

Design of 16 S rRNA-based Oligonucleotide Array Using Group-specific Non-unique Probes in Large Scale Bacteria Detection*

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Abstract With thousands of sequenced 16 S rRNA genes available, and advancements in oligonucleotide microarray technology, the detection of microorganisms in microbial communities consisting of hundreds of species may be possible. The existing algorithms developed for sequence-specific probe design are not suitable for applications in large-scale bacteria detection due to the lack of coverage, flexibility and efficiency. Many other strategies developed for group-specific probe design focus on how to find a unique group-specific probe that can specifically detect all target sequences of a group. Unique group-specific probe for each group can not always be found. Hence, it is necessary to design non-unique probes. Each probe can specifically detect target sequences of a different subgroup. Combination of multiple probes can achieve higher coverage. However, it is a time-consuming task to evaluate all possible combinations. A feasible algorithm using relative entropy and genetic algorithm (GA) to design group-specific non-unique probes was presented.

Key words 16 S rRNA, oligonucleotide microarray, group-specific probes

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Metagenomics is a new field combining molecular biology and genetics in an attempt to reveal the vast scope of biodiversity in a wide range of environment, as well as new functional capacities of individual cells and communities, and the complex evolutionary relationships between them^[1~4]. Apparently, revealing biodiversity in microbial communities is the first step^[1, 5]. The vast majority of microbial diversity had been missed by cultivation-based methods^[2]. The analysis of 16 S rRNA gene sequences is the most common approach to determine microbial diversity^[6].

With thousands of sequenced 16 S rRNA genes available, and advancements in oligonucleotide microarray technology, the detection of microorganisms in microbial communities consisting of hundreds of species may be possible. At present, the Ribosomal Database Project (RDP) has collected around 150 000 16 S rDNA sequences in bacteria superkingdom, and catalogued them at multiple hierarchical phylogenetic levels^[7]. Oligonucleotide

microarrays now afford an ideal tool for identifying sequence variants (even single-base-pair variant) in 16 S rRNA genes derived from diverse microorganisms simultaneously in a single assay^[8~10]. Many 16 S rRNA-based oligonucleotide microarrays have been designed to detect multiple pathogens simultaneously^[11, 12]. Such technology has the potential to revolutionize microbial detection^[13, 14].

A critical issue for oligonucleotide microarray design is to find appropriate oligonucleotide probes

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based on their hybridization characteristics. Many software packages, such as OligoWiz^[15], PROBEmer^[16], OligoArray^[17], OligoPicker^[18], ROSO^[19], Osprey^[20] and Picky^[21], were developed to generate sequence-specific probes for each gene of a specified genome. When target sequences are highly similar to each other, unique sequence-specific probe for each target sequence can not be found due to cross-hybridization. Klau *et al.*^[22] presented an exact approach to select non-unique probes whose hybridization patterns can then be deconvolved to identify the presence of particular sequences. These algorithms are applicable when probes for specific sequence identification are necessary.

For applications where detecting the presence of multiple bacteria species is required, all target sequences are clustered into several groups based on taxonomy. There exist multiple copies of 16 S rRNA gene in a single species. A cluster- or group-specific probe concept needs to be applied. Kumar *et al.*^[23] provided a software package ARB to evaluate sequence alignments and oligonucleotide probes with respect to three-dimensional structure of ribosomal RNA. DeSantis *et al.*^[24, 25] proposed an alignment compression algorithm, NAST (Nearest Alignment Space Termination), to find Operational Taxonomic Units (OTUs) for automated design of effective probes. These algorithms have been applied to design group-specific probes based on comprehensive analysis of construction of aligned target sequences.

Because target sequences of each group are homologous but not identical, finding a unique group-specific probe that can specifically detect all target sequences of a group is often difficult. To improve coverage, a set of probes should be selected to identify a group of sequences and each probe is specific for a particular subgroup. However, it is time-consuming to evaluate all possible combinations of probes. We propose a feasible approach to design group-specific non-unique probes. Firstly, the specificity scores in each site along the aligned sequences are calculated based on relative entropy and then are utilized to generate a number of probe candidates. All probe candidates are checked for G+C content, melting temperature (T_m), self-structure and homopolymers requirements. These filtering techniques can reduce the search space effectively. Secondly, genetic algorithm (GA) is used to select an optimized set of probes with high coverage and

specificity. Two 16 S rRNA datasets were analyzed by this approach and results demonstrated its high specificity and coverage.

