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Design and Evaluation of Useful Bacterium-Specific PCR Primers That Amplify Genes Coding for Bacterial 16S rRNA

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We report the design and evaluation of PCR primers 63f and 1387r for amplification of 16S rRNA genes from bacteria. Their specificity and efficacy were tested systematically with a variety of bacterial species and environmental samples. They were found to be more useful for 16S rRNA gene amplification in ecological and systematic studies than PCR amplimers that are currently more generally used.

Until recently, the estimation of bacterial biodiversity has been hampered by limitations associated with cultivating bacteria from natural environments. An uncultured fraction has been recognized to be a major component of all microbial communities (7). The application of molecular approaches to the characterization of bacterial communities has overcome the requirement for prior cultivation of community members. In particular, the analysis of 16S rRNA genes, aided by using PCR to amplify target sequences in environmental samples, has enabled molecular ecologists to provide better estimates of bacterial diversity (1). PCR primers for amplification of 16S rRNA genes are widely available (6, 13). However, a problem with several commonly used amplimers is that they have been constructed theoretically, using the (incomplete) database of 16S rRNA sequences from cultured organisms, and have not been tested systematically. Hence, empirical testing is essential to confirm PCR primer specificity prior to their use in PCRs with environmental samples.

Primers for PCR described by Lane (6), which have been used by many other workers (5, 8), consistently failed to work with some difficult samples generated in our work. DNA samples extracted from various sources, including deep sea sediment, oral bacteria, and bacteria isolated from epilithon (biofilms associated with stones in lotic habitats), were found to be poor templates for amplification of the 16S rRNA gene with amplimers such as 27f and either 1492r or 1392r (numbering is based on the Escherichia coli 16S rRNA gene [3]). We had attempted to optimize the PCR conditions for 27f-1392r by altering the annealing temperature, Mg²⁺ concentration, and DNA template concentration and also by including the PCR additives bovine serum albumin, Triton X-100, T4 gene 32 protein, polyethylene glycol 8000 and glycerol. Finally, we decided to redesign the amplimers, and when we used 63f and 1387r with the difficult DNA samples, we were successful.

The wide adoption of amplimers 27f, 1392r, and 1492r is empirically based, and although their utility for investigating the molecular ecology of natural bacterial communities is often assumed, to our knowledge they have not been systematically tested. In this communication, we describe a new set of amplimers which were designed to be universal for the domain *Bacteria* (14) and their testing on a range of pure cultures and difficult natural samples.

Unless otherwise noted, genomic DNA was extracted from pure cultures, reference strains, and environmental samples by a modification of the method of Ausubel et al. (2). DNA from marine sediment samples (1 to 2 g), collected as described previously (9), was extracted by a modification of the method of Rochelle et al. (10). After the lysozyme step, 100 μ l of proteinase K (18 mg/ml; Sigma) was added, and the solution was further incubated for 1 h at 37°C. Second, the phenolchloroform step was replaced by adding one-half volume of 7.5 M ammonium acetate, and the mixture was centrifuged at 11,220 × g_{av} for 20 min at 4°C. DNA from the three *Leptospira* strains was obtained as a thermolysate (2a).

Two PCR primers were designed (Oligo, version 3.4; National Biosciences Inc.) to amplify approximately 1,300 bp of a consensus 16S rRNA gene (6): forward primer 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387r (5'-GGG CGG WGT GTA CAA GGC-3') (Pharmacia). Primers 27f and 1392r (6) were also used.

The PCR mixtures (100 μ l) contained 20 pmol of each appropriate primer, 200 μ M each deoxynucleoside triphosphate, *Taq* extender PCR buffer (Stratagene Ltd.), 0.5 U of *Taq* extender (Stratagene), and 0.5 U of *Taq* polymerase (Boehringer). Approximately 200 to 300 ng of DNA from a test strain culture and subnanogram quantities of sediment DNA were added to PCRs. In addition, 2 μ g of T4 gene 32 protein (Pharmacia) was included in PCRs of sediment DNA. PCR was performed with one of the following thermal cyclers: Hybaid Omnigene, Omni-E, or TR1; Perkin-Elmer 460; or MJ Research PTC-100. All cyclers were programmed to perform 30 cycles consisting of 95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min followed by a final extension step of 5 min at 72°C. PCR products were visualized by agarose gel electrophoresis (11).

The specificities of the new primers were tested in PCRs

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TABLE 1. Species of bacteria and archaea tested in PCRs using amplimer 63f and 1387f to amplify 16S rRNA genes

Strain ^a	Woese grouping	PCR amplification ^b
Chlamydia psittaci ^c	Planctomyces and relatives	+
Synechocystis sp. strain PCC 6803	Cyanobacteria and chloroplasts	+
Synechococcus sp. strain WH 7803	Cyanobacteria and chloroplasts	+
Synechococcus sp. strain PCC 7942	Cyanobacteria and chloroplasts	+
Nostoc sp.	Cyanobacteria and chloroplasts	+
Anabaena sp. strain PCC 7120 ^c	Cyanobacteria and chloroplasts	+
Runella sp.	Flexibacter-Cytophaga-Bacteroides phylum	+
Prevotella oris	Flexibacter-Cytophaga-Bacteroides phylum	+
Prevotella veroralis	Flexibacter-Cytophaga-Bacteroides phylum	+
Prevotella buccalis	Flexibacter-Cytophaga-Bacteroides phylum	+
Prevotella intermedia	Flexibacter-Cytophaga-Bacteroides phylum	+
Cytophaga ochracea	Flexibacter-Cytophaga-Bacteroides phylum	+
Rhodobacter sphaeroides	α-Proteobacteria	+
Alcaligenes eutrophus JMP222	B-Proteobacteria	+
Myxococcus xanthus NCIMB 9412 ^c	δ-Proteobacteria	+
Desulfovibrio desulfuricans ^c	δ-Proteobacteria	+
Desulfovibrio salexigens ^c	δ-Proteobacteria	+
Desulfovibrio satemgens Desulfovibrio satemgens	δ-Proteobacteria	+
Desulforacter postaatei	8-Proteobacteria	+
Desulfobultus provionicus ^c	8-Proteobacteria	+
Walinella succinagenes ATCC 205/3	s-Proteobacteria	+
Fusohactarium nucleatum ^c	c-Proteobacteria	+
Campulohactor concisus ATCC 33237	c Proteobacteria	1
Campylobacter sputorum subsp. sputorum ATCC 35080	c Proteobacteria	1
Campylobacter spation and Subsp. spation ATCC 55760	e Protochastoria	1
Campylobacter reclus ATCC 35256	e Protochastoria	- -
Campylobacter curvus ATCC 55224	e-Froteobacteria	т 1
Eacharistic coli UD1016	E-FIOICODACICIIA	т 1
Asingto haster as loggesting AC9250	γ-Proteobacteria	+
ActinetoDacter calcoaceticus AC8250	γ-Proteobacteria	+
<i>Pseudomonas punda</i> UWCI ⁻	γ-Proteobacteria	+
Kiedstella pneumoniae	γ-Proteobacteria	+
Euloacterium nodatum ATCC 33099°	Clostridia and relatives	+
Eubacterium brachy ATCC 33089	Clostridia and relatives	+
Eubacterium timidum ATCC 33092, ATCC 33093	Clostridia and relatives	+
Eubacterium tardum SCo8, SC88P	Clostridia and relatives	+
Eubacterium exiguum ATCC 700122	Clostridia and relatives	+
Eubacterium minutum ATCC 700079, ATCC 700080	Clostridia and relatives	+
Eubacterium saburreum ATCC 332/1, ATCC 33318, ATCC 33319, ATCC 43850	Clostridia and relatives	+
<i>Pseudoramibacter alactolyticus</i> DSM 3980 (= ATCC 23263)	Clostridia and relatives	+
Ruminococcus torques ATCC 27/56	Clostridia and relatives	+
Veillonella atypica ATCC 17744	Clostridia and relatives	+
Veillonella dispar ATCC 17748	Clostridia and relatives	+
Veillonella parvula ATCC 10790	Clostridia and relatives	+
Veillonella caviae ATCC 33540	Clostridia and relatives	+
Veillonella criceti ATCC 17747	Clostridia and relatives	+
Veillonella rodentium ATCC 17743	Clostridia and relatives	+
Veillonella ratti ATCC 17746	Clostridia and relatives	+
Eubacterium lentum NCTC 11813, ^c ATCC 25559	Gram positive, high G+C	+
Micrococcus luteus	Gram positive, high G+C	+
Actinomyces naeslundii genosp. 1 ATCC 12104	Gram positive, high G+C	+
Actinomyces naeslundii genosp. 2 WVU 627	Gram positive, high G+C	+
Actinomyces israelii ATCC 12102, ATCC 10048, MTU 01003, MTU 01004	Gram positive, high G+C	+
Actinomyces gerencseriae ATCC 23860	Gram positive, high G+C	+
Actinomyces odontolyticus ATCC 17929	Gram positive, high G+C	+
Actinomyces meyeri ATCC 35568	Gram positive, high G+C	+
Actinomyces georgiae ATCC 49285	Gram positive, high G+C	+
Actinomyces viscosus ATCC 15987	Gram positive, high G+C	+
Propionibacterium acnes ATCC 11827	Gram positive, high G+C	+
Bacillus cereus NCTC 7464	Bacillus-Lactobacillus-Streptococcus subdivision	+
Bacillus popilliae NRRL B2309M ^c	Bacillus-Lactobacillus-Streptococcus subdivision	+
Peptostreptococcus anaerobius	Bacillus-Lactobacillus-Streptococcus subdivision	+
Lactobacillus delbrueckii subsp. delbrueckii ATCC 9649	Bacillus-Lactobacillus-Streptococcus subdivision	+
Lactobacillus corvniformis subsp. torauens ATCC 25600	Bacillus-Lactobacillus-Streptococcus subdivision	+
Streptococcus salivarius ATCC 7073	Bacillus-Lactobacillus-Streptococcus subdivision	+
Streptococcus sanouis ATCC 10556	Bacillus-Lactobacillus-Streptococcus subdivision	+
Fuhacterium sanhenum ATCC 49989	Gram positive low G+C	+
Leptospira inadai. ATCC 43289	Spirochaeta-Treponema-Borrelia subdivision	$+^{d}$
	Space and a sponorm borrow subdivision	

