaATG...CC GgGCaCcATC AAgCCgCACA CcAAGTT	78
Selected sequences for species-specific primers	TTGACCTT GTTGATGACG AAGAG	999
	AAGAGTTGCT TGAATTAGTT GAG	1001
Selected sequence for species-specific primer [b]	AGTTCAATC AACCCACABCA CTAA	1000

The sequence numbering refers to the *Streptococcus pyogenes* tuf gene fragment (SEQ ID NO. 1002).
Nucleotides in capitals are identical to the selected sequences or match those sequences.
Mismatches are indicated by lower-case letters.
Dots indicate gaps in the sequences displayed.
[a]The SEQ ID NO. refers to previous patent publication WO98/20157.
[b]This sequence is the reverse-complement of the selected primer.

Annex XXVI: Strategy for the selection stx_1-specific amplification primers and hybridization probe.

	Accession #	230 263 343 375 391 421	SEQ ID NO.:
stx_1	M19473a	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	M16625	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	M17358	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	Z36900	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTaGTGA CAGTAGCTAT ACCA	—
stx_1	L04539	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	M19437	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	M24352	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	X07903	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	Z36899	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	—
stx_1	Z36901	TTGATGTC AGAGGGATAG ATCCAGAGGA AGGGCG...TATCG CTTTGCTGAT TTTTCACATG TTACCTTT...GTTACAT TGTCTGGTGA CAGTAGCTAT ACCA	1076
stx_2	X61283	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	L11079	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	M21534	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	M36727	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X81415	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X81416	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X81417	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X81418	TAGgTaTa cGAGGGcTtG ATgtttAtcA gGaGCG...TACaG aTTTtCaGAT TTTgCACATa TatCaTTG...ATTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	E03962	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	E03959	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—

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Annex XXVI: Strategy for the selection stx_1-specific amplification primers and hybridization probe.

	Accession #	230 263	343 375	391 421	SEQ ID NO.:
stx_2	X07865	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	Y10775	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	Z37725	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	1077
stx_2	Z50754	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X67514	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	L11078	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	X65949	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACCG tTTTtCaGAT TTTaCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
stx_2	AF043627	TGGATaTa cGAGGGcTtG ATgtctAtcA gGcGCG...	TACaG aTTTtCaGAT TTTgCACATa TatCaGTG...	GTTtCca TGaCaacgGA CAGcAGtTAT ACCA	—
Selected sequence for amplification primer		ATGTC AGAGGGATAG ATCCAGAGGA AGG			1081
Selected sequence for hybridization probe			CG CTTTGCTGAT TTTTCACATG TTACC		1084
Selected sequence for amplification primer[a]				ACAT TGTCTGGTGA CAGTAGCTAT A	1080

The sequence numbering refers to the *Escherichia coli* stx_1 gene fragment (SEQ ID NO. 1076). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a]This sequence is the reverse-complement of the selected primer.

Annex XXVII: Strategy for the selection of stx_2-specific amplification primers and hybridization probe.

	Accession #	543 570 614 641 684 708	SEQ ID NO.:
stx_1	M19473	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gCGTcCTgCC tGAC	—
stx_1	M16625	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gCGTcCTgCC tGAC	—
stx_1	M17358	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gCGTcCTgCC tGAC	—
stx_1	Z36900	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAT	—
stx_1	L04539	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAT	—
stx_1	M19437	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAC	—
stx_1	M24352	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAC	—
stx_1	X07903	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAC	—
stx_1	Z36899	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTGgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAC	—
stx_1	Z36901	AGCga TgtTaCGgTT TGTtACTGTG ACA...CAAC ACTtgaTGAt ctcAgTGggC gTtcTTA...A AGgtTgAGtA gTGTcCTgCC tGAC	1076
stx_2	X61283	AGCAG TTCTGCGTTT TGTCACTGTC ACA...AGGC ACTGTCTGA. ..AACTGCTC CTGTGTA...G CGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	L11079	AGCAG TTCTGCGTTT TGTCACTGTC ACA...AGGC ACTGTCTGA. ..AACTGCTC CTGTGTA...G CGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	M21534	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	M36727	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	U72191	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	X81415	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	X81416	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	X81417	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	X81418	AGCAG TTCTGCGTTT TGTCACTGTC ACA...TGGC ACTGTCTGA. ..AACTGCTC CTGTTTA...G AGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	E03962	AGCAG TTCTGCGTTT TGTCACTGTC ACA...AGGC ACTGTCTGA. ..AACTGCTC CTGTGTA...G CGAATCAGCA ATGTGCTTCC GGAG	—
stx_2	E03959	AGCAG TTCTGCGTTT TGTCACTGTC ACA...AGGC ACTGTCTGA. ..AACTGCTC CTGTGTA...G CGAATCAGCA ATGTGCTTCC GGAG	—

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Annex XXVII: Strategy for the selection of stx_2-specific amplification primers and hybridization probe.

	Accession #	543	570 614	641 684	708	SEQ ID NO.:
stx_2	X07865	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	CG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	Y10775	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	CG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	Z37725	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	CG**AATCAGCA** **ATGTGCTTCC** GGAG	1077
stx_2	Z50754	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	CG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	X67514	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	CG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	L11078	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	AG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	X65949	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...AGGC **A**CTGTCT**GA**.	..**AA**CTGCTC **CTGT**GTA...G	AG**AATCAGCA** **ATGTGCTTCC** GGAG	—
stx_2	AF043627	AGC**AG** **TTCTGCGTTT** **TGTCACTGTC**	ACA...TGGC **ACTGTCTGA**.	..**AA**CTGCTC **CTGT**TTA...G	AG**AATCAGCA** **ATGTGCTTCC** GGAG	—
Selected sequence for amplification primer		**AG** **TTCTGCGTTT** TGTCACTGTC				1078
Selected sequence for hybridization probe			C ACTGTCTGA.	..AACTGCTC CTGT		1085
Selected sequence for amplification primer[a]					**AATCAGCA** **ATGTGCTTCC** **G**	1079

The sequence numbering refers to the *Escherichia coli* stx_2 gene fragment (SEQ ID NO. 1077). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.

[a]This sequence is the reverse-complement of the selected primer.

Annex XXVIII: Strategy for the selection of vanA-specific amplification primers from van sequences.

	Accession #	926 952 1230 1255	SEQ ID NO.:
vanA	X56895	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1139
vanA	M97297	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1141
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1051
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1052
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1053
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1054
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1055
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1056
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1057
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1049
vanA	—	GTCAAT AGCGCGGACG AATTGGACTA C...GT AGAGGTCTAG CCCGTGTGGA TATG	1050
vanB	U94526	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	1117
vanB	U94527	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	—
vanB	U94528	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	U94529	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	U94530	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	Z83305	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	U81452	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	U35369	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	—
vanB	U72704	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	L06138	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	—
vanB	L15304	GTAAAc gGtaCGGAaG AAcTtaACGC T...GC AGAGGgCTtG CCCGTGTtGA TCTT	—
vanB	U00456	GTAAAc AGtaCGGAaG AAcTaaACGC T...GC AGAGGgCTtG CtCGTGTtGA TCTT	—
vanD	AF130997	GTAtgc AagGCaGAaG AAcTGcAgGC A...GC AGAGGatTgG CCCGcaTtGA cCTG	—
vanE	AF136925	GTAgAa caaaaaagtG AtTTatAtAA A...GC AaAGGatTAG CgaGaaTcGA cTTT	—
Selected sequence for amplification primer		AAT AGCGCGGACG AATTGGAC	1090
Selected sequence for amplification primer[a]		GAGGTCTAG CCCGTGTGGA T	1089

The sequence numbering refers to the *Enterococcus faecium* vanA gene fragment (SEQ ID NO. 1139). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
[a]This sequence is the reverse-complement of the above selected primer.

Annex XXIX: Strategy for the selection of vanB-specific amplification primers from van sequences.

	Accession #	470 495 608 633	SEQ ID NO.:
vanA	X56895	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tAtTCATCAG GAA	1139

-continued

Annex XXIX: Strategy for the selection of vanB-specific amplification primers from van sequences.

	Accession #	470 495 608 633	SEQ ID NO.:
vanA	M97297	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1141
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1051
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1052
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1053
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1054
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1055
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1056
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1057
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1049
vanA	—	A CGCaATtGAA tCgGCAaGAC AATAT...ACG GaATCTTtCG tATtCATCAG GAA	1050
vanB	U94526	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	1117
vanB	U94527	C TGCGATAGAA GCAGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U94528	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U94529	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U94530	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	Z83305	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U81452	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U35369	C TGCGATAGAA GCAGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U72704	C TGCGATAGAA GCgGCAGGAC AATAT...ATG GTATCTTCCG CATCCATCAG GAA	—
vanB	L06138	C TGCGATAGAA GCAGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	L15304	C TGCGATAGAA GCgGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanB	U00456	C TGCGATAGAA GCAGCAGGAC AATAT...ACG GTATCTTCCG CATCCATCAG GAA	—
vanD	AF130997	C AGCaATcGAA GaAGCAaGAa AATAT...ACG GctTtTTaa gATtCATCAG GAA	—
vanE	AF136925	A AGCaATAGAc GaAGCttcAa AATAT...ATG GctTtTTCga CtatgAagAG AAA	—
Selected sequence for amplification primer		CGATAGAA GCAGCAGGAC AA	1095
Selected sequence for amplification primer[a]		GTATCTTCCG CATCCATCAG	1096

The sequence numbering refers to the *Enterococcus faecium* vanB gene fragment (SEQ ID NO. 1117). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
[a]This sequence is the reverse-complement of the above vanB sequence.

Annex XXX: Strategy for the selection of vanC-specific amplification primers from vanC sequences.

	Accession #	929 957 1064 1092	SEQ ID NO.:
vanC1	—	GT CGACGGTTTT TTTGATTTTG AAGAGAA...ACGGGTC TGGCTCGAAT CGATTTTTTC GT	1058
vanC1	—	GT CGACGGTTTT TTTGATTTTG AAGAGAA...ACGGGTC TGGCTCGAAT CGATTTTTTC GT	1059
vanC1	M75132	GT CGACGGTTTT TTTGATTTTG AAGAGAA...ACGGGTC TGGCTCGAAT CGATTTTTTC GT	1138
vanC2	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1060
vanC2	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1061
vanC2	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1062
vanC2	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1063
vanC2	L29638	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	—
vanC2	L29638	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	—
vanC3	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1064
vanC3	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	1065
vanC3	—	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGaC TTGCTCGCAT CGACTTTTTT GT	1066
vanC3	L29639	GT AGACGGCTTT TTCGATTTTG AAGAAAA...AAAGGTC TTGCTCGCAT CGACTTTTTT GT	—
Selected sequence for resistance primer		GACGGYTTT TTYGATTTTG AAGA	1101
Selected sequence for resistance primer[a]		GGTC TKGCTCGMAT CGAYTTTTT	1102

The sequence numbering refers to the vanC1 gene fragment (SEQ ID NO. 1138). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequence displayed. "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[a]This sequence is the reverse-complement of the selected sequence.

Annex XXXI: Strategy for the selection of Streptococcus pneumoniae-specific amplification primers and hybridization probes from pbp1a sequences.