1 Materials and methods

Two datasets were analyzed to examine the performance of our method. One dataset, which contained 73 familiar pathogens with a total of 3 637 16S rRNA sequences, was analyzed as an example of multiple pathogens discrimination. Another dataset, which contained all bacteria genera (997 bacteria genera, excluding unclassified samples) with a total of 48 351 16 S rRNA sequences collected by RDP, was prepared to examine the performance of our method in large scale bacteria detection. The presented algorithm involves two steps: finding probe candidates and selecting optimized group-specific probe set.

1.1 Finding probe candidates

All aligned 16 S rRNA sequences S (provided by RDP^[7]) are clustered into G groups. Group is defined as a set of target sequences based on taxonomy or other particular requirements. There are 73 groups for the first dataset and 997 groups for the second dataset in this paper. $S_i \subset S$, for i from 1 to G . Group S_i contains N_i sequences. The lengths of aligned sequences are identical. $S = \{S_{i,n} | i = 1, \dots, G; n = 1, \dots, N_i\}$ ($S_{i,n} = s_{i,n,1} s_{i,n,2} \dots s_{i,n,l}$). In a site l , $s_{i,n,l}$ take values from an alphabet with 5 different letters ('A', 'G', 'C', 'T' and '-' which represent a gap). For group S_i and $S_i^c (S_i \cup S_i^c = S)$, character frequency statistics is calculated in each site. Character distributions $P_{i,l}$ (for group S_i) and $Q_{i,l}$ (for group S_i^c) are obtained. A modified Kullback-Leibler Divergence (KL) is used to measure the distance of distributions. Since the original KL (Equation 1) can not reliably determine differences in the tails of a probability distribution, logarithm transformation (Equation 2) is applied to deal with these tail effects. The specificity scores in each site are obtained (Figure 1).

In order to pick out suitable sites for probe candidates generation, rejection sampling techniques are utilized to generate random variables that follow a probability distribution function $\theta(i, l)$ (Equation 2). The procedure for oligonucleotide probe candidates generation works as follows:

$$D(P_{i,l}, Q_{i,l}) = \sum P_{i,l}(X) \lg \left[\frac{P_{i,l}(X)}{Q_{i,l}(X)} \right] \quad (1)$$

$$\theta(i, l) = \lg(1 + D(P_{i,l}, Q_{i,l})) \quad (2)$$

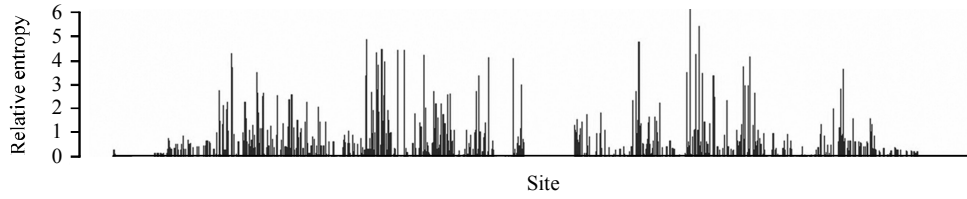


Fig. 1 Specificity scores based on relative entropy

For group S_i and group S_i^c , character ('A', 'G', 'C', 'T' and '-' which represent a gap) frequency statistics was applied in each site l . And then, character distributions $P_{i,l}$ (for group S_i) and $Q_{i,l}$ (for group S_i^c) are obtained. A modified Kullback-Leibler Divergence (KL) was used to calculate specific scores in each site. Here, group S_i is a set of 16 S rRNA gene sequences in *Acinetobacter baumannii*.

Step 1, we pick out a site l_0 randomly. Then, we generate a random number x uniformly distributed in $[0, 1]$. If $x \leq \theta(i, l_0) / \max_{l=1, \dots, L} \{\theta(i, l)\}$, we accept this site. Otherwise, we reject this site and go back to the start of step 1.

Step 2, we pick out one sequence $S_{i,n}$ from group S_i randomly. Regarding l_0 as central site, we find a word w of length L_w in $S_{i,n}$ (a word is defined as a subsequence without gap). Complementary to w a probe sequence is generated.