Continued on following page

Strain ^a	Woese grouping	PCR amplification ^b
Leptospira kirschneri ATCC 23469	Spirochaeta-Treponema-Borrelia subdivision	$+^d$
Leptospira wolbachii ATCC 43284	Spirochaeta-Treponema-Borrelia subdivision	$+^{d}$
Treponema denticola ATCC 35405, ATCC 33520	Spirochaeta-Treponema-Borrelia subdivision	+
Treponema vincentii ATCC 35580	Spirochaeta-Treponema-Borrelia subdivision	+
Treponema pallidum subsp. pallidum Nichols strain	Spirochaeta-Treponema-Borrelia subdivision	+
Treponema phagedenis biovar Kazan ATCC 27087, biovar Reiter	Spirochaeta-Treponema-Borrelia subdivision	+
Treponema socranskii subsp. socranskii ATCC 35536	Spirochaeta-Treponema-Borrelia subdivision	+
Treponema medium G7201	Spirochaeta-Treponema-Borrelia subdivision	+
Methanosarcina frisius	Euryarchaeota, Methanosarcinale	-
Halobacterium halobium NCMB 2090	Euryarchaeota, extreme halophile	-

^a Strains not identified as originating from specific culture collections were well-characterized strains from culture collections held by participating laboratories.

^b +, positive reaction, i.e., a single amplicon of the correct predicted size (ca. 1,300 bp); -, no PCR product.

^c The PCR product of this strain was sequenced to ensure that it represented the 16S rRNA gene from the organism.

^d The 16S rRNA gene from this Leptospira species was successfully amplified; however, the resulting amount was approximately 90% less than the signal generated from an amplification of the 16S rRNA gene of *E. coli*.

with template DNA from cultures of well-characterized species representing the major groups of the domain *Bacteria* (Table 1) (14). Since DNA from the *Thermotogales*, green non-sulfur bacteria, and *Fibrobacteria* groups was not available for testing, the sequence similarities between amplimers 63f and 1387r and the 16S rRNA genes of selected species from those groups were assessed by aligning the amplimer sequences with the appropriate prealigned sequences obtained from the Antwerp rRNA database (12) (Table 2). In the comparison of alignments shown in Tables 2 and 3, one main point emerged. Amplimers 63f and 1387r successfully amplified 16S rRNA genes from species showing higher levels of theoretical 5' mismatches than amplimer pair 27f-1392r, in some cases with 3' mismatches in 1387r.

In a range of experimental studies carried out in the participating laboratories, primers 63f and 1387r were used successfully and consistently to amplify 16S rRNA genes from template DNA extracted from a variety of organisms: organisms identified as belonging to the coryneform and *Micrococcus* genera (gram-positive, high-G+C bacteria), cultured for the first time from concrete; *Eubacterium* species cultured from dental abscesses; novel δ -proteobacteria (sulfate- and ironreducing bacteria); epilithic samples; and deep sea sediments. Conversely, amplimer pair 27f-1392r failed to amplify the 16S rRNA genes of many of these test samples.

Our results provide no clear theoretical explanation for why

amplimer pair 63f-1387r was so much more successful than 27f-1392r. One suggestion is that the latter amplimers are not optimal for PCR since 27f may form an intramolecular duplex with a 5' overhang and may thus be susceptible to the $5' \rightarrow 3'$ exonuclease activity of *Taq* polymerase. Any resultant removal of 5' nucleotides from 27f (possibly six in total) would affect the annealing temperature of the primer pair (ΔT_m of 13.6°C instead of 1.6°C) and also result in unfavorable intermolecular complementarity between 27f and 1392r, leading to binding of the 3' ends. An alternative explanation comes from a recent computer analysis of the potential of primers to hybridize with 16S rRNA genes (4). In this study, a primer designed for the conserved area of the 16S rRNA gene, which was also used for 63f, was found to have a greater hybridization potential than the conserved area used for 27f.

In conclusion, although 63f and 1387r showed some theoretical bias, in practice they were more successful than amplimer pair 27f-1392r and amplified 16S rRNA genes from a wider range of bacteria than other primers which are commonly used for bacterial community analysis. So far as we are aware, the other primers have not been tested in the systematic way described for 63f-1387r in this paper. The results presented here suggest that the latter primer pair may be better suited for this type of molecular ecological analysis, the aim of which is to minimize PCR bias, and underline the point that the theoretical design of PCR amplimers is only the beginning

 TABLE 2. Theoretical alignment of sequences of amplimers 63f and 1387r with database sequences of 16S rRNA genes from species not tested by PCR

0	11/	Sequence vs ^{<i>a</i>} :		
Strain	woese grouping	63f	1387r	
		CAGGCCTAACACATGCA-AGTC	CGGAACATGTGWGGCGGG	
Fibrobacter intestinales	Fibrobacter spp.	AGTGTCTTATACATGCA-AGTC	GCCTTGTACACACCGCCC	
Chlorobium limicola	Green non-sulfur	C <u>GT</u> GCCTAACACATGCA—AGTC	GCCTTGTACACACCGCCC	
Chloroflexus aurantiacus	Green non-sulfur	CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC	
Herpetosiphon aurantiacus	Green non-sulfur	CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC	
Thermomicrobium roseum	Green non-sulfur	CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC	
Deinococcus radiodurans	Radioresistant micrococci and relatives	CGTGCTTAAGACATGCA-AGTC	GCCTTGTACACACCGCCC	
Thermus aquaticus	Radioresistant micrococci and relatives	CGTGCCTAAGACATGCA-AGTC	GCCTTGTACACACCGCCC	
Thermus ruber	Radioresistant micrococci and relatives	T A T GCCTAA G ACATGCA-AGTC	GCCTTGTACACACCGCCC	
Fervidobacterium nodosum	Thermotogales	CGTGCCTAACACATGCA-AGTC	GCCTTGTACACACCGCCC	
Thermotoga elfii	Thermotogales	CGTGCTTAACACATGCA-AGTC	G GT TTGCACACACCGCCC	
Thermotoga maritima	Thermotogales	$C\overline{\mathbf{GT}}G\overline{\mathbf{C}}C$ TAACACATGCA—AGTC	GCCTTGTACACACCGCCC	

^a Nucleotides underlined and in boldface differ from corresponding nucleotides in amplimer.