	Accession #	453 505 678 706	SEQ ID NO.:
pbp1a	M90528	A TTGACTAcCC AAGCATaCAc TATGCtAAtG CtATTTCAAG TAATACAACC GA...TATATG ATGACaGAtA TGATGAAAAC CGT...	—
pbp1a	X67873	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGAAA TGATGAAAAC AGT...	—
pbp1a	AB006868	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC AGT...	—
pbp1a	AF046234	A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGAAA TGATGAAAAC TGT...	—
pbp1a		A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC TGT...	1014
pbp1a		A TCGACTAcCC AAGtATtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1017
pbp1a	AB006873	A TCGACTAcCC AAGtcTtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TATATG ATGACCGACA TGATGAAAAC AGT...	—
pbp1a	AF139883	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1169
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1004
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1007
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1008
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1009
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	1011
pbp1a	AF159448	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TATATG ATGACCGACA TGATGAAAAC AGT...	—
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1005
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1015
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1006
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	1012
pbp1a	X67867	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAATACAACA GA...TACATG ATGACCGAAA TGATGAAAAC TGT...	—
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1010
pbp1a	Z49094	A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	—
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1013
pbp1a		A TCGACTATCC AAGCATGCAT TATGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1016

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Annex XXXI: Strategy for the selection of Streptococcus pneumoniae-specific amplification primers and hybridization probes from pbp1a sequences.

pbp1a	X67870	A TCGACTATCC AAGtATGCAT TAcGCAAACG CCATTTCAAG TAAcACAACT GA...TATATG ATGACCGAAA TGATGAAAAC TGT...	—
pbp1a		A TTGACTATCC AAGtATtCAc TActCAAAtG CtATTTCAAG TAATACAACT GA...TATATG ATGACtGAAA TGATGAAAAC TGT...	1018
pbp1a	AJ002290	A TTGAtTAcCC AActATGgtc TATGCtAACG CtATTTCAAG TAATACAACT GA...TACATG ATGACtGAAA TGATGAAAAC AGT...	—
pbp1a	X67871	A TCGACTAcCC AAGtcTtCAc TActCAAAtG CCATTTCAAG TAAcACAACC GA...TACATG ATGACaGAAA TGATGAAAAC AGT...	—
Selected sequences for amplification primers		GACTATCC AAGCATGCAT TATG	1130
		ATG ATGACH-	1129
		GAMA TGATGAAAAC	
Selected sequence for hybridization probe		CAAACG CCATTTCAAG TAATACAAC	1197

The sequence numbering refers to the *Streptococcus pneumoniae* pbp1a gene fragment (SEQ ID NO. 1004). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dotes indicate gaps in the sequences displayed. "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "H" stands for A, C or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

	Accession #	756 783 813 840	SEQ ID SEQ ID NO.:
pbp1a	M90528	...GCTGGTAA aACtGGTACg TCTaACTATA...A ATACgGGTTA TGTAGCTCCG GAcGAAA	—
pbp1a	X67873	...GCTGGTAA aACAGGaACc TCTAACTATA...A CCtCTcaaTt TGTAGCaCCt GATGAAC	—
pbp1a	AB006868	...GCTGGTAA aACAGGaACc TCTAACTATA...A CCtCTcaaTt TGTAGCaCCt GAcGAAC	—
pbp1a	AF046234	...GCAGGTAA aACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	—
pbp1a		...GCAGGTAA aACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA	1014
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1017
pbp1a	AB006873	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA	—
pbp1a	AF139883	...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1169
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1004
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1007
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA	1008

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Annex XXXI: Strategy for the selection of Streptococcus pneumoniae-specific amplification primers and hybridization probes from pbp1a sequences.

pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1009
pbp1a		...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1011
pbp1a	AF159448	...GCTGGTAA aACAGGaACc TCTAACTATA...A ACACTGGCTA TGTAGCTCCA GATGAAA		—
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1005
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1015
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1006
pbp1a		...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA		1012
pbp1a	X67867	...GCTGGTAA GACAGGTACT TCTAACTACA...A ACACTGGCTA TGTAGCTCCA GATGAAA		—
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA		1010
pbp1a	Z49094	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA		—
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA		1013
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA		1016
pbp1a	X67870	...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA		—
pbp1a		...GCAGGTAA GACAGGTACT TCTAACTATA...A ACACTGGCTA CGTAGCTCCA GATGAAA		1018
pbp1a	AJ002290	...GCAGGTAA GACgGGTACa TCTAACTACA...A ACACTGGCTA C~~~~~~~~~ ~~~~~~~		—
pbp1a	X67871	...GCTGGTAA aACAGGTACc TCTAACTATA...A ACACTGGTTA CGTAGCTCCA GATGAAA		—
Selected sequence for hybridization probe		GGTAA GACAGGTACT TCTAACT	1193	
Selected sequence for amplification primer[a]		ACTGGYTA YGTAGCTCCA GATG	1131	

The sequence numbering refers to the *Streptococcus pneumoniae* pbp1a gene fragment (SEQ ID NO. 1004).
Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
"~" indicates incomplete sequence data.
"R" "Y" "W" and "S" designate nucleotide positions which are degenerated.
"R" stands for A or G; "Y" stands for C or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.
[a]This sequence is the reverse-complement of the selected primer.

Annex XXXII: Specific and ubiquitous primers for nucleic acid amplification (toxin sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Toxin gene: cdtA		
2123	5'-TCT ACC ACT GAA GCA TTA C	2129[a]	442-460
2124[b]	5'-TAG GTA CTG TAG GTT TAT TG	2129[a]	580-599
	Toxin gene: cdtB		
2126	5'-ATA TCA GAG ACT GAT GAG	2130[a]	2665-2682
2127[b]	5'-TAG CAT ATT CAG AGA ATA TTG T	2130[a]	2746-2767
	Toxin gene: stx_1		
1081	5'-ATG TCA GAG GGA TAG ATC CA	1076[a]	233-252
1080[b]	5'-TAT AGC TAC TGT CAC CAG ACA ATG T	1076[a]	394-418
	Toxin gene: stx_2		
1078	5'-AGT TCT GCG TTT TGT CAC TGT C	1077[a]	546-567
1079[b]	5'-CGG AAG CAC ATT GCT GAT T	1077[a]	687-705
	Toxin genes: stx_1, and stx_2		
1082	5'-TTG ARC RAA ATA ATT TAT ATG TG	1076[a]	278-300
1083[b]	5'-TGA TGA TGR CAA TTC AGT AT	1076[a]	781-800

[a]Sequences from databases.

[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXXIII: Molecular beacon internal hybridization probes for specific detection of toxin sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Toxin gene: cdtA		
2125[b]	5'-CAC GCG GAT TTT GAA TCT CTT CCT CTA GTA GCG CGT G	2129[c]	462-488
	Toxin gene: cdtB		
2128	5'-CAA CGC TGG AGA ATC TAT ATT TGT AGA AAC TGC GTT G	2130[c]	2714-2740
	Toxin gene: stx_1		
1084	5'-CCA CGC CGC TTT GCT GAT TTT TCA CAT GTT ACC GCG TGG	1076[c]	337-363
2012[d]	5'-CCG CGG ATT ATT AAA CCG CCC TTC CGC GG-MR-HEG-ATG TCA GAG GGA TAG ATC CA	1076[c]	248-264
	Toxin gene: stx_2		
1085	5'-CCA CGC CAC TGT CTG AAA CTG CTC CTG TG CGT GG	1077[c]	617-638

[a]Underlined nucleotides indicate the molecular beacon's stem.

[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[c]Sequences from databases.

[d]Scorpion primer.

Annex XXXIV: Specific and ubiquitous primers for nucleic acid amplification (van sequences).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Resistance gene: vanA		
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1087[c]	5'-CTC ACA GCC CGA AAC AGC CT	1049-1057[a]	699-718[b]
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1088[c]	5'-TGC CGT TTC CTG TAT CCG TC	1049-1057[a]	885-904[b]
1086	5'-CTA CTC CCG CCT TTT GGG TT	1049-1057[a]	513-532[b]
1089[c]	5'-ATC CAC ACG GGC TAG ACC TC	1049-1057[a]	933-952[b]
1090	5'-AAT AGC GCG GAC GAA TTG GAC	1049-1057[a]	629-649[b]
1091[c]	5'-AAC GCG GCA CTG TTT CCC AA	1049-1057[a]	734-753[b]
1090	5'-AAT AGC GCG GAC GAA TTG GAC	1049-1057[a]	629-649[b]
1089[c]	5'-ATC CAC ACG GGC TAG ACC TC	1049-1057[a]	933-952[b]
1092	5'-TCG GCA AGA CAA TAT GAC AGC	1049-1057[a]	662-682[b]
1088[c]	5'-TGC CGT TTC CTG TAT CCG TC	1049-1057[a]	885-904[b]
	Resistance gene: vanB		
1095	5'-CGA TAG AAG CAG CAG GAC AA	1117[d]	473-492
1096[c]	5'-CTG ATG GAT GCG GAA GAT AC	1117[d]	611-630
	Resistance genes: vanA, vanB		
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057, 1117[a]	437-456[b]
1113[c]	5'-ACC GAC CTC ACA GCC CGA AA	1049-1057, 1117[a]	705-724[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057, 1117[a]	437-456[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-837[b]
1115	5'-TTT CGG GCT GTG AGG TCG GBT GHG CG	1049-1057, 1117[a]	705-730[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-837[b]
1116	5'-TTT CGG GCT GTG AGG TCG GBT GHG CGG	1049-1057, 1117[a]	705-731[b]
1114[c]	5'-TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-837[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057, 1117[a]	437-456[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-840[b]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the vanA sequence fragment (SEQ ID NO. 1051).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]Sequences from databases.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
1115	5'-TTT CGG GCT GTG AGG TCG GBT GHG CG	1049-1057, 1117[a]	705-730[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-840[b]

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1116	5'-TTT CGG GCT GTG AGG TCG GBT GHG CGG	1049-1057, 1117[a]	705-731[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-840[b]
1119	5'-TTT CGG GCT GTG AGG TCG GBT GHG C	1049-1057, 1117[a]	705-729[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-840[b]
1120	5'-TTT CGG GCT GTG AGG TCG GBT GHG	1049-1057, 1117[a]	705-728[b]
1118[c]	5'-TTT TCW GAG CCT TTT TCC GGC TCG	1049-1057, 1117[a]	817-840[b]
1121	5'-TGT TTG WAT TGT CYG GYA TCC C	1049-1057, 1117[a]	408-429[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057, 1117[a]	806-830[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057, 1117[a]	437-456[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057, 1117[a]	806-830[b]
1123	5'-TTT CGG GCT GTG AGG TCG GBT G	1049-1057, 1117[a]	705-726[b]
1111[c]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1049-1057, 1117[a]	806-830[b]
1112	5'-GGC TGY GAT ATT CAA AGC TC	1049-1057, 1117[a]	437-456[b]
1124[c]	5'-GAT TTG RTC CAC YTC GCC RAC A	1049-1057, 1117[a]	757-778[b]
	Resistance gene: vanC1		
1103	5'-ATC CCG CTA TGA AAA CGA TC	1058-1059[a]	519-538[d]
1104[c]	5'-GGA TCA ACA CAG TAG AAC CG	1058-1059[a]	678-697[d]
	Resistance genes: vanC1, vanC2, vanC3		
1097	5'-TCY TCA AAA GGG ATC ACW AAA GTM AC	1058-1066[a]	607-632[d]
1098[c]	5'-TCT TCA AAA TCG AAA AAG CCG TC	1058-1066[a]	787-809[d]
1099	5'-TCA AAA GGG ATC ACW AAA GTM AC	1058-1066[a]	610-632[d]
1100[c]	5'-GTA AAK CCC GGC ATR GTR TTG ATT TC	1058-1066[a]	976-1001[d]
1101	5'-GAC GGY TTT TTY GAT TTT GAA GA	1058-1066[a]	787-809[d]
1102[c]	5'-AAA AAR TCG ATK CGA GCM AGA CC	1058-1066[a]	922-944[d]
	Resistance genes: vanC2, vanC3		
1105	5'-CTC CTA CGA TTC TCT TGA YAA ATC A	1060-1066, 1140[a]	487-511[e]
1106[c]	5'-CAA CCG ATC TCA ACA CCG GCA AT	1060-1066, 1140[a]	690-712[e]

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the vanA sequence fragment (SEQ ID NO. 1051).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]The nucleotide positions refer to the vanC1 sequence fragment (SEQ ID NO. 1058).
[e]The nucleotide positions refer to the vanC2 sequence fragment (SEQ ID NO. 1140).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
	Resistance gene: vanD		
1591	5'-ATG AGG TAA TAG AAC GGA TT	1594	797-837
1592[b]	5'-CAG TAT TTC AGT AAG CGT AAA	1594	979-999

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Resistance gene: vanE			
1595	5'-AAA TAA TGC TCC ATC AAT TTG CTG A	1599[a]	74-98
1596[b]	5'-ATA GTC GAA AAA GCC ATC CAC AAG	1599[a]	394-417
1597	5'-GAT GAA TTT GCG AAA ATA CAT GGA	1599[a]	163-186
1598[b]	5'-CAG CCA ATT TCT ACC CCT TTC AC	1599[a]	319-341
Sequencing primers (vanAB)			
1112	5'-GGC TGY GAT ATT CAA AGC TC	1139[a]	737-756
1111[b]	5'-CTT TTT CCG GCT CGW YTT CCT GAT G	1139[a]	1106-1130
Sequencing primers (vanA, vanX, vanY)			
1150	5'-TGA TAA TCA CAC CGC ATA CG	1141[a]	860-879
1151[b]	5'-TGC TGT CAT ATT GTC TTG CC	1141[a]	1549-1568
1152	5'-ATA AAG ATG ATA GGC CGG TG	1141[a]	1422-1441
1153[b]	5'-CTC GTA TGT CCC TAC AAT GC	1141[a]	2114-2133
1154	5'-GTT TGA AGC ATA TAG CCT CG	1141[a]	2520-2539
1155[b]	5'-CAG TGC TTC ATT AAC GTA GTC	1141[a]	3089-3109
Sequencing primers (vanC1)			
1110	5'-ACG AGA AAG ACA ACA GGA AGA CC	1138[a]	122-144
1109[b]	5'-ACA TCG TGA TCG CTA AAA GGA GC	1138[a]	1315-1337
Sequencing primers (vanC2, vanC3)			
1108	5'-GTA AGA ATC GGA AAA GCG GAA GG	1140[a]	1-23
1107[b]	5'-CTC ATT TGA CTT CCT CCT TTG CT	1140[a]	1064-1086

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XXXV: Internal hybridization probes for specific detection of van sequences.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Resistance gene: vanA			
1170	5'-ACG AAT TGG ACT ACG CAA TT	1049-1057[a]	639-658[b]
2292	5'-GAA TCG GCA AGA CAA TAT G	2293[c]	583-601
Resistance gene: vanB			
1171	5'-ACG AGG ATG ATT TGA TTG TC	1117[c]	560-579
2294	5'-AAA CGA GGA TGA TTT GAT TG	2296[a]	660-679
2295	5'-TTG AGC AAG CGA TTT CGG	2296[a]	614-631

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Annex XXXV: Internal hybridization probes for specific detection of van sequences.			
		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Resistance gene: vanD			
2297	5'-TTC AGG AGG GGG ATC GC	1594[c]	458-474

[a]These sequences were aligned to derive the corresponding primer.