To ensure the closer optimum hybridization condition in one chip including all oligos, the G+C content range to 40%~60%. Furthermore, probe lengths L_w of probe candidates are variable. By adjusting probe lengths, probe candidates whose melting temperatures are closer to a predefined optimum hybridization condition will be generated. Self-structure of probe is harmful to hybridization. Gibbs free energy (ΔG_T°) values predicted by RNAFold [26], a component of the Vienna RNA package, are measurement for probe self-structure stabilities. Probes with stable self-structure would be unacceptable. Furthermore, oligonucleotides containing either AAAAA, GGGGG, CCCCC, TTTTT or longer homopolymers are rejected [17]. By using several filtering techniques to reduce search space, a number of oligonucleotide probe candidates are obtained. 50 oligonucleotide probe candidates are actually generated for each group.

1.2 Selecting optimized group-specific probe set

Genetic algorithm is applied to select optimized group-specific probe set from 50 oligonucleotide probe candidates. The algorithm consists of the initialization process, evaluation process, crossover process and the mutation process.

The initialization process randomly generates 100 initial individuals. In an individual the probability for

each probe candidate to be selected is 0.1.

In the evaluation process classification precision (defined to evaluate coverage and cross-hybridization) and discrimination power (defined to evaluate specificity) of each individual will be calculated. The problem can be formalized as follows:

For a particular group $S_g = \{S_{i,n} | i=g, n=1, \dots, N_g\}$, $S_g \subset S$. There are $M_{g,d}$ ($0 \leq M_{g,d} \leq 50$) probes $p_1, p_2, \dots, p_{M_{g,d}}$ selected in an individual $P_{g,d}$, for d from 1 to 100.

To evaluate classification precision, we observe the hybridization of all probes in $P_{g,d}$ incident to any target in S . S_{PM} and S'_{PM} can be obtained. S_{PM} and S'_{PM} is defined as

$$S_{PM} = \{S_{i,n} | S_{i,n} \in S_g, \exists p_m \in P_{g,d}, \text{duplex}(S_{i,n}, p_m) = PM\}$$

$$S'_{PM} = \{S_{i,n} | S_{i,n} \in S_g^c, \exists p_m \in P_{g,d}, \text{duplex}(S_{i,n}, p_m) = PM\}$$

where PM denotes perfect match. S_{PM} is a set representing coverage and S'_{PM} is a set representing cross-hybridization. α is cardinality of the set S_{PM} and β is cardinality of the set S'_{PM} . Classification precision $C_{g,d}$ of this individual $P_{g,d}$ is defined as

$$|S_{PM}| = \alpha$$

$$|S'_{PM}| = \beta$$

$$|S_g| = N_g$$

$$C_{g,d} = \alpha / (N_g + \beta)$$

If $S_{PM} = S_g$ and $S'_{PM} = \emptyset$ (i.e. 100% coverage and no cross-hybridization), classification precision of this individual would achieve the maximum value 1.

To evaluate discrimination power, melting temperatures $T_{PM}(S_g, P_{g,d})$ and $T_{MM}(S_g^c, P_{g,d})$ are calculated using the NN (Nearest Neighbor) model with Na^+ and DNA concentrations set to 1 mol/L and 1 $\mu\text{mol/L}$ and are defined as

$$T(S_g, P_{g,d}) = \{T_m(S_{i,n}, p_m) | S_{i,n} \in S_g, p_m \in P_{g,d}\}$$

$$T_{PM}(S_g, P_{g,d}) = \{T_m(S_{i,n}, p_m) | S_{i,n} \in S_g, p_m \in P_{g,d}, \text{duplex}(S_{i,n}, p_m) = PM\}$$

$$T_{MM}(S_g, P_{g,d}) = \{T_m(S_{i,n}, p_m) | S_{i,n} \in S_g, p_m \in P_{g,d}, \text{duplex}(S_{i,n}, p_m) = MM\}$$

$$\mathbf{T}(\mathbf{S}_g, \mathbf{P}_{g,d}) = \mathbf{T}_{PM}(\mathbf{S}_g, \mathbf{P}_{g,d}) \cup \mathbf{T}_{MM}(\mathbf{S}_g, \mathbf{P}_{g,d})$$

where PM denotes perfect match and MM denotes mismatch. Given a threshold h , discrimination power $D_{g,d}$ of this individual $\mathbf{P}_{g,d}$ is defined as

$$t_{\min PM} = \min \{ \mathbf{T}_{PM}(\mathbf{S}_g, \mathbf{P}_{g,d}) \}$$

$$\mathbf{T}_{\text{non-cross}} = \{ t_r | t_r \in \mathbf{T}_{MM}(\mathbf{S}_g^c, \mathbf{P}_{g,d}), t_r < t_{\min PM} - h \}$$

$$D_{g,d} = \frac{|\mathbf{T}_{\text{non-cross}}|}{|\mathbf{T}_{MM}(\mathbf{S}_g^c, \mathbf{P}_{g,d})|}$$