Main Wilds grup 27 and 63f 1387 and 1392r Redokover pharmides ATH 2.4.1 e-Proteobacteria CAGGCCTAACACACGCAG-GTC CECTETTERACACGEGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG			Sequence vs primers ^a :			
ADAGTTGATCHTGGTC [†] CAGGCTAACACATGCAA-GTC CRETCHGGGGGG Rhadabacter sphaensides AH12.4.1 a-Proteobateria GAGATTTAATCCTGGCTAGGAAGACGCAAGCATGCAA-GTC GCTTGTACACACGCGGCGG Adadgenes europhis B-Proteobateria GAGATTTAATCCTGGCTCAGAATGAAGCGTGGGGGGGGGCGTAAGACACATGCAA-GTC GCTTGTACACACGCGGCGCG Complobacter oncisus (FDC 288) e-Proteobateria GAGATTTGATCCTGGCTCAGAAGTGAAGCCTGGGGGGGGG	Strain	woese group	271	f and 63f	1387r and 1392r	
Rhodbacter spharolide NTF 2.41a-ProteobacteriaGAGATTTAATCTGACTAGAATGAAGGACGGCAGACCTAACACATGCAA-GTCGCCTTATACCACCGCCCCCCCamplobacter oncisus (PLC 288)e-ProteobacteriaGAGATTTAATCCTGACTAGAATGAAGGCTGGGCAGGCCTAACACATGCAA-GTCGCCTTATACCACCGCCCCC9584)complobacter oncisus (CLC 312)e-ProteobacteriaGAGATTTAATCCTGACTAGAGTGAAGGCTGGGGGGGCCTAATACATGCAA-GTCGCCTTATACCACCGCCCCCT9584)e-ProteobacteriaGAGATTTAATCCTGACTAGAGTGAAGGCTGGGGGGGGCCTAATACATGCAA-GTCGCCTTATACCACCGCCCCCTCamplobacter oncisus (CLC 312)e-ProteobacteriaGAGATTTAATCCTGACTAGAGTGAAGGCTGGGGGGGGCCTAATACATGCAA-GTCGCCTTATACCACCGCCCCGTCamplobacter oncisus (CLC 312)e-ProteobacteriaGAGATTTAATCCTGACTAGAGTGAAGGCTGGGGGGGGCCTAATACATGCAA-GTCGCCTTATACCACCGCCCGTMooccaccu annutus DKIGC (ATCC mil)p-ProteobacteriaGAGATTTAATCCTGACTGAGAGGCGGGGGGGGGGGGGGG			$\textbf{AGAGTTTGATCMTGGCTC}^{\mathcal{D}}$	${\tt CAGGCCTAACACATGCAA-GTC}^c$	CRTGTGTGGCGGGCA ^d CGGAACATGTGMGGCGGG ^e	
Alcaligens europhus β-Proteobaterin AAGASTTTGATCOTGGCTGAARTTLAACCOTGGCGCACCTCACACACTCAA-OTC GTCTTGTACACACCGCCCT Comploblacter eurous (ATCC 5524 ⁺ ; VP e-Proteobaterin GAGASTTTGATCOTGGCTGAGGTGACGCGGCGCGCCTAARACATTGAACGTGGCGGCGGCGCCTAARACATGCAA-OTC GTCTTGTACCACCGCCCGT Complobater reus (CCUG 19168) e-Proteobaterini GAGASTTTGATCCTGGCTGAGGTGACGGGGGGCGCCTAARACATGCAA-OTC GTCTTGTACCACCGCCCGT Complobater reus (CCUG 19168) e-Proteobaterini GAGASTTTGATCCTGGCTGAGGTGACGGGGGGGCCTAAACATGCAA-OTC GTCTTGTACCACCGCCCGT Deudiotino dustriaus (TACC 2774) b-Proteobaterini GAGASTTTGATCCTGGCTGAGGTGGCGGGGGGCGTGTAACATGCAA-OTC GTCTTGTACACCGCCCCGT Maccoccus annuu DKIG2 (ATCC 2774) b-Proteobaterini GAGASTTTGATCCTGGCTGAGGCGGGGGGGGCTAACACATGCAA-OTC GCCTTGTACACCGCCCCGT Maccoccus annuu DKIG2 (ATCC 2774) b-Proteobaterini GAGASTTTGATCCTGGCTGAGGCGAACGCTGGCGGGGGGCCTAACACATGCAA-GTC GCCTTGTACACCGCCCCGT Paulos acterica ductorina dustriaus (Transcrinaus Constructure) b-Proteobaterini GAGASTTTGATCCTGGCTGGGGGGGGGGGCCTAAGACATGCAA-GTC GCCTTGTACACCGCCCCCGT Paulos actobacillus Supplococcus subitision GAGASTTTGATCCTGGCTGGGGGGGGGGGCCTAAGACATGCAA-GTC GCCTTGTACACACGCCCCCCGT Paulos actobacillus Supplococcus subitision GAGASTTTGATCCTGGCTGGGGGGGGGCCCTAAGACATGCAA-GTC GCCTTGTACACACGCCCCCCGT B	Rhodobacter sphaeroides ATH 2.4.1	α-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAATGAAC	GCTGGCGGCAGGCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Campibbacter concisus (EPC 288) i-Proteobasteria GAGASTTTEATCOTGGCTGAGGTGGCGTGGCGTGGCTAAACATTGCA-GTC GTCTTGTACEACCCCCCGT Ossal Campibbacter reuss (CCUG 19168) i-Proteobasteria GAGASTTTGATCOTGGCTGAGGTGGCGTGGCGGTGGCCTAAACATTGCA-GTC GTCTTGTACEACCCCCCGT Campibbacter reuss (CCUG 3054) i-Proteobasteria GAGASTTTGATCOTGGCTGGGGGGGGGCGCGTAAACATGCAA-GTC GTCTTGTACEACCCCCCCT Dauliforbito desultarica i-Proteobasteria GAGASTTTGATCOTGGCTGGGGGGGGGCGCTAAACATGCAA-GTC GTCTTGTACEACCCCCCCT Macaccus i-Proteobasteria GAGASTTTGATCOTGGCTGGGGGGGGGGCGTAAACATGCAA-GTC GCTTGTACACCCCCCCCT Annotactus i-Proteobasteria GAGASTTTGATCCTGGCTGGGGGGGGGGCGTAAACATGCAACTGC GCTTGTACACCCCCCCCT Annotactus i-Proteobasteria GAGASTTTGATCCTGGCTGGGGGGGGGGCGTAAACATGCAA-GTC GCTTGTACACCCCCCCCT Actine constructus i-Proteobasteria AAGASTTTGATCCTGGCTGAGGGCGGGGGGGGCGTAAACATGCAA-GTC GCTTGTACACCCCCCCCT Actine cancel Relifica AAGASTTTGATCCTGGCTGAGGGCAGGCGCGAGGCGTAAGACATGGAA-GTC GCTTGTACACCCCCCCCT GCTTGTACACCCCCCCCT Actine cancel Relifica AAGASTTGATCCTGGCGCGGGGGGGGGGGGGGGGGGGGGG	Alcaligenes eutrophus	β-Proteobacteria	AAGAGTTTGATCCTGGCTCAGATTNAAC	GCTGGCGGCATGCCTTACACATGCAA-GTC	G T CTTGTACACCCGCCCGT	
Campiphotecre curus (ATCC 3524 ⁷ ; VPIe-ProteobateriaGAGACTITUATCCTOGCTAGACTAGACCTGCGCGGCGGCGCCCATAGACATCCAA-CTCGCTTGTACCACCCCCCTCampiphotecre retus (CCUG 19168)e-ProteobateriaGAGACTITUATCCTOGCTAGACTAGACGTGGCGGGGGCGCCATAGACATCGAA-CTCGCTTGTACCACCCCCCCTCampiphotecre retus (CUG 3054)e-ProteobateriaGAGACTITUATCCTOGCTAGACTGAACGCGGCGGGCGTCATACACATGCAA-CTCGCTTGTACCACCCCCCCTDeutifobioter postgateib-ProteobateriaGAGACTITUATCCTOGCTAGACTGAACGCGGCGGGCGTCATACACATGCAA-CTCGCTTGTACCACCCCCCCTDeutifobioter postgateib-ProteobateriaGAGACTITUATCCTOGCTAGAATGAACCTGGCGGGGGGCGTAACACATGCAA-CTCGCTTGTACCACCCCCCCTAscoccus antinis DK1622 (ATCC au)b-ProteobateriaGAGACTITUATCCTOGCTAGAACGCGGCGGGGGGCGTAACACATGCAA-GTCGCTTGTACCACCCCCCCTPeudoambinecre alcolpticus DSM 3980, T Proteobateria-AGACTITUATCCTOGCTAGGACGACCGCGGGGGGGCGTAATCACATGCAA-GTCGCCTTGTACCACCCCCCCTRecillus cereus 1481072 serotype 1 Catcholacillus comptiorisBacillus-Lactobacillus-Streptococcus sublivisionGAGACTITUATCCTGGCTAGGACGACGCGGGGGGGCGCAATACACTGCAA-GTCGCTTGTACCACCCCCCCTRecillus popiliaBacillus-Lactobacillus-Streptococcus sublivisionGAGACTITUATCCTGGCTAGGACGACGGCGGGGGGCGCAATACACTGACAGCGGGGGGGCGCCCAATACACATGCAA-GTCGCTTGTACACACCGCCCGTStrain CaberBacillus-Lactobacillus-Streptococcus sublivisionGAGACTITUATCCTGGCTAGGACGACGGCGGGGGGCGCAATACACTGCAA-GTCGCTTGTACACACCGCCCGTStrain CaberBacillus-Lactobacillus-Streptococcus sublivisionGAGACTITUATCCTGGCTAGGACGGCGGGCGGCGCCAATACACTGCAA-GTCGCTTGTACACACCGCCCGTStrain CaberBacillus-Lactobacillus-Streptococcus sublivisionGAGACTITUATCCT	Campylobacter concisus (FDC 288)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAAC	GCTGGTGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA—GTC	G <u>T</u> CTTGTAC <u>T</u> CACCGCCCGT	
Campiobacter rectus (CCUG 19168)e-ProteobacteriaGAGACTITGATCCTGGCTCAAGATGAACGCTGCCGGGGTGCTAAACACGA-GTCGCTTTATCCTAGCCACGCCCCTDesulfobicion (SAUSA)e-ProteobacteriaGAGACTITGATCCTGGCTCAAGATGAACGCTGCCGGGGTGCTAAACACATGCA-GTCGCTTTATACCACGCCCCCCDesulfobicion (SAUCC 2774)b-ProteobacteriaGAGACTITGATCCTGGCTCAAGATGAACGTGCGGGGGTGCTAAACACACGA-GTCGCCTTTATACCACGCCCCCCAchecotoace clacaceicus-ProteobacteriaAGACTITGATCCTGGCTCAAGATGAACGTGCGGGGGGTGCCTAAACACACGA-GTCGCCTTTATACACCCCCCCCCCCEscherichia col-Proteobacteria-AGACTITGATCATGGCTCAGGATGAACGTGGCGGCGTAACACACGCA-GTCGCCTTGTAACACCCCCCCCCCPendoaminteer alactoficius DSM 3980, ACICC 1143)-Proteobacteria-AGACTITGATCATGGCTCAGGATGAACGCTGGCGGGTGCCTAAACACACGCA-GTCGCCTTGTAACACCCCCCCCGTCCL 23263Bacillus-Lactobacillus-Streptococcus subdivisionGAGACTITGATCCTGGCTCAGGACGACGCTGCGCGTGCCTAAACACACGCAA-GTCGCCTTGTAACACCCCCCCCGTCL actobacillus ConvigionisBacillus-Lactobacillus-Streptococcus subdivisionGAGACTITGATCCTGGCTCAGGACGACGCTGGCGGTGCCTAAACACGCAA-GTCGCCTTGTAACACACCGCCCCGTStraito CAverBacillus-Lactobacillus-Streptococcus subdivisionGAGACTITGATCCTGGCTCAGGACGACGCTGGCGGTGCCTAAACACGCAA-GTCGCCTTGTAACACACCGCCCCGTStraito CAverBacillus-Lactobacillus-Streptococcus subdivisionGAGACTITGATCCTGGCTCAGGACGACGCTGGCGGTGCCTAAACACGCAA-GTCGCCTTGTAACACCCGCCCGTStraito CAverBacillus-Lactobacillus-Streptococcus subdivisionGAGACTITGATCCTGGCTCAGGACGACGCTGGCGGTGCCTAAACACCGCAA-GTCGCCTTGTAACACCCGCCCGTStraito CAverBacillus-Lactobacillus-Streptococcus subdivisionGAGACTTTGATCCTGGCTCAGGACGACGCTGGCGGGTGCCTAAAC	<i>Campylobacter curvus</i> (ATCC 35224 ^T ; VPI 9584)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAAC	CCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA-GTC	$G\underline{\mathbf{T}}CTTGTAC\underline{\mathbf{T}}CACNGCCCGT$	
