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

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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, Mg^{2+} 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 μl of proteinase K (18 mg/ml; Sigma) was added, and the solution was further incubated for 1 h at 37°C. Second, the phenol-chloroform step was replaced by adding one-half volume of 7.5 M ammonium acetate, and the mixture was centrifuged at $11{,}220 \times g_{av}$ 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 μl) contained 20 pmol of each appropriate primer, 200 μM 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 μg 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	β-Proteobacteria	+
Myxococcus xanthus NCIMB 9412[c]	δ-Proteobacteria	+
Desulfovibrio desulfuricans[c]	δ-Proteobacteria	+
Desulfovibrio salexigens[c]	δ-Proteobacteria	+
Desulfovibrio sapovorans	δ-Proteobacteria	+
Desulfobacter postgatei	δ-Proteobacteria	+
Desulfobulbus propionicus[c]	δ-Proteobacteria	+
Wolinella succinogenes ATCC 29543	ε-Proteobacteria	+
Fusobacterium nucleatum[c]	ε-Proteobacteria	+
Campylobacter concisus ATCC 33237	ε-Proteobacteria	+
Campylobacter sputorum subsp. *sputorum* ATCC 35980	ε-Proteobacteria	+
Campylobacter rectus ATCC 33238	ε-Proteobacteria	+
Campylobacter curvus ATCC 35224	ε-Proteobacteria	+
Campylobacter showae ATCC 51146	ε-Proteobacteria	+
Escherichia coli HB101[c]	γ-Proteobacteria	+
Acinetobacter calcoaceticus AC8250	γ-Proteobacteria	+
Pseudomonas putida UWC1[c]	γ-Proteobacteria	+
Klebsiella pneumoniae	γ-Proteobacteria	+
Eubacterium nodatum ATCC 33099[c]	Clostridia and relatives	+
Eubacterium brachy ATCC 33089	Clostridia and relatives	+
Eubacterium timidum ATCC 33092, ATCC 33093	Clostridia and relatives	+
Eubacterium tardum SC68,[c] SC88P	Clostridia and relatives	+
Eubacterium exiguum ATCC 700122	Clostridia and relatives	+
Eubacterium minutum ATCC 700079, ATCC 700080	Clostridia and relatives	+
Eubacterium saburreum ATCC 33271, ATCC 33318, ATCC 33319, ATCC 43850	Clostridia and relatives	+
Pseudoramibacter alactolyticus DSM 3980 (= ATCC 23263)	Clostridia and relatives	+
Ruminococcus torques ATCC 27756	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 coryniformis subsp. *torquens* ATCC 25600	*Bacillus-Lactobacillus-Streptococcus* subdivision	+
Streptococcus salivarius ATCC 7073	*Bacillus-Lactobacillus-Streptococcus* subdivision	+
Streptococcus sanguis ATCC 10556	*Bacillus-Lactobacillus-Streptococcus* subdivision	+
Eubacterium saphenum ATCC 49989	Gram positive, low G+C	+
Leptospira inadai ATCC 43289	*Spirochaeta-Treponema-Borrelia* subdivision	+[d]

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TABLE 1—*Continued*

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 iron-reducing 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′→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

Strain	Woese grouping	Sequence vs[a]: 63f	Sequence vs[a]: 1387r
		CAGGCCTAACACATGCA–AGTC	**CGGAACATGTGWGGCGGG**
Fibrobacter intestinales	*Fibrobacter* spp.	**AG**T**G**T**C**TT**A**T**A**CATGCA–AGTC	GCCTTGTACACACCGCCC
Chlorobium limicola	Green non-sulfur	C**GT**GCCTAACACATGCA–AGTC	GCCTTGTACACACCGCCC
Chloroflexus aurantiacus	Green non-sulfur	C**GT**GCCTAA**TG**CATGCA–AGTC	GCCTTGTACACACCGCCC
Herpetosiphon aurantiacus	Green non-sulfur	C**GT**GCCTAA**TG**CATGCA–AGTC	GCCTTGTACACACCGCCC
Thermomicrobium roseum	Green non-sulfur	C**GT**GCCTAA**TG**CATGCA–AGTC	GCCTTGTACACACCGCCC
Deinococcus radiodurans	Radioresistant micrococci and relatives	C**GT**GC**T**TAA**G**ACATGCA–AGTC	GCCTTGTACACACCGCCC
Thermus aquaticus	Radioresistant micrococci and relatives	C**GT**GCCTAA**G**ACATGCA–AGTC	GCCTTGTACACACCGCCC
Thermus ruber	Radioresistant micrococci and relatives	**T**A**T**GCCTAA**G**ACATGCA–AGTC	GCCTTGTACACACCGCCC
Fervidobacterium nodosum	*Thermotogales*	C**GT**G**C**CTAACACATGCA–AGTC	GCCTTGTACACACCGCCC
Thermotoga elfii	*Thermotogales*	C**GT**G**CT**TAACACATGCA–AGTC	G**GT**TTGCACACACCGCCC
Thermotoga maritima	*Thermotogales*	C**GT**G**C**CTAACACATGCA–AGTC	GCCTTGTACACACCGCCC