If $\mathbf{T}_{\text{non-cross}} = \mathbf{T}_{MM}(\mathbf{S}_g^c, \mathbf{P}_{g,d})$, (i.e. $\forall t_r \in \mathbf{T}_{MM}(\mathbf{S}_g^c, \mathbf{P}_{g,d}), t_r < t_{\min PM} - h$) and the specified threshold h is 10, melting temperatures of specific probe-target hybridizations are at least 10°C higher than that of non-specific probe-target hybridizations. Discrimination power of this individual would achieve the maximum value 1. The designed group-specific probes can achieve a high specificity.

The fitness value is defined as follows:

$$\text{Fitness}(\mathbf{P}_{g,d}) = \begin{cases} 10 * C_{g,d} + 2 * D_{g,d} + (50 - M_{g,d}) / 50, & M_{g,d} \neq 0 \\ 0, & M_{g,d} = 0 \end{cases}$$

Selection applies the Roulette Wheel method to allow the individuals with a high fitness value to have a higher chance to be selected to mate. Through the crossover process 40 new individuals are generated. And then, 4% genes in their genome will mutate. All those 140 individuals are arranged according to fitness

values. The best 100 individuals will survive in the next generation. If the fitness value of the best individual has no change in continual 50 generations, the genetic algorithm will be terminated.

2 Results

2.1 Probe design for multiple pathogens discrimination

Our algorithm is applied to select group-specific non-unique probes to discriminate 73 bacteria species. The optimized probe set $\mathbf{P}_i$ for each species $\mathbf{S}_i$ is enumerated in Table 1. For group $\mathbf{S}_i$, results are assessed using the two main performance indices of coverage (the number of sequences in group $\mathbf{S}_i$ that can be detected) and cross-hybridization (the number of sequences in group $\mathbf{S}_i^c$ that probes in $\mathbf{P}_i$ are complementary to). Our method can find group-specific probe sets (100% coverage and no cross-hybridization) for 64 of 73 bacteria species. Although for any of the other 9 bacteria species our algorithm can not find a probe set with 100% coverage and no cross-hybridization, a solution with relatively high coverage and low cross-hybridization can be provided.

Table 1 Group-specific probe sets for 73 bacterial species

Species	Targets	Probe sets	Coverage/%	Cross hybridization	Specificity	
					min[$T_m(PM)$]	max[$T_m(MM)$]
<i>Acinetobacter baumannii</i>	15	CGCCACTAAAGCCTCAAAG	100	0	70.7	56.0
<i>Actinomadura madurae</i>	5	GTTTTCCGGCAATGTCAAGC GTGGCTTTTACGACAGAC	100	0	72.6	68.2
<i>Actinomadura pelletieri</i>	2	CGTATCCACCGCAAACC	100	0	72.2	67.6
<i>Actinomyces bovis</i>	2	GAGAAACCACGTCTCCGT	100	0	72.8	60.3
<i>Actinomyces israelii</i>	3	GCTTCATAACCCGGCT TGCTTCTTCACCCATTACC	100	0	70.2	59.3
<i>Actinomyces naeslundii</i>	11	CACAAGGAGGAAACCC GGAAGACCCGAAAAGG CAACCGACCCACAAACGA	100	0	70.6	59.1
<i>Aeromonas hydrophila</i>	60	GTCACAGTCAGCAGATATTAG CTGTGTTCTGATTCCCGA	100	0	70.7	62.4
<i>Afipia</i> sp.	5	AAGGGAAAGCCAGATCTC CTACACTCGCAGTTCCAC	100	0	70.7	60.3
<i>Arcanobacterium haemolyticum</i>	2	TACCCTCAACTTTTCGCCT	100	0	70.1	55.5
<i>Bacillus anthracis</i>	155	CTAGGGTTGTGTCAGAGG	98.06	121	70.5	63.8
<i>Bacillus cereus</i>	298	GCAACTAAGATCAAGGGTT	97.65	143	70.0	65.4
<i>Bacteroides fragilis</i>	32	TTCACAGCGGTGATTGCT	100	0	70.1	47.3
<i>Bartonella henselae</i>	11	GTGCCCAACCAAATGCTG	100	0	71.4	62.5
<i>Bartonella quintana</i>	7	CCACACTCGAGACATCC	100	0	73.3	62.3
<i>Bartonella vinsonii</i>	9	TAAATATCCGCCTACGTGCG	88.89	0	70.8	70.7
<i>Bordetella pertussis</i>	7	TCTTTCTTTCCGAACCGCC	100	0	72.6	52.1
<i>Borrelia burgdorferi</i>	104	GGCAGTCTTATCTGAGT	100	0	70.5	60.4