Campibbacter showe (CCUG 3054) e-Proteobacteria GAACATTTACCCTGGCTCAAATGAACGCTGGCGGGGTGCTAACACATGCAA-GTC GCTTTACQCACCGCCCAT Desulfobiter postpatie Proteobacteria GAACATTTACCCTGGCTCAAATGAACGCTGGCGGGGTGCTAACACATGCAA-GTC GCTTTACACACGCCCCCT Mycocccus suntimus DK1622 (ATCC null) Proteobacteria GAACATTTACCAGCGTGCGGGGGGCTAACACATGCAA-GTC GCCTTTACACACGCCCCGT Acinetobacter calcoaceicus -yProteobacteria AAACATTTACATGGCTCAAGTAGAACGTGGCGGGGGTGCTAAACATGCAA-GTC GCCTTGTACACACGCCCCGT Peudoannibacter alacodocius Proteobacteria AAACATTTAATCCTGGCTCAAATGGAACGTGGCGGGGTGCTAAACATGCAA-GTC GCCTTGTACACACGCCCCGT Peudoannibacter alacodocius Proteobacteria AAACATTTAATCCTGGCTCAAGATGAACGCTGGCGGGGTGCTAAAACATGCAA-GTC GCCTTGTACACACCGCCCGT Racillus cercus F4S10/72 serotype 1 Bacillus-Lactobacillus-Steptococcus subdivision GAACATTTAATCCTGGCTCAAGACGACGCGGGGGGCCTAAACATGCAA-GTC GCCTTGTACACCCCCCGT Bacillus catobacillus-Steptococcus subdivision GAACATTTAATCCTGGCTCAAGACGACGCTGGGGGGGGCCTAATACATGCAA-GTC GCCTTGTACACCCCCCGT Steptococcus sanguis (NCTC 7863; ATCC Bacillus-Lactobacillus-Steptococcus subdivision GAACATTTGATCCTGGCTCAGGACGACGCTGGCGGGGGCCTAATACATGCAA-GTC GCCTTGTACACCCCCCGT Steptococcus sanguis (NCTC 7863; ATCC Bacillus-Lactobacillus-Steptococcus subdivision<	Campylobacter rectus (CCUG 19168)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAAC	GCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA—GTC	$G\underline{\mathbf{T}}CTTGTAC\underline{\mathbf{T}}CACCGCCCGT$	
Desulforbacter postgari& ProteobacteriaGAGATTITATACTCGGCTCAGAATGAAGCGTGGCGTGCTTAACACATGCAA-GTCONTITATACACACGCCCCTDesulforbicon (CTC 2774)& ProteobacteriaGAGATTIGATCGGGTCAGAATGAAGCGTGGCGGTGGCTTAACACATGCAA-GTGCCTTGTACACACGCCCCGTAcineobacter calcoacteria& ProteobacteriaAAGATTIGATCGGGTCGGAGTGGGGGGGGGGGGGGTGGCTAACACATGGAA-GTGCCTTGTACACACGCCCCGTExcherichia coli& ProteobacteriaAAGATTIGATCGGGTCGGGGGGGGGGGGGGGGGGGGGGGG	Campylobacter showae (CCUG 3054)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAAC	GCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA—GTC	G T CTTGTAC T CACCGCCCGT	
Desulfoxibio desulfuriams (ATCC 2774) b-Proteobacteria GAGGATTIGATTCAGGCTGGCIGGCIGGCIGGCATGCAATGCA	Desulfobacter postgatei	δ-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAATGAAC	GCTGGCGGC <u>GT</u> GC <u>T</u> TAACACATGCAA-GTC	GNNTTGTACACACCGCCNGT	
Myzococcus samihus DK1622 (ATCC null) 6-Protobacteria GAGATTGATCCTGGCTCAGACGACGCGCGCGCGCGCGCGC	Desulfovibrio desulfuricans (ATCC 27774)	δ-Proteobacteria	GAGAGTNTGAT <u>T</u> CTGGCTCAGATTGAAC	GCTGGCGGC <u>GT</u> GC <u>T</u> TAACACATGCAA-GTC	GCCTTGTACACCCGCCCGT	
Acinetobacter calcoacticus y-Protobacteria ACAGTTTGATCGTGGCTCAGATTGAACGCTGGGGGGGGCGTTAACCATGCAA-GTC GCCTTGTACCACCGCCCCCT Descherichia coli y-Protobacteria AAGGTTTGATCATGGCTCAGATTGAACGTGGGGGGGGGG	Myxococcus xanthus DK1622 (ATCC null)	δ-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAACGAAC	GCTGGCGGC <u>GT</u> GCCTAACACATGCAA-GTC	GCCTTGTACACCCGCCCGT	
Escherichia coliY-ProteobacteriaAAGATTTGATTCATGGGCCAGGGTTAACCATGGCA-GTCCCTTGTACACCGCCCCGTPseudomibuer alactobricus DSM 3980Y-Proteobacteria-AGAGTTTGATCTGGCTCAGGACGAGCGGGGGGGGGGGGG	Acinetobacter calcoaceticus	γ-Proteobacteria	AAGAGTTTGATCATGGCTCAGATTGAAC	GCTGGCGGCAGGC <u>T</u> TAACACATGCAA—GTC	GCCTTGTACACCCGCCCGT	
Pseudonumbacter alacolyticus DSM 3980, ATCC 2326 -Proteobacteria -AGAGTTTGATZMTGGCTCAGGAGAGAGCGTGGCGGTGCTAAGACAGTGGAGAGAGGTGGCGGGGCCTAAGACAGTGGCAGAGAGGGGGGGCGTGCCTAAGACAGTGGCAGAGAGGGGGGGG	Escherichia coli	y-Proteobacteria	AAGAGTTTGATCATGGCTCAGATTGAAC	GCTGGCGGCAGGCCTAACACATGCAA-GTC	CCTTGTACACACCGCCCGTC	
Bacillus cereus F4810/2 serotype 1 Bacillus-Lactobacillus-Sneptococcus subdivision GAGGTTTGATCCTGGCTCAGGAGGAGCGTGGCGGGTGCCTAAGACATGCAA-GTC GCCTTGTACACACCGCCGCT Bacillus popiliae Bacillus-Lactobacillus-Sneptococcus subdivision GAGGTTTGATCCTGGCTCAGGAGGAGCGTGGCGGGTGCCTAAGACATGCAA-GTC GCCTTGTACACACCGCCGCT Lactobacillus coryniformis Bacillus-Lactobacillus-Sneptococcus subdivision GAGGTTTGATCCTGGCTCAGGACGACGCTGGCGGGTGCCTAAGACATGCAA-GTC GCCTTGTACACACCGCCGCT Sneptococcus subdiviso Bacillus-Lactobacillus-Sneptococcus subdivision GAGGTTTGATCCTGGCTCAGGACGACGCTGGCGGGTGCCTAATACATGCAA-GTC GCCTTGTACACACCGCCGCT 13419) Sneptococcus subdivison GAGGTTTGATCCTGGCTCAGGACGACGCTGGCGGGTGCTTAACACATGCAA-GTC GCCTTGTACACACCGCCGCT Eubacterium angustum Clostridia and relatives GAGGTTTGATCCTGGCTCAGGACGGTGGCGGGGCGCTGAACACATGCAA-GTC GCCTTGTACACACCGCCCGCT Eubacterium fentum Clostridia and relatives GAGGTTTGATCCTGGCTCAGGACGGGGGCGCCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGCT Eubacterium nylanophilum Clostridia and relatives GAGGTTTGATCCTGGCTCAGGATGACGCTGGCGGGCGCCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGCT Prevotella intermedia Flexibacter-Cytophage-Bacteroides phylum GAGGTTTGATCCTGGCTCAGGATGAACGCTGGCGGTGCGTAACACATGCAA-GTC GCCTTGTACACACCGCCCGCT GCCTTGTACACACCGCCGCGCT	Pseudoramibacter alactolyticus DSM 3980, ATCC 23263	γ-Proteobacteria	-AGAGTTTGAT <u>Y</u> MTGGCTCAGGACGAAC	GCTGGCGG <u>T</u> A <u>T</u> GC <u>T</u> TAACACATGCAA—GTC	$G\underline{\mathbf{T}}$ CTTGTACACACCGCCCGT	
Bacillus popiliací Bacillus Jacobacillus Streptococcus subdivision GAGACTTGAATCCTGGCTCAGGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GTCTTGTACACACGCCCCGT Lactobacillus delbracckii sublis-Lactobacillus-Streptococcus subdivision GAGACTTGGTCAGGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GCCTTGTACACACGCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGACGGTGGCGGCGTGCTAATACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGAGCGGCGGCGCTAACACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGGCGGCGGCGTAACACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGGCGGCGGCGGCGTAACACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sublivinis GGAGATTGATCCTGGCTCAGGACGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCG	Bacillus cereus F4810/72 serotype 1 (NCTC 11143)	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTTGATCCTGGCTCAGGATGAAC	GCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA—GTC	GCCTTGTACACACCGCCCGT	
Lactobacillus conniformis Lactobacillus-Lactobacillus-Streptococcus subdivisionGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGGCGCCTAATACATGCAA-GTCGCCTTGTACACACGCGCCGT GCCTTGTACACACGCCCCGTStreptococcus salivarius C699 (ATCC 13419)Bacillus-Lactobacillus-Streptococcus subdivisionGAGAGTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAA-GTC GAGAGTTGATCCTGGCTCAGGACGAACGCTGGCGGCGCGCGC	Bacillus popilliae	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTTGATCCTGGCTCAGGACGAAC	GCTGGCGGC GT GCCTAA G ACATGCAA-GTC	G T CTTGTACACACCGCCCGT	
Lactobacillus delbrueckii Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTIGATCCTGGCTAGGAGAGAGCGTGGCGGGCGTGCCTAATACATGCAA-GTC GCCTTGTACACACGCGCCGT Streptococcus saluvirius C699 (ATCC Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTIGATCCTGGCTCAGGACGACGCTGGCGGCGTGCCTAATACATGCAA-GTA GCCTTGTACACACGCGCCGT Streptococcus saluvirius C699 (ATCC Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTIGATCCTGGCTCAGGACGACGCTGGCGGCGTGCTAACACATGCAA-GTC GCCTTGTACACACGCCCCGT Streptococcus sanguis (NCTC 7863; ATCC Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTIGATCCTGGCTCAGGACGACGCTGGCGGCGTGCTAACACATGCAA-GTC GCCTTGTACACACGCCCGT Eubacterium lentum Clostridia and relatives GAGAGTTIGATCCTGGCTCAGGACGACGCTGGCGGCGTGCTAACACATGCAA-GTC GCCTTGTACACACGCCCGT Eubacterium lentum Clostridia and relatives GAGAGTTIGATCCTGGCTCAGGACGACGCTGGCGGCGTAGCACATGCAA-GTC GCCTTGTACACACCGCCCGT Prevotella intermedia Flexibacter-Cytophaga-Bacteroides phylum GAGAGTTTGATCCTGGCTCAGGATNAACCCTGGCGGCGTCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT Prevotella vervalis Flexibacter-Cytophaga-Bacteroides phylum GAGAGTTTGATCCTGGCTCAGGATNAACCGTGGCGGCGTCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT Prevotella vervalis Flexibacter-Cytophaga-Bacteroides phylum GAGAGTTTGATCCTGGCTCAGGATNAACCGTGGCGGCGTCTAACACATGCAA-GTC	Lactobacillus corvniformis	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTTGATCCTGGCTCAGGACGAAC	GCTGGCGGCGTGCTAATACATGCAA-GTC	GCCTTGTACACACCGCNCGT	