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

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

Strain	Woese group	Sequence vs primers[a]: 27f and 63f	1387r and 1392r
		AGAGTTTGATCMTGGCTC[b]	**CAGGCCTAACACATGCAA–GTC**[c]	**CRTGTGTGGCGGGCA**[d] **CGGAACATGTGMGGCGGG**[e]
Rhodobacter sphaeroides ATH 2.4.1	α-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCAGGCCTAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Alcaligenes eutrophus	β-Proteobacteria	AAGAGTTTGATCCTGGCTCAGATTnAACGCTGGCGGCA**T**GCC**T**TACACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Campylobacter concisus (FDC 288)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGTGGC**GT**GCCTAA**T**ACATGCAA–GTC	G**T**CTTGTAC**T**CACCGCCCGT
Campylobacter curvus (ATCC 35224[T]; VPI 9584)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	G**T**CTTGTAC**T**CACNGCCCGT
Campylobacter rectus (CCUG 19168)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	G**T**CTTGTAC**T**CACCGCCCGT
Campylobacter showae (CCUG 3054)	ε-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	G**T**CTTGTAC**T**CACCGCCCGT
Desulfobacter postgatei	δ-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA–GTC	GNNTTGTACACACCGCCNGT
Desulfovibrio desulfuricans (ATCC 27774)	δ-Proteobacteria	GAGAGTNTGAT**T**CTGGCTCAGATTGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Myxococcus xanthus DK1622 (ATCC null)	δ-Proteobacteria	GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GT**GCCTAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Acinetobacter calcoaceticus	γ-Proteobacteria	AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Escherichia coli	γ-Proteobacteria	AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA–GTC	CCTTGTACACACCGCCCGTC
Pseudoramibacter alactolyticus DSM 3980, ATCC 23263	γ-Proteobacteria	-AGAGTTTGAT**Y**MTGGCTCAGGACGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Bacillus cereus F4810/72 serotype 1 (NCTC 11143)	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Bacillus popilliae	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**G**ACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Lactobacillus coryniformis	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	GCCTTGTACACACCGCNCGT
Lactobacillus delbrueckii subsp. *delbrueckii* strain Calvert	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTNGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Streptococcus salivarius C699 (ATCC 13419)	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA–GT**A**	GCNTNGTACACACCGCCCGT
Streptococcus sanguis (NCTC 7863; ATCC 15300)	*Bacillus-Lactobacillus-Streptococcus* subdivision	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**NCNTGCAA–GTN	GCCTTGTACACACCGCCCGT
Eubacterium angustum	Clostridia and relatives	GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Eubacterium fossor	Clostridia and relatives	GAGAGTT**C**GATCCTGGCTCAGGATGAACGCTGGCGGC**GC**GCCTAACACATGCAA–GTC	GCNTTGTACACACCGCCCGT
Eubacterium lentum	Clostridia and relatives	-AGAGTTTGATNNTGGCTCAGGATGAACGCTGGCGGC**GT**GCCTAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Eubacterium limosum	Clostridia and relatives	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Eubacterium xylanophilum	Clostridia and relatives	GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA–GTC	G**T**CTTGTACACACCGCNCGT
Nostoc muscorum	Cyanobacteria and chloroplasts	GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Prevotella buccalis	*Flexibacter-Cytophaga-Bacteroides* phylum	TAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Prevotella intermedia	*Flexibacter-Cytophaga-Bacteroides* phylum	GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTA**T**AGGC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Prevotella oris	*Flexibacter-Cytophaga-Bacteroides* phylum	GAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Prevotella veroralis	*Flexibacter-Cytophaga-Bacteroides* phylum	GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGC**T**TAACACATGCAA–GTC	GCCTTGTACACACCGCCCGT
Actinomyces viscosus	Gram positive, high G+C	GAGAGTTNNATCCTGGCTCAGGACGAANGCTNGCGGC**GT**GC**T**TAACACATGCAA–GTC	GGCTTGTACACACCGCCCGT
Propionibacterium acnes	Gram positive, high G+C	GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA–GTC	G**T**CTTGTACACACCGCCCGT
Chlamydia psittaci	*Planctomyces* and relatives	GAGA**A**TTTGATC**T**TGG**T**TCAGATTGAACGCTGGCGGC**GT**G**GA**T**G**A**GG**CATGCAA–GTC	GCCTTGTACACACCGCCCGT
Leptospira inadai ATCC 43289	*Spirochaeta-Treponema-Borrelia* subdivision	????????????????????????STARCGTTGGCGGC**GCGT**C**TT**A**A**ACATGCAA–GTC	**A**CCTTGTACACACCGCCCGT
Leptospira kirschneri ATCC 23469	*Spirochaeta-Treponema-Borrelia* subdivision	????????????????????????STARCGCTGGCGGC**GCGT**C**TT**A**A**ACAT**T**C**CA**AGTC	**A**CCTTGTACACACCGCCCGT
Leptospira wolbachii ATCC 43284	*Spirochaeta-Treponema-Borrelia* subdivision	????????????????????????STARCGCTGGCGGC**GCGT**C**TT**A**A**ACATGCA–A**C**TC	**A**CCTTGTACACACCGCCCGT
Treponema denticola	*Spirochaeta-Treponema-Borrelia* subdivision	GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GC**GT**C**TT**A**A**G**CATGCAA–GTC	GCCTTGTACACACCGCCCGT
Treponema phagedenis	*Spirochaeta-Treponema-Borrelia* subdivision	GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GC**GT**C**TT**A**A**G**CATGCAA–GTC	GCCTTGTACACACCGCCCGT