[b]The nucleotide positions refer to the vanA sequence fragment (SEQ ID NO. 1051).

[c]Sequences from databases.

Annex XXXVI: Specific and ubiquitous primers for nucleic acid amplification (pbp sequences).			
		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence	SEQ ID NO.	Nucleotide position
Resistance gene: pbp1a			
1129	5'-ATG ATG ACH GAM ATG ATG AAA AC	1004-1018[a]	681-703[b]
1131[c]	5'-CAT CTG GAG CTA CRT ARC CAG T	1004-1018[a]	816-837[b]
1130	5'-GAC TAT CCA AGC ATG CAT TAT G	1004-1018[a]	456-477[b]
1131	5'-CAT CTG GAG CTA CRT ARC CAG T	1004-1018[a]	816-837[b]
2015	5'-CCA AGA AGC TCA AAA ACA TCT G	2047[d]	909-930
2016[c]	5'-TAD CCT GTC CAW ACA GCC AT	2047[d]	1777-1796
Sequencing primers (pbp1a)			
1125	5'-ACT CAC AAC TGG GAT GGA TG	1169[d]	873-892
1126[c]	5'-TTA TGG TTG TGC TGG TTG AGG	1169[d]	2140-2160
1125	5'-ACT CAC AAC TGG GAT GGA TG	1169[d]	873-892
1128[c]	5'-GAC GAC YTT ATK GAT ATA CA	1169[d]	1499-1518
1127	5'-KCA AAY GCC ATT TCA AGT AA	1169[d]	1384-1403
1126[c]	5'-TTA TGG TTG TGC TGG TTG AGG	1169[d]	2140-2160
Sequencing primers (pbp2b)			
1142	5'-GAT CCT CTA AAT GAT TCT CAG GTG G	1172[d]	1-25
1143[c]	5'-CAA TTA GCT TAG CAA TAG GTG TTG G	1172[d]	1481-1505
1142	5'-GAT CCT CTA AAT GAT TCT CAG GTG G	1172[d]	1-25
1145[c]	5'-AAC ATA TTK GGT TGA TAG GT	1172[d]	793-812
1144	5'-TGT YTT CCA AGG TTC AGC TC	1172[d]	657-676
1143[c]	5'-CAA TTA GCT TAG CAA TAG GTG TTG G	1172[d]	1481-1505

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Annex XXXVI: Specific and ubiquitous primers for nucleic acid amplification (pbp sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Sequencing primers (pbp2x)		
1146	5'-GGG ATT ACC TAT GCC AAT ATG AT	1173[d]	219-241
1147[c]	5'-AGC TGT GTT AGC VCG AAC ATC TTG	1173[d]	1938-1961
1146	5'-GGG ATT ACC TAT GCC AAT ATG AT	1173[d]	219-241
1149[c]	5'-TCC YAC WAT TTC TTT TTG WG	1173[d]	1231-1250
1148	5'-GAC TTT GTT TGG CGT GAT AT	1173[d]	711-730
1147[c]	5'-AGC TGT GTT AGC VCG AAC ATC TTG	1173[d]	1938-1961

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the pbp1a sequence fragment (SEQ ID NO. 1004).
[c]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[d]Sequences from databases.

Annex XXXVII: Internal hybridization probes for specific detection of pbp sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: pbp1a		
1132	5'-AGT GAA AAR ATG GCT GCT GC	1004-1018[a]	531-550[b]
1133	5'-CAT CAA GAA CAC TGG CTA YGT AG	1004-1018[a]	806-828[b]
1134	5'-CTA GAT AGA GCT AAA ACC TTC CT	1004-1018[a]	417-439[b]
1135	5'-CAT TAT GCA AAC GCC ATT TCA AG	1004-1018[a]	471-493[b]
1192	5'-GGT AAA ACA GGA ACC TCT AAC T	1004-1018[a]	759-780[b]
1193	5'-GGT AAG ACA GGT ACT TCT AAC T	1004-1018[a]	759-780[b]
1194	5'-CAT TTC AAG TAA TAC AAC AGA ATC	1004-1018[a]	485-508[b]
1195	5'-CAT TTC AAG TAA CAC AAC TGA ATC	1004-1018[a]	485-508[b]
1196	5'-GCC ATT TCA AGT AAT ACA ACA GAA	1004-1018[a]	483-506[b]
1197	5'-CAA ACG CCA TTT CAA GTA ATA CAA C	1004-1018[a]	478-502[b]
1094	5'-GGT AAA ACA GGT ACT TCT AAC TA	1004-1018[a]	759-781[b]
1214	5'-GGT AAA ACA GGT ACC TCT AAC TA	1004-1018[a]	759-781[b]
1216	5'-GGT AAG ACT GGT ACA TCA AAC TA	1004-1018[a]	759-781[b]
1217	5'-CAA ATG CCA TTT CAA GTA ACA CAA C	1004-1018[a]	478-502[b]
1218	5'-CAA ACG CCA TTT CAA GTA ACA CAA C	1004-1018[a]	478-502[b]
1219	5'-CAA ATG CTA TTT CAA GTA ATA CAA C	1004-1018[a]	478-502[b]

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Annex XXXVII: Internal hybridization probes for specific detection of pbp sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
1220	5'-CAA ACG CCA TTT CAA GTA ATA CGA C	1004-1018[a]	478-502[b]
2017	5'-ACT TTG AAT AAG GTC GGT CTA G	2047[c]	1306-1327
2018	5'-ACA CTA AAC AAG GTT GGT TTA G	2063	354-375
2019	5'-ACA CTA AAC AAG GTC GGT CTA G	2064	346-367
2020	5'-GTA GCT CCA GAT GAA ATG TTT G	2140[c]	1732-1753
2021	5'-GTA GCT CCA GAC GAA ATG TTT G	2057	831-852
2022	5'-GTA GCT CCA GAT GAA ACG TTT G	2053[c]	805-826
2023	5'-GTA ACT CCA GAT GAA ATG TTT G	2056	819-840
2024	5'-AGT GAA AAG ATG GCT GCT GC	2048[c]	1438-1457
2025	5'-AGT GAG AAA ATG GCT GCT GC	2047[c]	1438-1457
2026	5'-TCC AAG CAT GCA TTA TGC AAA CG	2047[c]	1368-1390
2027	5'-TCG GTC TAG ATA GAG CTA AAA CG	2047[c]	1319-1341
2028	5'-TAT GCT CTT CAA CAA TCA CG	2047[c]	1267-1286
2029	5'-AGC CGT TGA GAC TTT GAA TAA G	2047[c]	1296-1317
2030	5'-CTT AAT GGT CTT GGT ATC G	2047[c]	1345-1366
2031	5'-CGT GAC TGG GGT TCT GCT ATG A	2049[c]	1096-1117
2032	5'-CGT GAC TGG GGA TCA TCA ATG A	2047[c]	1096-1117
2033	5'-CGT GAC TGG GGT TCT GCC ATG A	2057	195-216
2034	5'-ATC AAG AAC ACT GGC TAT GTA G	2050[c]	787-808
2035	5'-ATC AAG AAC ACT GGC TAC GTA G	2051[c]	787-808
2036	5'-ATC AAG AAC ACT GGT TAC GTA G	2047	1714-1735
2037	5'-ATC AAA AAT ACT GGT TAT GTA G	2057	813-834
2038	5'-ATC AAG AAT ACT GGC TAC GTA G	2052[c]	757-778
2039	5'-ATC AAA AAC ACT GGC TAT GTA G	2053[c]	787-808

[a]These sequences were aligned to derive the corresponding primer.
[b]The nucleotide positions refer to the pbp1a sequence fragment (SEQ ID NO. 1004).
[c]Sequence from databases.

Annex XXXVIII: Strategy for the selection of vanAB-specific amplification primers and vanA- and vanB-specific hybridization probes from van sequences.

	Accession #	734 759 936 961	SEQ ID NO.:
vanA	X56895	GTAGGC**T** GCG**ATAT**TC**A** **AAGCT**CAGC...CGGA**A**CG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1139
vanA	M97297	GTAGGC**T** GCG**ATAT**TC**A** **AAGCT**CAGC...CGGA**A**CG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1141

-continued

Annex XXXVIII: Strategy for the selection of vanAB-specific amplification primers and vanA- and vanB-specific hybridization probes from van sequences.

vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1051
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1052
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1053
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1054
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1055
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1056
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1057
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1049
vanA		GTAGGC**T** GCG**ATATTCA** **AAGCT**CAGC...CGGAACG**AATT** GGAC**TA**CGC**A** **ATT**GAA...	1050
vanB	U94526	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	1117
vanB	U94527	GTAGGC**T** GCG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **aaA**CgctGCg **ATa**GAA...	—
vanB	U94528	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	U94529	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	U94530	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	Z83305	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	U81452	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	U35369	GTAGGC**T** GCG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **aaA**CgctGCg **ATa**GAA...	—
vanB	U72704	GTGGGC**T** GCG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	L06138	GTAGGC**T** GCG**ATATTCA** **AAGCT**CCGC...CGGAaGAAcT aaACgctGCg ATaGAA...	—
vanB	L15304	GTGGGC**T** GTG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **taA**CgctGCg **ATa**GAA...	—
vanB	U00456	GTAGGC**T** GCG**ATATTCA** **AAGCT**CCGC...CGG**AaGAAcT** **aaA**CgctGCg **ATa**GAA...	—
vanD	AF130997	GTGGGaT GCG**ATATTCA** **AAGCT**CCGT...CAG**AaGAAcT** **GcA**ggcaGC**A** **AT**cGAA...	—
vanE	AF136925	GTAGGtT GTGgTATcgg AgctgCAGC...AAAgtGAtTT atAtaAaGC**A** **ATa**GAC...	—
Selected sequence for amplification primer		GGC**T** **GYGATATTCA** **AA**GCTC	1112
Selected sequence for hybridization probe		ACGAATT GGACTACGC**A** **ATT** (vanA)	1170

The sequence numbering refers to the *Enterococcus faecium* vanA gene fragment (SEQ ID NO. 1139).
Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
"R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

	Accession #	1038 1063 1103 1133	SEQ ID NO.:
vanA	X56895	GAAAC**agt** GccGcG**TTag** **TTGTt**GGC...ATT C**ATCAGGAAG** **TCGAGCCGGA** **AAAA**GGCT	1139
vanA	M97297	GAAAC**agt** GccGcg**TTag** **TTGTt**GGC...ATT C**ATCAGGAAG** **TCGAGCCGGA** **AAAA**GGCT	1141

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Annex XXXVIII: Strategy for the selection of vanAB-specific amplification primers and vanA- and vanB-specific hybridization probes from van sequences.

vanA		GAAACagt GccGcgTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1051
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1052
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1053
vanA		GAAACagt GccGcgTTag TTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1054
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1055
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1056
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1057
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1049
vanA		GAAACagt GccGcgTTag cTGTtGGC...ATT CATCAGGAAG TCGAGCCGGA AAAAGGCT	1050
vanB	U94526	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	1117
vanB	U94527	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U94528	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U94529	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U94530	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	Z83305	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U81452	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U35369	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U72704	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGAT	—
vanB	L06138	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	L15304	GGAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanB	U00456	GAAACGAG GATGATTTGA TTGTCGGC...ATC CATCAGGAAA ACGAGCCGGA AAAAGGCT	—
vanD	AF130997	GAAACGga aATGATcTcA TgGctGGC...ATT CATCAGGAAG cacAGCCGGA aAAGGGAT	—
vanE	AF136925	GGAA...t GAacAaTTGg TcGTtGGA...TAT gAagAGaAAt ACaA...... ......TT	—
Selected sequence for hybridization probe		ACGAG GATGATTTGA TTGTC (vanB)	1171
Selected sequence for amplification prime[a]		CATCAGGAAR WCGAGCCGGA AAAAG	1111

The sequence numbering refers to the *Enterococcus faecium* vanA gene fragment (SEQ ID NO. 1139).
Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed.
"R" and "W" designate nucleotide positions which are degenerated.
"R" stands for A or G; "W" stands for A or T

[a]This sequence is the reverse-complement of the above selected primer.