Streptococcus salivarius C699 (ATCC 1349) Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTGATCCTGGCTCAGGACGAACGCTGGCGCGTGCCTAATACATGCAA-GTA GAGAGTTGATCCTGGCTCAGGACGAACGCTGGCGCGCGCG	Lactobacillus delbrueckii subsp. delbrueckii strain Calvert	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTNGATCCTGGCTCAGGACGAAC	GCTGGCGGCGTGCCTAATACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Streptococcus sanguis (NCTC 7863; ATCC 15300) Bacillus-Lactobacillus-Streptococcus subdivision GAGAGTTGATCCTGGCTCAGGAGAGACGCTGCGCGCGTGCTTAACACATGCAA-GTC GAGAGTTGATCCTGGCTCAGGATGAACGCTGGCGGCGCGCCTAACACATGCAA-GTC Eubacterium linusum GCCTTGTACACACGCCCGT GCTTGTACACACGCCCGT Eubacterium angustum Clostridia and relatives GAGAGTTGATCCTGGCTCAGGATGAACGCTGGCGGCGCGCCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACGCCCGT Eubacterium linusum Clostridia and relatives -AGAGTTGATCCTGGCTCAGGACGACGCTGGCGGGCGTGCCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT Subacterium sylanophilum Clostridia and relatives GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGGTGCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT Nostoc muscorum Cyanobacteria and chloroplasts GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT Prevotella intermedia Flexibacter-Cytophaga-Bacteroides phylum Prevotella veroralis Flexibacter-Cytophaga-Bacteroides phylum GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGGCTAACGACTGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT Prevotella veroralis Flexibacter-Cytophaga-Bacteroides phylum Prevotella veroralis GAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGCATGCAA-GTC GCCTTGTACACACCGCCCGT GCCTTGTACACACCGCCCGT Chlamydia psittaci Flexibacter-Cytophaga-Bacteroides phylum Propioninacterium anees GAGAGTTTGATCCTGGCTCA	Streptococcus salivarius C699 (ATCC 13419)	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTTGATCCTGGCTCAGGACGAAC	GCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA-GT <u>A</u>	GCNTNGTACACACCGCCCGT	
Eubacterium angustumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGCTTAACACATGCAA-GTCGTCTTGTACACACGCCCGTEubacterium fossorClostridia and relativesGAGAGTTGATCCTGGCTCAGGATGAACGCTGGCGCGCGCCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTEubacterium limosumClostridia and relatives-AGAGTTTGATCCTGGCTCAGGACGACGCTGGCGGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTEubacterium limosumClostridia and relativesGAGAGTTGATCCTGGCTCAGGACGACGCTGGCGGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTFuebacterium sylanophilumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella intermediaFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTACGGCGTTAACCACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTATAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGCTCAGGATACAGGCTTAACCACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGCTGCGCGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGCGCGGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGACGACGCTGCGGCGGCGTGTAACCACTGCAA-GTCGCCTTGTACACACCGCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGAGACGACGCTGCGGCGGCGTTAACCACAGCAA-GTCGCCT	Streptococcus sanguis (NCTC 7863; ATCC 15300)	Bacillus-Lactobacillus-Streptococcus subdivision	GAGAGTTTGATCCTGGCTCAGGACGAAC	:GCTGGCGGC <u>GT</u> GCCTAA <u>T</u> NCNTGCAA-GTN	GCCTTGTACACACCGCCCGT	
Eubacterium fossorClostridia and relativesGAGAGTTQGATCCTGGCTCAGGATGAACGCTGGCGGCGCCTAACACATGCAA-GTCGCNTTGTACACACCGCCGTEubacterium limosumClostridia and relativesGAGAGTTTGATCATGGACAGGCGGCGTGCCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTEubacterium sylanophilumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTAACACATGCAA-GTCGTCTTGTACACACCGCCCGTNosco muscorumCyanobacteria and chloroplastsGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella intermediaFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGCGTGGCGGCGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTLeptospira kirschneri ATCC 23469Spirochaeta-Treponema-Borrelia subdivision?????????????????????????????????	Eubacterium angustum	Clostridia and relatives	GAGAGTTTGATCCTGGCTCAGGATGAAC	GCTGGCGGC GT GC T TAACACATGCAA—GTC	G T CTTGTACACACCGCCCGT	
Eubacterium lentumClostridia and relatives-AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGTGCTAACACATGCAA-GTCGCCTTGTACACACCGCCGCTEubacterium linosumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGACGCTGGCGTGCTAACACATGCAA-GTCGTCTTGTACACACCGCCCGTEubacterium xylanophilumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGACGCTGGCGGTGCTAACACATGCAA-GTCGTCTTGTACACACCCGCCCGTNostoc muscorumCyanobacteria and chloroplastsGAGAGTTTGATCCTGGCTCAGGATGACGCTGGCGGTGCTAACACATGCAA-GTCGCCTTGTACACACCCGCCCGTPrevotella buccalisFlexibacter-Cytophaga-Bacteroides phylumTAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTATGAGCTATACACATGCAA-GTCGCCTTGTACACACCCGCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCCGCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCCGCCGTActinomyces viscosusGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAA-GTCGCCTTGTACACACCCGCCGTChlamydia psitaciPlanctomyces and relativesGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGCGCGTGTAACACATGCAA-GTCGCCTTGTACACACCCCCCGTLeptospira inadai ATCC 43289Spirochaeta-Treponema-Borrelia subdivision????????????????????????????STARCGTGGCGCGCGCTGTTAACACATGCAA-GTCACCTTGTACACACCCCCCGTTerponema denticolaSpirochaeta-Treponema-Borrelia subdivision?????????????????????????????????	Eubacterium fossor	Clostridia and relatives	GAGAGTTCGATCCTGGCTCAGGATGAAC	CCTGGCGGCGCCCTAACACATGCAA-GTC	GCNTTGTACACACCGCCCGT	
Eubacterium limosumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGGTATGCTTAACACATGCAA-GTCGTCTTGTACACACCGCCCCTEubacterium xylanophilumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGACGACGCGGGGTGCTTAACACATGCAA-GTCGTCTTGTACACACCGCCCCTNostoc muscorumCyanobacteria and chloroplastsGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGTATGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella intermediaFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCGTACAGCGTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGACGAACGCTGCGCGCGCG	Eubacterium lentum	Clostridia and relatives		GCTGGCGGCGTGCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Eubacterium xylanophilumClostridia and relativesGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGGCGTCTTAACACATGCAA-GTCGTCTTGTACACACCGCCCGTNostoc muscorumCyanobacteria and chloroplastsGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGGGTATGCTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella buccalisFlexibacter-Cytophaga-Bacteroides phylumTAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCGACGTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGACGAAGCTGCGGCGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGCGTGGCGGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPropionibacterium ancesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGCGTGGCGGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTLeptospira inadai ATCC 43289Spirochaeta-Treponema-Borrelia subdivision?????????????????????????????????	Eubacterium limosum	Clostridia and relatives	GAGAGTTTGATCCTGGCTCAGGACGAAC	GCTGGCGGTATGCTTAACACATGCAA-GTC	GTCTTGTACACACCCCCCCT	