[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.

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

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REFERENCES

1. **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.
2. **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.

3. **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.
4. **Brunk, C. F., E. Avaniss-Aghajani, and C. A. Brunk.** 1996. A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences. Appl. Environ. Microbiol. **62:**872–879.
5. **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.
7. **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.
8. **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.
9. **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.
10. **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.
11. **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.
12. **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.
13. **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 CH_4 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**
AGTGT**T**CTT**T**A**T**ACATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GCCTAACACATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GCCTAA**TG**CATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GCCTAA**TG**CATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GCCTAA**TG**CATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GC**T**TAA**G**ACATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**GCCTAA**G**ACATGCA-AGTC	GCCTTGTACACACCGCCC
TA**T**GCCTAA**G**ACATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**G**C**CTAACACATGCA-AGTC	GCCTTGTACACACCGCCC
C**GT**G**CT**TAACACATGCA-AGTC	G**GT**TTGCACACACCGCCC
C**GT**G**C**CTAACACATGCA-AGTC	GCCTTGTACACACCGCCC

TABLE 2

Sequence vs primers[a]:	
27f and 63f	1387r and 1392r
AGAGTTTGATCMTGGCTC[b] **CAGGCCTAACACATGCAA-GTC**[c]	**CRTGTGTGGCGGGCA**[d] **CGGAACATGTGWGGCGGG**[e]
GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCAGGCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
AAGAGTTTGATCCTGGCTCAGATTnAACGCTGGCGGCA**T**GCCT**T**ACACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAGTGGTGGC**GT**GCCTAA**T**ACATGCAA-GTC	G**T**CTTGTAC**T**CACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	G**T**CTTGTAC**T**CACNGCCCGT
GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	G**T**CTTGTAC**T**CACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	G**T**CTTGTAC**T**CACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA-GTC	GNNTTGTACACACCGCCNGT
GAGAGTNTGAT**T**CTGGCTCAGATTGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GT**GCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAA-GTC	CCTTGTACACACCGCCCGTC
-AGAGTTTGAT**Y**MTGGCTCAGGACGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**G**ACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	GCCTTGTACACACCGCNCGT
GAGAGTTNGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**ACATGCAA-GT**A**	GCNTNGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GCCTAA**T**NCNTGCAA-GTN	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGAGTT**C**GATCCTGGCTCAGGATGAACGCTGGCGGC**GC**GCCTAACACATGCAA-GTC	GCNTTGTACACACCGCCCGT
-AGAGTTTGATNNTGGCTCAGGATGAACGCTGGCGGC**GT**GCCTAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA-GTC	G**T**CTTGTACACACCGCNCGT
GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGG**T**A**T**GC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
TAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTA**T**AGGC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATNAACGCTGGCTACAGGC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGC**T**TAACACATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTNNATCCTGGCTCAGGACGAANGCTNGCGGC**GT**GC**T**TAACACATGCAA-GTC	GGCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGC**GT**GC**T**TAACACATGCAA-GTC	G**T**CTTGTACACACCGCCCGT
GAGA**A**TTTGATC**T**TGG**T**TCAGATTGAACGCTGGCGGC**GT**G**GA**T**G**A**GG**CATGCAA-GTC	GCCTTGTACACACCGCCCGT
????????????????????????STARCGTTGGCGGC**GCGT**C**TT**A**A**ACATGCAA-GTC	**A**CCTTGTACACACCGCCCGT
????????????????????????STARCGCTGGCGGC**GCGT**C**TT**A**A**ACAT**TC**C**A**AGTC	**A**CCTTGTACACACCGCCCGT
????????????????????????STARCGCTGGCGGC**GCGT**C**TT**A**A**ACATGCA-A**C**TC	**A**CCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GC**G**T**CT**T**AA**G**CATGCAA-GTC	GCCTTGTACACACCGCCCGT
GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGC**GC**G**T**CT**T**AA**G**CATGCAA-GTC	GCCTTGTACACACCGCCCGT

TABLE 3