Annex XXXIX: Internal hybridization probe for specific detection of mecA.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: mecA		
1177	5'-GCT CAA CAA GTT CCA GAT TA	1178[a]	1313-1332

[a]Sequence from databases.

Annex XL: Specific and ubiquitous primers for nucleic acid amplification (hexA sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Streptococcus pneumoniae*		
1179	5'-ATT TGG TGA CGG GTG ACT TT	1183[a]	431-450
1181[b]	5'-AGC AGC TTA CTA GAT GCC GT	1183-1191[c]	652-671[d]
	Sequencing primers		
1179	5'-ATT TGG TGA CGG GTG ACT TT	1183[a]	431-450
1182[b]	5'-AAC TGC AAG AGA TCC TTT GG	1183[a]	1045-1064

[a]Sequences from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the hexA sequence fragment (SEQ ID NO. 1183).

Annex XLI: Internal hybridization probe for specific detection of hexA sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Streptococcus pneumoniae*		
1180[a]	5'-TCC ACC GTT GCC AAT CGC A	1183-1191[b]	629-647[c]

[a]This sequences is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[b]These sequences were aligned to derive the corresponding primer.
[c]The nucleotide positions refer to the hexA sequence fragment (SEQ ID NO. 1183).

Annex XLII: Strategy for the selection of *Streptococcus pneumoniae* species-specific amplification primers and hybridization probe from hexA sequences.

	428 453 626
S. pneumoniae	TGG **ATTTGGTGAC GGGTGACTTT** TAT...ATT**TG CGATTGGCAA CGGTGGA**GCA
S. pneumoniae	~~~ ~~~~~~**TGAC GGGTGACTTT** TAT...ATT**TG CGATTGGCAA CGGTGGA**GCA
S. pneumoniae	~~~ ~~~~~~**TGAC GGGTGACTTT** TAT...ATT**TG CGATTGGCAA CGGTGGA**GCA
S. pneumoniae	~~~ ~~~~~~**TGAC GGGTGACTTT** TAT...ATT**TG CGATTGGCAA CGGTGGA**GCA
S. pneumoniae	~~~ ~~~~~~**TGAC GGGTGACTTT** TAT...ATT**TG CGATTGGCAA CGGTGGA**GCA
S. oralis	~~~ ~~~~~~~~~~**GGGTGACTTT** TAT...ATCc**a CGAcTGGCAg CtGTGGA**GCA
S. mitis	~~~ ~~~~GGTGAC **GGGTGACTTT** TAT...ATTc**a CGATTGGCAg CtGTGGA**GCA
S. mitis	~~~ ~~~~~~**TGAC GGGTGACTTT** CAG...GCG**aG gagcTGtCtc CtaTGGA**GCG
S. mitis	~~~ ~~~~~~**TGAC GGGTGACTTT** CAG...GCG**aG gaAcTGtCtc CtaTGGA**GCG
Selected sequence for amplification primer	**ATTTGGTGAC GGGTGACTTT**
Selected sequences for amplification primers[a]	
Selected sequence for hybridization probe[a]	**TG CGATTGGCAA CGGTGGA**

	674 1042 1067	SEQ ID NO.:
S. pneumoniae	**AACGGCATCT AGTAAGCTGC T**CCA...AATCC**AAAG GATCTCTTGC AGTTGGC**	1183
S. pneumoniae	**AACGGCATCT AGTAAGCTGC T**CCA...AATCC**AAAG GATCTCTTG**~ ~~~~~~~	1184
S. pneumoniae	**AACGGCATCT AGTAAGCTGC T**CCA...AATCC**AAAG GATCTCT**~~~ ~~~~~~~	1185
S. pneumoniae	**AACGGCATCT AGTAAGCTGC T**CCA...AATCC**AAAG GATCTCTT**~~ ~~~~~~~	1186
S. pneumoniae	**AACGGCATCT AGTAAGCTGC T**CCG...AATCC**AAAG GATCTCTT**~~ ~~~~~~~	1187
S. oralis	Ag**CGGCAgCT AGTAAGCTcC T**CCA...~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~	1188
S. mitis	Ag**CGGCATCT AGTAAaCTGC T**TCA...AATCC**AAAG GATCTCTT**~~ ~~~~~~~	1189
S. mitis	**TcaGGCAgCa gGgAAaCTGC T**GGA...~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~	1190
S. mitis	**TcaGGCAgCg gGgAAatTGC T**AGA...AATCC**AAAG GATCTCTT**~~ ~~~~~~~	1191
Selected sequence for amplification primer		1179
Selected sequences for amplification primers[a]	**ACGGCATCT AGTAAGCTGC T** **CCAAAG GATCTCTTGC AGTT**	1181 1182
Selected sequence for hybridization probe[a]		1180

The sequence numbering refers to the *Streptococcus pneumoniae* hexA gene fragment (SEQ ID NO. 1183). Nucleotides in capitals are identical to the selected sequences or match those sequences. Mismatches are indicated by lower-case letters. Dots indicate gaps in the sequences displayed. "~" indicate incomplete sequence data.

[a]This sequence is the reverse-complement of the selected primer.

Annex XLIII: Specific and ubiquitous primers for nucleic acid amplification (pcp sequence).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: *Streptococcus pyogenes*			
1211	5'-ATT CTT GTA ACA GGC TTT GAT CCC	1215[a]	291-314
1210[b]	5'-ACC AGC TTG CCC AAT ACA AAG G	1215[a]	473-494

[a]Sequences from databases.

[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex XLIV: Specific and ubiquitous primers for nucleic acid amplification of *S. saprophyticus* sequences of unknown coding potential.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: *Staphylococcus saprophyticus*			
1208	5'-TCA AAA AGT TTT CTA AAA AAT TTA C	74, 1093, 1198[b]	169-193[c]
1209[a]	5'-ACG GGC GTC CAC AAA ATC AAT AGG A	74, 1093, 1198[b]	355-379[c]

[a]This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[b]These sequences were aligned to derive the corresponding primer.

[c]The nucleotide positions refer to the *S. saprophyticus* unknown gene sequence fragment (SEQ ID NO. 1198).

Annex XLV: Molecular beacon internal hybridization probes for specific detection of antimicrobial agents resistance gene sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance gene: gyrA			
2250	5'-CCG TCG GAT GGT GTC GTA TAC CGC GGA GTC GCC GAC GG	1954[b]	218-243
2251	5'-CGG AGC CGT TCT CGC TGC GTT ACA TGC TGG TGG CTC CG	1954[b]	259-286
Resistance gene: mecA			
1231	5'-GCG AGC CCG AAG ATA AAA AAG AAC CTC TGC TGC TCG C	1178[b]	1291-1315
Resistance gene: parC			
1938[b]	5'-CCG CGC ACC ATT GCT TCG TAC ACT GAG GAG TCT CCG CGC GG	1321[c]	232-260
1939	5'-CGA CCC GGA TGG TAG TAT CGA TAA TGA TCC GCC AGC GGC CGG GTC G	1321[c]	317-346
1955[b]	5'-CGC GCA ACC ATT GCT TCG TAC ACT GAG GAG TCT GCG CG	1321[c]	235-260
Resistance gene: vanA			
1239	5'-GCG AGC GCA GAC CTT TCA GCA GAG GAG GCT CGC	1051	860-880
1240	5'-GCG AGC CGG CAA GAC AAT ATG ACA GCA AAA TCG CTC GC	1051	663-688
Resistance gene: vanB			
1241	5'-GCG AGC GGG GAA CGA GGA TGA TTT GAT TGG CTC GC	1117	555-577

-continued

Annex XLV: Molecular beacon internal hybridization probes for specific detection of antimicrobial agents resistance gene sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: vanD		
1593	5'-CCG AGC GATTTA CCG GAT ACT TGG CTG ICG CTC GG	1594	835-845

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]Sequence from databases.

Annex XLVI: Molecular beacon internal hybridization probe for specific detection of *S. aureus* gene sequences of unknown coding potential.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *S. aureus*		
1232	5'-GGA GCC GCG CGA TTT TAT AAA TGA ATG TTG ATA ACC GGC TCC	1244	53-80

[a]Underlined nucleotides indicate the molecular beacon's stem.

Annex XLVII: Molecular beacon internal hybridization probes for specific detection of tuf sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Chlamydia pneumoniae*		
2091	5'-CGC GAC TTG AGA TGG AAC TTA GTG AGC TTC TTG GTC GCG	20	157-183
2092	5'-CGC GAC GAA AGA ACT TCC TGA AGG TCG TGC AGG TCC AG	20	491-516
	Bacterial species: *Chlamydia trachomatis*		
2213	5'-CGT GCC ATT GAC ATG ATT TCC GAA GAA GAC GCT GAA GGC ACG	1739[b]	412-441
	Bacterial species: *Enterococcus faecalis*		
1236	5'-GCG AGC CGT GGT GAA GTT CGC GTT GGT GGC TCG C	883	370-391

-continued

	Bacterial species: *Enterococcus faecium*		
1235	5'-GCG AGC CGA AGT TGA AGT TGT TGG TAT TGC TGG CTC GC	64	412-437
	Bacterial species: *Legionella pneumophila*		
2084[c]	5'-CAC GCG TCA ACA CCC GTA CAA GTC GTC TTT TGC GCG TG	112	461-486
	Bacterial species: *Mycoplasma pneumoniae*		
2096[c]	5'-CGC GAC CGG TAC CAC GGC CAG TAA TCG TGT CGC G	2097[b]	658-679
	Bacterial species: *Neisseria gonorrhoeae*		
2177	5'-GGC ACG GAC AAA CCA TTC CTG CTG CCT ATC GAA ACG TGT TCC CGT GCC	126	323-357
2178	5'-GGC ACG ACA AAC CAT TCC TGC TGC CTA TCG AAC GTG CC	126	323-348
2179	5'-GGC AGC TCT ACT TCC GTA CCA CTG ACG TAA CCG GCT GCC	126	692-718

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]Sequence from databases.
[c]This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence[a]	SEQ ID NO.	Nucleotide position
	Bacterial species: *Pseudomonas aeruginosa*		
2122	5'-CCG AGC GAA TGT AGG AGT CCA GGG TCT CTG CTC GG	153, 880, 2138[b,c]	280-302[d]
	Bacterial species: *Staphylococcus aureus*		
2186	5'-ACG CGC TCA AAG CAG AAG TAT ACG TAT TAT CAA AAG ACG CGC GT	1728	615-646
	Bacterial group: *Staphylococcus* sp. other than *S. aureus*		
1233	5'-GCG AGC GTT ACT GGT GTA GAA ATG TTC CGG CTC GC	878	372-394
	Fungal species: *Candida albicans*		
2073	5'-CCG AGC AAC ATG ATT GAA CCA TCC ACC AAC TGG CTC GG	408	404-429
	Fungal species: *Candida dubliniensis*		
2074	5'-CCG AGC AAC ATG ATT GAA GCT TCC ACC AAC TGG CTC GG	414	416-441
	Fungal species: *Candida glabrata*		
2110[b]	5'-GCG GGC CCT TAA CGA TTT CAG CGA ATC TGG ATT CAG CCC GC	417	307-335
2111	5'-GCG GGC ATG TTG AAG CCA CCA CCA ACG CTT CCT GGC CCG C	417	419-447

-continued

	Fungal species: *Candida krusei*		
2112[b]	5'-GCG GGC TTG ATG AAG TTT GGG TTT CCT TGA CAA TTG CCC GC	422	318-347
2113	5'-GCG GGC ACA AGG GTT GGA CTA AGG AAA CCA AGG CAG CCC GC	422	419-447
2114	5'-GCG GGC ATC GAT GCT ATT GAA CCA CCT GTC AGA CCG CCC GC	422	505-533

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]Sequence from databases.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the *P. aeruginosa* tuf sequence fragment (SEQ ID NO. 153).

		Originating DNA fragment	
SEQ ID NO.	Nucleotide sequence[a]	SEQ ID NO.	Nucleotide position
	Fungal species: *Candida lusitaniae*		
2115[b]	5'-GCG GGC GGT AAG TCC ACC GGT AAG ACC TTG TTG GCC CGC	424	304-330
2116	5'-GCG GGC GTA AGT CAC CGG TAA GAC CTT GTT GGC CCG C	424	476-502
2117	5'-GCG GGC GAC GCC ATT GAG CCA CCT TCG AGA GCC CGC	424	512-535
	Fungal species: *Candida parapsilosis*		
2118[b]	5'-GCG GGC TCC TTG ACA ATT TCT TCG TAT CTG TTC TTG GCC CGC	426	301-330
	Fungal species: *Candida tropicalis*		
2119	5'-GCG GGC TTA CAA CCC TAA GGC TGT TCC ATT CGT TGC CCG C	429	357-384
2120	5'-GCG GGC AGA AAC CAA GGC TGG TAA GGT TAC CGG AGC CCG C	429	459-487
	Fungal species: *Cryptococcus neoformans*		
2106	5'-GCG AGC AGA GCA CGC CCT CCT CGC CGC TCG C	623, 1985, 1986[c]	226-244[d]
2107	5'-GCG AGC TCC CCA TCT CTG GTT GGC ACG CTC GC	623, 1985, 1986[c]	390-408[d]
	Bacterial genus: *Legionella* sp.		
2083	5'-CCG CCG ATG TTC CGT AAA TTA CTT GAI GAA GGT CGA GCC GGC GG	111-112[d]	488-519[e]

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]This sequence is from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the *C. neoformans* tuf (EF-1) sequence fragment (SEQ ID NO. 623).
[e]The nucleotide positions refer to the *L. pneumophila* tuf (EF-1) sequence fragment (SEQ ID NO. 112).