Nosico muscorumCyanobacteria and chloroplastsGAGAGTTTGATCCTGGCTCAGGATAGACGCTGCGGGTATGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCCTPrevotella buccalisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTActinomyces viscosusGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGACGCTGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGC	Eubacterium xylanophilum	Clostridia and relatives	GAGAGTTTTGATCCTGGCTCAGGATGAAC	GCTGGCGGC GT GC T TAACACATGCAA—GTC	GTCTTGTACACACCGCNCGT	
Prevotella buccalisFlexibacter-Cytophaga-Bacteroides phylumTAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCACACAGTGCAA-GTCGCCTTGTACACACGCGCCGTPrevotella intermediaFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACGCGCCGTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTActinomyces viscosusGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGGCGGCGTGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTChlamydia psittaciPlanctomyces and relativesGAGATTTGATCCTGGCTCAGGACGGCGGGGGGGGGGGGG	Nostoc muscorum	Cyanobacteria and chloroplasts	GAGAGTTTTGATCCTGGCTCAGGATGAAC	GCTGGCGGTATGCTTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Prevotella intermediaFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCATGCAA-GTCGCCTTGTACACACGCCCCCTPrevotella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCCTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGCGCGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCCGTActinomyces viscosusGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGGATGAGACGAA-GTCGCCTTGTACACACCGCCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGGATGAGGCATGCAA-GTCGCCTTGTACACACCGCCCCGTChlamydia psitaciPlanctomyces and relativesGAGAATTTGATCCTGGTTCAGACGCTGCGGGCGGCGGGGGGGG	Prevotella buccalis	Flexibacter-Cytophaga-Bacteroides phylum	TAGAGTTTGATCCTGGCTCAGGATNAAC	CCTACCTACACGCTTAACACATCCAA-GTC	GCCTTGTACACACCGCCCGT	
Prevoiella orisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGGTTAACACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPrevotella veroralisFlexibacter-Cytophaga-Bacteroides phylumGAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGCTTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTActinomyces viscosusGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGGCTGGCGGCGTGTAACACATGCAA-GTCGCCTTGTACACACCGCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGGCTGGCGGCGTGGATGAACACATGCAA-GTCGCCTTGTACACACCGCCCGTChlanydia psittaciPlanctomyces and relativesGAGAATTTGATCCTGGTTCAGAATGGAAGCGTGGCGGCGGCGTGAAACACATGCAA-GTCGCCTTGTACACACCGCCCGTLeptospira inadai ATCC 43289Spirochaeta-Treponema-Borrelia subdivision?????????????????????????????????	Prevotella intermedia	Flexibacter-Cytophaga-Bacteroides phylum	GAGAGTTTGATCCTGGCTCAGGATNAAC	CCTAGCTATAGGCTTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Prevoided veroralis Flexibacter-Oytophaga-Bacteroides phylum GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCACAGTGCAA-GTC GCCTTGTACACACGCGCCCCT Actinomyces viscosus Gram positive, high G+C GAGAGTTTGATCCTGGCTCAGGACGAAGCGCTGGCGGCGTGCTTAACACATGCAA-GTC GCCTTGTACACACGCCCCCGT Propionibacterium acnes Gram positive, high G+C GAGAGTTTGATCCTGGCTCAGGACGACGCTGGCGGCGTGCTTAACACATGCAA-GTC GCCTTGTACACACCGCCCGT Chlamydia psittaci Planctomyces and relatives GAGAGTTTGATCCTGGTTCAGATTGAACGCTGGCGGCGGCGTGGAAGGCATGCAA-GTC GCCTTGTACACACCGCCCGT Leptospira inadai ATCC 43289 Spirochaeta-Treponema-Borrelia subdivision ????????????????????????????????????	Prevotella oris	Flexibacter-Cytophaga-Bacteroides phylum	GAGAGTTTTGATCCTGGCTCAGGATNAAC	GCTGGCTACAGGC T TAACACATGCAA—GTC	GCCTTGTACACACCGCCCGT	
Actinomyces viscosusGram positive, high G+CGAGAGTTNNATCCTGGCTCAGGACGAAGCTTGGCGGCGTGCTTAACACATGCAA-GTCGGCTTGTACACACCGCCCGTPropionibacterium acnesGram positive, high G+CGAGAGTTTGATCCTGGCTCAGGACGAAGCTTGGCGGCGTGCTTAACACATGCAA-GTCGTCTTGTACACACCGCCCGTChlamydia psittaciPlanctomyces and relativesGAGATTTGATCCTGGTTCAGATGAAGCGTGGCGGCGTGGATGAAGGCATGCAA-GTCGCCTTGTACACACCGCCCGTLeptospira inadai ATCC 43289Spirochaeta-Treponema-Borrelia subdivision?????????????????????????????????	Prevotella veroralis	Flexibacter-Cytophaga-Bacteroides phylum	GAGAGTTTGATCCTGGCTCAGGATNAAC	CCTACCTACACCCTTAACACATCCAA-GTC	GCCTTGTACACACCGCCCGT	
Propionilacterium acnes Gram positive, high G+C GAGAGTTTGATCCTGGCTCAGGACGACGCTGGCGGCGCGTCTTAACACATGCAA-GTC GTCTTGTACACACGCGCCCCT Chlamydia psittaci Planctomyces and relatives GAGAGTTTGATCCTGGCTCAGGACGCTGGCGGCGGCGTGGATGAA-GTC GCCTTGTACACACGCGCCCCT Leptospira inadai ATCC 43289 Spirochaeta-Treponema-Borrelia subdivision ??????????????STARCGTTGGCGGCGCGCGTCTTAAACATGCAA-GTC ACCTTGTACACACCGCCCCT Leptospira wolbachii ATCC 43284 Spirochaeta-Treponema-Borrelia subdivision ??????????????STARCGCTGGCGGCGCGCTCTTAAACATTCCAAGTC ACCTTGTACACACCGCCCCT Treponema denticola Spirochaeta-Treponema-Borrelia subdivision ???????????????????STARCGCTGGCGGCGCGTCTTAAACATGCAA-GTC ACCTTGTACACACCGCCCCCT Treponema denticola Spirochaeta-Treponema-Borrelia subdivision ??????????????????????????STARCGCTGGCGGCGCGTCTTAAACATGCAA-GTC GCCTTGTACACACCGCCCCCT Treponema phagedenis Spirochaeta-Treponema-Borrelia subdivision GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCGCGTCTTAAACAATGCAA-GTC GCCTTGTACACACCGCCCCCT	Actinomyces viscosus	Gram positive, high G+C	GAGAGTTNNATCCTGGCTCAGGACGAAN	IGCTNGCGGCGTGCTTAACACATGCAA-GTC	GGCTTGTACACACCGCCCGT	
Chlanydia psittaci Planctomyces and relatives GAGAATTGATCTTGGTTCAGACTGACGCGGGCGGGGGGGG	Propionibacterium acnes	Gram positive, high $G+C$	GAGAGTTTGATCCTGGCTCAGGACGAAC	GCTGGCGGCGTGCTTAACACATGCAA-GTC	GTCTTGTACACACCGCCCGT	
Leptospira inadai ATCC 43289 Spirochaeta-Treponema-Borrelia subdivision ????????????????????????????????????	Chlamydia psittaci	<i>Planctomyces</i> and relatives	GAGAATTTGATCTTGGTTCAGATTGAAC	GCTGGCGGCGTGGATGAGGCATGCAA-GTC	GCCTTGTACACACCGCCCGT	
Deptophra kinschneri ATCC 23469 Spirochaeta-Treponema-Borrelia subdivision Spirochaeta-Treponema-Borrelia subdivision CCTTGTACACACCGCCCCT Leptospira kinschneri ATCC 43284 Spirochaeta-Treponema-Borrelia subdivision Spirochaeta-Treponema-Borrelia subdivision CCTTGTACACACCGCCCCT Treponema denticola Spirochaeta-Treponema-Borrelia subdivision Spirochaeta-Treponema-Borrelia subdivision CCTTGTACACACCGCCCCT ACCTTGTACACACCGCCCCCT Treponema phagedenis Spirochaeta-Treponema-Borrelia subdivision GAGAGTTTGATCCTGGCTCAGAACGACGCGCGCGCTCTTAACATGCAA-GTC GCCTTGTACACACCGCCCCCT	Leptospira inadai ATCC 43289	Spirochaeta-Treponema-Borrelia subdivision	22222222222222222222222222222222222222	GTTGGCGGCGCGCGTCTTAAACATGCAA-GTC	ACCTTGTACACACCCCCCCGT	
Leptospira wolbachii ATCC 43284 Spirochaeta-Treponema-Borrelia subdivision ????????????????????????????????????	Leptospira kirschneri ATCC 23469	Spirochaeta-Treponema-Borrelia subdivision	22222222222222222222222222222222222222	GCTGGCGGCGCGCGTCTTAAACATTCCAACTC	ACCTTGTACACACCCGCCCGT	
Treponema denticola Spirochateta-Treponema-Borrelia subdivision GAGAGTTTGATCCTGGCTCAGAACGCTGGCGGCGCGTCTTAAGCATGCAA-GTC GCCTTGTACACACCGCCCCT Treponema phagedenis Spirochateta-Treponema-Borrelia subdivision GAGAGTTTGATCCTGGCTCAGAACGCTGGCGGCGCGTCTTAAGCATGCAA-GTC GCCTTGTACACACCGCCCCT	Leptospira wolbachii ATCC 43284	Spirochaeta-Treponema-Borrelia subdivision	22222222222222222222222222222222222222	GCTGGCGGCGCGCGTCTTAAACATGCA_ACTC	ACCTTGTACACACCCCCCCT	
Treponema phagedenis Spirochaeta-Treponema-Borrelia subdivision GAGAGTTTGATCCTGGCTCAGAACGCTGGCGGCGCGCGCG	Treponema denticola	Spirochaeta-Treponema-Borrelia subdivision	GAGAGTTTGATCCTGGCTCAGAACGAAC	GCTGGCGGCGCGTCTTAAGCATGCAA_GTC	GCCTTGTACACACCCGCCCGT	
	Treponema phagedenis	Spirochaeta-Treponema-Borrelia subdivision	GAGAGTTTGATCCTGGCTCAGAACGAAC	CCTGGCGGCGCGTCTTAAGCATGCAA-GTC	GCCTTGTACACACCGCCCGT	