-continued

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Fungal genus: *Candida* sp.		
2108	5'-GCG GGC AAC TTC RTC AAG AAG GTT GGT TAC AAC CCG CCC GC	414, 417, 422, 424, 426, 429, 624[b]	52-80[c]
2109	5'-GCGP GGC CCA ATC TCT GGT TGG AAY GGT GAC AAG CCC GC	Same as SEQ ID NO. 2108	100-125[c]
	Bacterial group: *Pseudomonads*		
2121	5'-CGA CCG CIA GCC GCA CAC CAA GTT CCG GTC G	153-155, 205, 880, 2137[d], 2138[d,b]	598-616[e]

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]These sequences were aligned to derive the corresponding primer.
[c]The nucleotide positions refer to the *C. albicans* tuf (EF-1) sequence fragment (SEQ ID NO. 624).
[d]Sequence from databases.
[e]The nucleotide positions refer to the *P. aeruginosa* tuf sequence fragment (SEQ ID NO. 153).

Annex XLVIII: Molecular beacon internal hybridization probes for specific detection of ddl and mtl gene sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Bacterial species: *E. faecium* (ddl)		
1237	5'-GCG AGC CGC GAA ATC GAA GTT GCT GTA TTA GGG CTC GC	1242[b]	334-359
	Bacterial species: *E. faecalis* (mtl)		
1238	5'-GCG AGC GGC GTT AAT TTT GGC ACC GAA GAA GAG CTC GC	1243[b]	631-656

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]Sequence from databases.

Annex XLIX: Internal hybridization probe for specific detection of *S. aureus* sequences of unknown coding potential.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Bacterial species: *Staphylococcus aureus*		
1234	5'-ACT AAA TAA ACG CTC ATT CG	1244	35-54

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Resistance gene: aac(2')-Ia		
1344	5'-AGC AGC AAC GAT GTT ACG CAG CAG	1348[a]	163-186
1345[b]	5'-CCC GCC GAG CAT TTC AAC TAT TG	1348[a]	392-414
1346	5'-GAT GTT ACG CAG CAG GGC AGT C	1348[a]	172-193
1347[b]	5'-ACC AAG CAG GTT CGC AGT CAA GTA	1348[a]	467-490
	Resistance gene: aac(3')-Ib		
1349	5'-CAG CCG ACC AAT GAG TAT CTT GCC	1351[a]	178-201
1350[b]	5'-TAA TCA GGG CAG TTG CGA CTC CTA	1351[a]	356-379
	Resistance gene: aac(3')-IIb		
1352	5'-CCA CGC TGA CAG AGC CGC ACC G	1356[a]	383-404
1353[b]	5'-GGC CAG CTC CCA TCG GAC CCT G	1356[a]	585-606
1354	5'-CAC GCT GAC AGA GCC GCA CCG	1356[a]	384-404
1355[b]	5'-ATG CCG TTG CTG TCG AAA TCC TCG	1356[a]	606-629
	Resistance gene: aac(3')-IVa		
1357	5'-GCC CAT CCA TTT GCC TTT GC	1361[a]	295-314
1358[b]	5'-GCG TAC CAA CTT GCC ATC CTG AAG	1361[a]	517-540
1359	5'-TGC CCC TGC CAC CTC ACT C	1361[a]	356-374
1360[b]	5'-CGT ACC AAC TTG CCA TCC TGA AGA	1361[a]	516-539
	Resistance gene: aac(3')-VIa		
1362	5'-CGC CGC CAT CGC CCA AAG CTG G	1366[a]	285-306
1363[b]	5'-CGG CAT AAT GGA GCG CGG TGA CTG	1366[a]	551-574
1364	5'-TTT CTC GCC CAC GCA GGA AAA ATC	1366[a]	502-525
1365[b]	5'-CAT CCT CGA CGA ATA TGC CGC G	1366[a]	681-702
	Resistance gene: aac(6')-Ia		
1367	5'-CAA ATA TAC TAA CAG AAG CGT TCA	1371[a]	56-79
1368[b]	5'-AGG ATC TTG CCA ATA CCT TTA T	1371[a]	269-290
1379	5'-AAA CCT TTG TTT CGG TCT GCT AAT	1371[a]	153-176
1380[b]	5'-AAG CGA TTC CAA TAA TAC CTT GCT	1371[a]	320-343

-continued

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: aac(6')-Ic		
1372	5'-GCT TTC GTT GCC TTT GCC GAG GTC	1376[a]	157-180
1373[b]	5'-CAC CCC TGT TGC TTC GCC CAC TC	1376[a]	304-326
1374	5'-AGA TAT TGG CTT CGC CGC ACC ACA	1376[a]	104-127
1375[b]	5'-CCC TGT TGC TTC GCC CAC TCC TG	1376[a]	301-323
	Resistance gene: ant(3')-Ia		
1377	5'-GCC GTG GGT CGA TGT TTG ATG TTA	1381[a]	100-123
1378[b]	5'-GCT CGA TGA CGC CAA CTA CCT CTG	1381[a]	221-244
1379	5'-AGC AGC AAC GAT GTT ACG CAG CAG	1381[a]	127-150
1380[b]	5'-CGC TCG ATG ACG CCA ACT ACC TCT	1381[a]	222-245
	Resistance gene: ant(4')-Ia		
1382	5'-TAG ATA TGA TAG GCG GTA AAA AGC	1386[a]	149-172
1383[b]	5'-CCC AAA TTC GAG TAA GAG GTA TT	1386[a]	386-408
1384	5'-GAT ATG ATA GGC GGT AAA AAG C	1386[a]	151-172
1385[b]	5'-TCC CAA ATT CGA GTA AGA GGT A	1386[a]	388-409
	Resistance gene: aph(3')-Ia		
1387	5'-TTA TGC CTC TTC CGA CCA TCA AGC	1391[a]	233-256
1338[b]	5'-TAC GCT CGT CAT CAA AAT CAC TCG	1391[a]	488-511
1389	5'-GAA TAA CGG TTT GGT TGA TGC GAG	1391[a]	468-491
1390[b]	5'-ATG GCA AGA TCC TGG TAT CGG TCT	1391[a]	669-692
	Resistance gene: aph(3')-IIa		
1392	5'-TGG GTG GAG AGG CTA TTC GGC TAT	1396[a]	43-66
1393[b]	5'-CAG TCC CTT CCC GCT TCA GTG AC	1396[a]	250-272
1394	5'-GAC GTT GTC ACT GAA GCG GGA AGG	1396[a]	244-267
1395[b]	5'-CTT GGT GGT CGA ATG GGC AGG TAG	1396[a]	386-409
	Resistance gene: aph(3')-IIIa		
1397	5'-GTG GGA GAA AAT GAA AAC CTA T	1401[a]	103-124
1398[b]	5'-ATG GAG TGA AAG AGC CTG AT	1401[a]	355-374
1399	5'-ACC TAT GAT GTG GAA CGG GAA AAG	1401[a]	160-183
1400[b]	5'-CGA TGG AGT GAA AGA GCC TGA TG	1401[a]	354-376
	Resistance gene: aph(3')-VIa		
1402	5'-TAT TCA ACA ATT TAT CGG AAA CAG	1406[a]	18-41
1403[b]	5'-TCA GAG AGC CAA CTC AAC ATT TT	1406[a]	175-197
1404	5'-AAA CAG CGT TTT AGA GCC AAA TAA	1406[a]	36-59
1405[b]	5'-TTC TCA GAG AGC CAA CTC AAC ATT	1406[a]	177-200
	Resistance gene: blaCARB		
1407	5'-CCC TGT AAT AGA AAA GCA AGT AGG	1411[a]	351-374
1408[b]	5'-TTG TCG TAT CCC TCA AAT CAC C	1411[a]	556-577
1409	5'-TGG GAT TAC AAT GGC AAT CAG CG	1411[a]	205-227
1410[b]	5'-GGG AAT AGT CAC AGA TCT GCT T	1411[a]	329-353

-continued

Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: blaCMY-2		
1412	5'-GAG AAA ACG CTC CAG CAG GGC	1416[a]	793-813
1413[b]	5'-CAT GAG GCT TTC ACT GCG GGG	1416[a]	975-995
1414	5'-TAT CGT TAA TCG CAC CAT CAC	1416[a]	90-110
1415[b]	5'-ATG CAG TAA TGC GGC TTT ATC	1416[a]	439-459
	Resistance genes: blaCTX-M-1, blaCTX-M-2		
1417	5'-TGG TTA ACT AYA ATC CSA TTG CGG A	1423[a]	314-338
1418[b]	5'-ATG CTT TAC CCA GCG TCA GAT T	1423[a]	583-604
	Resistance gene: blaCTX-M-1		
1419	5'-CGA TGA ATA AGC TGA TTT CTC ACG	1423[a]	410-433
1420[b]	5'-TGC TTT ACC CAG CGT CAG ATT ACG	1423[a]	580-603
1421	5'-AAT TAG AGC GGC AGT CGG GAG GAA	1423[a]	116-139
1422[b]	5'-GAA ATC AGC TTA TTC ATC GCC ACG	1423[a]	405-428
	Resistance gene: blaCTX-M-2		
1424	5'-GTT AAC GGT GAT GGC GAC GCT AC	1428[a]	30-52
1425[b]	5'-GAA TTA TCG GCG GTG TTA ATC AGC	1428[a]	153-176
1426	5'-CAC GCT CAA TAC CGC CAT TCC A	1428[a]	510-531
1427[b]	5'-TTA TCG CCC ACT ACC CAT GAT TTC	1428[a]	687-710
	Resistance gene: blaIMP		
1429	5'-TTT ACG GCT AAA GAT ACT GAA AAG T	1433[a]	205-229
1430[b]	5'-GTT TAA TAA AAC AAC CAC CGA ATA AT	1433[a]	513-538
1431	5'-TAA TTG ACA CTC CAT TTA CGG CTA A	1433[a]	191-215
1432[b]	5'-ACC GAA TAA TAT TTT CCT TTC AGG CA	1433[a]	497-522
	Resistance gene: blaOXA2		
1434	5'-CAC AAT CAA GAC CAA GAT TTG CGA T	1438[a]	319-343
1435[b]	5'-GAA AGG GCA GCT CGT TAC GAT AGA G	1438[a]	532-556
	Resistance gene: blaOXA10		
1436	5'-CAG CAT CAA CAT TTA AGA TCC CCA	1439[a]	194-217
1437[b]	5'-CTC CAC TTG ATT AAC TGC GGA AAT TC	1439[a]	479-504
	Resistance gene: blaPER-1		
1440	5'-AGA CCG TTA TCG TAA ACA GGG CTA AG	1442[a]	281-306
1441[b]	5'-TTT TTT GCT CAA ACT TTT TCA GGA TC	1442[a]	579-604
	Resistance gene: blaPER-2		
1443	5'-CTT CTG CTC TGC TGA TGC TTG GC	1445[a]	32-54
1444[b]	5'-GGC GAC CAG GTA TTT TGT AAT ACT GC	1445[a]	304-329
	Resistance genes: blaPER-1, blaPER-2		
1446	5'-GGC CTG YGA TTT GTT ATT TGA ACT GGT	1442[a]	414-440
1447[b]	5'-CGC TST GGT CCT GTG GTG GTT TC	1442[a]	652-674
1448	5'-GAT CAG GTG CAR TAT CAA AAC TGG AC	1442[a]	532-557
1449[b]	5'-AGC WGG TAA CAA YCC TTT TAA CCG CT	1442[a]	671-696

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: blaSHV		
1883	5'-AGC CGC TTG AGC AAA TTA AAC TA	1900[a]	71-93
1884[b]	5'-GTA TCC CGC AGA TAA ATC ACC AC	1900[a]	763-785
1885	5'-AGC GAA AAA CAC CTT GCC GAC	1900[a]	313-333
1884[b]	5'-GTA TCC CGC AGA TAA ATC ACC AC	1900[a]	763-785
	Resistance gene: blaTEM		
1906	5'-CCT TAT TCC CTT TTT TGC GG	1927[a]	27-46
1907[b]	5'-CAC CTA TCT CAG CGA TCT GTC T	1927[a]	817-838
1908	5'-AAC AGC GGT AAG ATC CTT GAG AG	1927[a]	148-170
1907[b]	5'-CAC CTA TCT CAG CGA TCT GTC T	1927[a]	817-838
	Resistance gene: catI		
2145	5'-GCA AGA TGT GGC GTG TTA CGG T	2147[a]	363-384
2146[b]	5'-GGG GCG AAG AAG TTG TCC ATA TT	2147[a]	484-506
	Resistance gene: catII		
2148	5'-CAG ATT AAA TGC GGA TTC AGC C	2150[a]	67-88
2149[b]	5'-ATC AGG TAA ATC ATC AGC GGA TA	2150[a]	151-173
	Resistance gene: catIII		
2151	5'-ATA TTT CAG CAT TAC CTT GGG TT	2153[a]	419-441
2152[b]	5'-TAC ACA ACT CTT GTA GCC GAT TA	2153[a]	603-625
	Resistance gene: catP		
2154	5'-CGC CAT TCA GAG TTT AGG AC	2156[a]	178-197
2155[b]	5'-TTC CAT ACC GTT GCG TAT CAC TT	2156[a]	339-361
	Resistance gene: cat		
2157	5'-CCA CAG AAA TTG ATA TTA GTG TTT TAT	2159[a]	89-115
2158[b]	5'-TCG CTA TTG TAA CCA GTT CTA	2159[a]	201-221
2160	5'-TTT TGA ACA CTA TTT TAA CCA GC	2162[a]	48-70
2161[b]	5'-GAT TTA ACT TAT CCC AAT AAC CT	2162[a]	231-253
	Resistance gene: dfrA		
1450	5'-ACC ACT GGG AAT ACA CTT GTA ATG GC	1452[a]	106-131
1451[b]	5'-ATC TAC CTG GTC AAT CAT TGC TTC GT	1452[a]	296-321
	Resistance gene: dhfrIa		
1457	5'-CAA AGG TGA ACA GCT CCT GTT T	1461[a]	75-96
1458[b]	5'-TCC GTT ATT TTC TTT AGG TTG GTT AAA	1461[a]	249-275
1459	5'-AAG GTG AAC AGC TCC TGT TT	1461[a]	77-96
1560[b]	5'-GAT CAC TAC GTT CTC ATT GTC A	1461[a]	207-228
	Resistance genes: dhfrIa, dhfrXV		
1453	5'-ATC GAA GAA TGG AGT TAT CGG RAA TG	1461[a]	27-52
1454[b]	5'-CCT AAA AYT RCT GGG GAT TTC WGG A	1461[a]	384-408
1455	5'-CAG GTG GTG GGG AGA TAT ACA AAA	1461[a]	290-313
1456[b]	5'-TAT GTT AGA SRC GAA GTC TTG GKT AA	1461[a]	416-441

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: dhfrIb		
1466	5'-AAG CAT TGA CCT ACA ATC AGT GT	1470[a]	98-120
1467[b]	5'-AAT ACA ACT ACA TTG TCA TCA TTT GAT	1470[a]	204-230
1468	5'-CGT TAC CCG CTC AGG TTG GAC ATC AA	1470[a]	183-208
1469[b]	5'-CAT CCC CCT CTG GCT CGA TGT CG	1470[a]	354-376
	Resistance gene: dhfrV		
1471	5'-GAT AAT GAC AAC GTA ATA GTA TTC CC	1475[a]	208-233
1472[b]	5'-GCT CAA TAT CAA TCG TCG ATA TA	1475[a]	342-364
1473	5'-TTA AAG CCT TGA CGT ACA ACC AGT GG	1475[a]	95-120
1474[b]	5'-TGG GCA ATG TTT CTC TGT AAA TCT CC	1475[a]	300-325
	Resistance genes: dhfrIb, dhfrV		
1462	5'-GCA CTC CCY AAT AGG AAA TAC GC	1470[a]	157-179
1463[b]	5'-AGT GTT GCT CAA AAA CAA CTT CG	1470[a]	405-427
1464	5'-ACG TTY GAA TCT ATG GGM GCA CT	1470[a]	139-161
1465[b]	5'-GTC GAT AAG TGG AGC GTA GAG GC	1470[a]	328-350
	Resistance gene: dhfrVI		
1476	5'-GGC GAG CAG CTC CTA TTC AAA G	1480[a]	79-100
1477[b]	5'-TAG GTA AGC TAA TGC CGA TTC AAC A	1480[a]	237-261
1478	5'-GAG AAT GGA GTA ATT GGC TCT GGA TT	1480[a]	31-56
1479[b]	5'-GCG AAA TAC ACA ACA TCA GGG TCA T	1480[a]	209-233
	Resistance gene: dhfrVII		
1485	5'-AAA ATG GCG TAA TCG GTA ATG GC	1489[a]	32-54
1486[b]	5'-CAT TTG AGC TTG AAA TTC CTT TCC TC	1489[a]	189-214
1487	5'-AAT CGA AAA TAT GCA GTA GTG TCG AG	1489[a]	166-191
1488[b]	5'-AGA CTA TTG TAG ATT TGA CCG CCA	1489[a]	294-317
	Resistance genes: dhfrVII, dhfrXVII		
1481	5'-RTT ACA GAT CAT KTA TAT GTC TCT	1489[a]	268-291
1482[b]	5'-TAA TTT ATA TTA GAC AWA AAA AAC TG	1489[a]	421-446
1483	5'-CAR YGT CAG AAA ATG GCG TAA TC	1489[a]	23-45
1484[b]	5'-TKC AAA GCR WTT TCT ATT GAA GGA AA	1489[a]	229-254
	Resistance gene: dhfrVIII		
1490	5'-GAC CTA TGA GAG CTT GCC CGT CAA A	1494[a]	144-168
1491[b]	5'-TCG CCT TCG TAC AGT CGC TTA ACA AA	1494[a]	376-401
1492	5'-CAT TTT AGC TGC CAC CGC CAA TGG TT	1494[a]	18-43
1493[b]	5'-GCG TCG CTG ACG TTG TTC ACG AAG A	1494[a]	245-269
	Resistance gene: dhfrIX		
1495	5'-TCT CTA AAC ATG ATT GTC GCT GTC	1499[a]	7-30
1496[b]	5'-CAG TGA GGC AAA AGT TTT TCT ACC	1499[a]	133-156
1497	5'-CGG ACG ACT TCA TGT GGT AGT CAG T	1499[a]	171-195
1498[b]	5'-TTT GTT TTC AGT AAT GGT CGG GAC CT	1499[a]	446-471

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: dhfrXII		
1500	5'-ATC GGG TTA TTG GCA ATG GTC CTA	1504[a]	50-73
1501[b]	5'-GCG GTA GTT AGC TTG GCG TGA GAT T	1504[a]	201-225
1502	5'-GCG GGC GGA GCT GAG ATA TAC A	1504[a]	304-325
1503[b]	5'-AAC GGA GTG GGT GTA CGG AAT TAC AG	1504[a]	452-477
	Resistance gene: dhfrXIII		
1505	5'-ATT TTT CGC AGG CTC ACC GAG AGC	1507[a]	106-129
1506[b]	5'-CGG ATG AGA CAA CCT CGA ATT CTG CTG	1507[a]	413-439
	Resistance gene: dhfrXV		
1508	5'-AGA ATG TAT TGG TAT TTC CAT CTA TCG	1512[a]	215-241
1509[b]	5'-CAA TGT CGA TTG TTG AAA TAT GTA AA	1512[a]	336-361
1510	5'-TGG AGT GCC AAA GGG GAA CAA T	1512[a]	67-88
1511[b]	5'-CAG ACA CAA TCA CAT GAT CCG TTA TCG	1512[a]	266-292
	Resistance gene: dhfrXVII		
1513	5'-TTC AAG CTC AAA TGA AAA CGT CC	1517[a]	201-223
1514[b]	5'-GAA ATT CTC AGG CAT TAT AGG GAA T	1517[a]	381-405
1515	5'-GTG GTC AGT AAA AGG TGA GCA AC	1517[a]	66-88
1516[b]	5'-TCT TTC AAA GCA TTT TCT ATT GAA GG	1517[a]	232-257
	Resistance gene: embB		
2102	5'-CAC CTT CAC CCT GAC CGA CG	2105[a]	822-841
2103[b]	5'-CGA ACC AGC GGA AAT AGT TGG AC	2105[a]	948-970
	Resistance genes: ereA, ereA2		
1528	5'-AAC TTG AGC GAT TTT CGG ATA CCC TG	1530[a]	80-105
1529[b]	5'-TTG CCG ATG AAA TAA CCG CCG ACT	1530[a]	317-340
	Resistance gene: ereB		
1531	5'-TCT TTT TGT TAC GAC ATA CGC TTT T	1535[a]	152-176
1532[b]	5'-AGT GCT TCT TTA TCC GCT GTT CTA	1535[a]	456-479
1533	5'-CAG CGG ATA AAG AAG CAC TAC ACA TT	1535[a]	461-486
1534[b]	5'-CCT CCT GAA ATA AAG CCC GAC AT	1535[a]	727-749
	Resistance gene: gyrA		
1340	5'-GAA CAA GGT ATG ACA CCG GAT AAA T	1299[a]	163-188
1341[b]	5'-GAT AAC TGA AAT CCT GAG CCA TAC G	1299[a]	274-299
1936	5'-TAC CAC CCG CAC GGC	1954[a]	205-219
1937[b]	5'-CGG AGT CGC CGT CGA TG	1954[a]	309-325
1942	5'-GAC TGG AAC AAA GCC TAT AAA AAA TCA	1954[a]	148-174
1937[b]	5'-CGG AGT CGC CGT CGA TG	1954[a]	309-325
2040	5'-TGT GAC CCC AGA CAA ACC C	2054[a]	33-51
2041[b]	5'-GTT GAG CGG CAG CAC TAT CT	2054[a]	207-226
	Resistance gene: inhA		
2098	5'-CTG AGT CAC ACC GAC AAA CGT C	2101[a]	910-931
2099[b]	5'-CCA GGA CTG AAC GGG ATA CGA A	2101[a]	1074-1095

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Resistance genes: linA, linA'			
1536	5'-AGA TGT ATT AAC TGG AAA ACA ACA A	1540[a]	99-123
1537[b]	5'-CTT TGT AAT TAG TTT CTG AAA ACC A	1540[a]	352-376
1538	5'-TTA GAA GAT ATA GGA TAC AAA ATA GAA G	1540[a]	187-214
1539[b]	5'-GAA TGA AAA AGA AGT TGA GCT T	1540[a]	404-425
Resistance gene: linB			
1541	5'-TGA TAA TCT TAT ACG TGG GGA ATT T	1545[a]	246-270
1542[b]	5'-ATA ATT TTC TAA TTG CCC TGT TTC AT	1545[a]	359-384
1543	5'-GGG CAA TTA GAA AAT TAT TTA TCA GA	1545[a]	367-392
1544[b]	5'-TTT TAC TCA TGT TTA GCC AAT TAT CA	1545[a]	579-604
Resistance gene: mefA			
1546	5'-CAA GAA GGA ATG GCT GTA CTA C	1548[a]	625-646
1547[b]	5'-TAA TTC CCA AAT AAC CCT AAT AAT AGA	1548[a]	816-842
Resistance gene: mefE			
1549	5'-GCT TAT TAT TAG GAA GAT TAG GGG GC	1551[a]	815-840
1550[b]	5'-TAG CAA GTG ACA TGA TAC TTC CGA	1551[a]	1052-1075
Resistance genes: mefA, mefE			
1552	5'-GGC AAG CAG TAT CAT TAA TCA CTA	1548[a]	50-73
1553[b]	5'-CAA TGC TAC GGA TAA ACA ATA CTA TC	1548[a]	318-343
1554	5'-AGA AAA TTA AGC CTG AAT ATT TAG GAC	1548[a]	1010-1035
1555[b]	5'-TAG TAA AAA CCA ATG ATT TAC ACC G	1548[a]	1119-1143
Resistance genes: mphA, mphK			
1556	5'-ACT GTA CGC ACT TGC AGC CCG ACA T	1560[a]	33-57
1557[b]	5'-GAA CGG CAG GCG ATT CTT GAG CAT	1560[a]	214-237
1558	5'-GTG GTG GTG CAT GGC GAT CTC T	1560[a]	583-604
1559[b]	5'-GCC GCA GCG AGG TAC TCT TCG TTA	1560[a]	855-878
Resistance gene: mupA			
2142	5'-GCC TTA ATT TCG GAT AGT GC	2144[a]	1831-1850
2143[b]	5'-GAG AAA GAG CCC AAT TAT CTA ATG T	2144[a]	2002-2026
Resistance gene: parC			
1342	5'-GAT GTT ATT GGT CAA TAT CAT CCA	1321[a]	205-229
1343[b]	5'-AAG AAA CTG TCT CTT TAT TAA TAT CAC GT	1321[a]	396-425
1934	5'-GAA CGC CAG CGC GAA ATT CAA AAA G	1781	67-91
1935[b]	5'-AGC TCG GCA TAC TTC GAC AGG	1781	277-297
2044	5'-ACC GTA AGT CGG CCA AGT CA	2055[a]	176-195
2045[b]	5'-GTT CTT TCT CCG TAT CGT C	2055[a]	436-454
Resistance gene: ppflo-like			
2163	5'-ACC TTC ATC CTA CCG ATG TGG GTT	2165[a]	922-945
2164[b]	5'-CAA CGA CAC CAG CAC TGC CAT TG	2165[a]	1136-1158