TABLE 3. Alignment of sequences of amplimers 63f-1387r and 27f-1392r with the corresponding regions of 16S rDNA genes of representative species used in PCR

^a Primer sequences are written 5'→3'. Sequence data on listed species were obtained from the Antwerp ribosomal database. Nucleotides underlined and in boldface differ from corresponding nucleotides in amplimer.
 ^b Amplimer 27f sequence.
 ^c Amplimer 63f sequence.
 ^d Amplimer 1387r sequence.
 ^e Amplimer 1392r sequence.

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and that systematic empirical testing of the amplimers is of paramount importance.

We thank Nyree West and Julie Scanlan for the kind gift of DNA from the five representatives of the cyanobacteria and chloroplast group and Guy Baranton for the three *Leptospira* strains.

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REFERENCES

- Amann, R. I., W. Ludwig, and K.-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143–169.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1989. Current protocols in molecular biology. Greene Publishing Associates-Wiley Interscience, New York, N.Y.
- 2a.Baranton, G. Personal communication.
- Brosius, J., J. L. Palmer, H. P. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 75:4801–4805.
- Brunk, C. F., E. Avaniss-Aghajani, and C. A. Brunk. 1996. A computer analysis of primer and probe hybridization potential with bacterial smallsubunit rRNA sequences. Appl. Environ. Microbiol. 62:872–879.
- Delong, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA 89:5685–5689.
- 6. Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115-175. In E. Stackebrandt

and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chichester, United Kingdom.