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: rpoB		
2065	5'-CCA GGA CGT GGA GGC GAT CAC A	2072[a]	1218-1239
2066[b]	5'-CAC CGA CAG CGA GCC GAT CAG A	2072[a]	1485-1506
	Resistance gene: satG		
1581	5'-AAT TGG GGA CTA CAC CTA TTA TGA TG	1585[a]	93-118
1582[b]	5'-GGC AAA TCA GTC AGT TCA GGA GT	1585[a]	310-332
1583	5'-CGA TTG GCA ACA ATA CAC TCC TG	1585[a]	294-316
1584[b]	5'-TCA CCT ATT TTT ACG CCT GGT AGG AC	1585[a]	388-413
	Resistance gene: sulII		
1961	5'-GCT CAA GGC AGA TGG CAT TCC C	1965[a]	222-243
1962[b]	5'-GGA CAA GGC GGT TGC GTT TGA T	1965[a]	496-517
1963	5'-CAT TCC CGT CTC GCT CGA CAG T	1965[a]	237-258
1964[b]	5'-ATC TGC CTG CCC GTC TTG C	1965[a]	393-411
	Resistance gene: tetB		
1966	5'-CAT GCC AGT CTT GCC AAC G	1970[a]	66-84
1967[b]	5'-CAG CAA TAA GTA ATC CAG CGA TG	1970[a]	242-264
1968	5'-GGA GAG ATT TCA CCG CAT AG	1970[a]	457-476
1969[b]	5'-AGC CAA CCA TCA TGC TAT TCC A	1970[a]	721-742
	Resistance gene: tetM		
1586	5'-ATT CCC ACA ATC TTT TTT ATC AAT AA	1590[a]	361-386
1587[b]	5'-CAT TGT TCA GAT TCG GTA AAG TTC	1590[a]	501-524
1588	5'-GTT TTT GAA GTT AAA TAG TGT TCT T	1590[a]	957-981
1589[b]	5'-CTT CCA TTT GTA CTT TCC CTA	1590[a]	1172-1192
	Resistance gene: vatB		
1609	5'-GCC CTG ATC CAA ATA GCA TAT A	1613[a]	11-32
1610[b]	5'-CCT GGC ATA ACA GTA ACA TTC TG	1613[a]	379-401
1611	5'-TGG GAA AAA GCA ACT CCA TCT C	1613[a]	301-322
1612[b]	5'-ACA ACT GAA TTC GCA GCA ACA AT	1613[a]	424-446
	Resistance gene: vatC		
1614	5'-CCA ATC CAG AAG AAA TAT ACC C	1618[a]	26-47
1615[b]	5'-ATT AGT TTA TCC CCA ATC AAT TCA	1618[a]	177-200
1616	5'-ATA ATG AAT GGG GCT AAT CAT CGT AT	1618[a]	241-266
1617[b]	5'-GCC AAC AAC TGA ATA AGG ATC AAC	1618[a]	463-486
	Resistance gene: vga		
1619	5'-AAG GCA AAA TAA AAG GAG CAA AGC	1623[a]	641-664
1620[b]	5'-TGT ACC CGA GAC ATC TTC ACC AC	1623[a]	821-843
1621	5'-AAT TGA AGG ACG GGT ATT GTG GAA AG	1623[a]	843-868
1622[b]	5'-CGA TTT TGA CAG ATG GCG ATA ATG AA	1623[a]	975-1000
	Resistance gene: vgaB		
1624	5'-TTC TTT AAT GCT CGT AGA TGA ACC TA	1628[a]	354-379
1625[b]	5'-TTT TCG TAT TCT TCT TGT TGC TTT C	1628[a]	578-602

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
1626	5'-AGG AAT GAT TAA GCC CCC TTC AAA AA	1628[a]	663-688
1627[b]	5'-TTA CAT TGC GAC CAT GAA ATT GCT CT	1628[a]	849-874
	Resistance genes: vgb, vgh		
1629	5'-AAG GGG AAA GTT TGG ATT ACA CAA CA	1633[a]	73-98
1630[b]	5'-GAA CCA CAG GGC ATT ATC AGA ACC	1633[a]	445-468
1631	5'-CGA CGA TGC TTT ATG GTT TGT	1633[a]	576-596
1632[b]	5'-GTT AAT TTG CCT ATC TTG TCA CAC TC	1633[a]	850-875
	Resistance gene: vgbB		
1634	5'-TTA ACT TGT CTA TTC CCG ATT CAG G	1882[a]	23-47
1635[b]	5'-GCT GTG GCA ATG GAT ATT CTG TA	1882[a]	267-289
1636	5'-TTC CTA CCC CTG ATG CTA AAG TGA	1882[a]	155-178
1637[b]	5'-CAA AGT GCG TTA TCC GAA CCT AA	1882[a]	442-464
	Sequencing primers Resistance gene: gyrA		
1290	5'-GAY TAY GCI ATG ISI GTI ATH GT	1299[a]	70-83
1292[b]	5'-ARI SCY TCI ARI ATR TGI GC	1299[a]	1132-1152
1291	5'-GCI YTI CCI GAY GTI MGI GAY GG	1299[a]	100-123
1292[b]	5'-ARI SCY TCI ARI ATR TGI GC	1299[a]	1132-1152
1293	5'-ATG GCT GAA TTA CCT CAA TC	1299[a]	1-21
1294[b]	5'-ATG ATT GTT GTA TAT CTT CTT CAA C	1299[a]	2626-2651
1295[b]	5'-CAG AAA GTT TGA AGC GTT GT	1299[a]	1255-1275
1296	5'-AAC GAT TCG TGA GTC AGA TA	1299[a]	1188-1208
1297	5'-CGG TCA ACA TTG AGG AAG AGC T	1300[a]	29-51
1298[b]	5'-ACG AAA TCG ACC GTC TCT TTT TC	1300[a]	415-437
	Resistance gene: gyrB		
1301	5'-GTI MGI AWI MGI CCI GSI ATG TA	1307[a]	82-105
1302[b]	5'-TAI ADI GGI GGI KKI GCI ATR TA	1307[a]	1600-1623
1303	5'-GGI GAI GAI DYI MGI GAR GG	1307[a]	955-975
1304[b]	5'-CIA RYT TIK YIT TIG TYT G	1307[a]	1024-1043
1305	5'-ATG GTG ACT GCA TTG TCA GAT G	1307[a]	1-23
1306[b]	5'-GTC TAC GGT TTT CTA CAA CGT C	1307[a]	1858-1888
	Resistance gene: parC		
1308	5'-ATG TAY GTI ATI ATG GAY MGI GC	1320[a]	67-90
1309[b]	5'-ATI ATY TTR TTI CCY TTI CCY TT	1320[a]	1993-2016
1310	5'-ATI ATI TSI ATI ACY TCR TC	1320[a]	1112-1132
1311[b]	5'-GAR ATG AAR ATI MGI GGI GAR CA	1320[a]	1288-1311
1312	5'-AAR TAY ATI ATI CAR GAR MGI GC	1321[a]	67-90
1313[b]	5'-AMI AYI CKR TGI GGI TTI TTY TT	1321[a]	2212-2235
1314	5'-TAI GAI TTY ACI GAI SMI CAR GC	1321[a]	1228-1251
1315[b]	5'-ACI ATI GCI TCI GCY TGI KSY TC	1321[a]	1240-1263

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Annex L: Specific and ubiquitous primers for nucleic acid amplification (antimicrobial agents resistance genes sequences).

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
1316	5'-GTG AGT GAA ATA ATT CAA GAT T	1321[a]	1-23
1317[b]	5'-CAC CAA AAT CAT CTG TAT CTA C	1321[a]	2356-2378
1318	5'-ACC TAY TCS ATG TAC GTR ATC ATG GA	1320[a]	58-84
1319[b]	5'-AGR TCG TCI ACC ATC GGY AGY TT	1320[a]	832-855
	Resistance gene: parE		
1322	5'-RTI GAI AAY ISI GTI GAY GAR G	1328[a]	133-155
1325[b]	5'-RTT CAT YTC ICC IAR ICC YTT	1328[a]	1732-1752
1323	5'-ACI AWR SAI GGI GGI ACI CAY G	1328[a]	829-850
1324[b]	5'-CCI CCI GCI SWR TCI CCY TC	1328[a]	1280-1302
1326	5'-TGA TTC AAT ACA GGT TTT AGA G	1328[a]	27-49
1327[b]	5'-CTA GAT TTC CTC CTC ATC AAA T	1328[a]	1971-1993

[a]Sequence from databases.
[b]These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex LI: Internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Nucleotide position
	Resistance gene: aph3'VIa		
2252	5'-CCA CAT ACA GTG TCT CTC	1406[a]	149-166
	Resistance gene: blaSHV		
1886	5'-GAC GCC CGC GCC ACC ACT	1900[a]	484-501
1887	5'-GAC GCC CGC GAC ACC ACT A	1899[a]	514-532
1888	5'-GAC GCC CGC AAC ACC ACT A	1901[a]	514-532
1889	5'-GTT CGC AAC TGC AGC TGC TG	1899[a]	593-612
1890	5'-TTC GCA ACG GCA GCT GCT G	1899[a]	594-612
1891	5'-CCG GAG CTG CCG AIC GGG	1902[a]	692-709
1892	5'-CGG AGC TGC CAA RCG GGG	1903[a]	693-710
1893	5'-GGA GCT GGC GAR CGG GGT	1899[a]	694-711
1894	5'-GAC CGG AGC TAG CGA RCG	1904[a]	690-707
1895	5'-CGG AGC TAG CAA RCG GGG T	1905[a]	693-711
1896	5'-GAA ACG GAA CTG AAT GAG GCG	1899[a]	484-504
1897	5'-CAT TAC CAT GGG CGA TAA CAG	1899[a]	366-386
1898	5'-CCA TTA CCA TGA GCG ATA ACAG	1899[a]	365-386

-continued

Annex LI: Internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Resistance gene: blaTEM		
1909	5'-ATG ACT TGG TTA AGT ACT CAC C	1928[a]	293-314
1910	5'-ATG ACT TGG TTG AGT ACT CAC C	1927[a]	293-314
1911	5'-CCA TAA CCA TGG GTG ATA ACA C	1928[a]	371-392
1912	5'-CCA TAA CCA TGA GTG ATA ACA C	1927[a]	371-392
1913	5'-CGC CTT GAT CAT TGG GAA CC	1928[a]	475-494
1914	5'-CGC CTT GAT CGT TGG GAA CC	1927[a]	475-494
1915	5'-CGC CTT GAT AGT TGG GAA CC	1929[a]	475-494
1916	5'-CGT GGG TCT TGC GGT ATC AT	1927[a]	712-731
1917	5'-CGT GGG TCT GGC GGT ATC AT	1930[a]	712-731
1918	5'-GTG GGT CTC ACG GTA TCA TTG	1927[a]	713-733
1919	5'-CGT GGG TCT CTC GGT ATC ATT	1931[a]	712-732
1920	5'-CGT GGI TCT CGC GGT ATC AT	1927[a]	712-731
1921	5'-CGT GGG TCT AGC GGT ATC ATT	1932[a]	713-733
1922	5'-GTT TTC CAA TGA TTA GCA CTT TTA	1927[a]	188-211
1923	5'-GTT TTC CAA TGA TAA GCA CTT TTA	1927[a]	188-211
1924	5'-GTT TTC CAA TGC TGA GCA CTT TT	1932[a]	188-210
1925	5'-CGT TTT CCA ATG ATG AGC ACT TT	1927[a]	187-209
1926	5'-GTT TTC CAA TGG TGA GCA CTT TT	1933[a]	188-210
2006	5'-TGG AGC CGG TGA GCG TGG	1927[a]	699-716
2007	5'-TGG AGC CAG TGA GCG TGG	2010[a]	699-716
2008	5'-TCT GGA GCC GAT GAG CGT G	1929[a]	697-715
2009	5'-CTG GAG CCA GTA AGC GTG G	2011[a]	698-716
2141	5'-CAC CAG TCA CAG AAA AGC	1927[a]	311-328
	Resistance gene: dhfrIa		
2253	5'-CAT TAC CCA ACC GAA AGT A	1461[a]	158-176
	Resistance gene: embB		
2104	5'-CTG GGC ATG GCI CGA GTC	2105[a]	910-927
	Resistance gene: gyrA		
1333	5'-TCA TGG TGA CTT ATC TAT TTA TG	1299[a]	240-263
1334	5'-CAT CTA TTT ATA AAG CAA TGG TA	1299[a]	251-274
1335	5'-CTA TTT ATG GAG CAA TGG T	1299[a]	254-273
1940	5'-GTA TCG TTG GTG ACG TAA T	1299[a]	206-224

-continued

Annex LI: Internal hybridization probes for specific detection of antimicrobial agents resistance genes sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
1943	5'-GCT GGT GGA CGG CCA G	1954[a]	279-294
1945	5'-CGG CGA CTA CGC GGT AT	1954[a]	216-232
1946	5'-CGG CGA CTT CGC GGT AT	1954[a]	216-232
1947	5'-CGG TAT ACG GCA CCA TCG T	1954[a]	227-245
1948	5'-GCG GTA TAC AAC ACC ATC G	1954[a]	226-244
1949	5'-CGG TAT ACG CCA CCA TCG T	1954[a]	227-245
2042	5'-CAC GGG GAT TTC TCT ATT TA	2054[a]	103-122
2043	5'-CAC GGG GAT TAC TCT ATT TA	2054[a]	103-122
	Resistance gene: inhA		
2100	5'-GCG AGA CGA TAG GTT GTC	2101[a]	1017-1034
	Resistance gene: parC		
1336	5'-TGG AGA CTA CTC AGT GT	1321[a]	232-249
1337	5'-TGG AGA CTT CTC AGT GT	1321[a]	232-249
1338	5'-GTG TAC GGA GCA ATG	1321[a]	245-260
1339	5'-CCA GCG GAA ATG CGT	1321[a]	342-357
1941	5'-GCA ATG GTC CGT TTA AGT	1321[a]	253-270
1944	5'-TTT CGC CGC CAT GCG TTA C	1781	247-265
1950	5'-GGC GAC ATC GCC TGC	1781	137-151
1951	5'-GGC GAC AGA GCC TGC TA	1781	137-153
1952	5'-CCT GCT ATG GAG CGA TGG T	1781	147-165
1953	5'-CGC CTG CTA TAA AGC GAT GGT	1781	145-165
2046	5'-ACG GGG ATT TTT CTA TCT AT	2055[a]	227-246
	Resistance gene: rpoB		
2067	5'-AGC TGA GCC AAT TCA TGG	2072[a]	1304-1321
2068	5'-ATT CAT GGA CCA GAA CAA C	2072[a]	1314-1332
2069	5'-CGC TGT CGG GGT TGA CCC	2072[a]	1334-1351
2070	5'-GTT GAC CCA CAA GCG CCG	2072[a]	1344-1361
2071	5'-CGA CTG TCG GCG CTG GGG	2072[a]	1360-1377
	Resistance gene: tetM		
2254	5'-ACC TGA ACA GAG AGA AAT G	1590[a]	1062-1080

[a]Sequence from databases.

Annex LII: Molecular beacon internal hybridization probes for specific detection of atpD sequences.

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Bacterial species: *Bacteroides fragilis*		
2136	5'-CCA ACG CGT CCT CAA TCA TTT CTA ACT TCT ATG GCC GGC GTT GG	929	353-382
	Bacterial species: *Bordetella pertussis*		
2182	5'-GCG CGC CAA CGA CTT CTA CCA CGA AAT GGA AGA GTC GCG CGC	1672	576-605
	Bacterial group: *Campylobacter jejuni* and *C. coli*		
2133	5'-CCA CGC ACA WAA ACT TGT TTT AGA AGT AGC AGC WCA GCG TGG	1576, 1600, 1849, 1863, 2139[b,c]	44-73[d]
	Fungal species: *Candida glabrata*		
2078	5'-CCG AGC CTT GGT CTT CGG CCA AAT GAA CGC TCG G	463	442-463
	Fungal species: *Candida krusei*		
2075	5'-CCG AGC CAG GTT CTG AAG TCT CTG CAT TAT TAG GTG CTC GG	468	720-748
	Fungal species: *Candida lusitaniae*		
2080	5'-CCG AGC CGA AGA GGG CCA AGA TGT CGC TCG G	470	520-538
	Fungal species: *Candida parapsilosis*		
2079	5'-CCG AGC GTT CAG TTA CTT CAG TCC AAG CCG GCT CGG	472	837-860
	Fungal species: *Candida tropicalis*		
2077	5'-CCG AGC AAC CGA TCC AGC TCC AGC TAC GCT CGG	475	877-897
	Bacterial species: *Klebsiella pneumoniae*		
2281	5'-CCC CCA GCT GGG CGG CGG TAT CGA TGG GGG	317	40-59

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]Sequence from databases.
[c]These sequences were aligned to derive the corresponding primer.
[d]The nucleotide positions refer to the *C. jejuni* atpD sequence fragment (SEQ ID NO. 1576).

SEQ ID NO.	Nucleotide sequence[a]	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
	Fungal genus: *Candida* sp.		
2076	5'-CCG AGC YGA YAA CAT TTT CAG ATT CAC CCA RGC GCT CGG	460-478, 663[b]	697-723[c]

[a]Underlined nucleotides indicate the molecular beacon's stem.
[b]These sequences were aligned to derive the corresponding primer.
[c]The nucleotide positions refer to the *C. albicans* atpD sequence fragment (SEQ ID NO. 460).

Annex LIII: Internal hybridization probes for specific detection of atpD sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: *Acinetobacter baumannii*			
2169	5'-CCC GTT TGC GAA AGG TGG	243	304-321
Bacterial species: *Klebsiella pneumoniae*			
2167	5'-CAG CAG CTG GGC GGC GGT	317	36-53

Annex LIV: Internal hybridization probes for specific detection of ddl and mtl sequences.

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment SEQ ID NO.	Originating DNA fragment Nucleotide position
Bacterial species: *Enterococcus faecium* (ddl)			
2286	5'-AGT TGC TGT ATT AGG AAA TG	2288[a]	784-803
2287	5'-TCG AAG TTG CTG TAT TAG GA	2288[a]	780-799
Bacterial species: *Enterococcus faecalis* mtl)			
2289	5'-CAC CGA AGA AGA TGA AAA AA	1243[a]	264-283
2290	5'-TGG CAC CGA AGA AGA TGA	1243[a]	261-278
2291	5'-ATT TTG GCA CCG AAG AAG A	1243[a]	257-275

[a]Sequence from databases.

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20100267012A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. A composition for the detection of a vancomycin resistant pathogen in a sample using a nucleic acid amplification assay, wherein said composition comprises a primer pair for the amplification of vanA sequences, said primer pair comprising a forward and a reverse oligonucleotides, wherein said forward and reverse oligonucleotides are between 13 and 30 nucleotides, and wherein said forward and reverse oligonucleotides each comprises a binding region that is complementary to primer binding sites present on opposite strands of the pathogen's DNA, wherein said primer pair is adapted to amplify a target region of a vancomycin resistance gene of said pathogen's DNA between and including said primer binding sites to produce a detectable vanA vancomycin amplification product, wherein at least one of said oligonucleotides comprises, or is fully complementary to, at least 11 consecutive nucleotides of SEQ ID NO: 1090 and SEQ ID NO: 1091, or the complements thereof.

2. (canceled)

3. (canceled)

4. The composition of claim **1**, further comprising at least one internal hybridization probe, wherein said internal hybridization probe can hybridize under stringent conditions to said vanA vancomycin amplification product.

5. The composition of claim **4**, wherein said internal hybridization probe is a molecular beacon.

6. The composition of claim **4**, wherein said internal hybridization probe comprises at least 10 consecutive nucleotides of SEQ ID NO: 2299.

7. (canceled)

8. The composition of claim **4**, further comprising:

an internal control DNA; and

an internal control probe,

wherein said internal control DNA is amplified using the said forward and reverse oligonucleotides to produce a control amplification product in said nucleic acid amplification assay,

and wherein said internal control probe hybridizes to said internal control DNA under the same conditions as said internal hybridization probe hybridizes to said vancomycin amplification product.

9. The composition of claim **8**, wherein said internal control probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2301.

10. A kit comprising the composition of claim **1**.

11. A method to detect the presence of vancomycin-resistant organisms in a sample comprising nucleic acids, comprising:

annealing the nucleic acids of said sample with the composition of claim **4**;

amplifying said sample nucleic acids with said annealed primer pair; and determining the amount of internal hybridization probe that is annealed to the vanA vancomycin amplification product and the target region.

12. The method of claim **11**, wherein said primer pair consists of SEQ ID NO's 1090 and 1091, or the complements thereof.

13. The method of claim **11**, wherein said primer pair and said internal hybridization probe are placed in the same physical enclosure.

14. (canceled)

15. The method of claim **11** wherein amplification step comprises a method selected from the group consisting of:

(a) polymerase chain reaction (PCR),

(b) ligase chain reaction,

(c) nucleic acid sequence-based amplification,

(d) self-sustained sequence replication,

(e) strand displacement amplification,

(f) branched DNA signal amplification,

(g) nested PCR, and

(h) multiplex PCR.

16. The method of claim **15**, wherein said amplification step comprises PCR.

17. (canceled)

18. The method of claim **11**, wherein said internal hybridization probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2299.

19. The composition of claim **1**, wherein said forward or said reverse oligonucleotide consists of SEQ ID NO: 1090.

20. The composition of claim **1**, wherein said forward or said reverse oligonucleotide consists of SEQ ID NO: 1091.

21. The composition of claim **1**, wherein said forward and reverse oligonucleotides consist of SEQ ID NO: 1090 and SEQ ID NO: 1091, respectively.

22. The composition of claim **21**, further comprising an internal hybridization probe, wherein said internal hybridization probe can hybridize under stringent conditions to said vancomycin amplification product.

23. The composition of claim **22**, wherein said internal hybridization probe is a molecular beacon.

24. The composition of claim **22**, wherein said internal hybridization probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2299.

25. The composition of claim **22**, further comprising:

an internal control DNA; and

an internal control probe,

wherein said internal control DNA is amplified under the same conditions and using said forward and reverse primers to produce a control amplification product, and wherein said internal control probe hybridizes to said internal control DNA under the same conditions as said internal hybridization probe hybridizes to said vanA vancomycin amplification product.

26. The composition of claim **25**, wherein said internal control DNA is SEQ ID NO: 2302.

27. The composition of claim **25**, wherein said internal control probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2301.

28. The composition of claim **8**, wherein said internal control DNA is SEQ ID NO: 2302.

29. The composition of claim **1**, further comprising a second amplification primer pair for the amplification of vanB sequences, said second primer pair comprising a second forward and a second reverse oligonucleotide, wherein said second forward and second reverse oligonucleotides are each between 13 and 30 nucleotides and wherein each of said forward and reverse oligonucleotides comprises a binding region that is complementary to primer binding sites present on opposite strands of the pathogen's DNA, wherein said primer pair is adapted to amplify a vancomycin resistance gene of said pathogen's DNA between and including said primer binding sites to produce a detectable vanB vancomycin amplification product, wherein at least one of said oligonucleotides of said second primer pair is selected from the group consisting of: SEQ ID NO: 1095 and SEQ ID NO: 1096, or the complements thereof.

30. The composition of claim **29**, wherein said second forward and second reverse oligonucleotides of said second primer pair consist of SEQ ID NO: 1095 and SEQ ID NO: 1096, respectively.

31. The composition of claim **29** further comprising an internal hybridization probe, wherein said internal hybridization probe can hybridize under stringent conditions to said vanA vancomycin amplification product or said vanB vancomycin amplification product.

32. The composition of claim **31**, wherein said internal hybridization probe is a molecular beacon.

33. The composition of claim **31**, wherein said internal hybridization probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2299.

34. The composition of claim **31**, wherein said internal hybridization probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2300.

35. The composition of claim **29**, further comprising:

an internal control DNA; and

an internal control probe,

wherein said internal control DNA is amplified under the same conditions and using the same forward and reverse oligonucleotides to produce a control amplification product, and wherein said internal control probe hybridizes to said internal control DNA under the same conditions as said internal hybridization probe hybridizes to said vanA or vanB vancomycin amplification product.

36. The composition of claim **36**, wherein said internal control DNA comprises SEQ ID NO: 2302.

37. The composition of claim **36**, wherein said internal control probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2301.

38. The method of claim **11**, further comprising annealing the sample nucleic acids with:

an internal control DNA; and

an internal control probe,

wherein said internal control DNA is amplified under the same conditions and using the same forward and reverse oligonucleotides as to produce a control amplification product, and wherein said internal control probe hybridizes to said internal control DNA under the same conditions as said internal hybridization probe hybridizes to said vanA vancomycin amplification product.

39. The method of claim **38**, wherein said internal control probe comprises at least 10 consecutive nucleotides of the sequence of SEQ ID NO: 2301.

40. The method of claim **38**, wherein said internal control DNA comprises of SEQ ID NO: 2302.

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