- Parkes, R. J., B. A. Cragg, J. C. Fry, R. A. Herbert, and J. W. T. Wimpenny. 1990. Bacterial biomass and activity in deep sediment layers from the Peru margin. Philos. Trans. R. Soc. Lond. B 133:139–153.
- Reysenbach, A.-L., G. S. Wickham, and N. R. Pace. 1994. Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. Appl. Environ. Microbiol. 60:2113–2119.
- Rochelle, P. A., J. C. Fry, R. J. Parkes, and A. J. Weightman. 1992. DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sediment communities. FEMS Microbiol. Lett. 100:59–66.
- Rochelle, P. A., J. A. K. Will, J. C. Fry, G. J. S. Jenkins, R. J. Parkes, C. M. Turley, and A. J. Weightman. 1995. Extraction and amplification of 16S rRNA genes from deep marine sediments and seawater to assess bacterial community diversity, p. 219–239. *In* J. D. van Elsas and J. T. Trevors (ed.), Nucleic acids in the environment: methods and applications. Springer-Verlag, Berlin, Germany.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Van de Peer, Y., J. Jansen, P. De Rijk, and R. De Wachter. 1997. Database on the structure of small ribosomal subunit RNA. Nucleic Acids Res. 25: 111–116.
- Wheeler Alm, E., D. B. Oerther, N. Larsen, D. A. Stahl, and L. Raskin. 1996. The Oligonucleotide Probe Database. Appl. Environ. Microbiol. 62:3557– 3559.
- 14. Woese, C. R. 1987. Bacterial evolution. Microbiol. Rev. 51:221–271.

ERRATA

Methane and Trichloroethylene Oxidation by an Estuarine Methanotroph, *Methylobacter* sp. Strain BB5.1

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Volume 63, no. 11, p. 4618, Table 1: footnote *b* should read "Micromoles of CH₄ or nanomoles of TCE hour⁻¹ milligram of total protein⁻¹."

Page 4619, Fig. 3A and B, y axes: "nmol" should read "µmol."

Design and Evaluation of Useful Bacterium-Specific PCR Primers That Amplify Genes Coding for Bacterial 16S rRNA

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Volume 64, no. 2, p. 795: The article byline should read as given above. p. 797 and 798, Tables 2 and 3, respectively: columns 3 and 4 (the sequence data) in each table should read as shown below.

Sequence vs ^a :			
63f	1387r		
CAGGCCTAACACATGCA-AGTC	CGGAACATGTGWGGCGGG		
AGTGTCTTATACATGCA-AGTC	GCCTTGTACACACCGCCC		
CGT GCCTAACACATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCCTAATGCATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCTTAAGACATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCTAAGACATGCA-AGTC	GCCTTGTACACACCGCCC		
TATGCCTAAGACATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCTAACACATGCA-AGTC	GCCTTGTACACACCGCCC		
CGTGCTTAACACATGCA-AGTC	GGT TTGCACACACCGCCC		
CGTGCCTAACACATGCA-AGTC	GCCTTGTACACACCGCCC		

TABLE 2

Sequence vs primers ^a :			
271	f and 63f	1387r and 1392r	
AGAGTTTGATCMTGGCTC ^b	CAGGCCTAACACATGCAA-GTC ^C	CRTGTGTGGCGGGCA ^d CGGAACATGTGWGGCGGG ^e	
GAGAGTTTGATCCTGGCTCAGAATGAA	CGCTGGCGGCAGGCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
AAGAGTTTGATCCTGGCTCAGATTnAA	CGCTGGCGGCA T GCCT T ACACATGCAA-GTC	G T CTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAGTGAA	CGCTGGTGGC GT GCCTAA T ACATGCAA-GTC	G T CTTGTAC T CACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAGTGAA	CGCTGGCGGC GT GCCTAA T ACATGCAA-GTC	GTCTTGTACTCACNGCCCGT	
GAGAGTTTGATCCTGGCTCAGAGTGAA	CGCTGGCGGCGTGCTAATACATGCAA-GTC	GTCTTGTACTCACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAGTGAA	CGCTGGCGGC GT GCCTAA T ACATGCAA-GTC	GTCTTGTACTCACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAATGAA	CGCTGGCGGCGTGCTTAACACATGCAA-GTC	GNNTTGTACACCGCCNGT	
GAGAGTNTGATTCTGGCTCAGATTGAA	CGCTGGCGGCGTGCTTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAACGAA	CGCTGGCGGCGTGCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
AAGAGTTTGATCATGGCTCAGATTGAA	CGCTGGCGGCAGGC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
AAGAGTTTGATCATGGCTCAGATTGAA	CGCTGGCGGCAGGCCTAACACATGCAA-GTC	CCTTGTACACCGCCCGTC	
-AGAGTTTGATYMTGGCTCAGGACGAA	CGCTGGCGG T A T GC T TAACACATGCAA-GTC	G T CTTGTACACCCCCCCGT	
GAGAGTTTGATCCTGGCTCAGGATGAA	CGCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	CGCTGGCGGC <u>GT</u> GCCTAA <u>G</u> ACATGCAA-GTC	G <u>T</u> CTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	CGCTGGCGGC GT GCCTAA T ACATGCAA-GTC	GCCTTGTACACACCGCNCGT	
GAGAGTTNGATCCTGGCTCAGGACGAA	CGCTGGCGGC GT GCCTAA T ACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	CGCTGGCGGC <u>GT</u> GCCTAA <u>T</u> ACATGCAA-GT <u>A</u>	GCNTNGTACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	GCCTTGTACACACCGCCCGT		
GAGAGTTTGATCCTGGCTCAGGATGAA	CGCTGGCGGC <u>GT</u> GC <u>T</u> TAACACATGCAA-GTC	G <u>T</u> CTTGTACACACCGCCCGT	
$GAGAGTT\underline{\mathbf{C}}GATCCTGGCTCAGGATGAA$	CGCTGGCGGC <u>GC</u> GCCTAACACATGCAA-GTC	GCNTTGTACACCGCCCGT	
-AGAGTTTGATNNTGGCTCAGGATGAA	CGCTGGCGGC <u>GT</u> GCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	CGCTGGCGG <u>T</u> A <u>T</u> GC <u>T</u> TAACACATGCAA-GTC	G <u>T</u> CTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGATGAA	CGCTGGCGGC <u>GT</u> GC <u>T</u> TAACACATGCAA-GTC	G <u>T</u> CTTGTACACACCGCNCGT	
GAGAGTTTGATCCTGGCTCAGGATGAA	CGCTGGCGG T A T GC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
TAGAGTTTGATCCTGGCTCAGGATNAA	CGCTAGCTACAGGC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGATNAA	CGCTAGCTA T AGGC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGATNAA	CGCTGGCTACAGGC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGATNAA	CGCTAGCTACAGGC T TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT	
GAGAGTTNNATCCTGGCTCAGGACGAA	NGCTNGCGGC <u>GT</u> GC <u>T</u> TAACACATGCAA-GTC	GGCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGGACGAA	CGCTGGCGGC GT GC T TAACACATGCAA-GTC	G T CTTGTACACACCGCCCGT	
$GAGA\underline{A}TTTGATC\underline{T}TGG\underline{T}TCAGATTGAA$	CGCTGGCGGC <u>GT</u> G <u>GA</u> T <u>G</u> A <u>GG</u> CATGCAA-GTC	GCCTTGTACACCGCCCGT	
???????????????????????????STARCGTTGGCGGCG <u>GCGT</u> C <u>TT</u> A <u>A</u> ACATGCAA-GTC		<u>A</u> CCTTGTACACACCGCCCGT	
??????????????????????????STARCGCTGGCGGC <u>GCGT</u> C <u>TTAA</u> ACAT <u>T</u> C <u>CA</u> AGTC		A CCTTGTACACACCGCCCGT	
??????????????????????STAR	CGCTGGCGGC <u>GCGT</u> C <u>TT</u> A <u>A</u> ACATGCA-A <u>C</u> TC	<u>A</u> CCTTGTACACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAACGAA	CGCTGGCGGC <u>GC</u> G <u>T</u> CT <u>T</u> AA <u>G</u> CATGCAA-GTC	GCCTTGTACACCGCCCGT	
GAGAGTTTGATCCTGGCTCAGAACGAA	CGCTGGCGGC <u>GC</u> G <u>T</u> CT <u>T</u> AA <u>G</u> CATGCAA-GTC	GCCTTGTACACACCGCCCGT	