Species	Targets	Probe sets	Coverage/%	Cross hybridization	Continued Specificity	
					min[$T_m(PM)$]	max[$T_m(MM)$]
<i>Brucella</i> sp.	9	GTGTTCCACCCAATATCTAC GAATTTACCTCTACACTCGG	100	0	71.1	71.4
<i>Burkholderia mallei</i>	42	CTAAGGAAATGAATCCCCAACA	100	0	70.9	53.3
<i>Campylobacter fetus</i>	30	GGTATTCCTGGTGATCTCTA	100	0	71.7	57.3
<i>Campylobacter jejuni</i>	33	TACCGTCAGAATTCTTCCCTAA	100	0	71.4	60.9
<i>Chlamydia trachomatis</i>	31	CGTCAGGTATAAATTAGAAAAGCGC	100	0	73.1	52.1
<i>Clostridium botulinum</i>	66	GGCTATGCAAGGGATGTC TTGGAATGCAGCACTCAGG	87.88	0	73.5	67.9
<i>Clostridium difficile</i>	15	GCCTTTCACCTCTGACTT	100	0	71.1	60.5
<i>Clostridium novyi</i>	16	CTCAGTTACGAGTAATTCAGG TCAAATGCAGCGCCAG	100	2	70.9	67.0
<i>Clostridium perfringens</i>	47	CGATTAAGAGTAATGCAAGGG	100	0	70.8	52.3
<i>Clostridium sordellii</i>	6	CCGATTAGGGAGAGGT	100	0	70.6	57.0
<i>Corynebacterium diphtheriae</i>	10	GTACCGTCACAAAAGCTTCG	100	0	70.8	58.7
<i>Coxiella burnetii</i>	14	CATCTCTGACAAGTTCGA	100	0	70.7	57.1
<i>Dermatophilus congolensis</i>	3	CACTTTCGCTTCTCCCC	100	0	73.1	59.4
<i>Edwardsiella tarda</i>	17	TTGCCAGTCTTGATGC	100	0	70.6	61.5
<i>Escherichia coli</i> O157 : H7	23	TTAACTTTACTCCCTTCCTCC	100	0	73.0	65.4
<i>Francisella tularensis</i>	48	CTCCCCAACTAAAGTGCT	100	0	71.6	59.3
<i>Gardnerella vaginalis</i>	2	CCGTAAAGCGATGGGCTT	100	0	71.7	55.2
<i>Haemophilus ducreyi</i>	12	GCGCCAGTCTTAAAGACC CATCACTACATGCTGGC	100	0	71.6	60.4
<i>Haemophilus influenzae</i>	369	CTTCCTCAATACCGAAAGAAC CAGGGCTTTCACACCT	100	0	70.3	67.0
<i>Helicobacter pylori</i>	59	ACAAC TAGCATCCATCGTTTAGG	100	0	74.5	62.5
<i>Legionella pneumophila</i>	22	ACCATCACATGCTGGCA	100	0	70.3	65.2
<i>Leptospira interrogans</i>	72	GGTTCGTTACTGAGGGTTAAAA	100	0	70.0	58.1
<i>Listeria monocytogenes</i>	21	CCAGAGTGGTCAAAGGA	100	0	70.7	53.7
<i>Morganella morganii</i>	24	CTGACCAGTATCAGATGC CGGTGCTTCCTTCTGT GTATCGCCTTCCTCC	95.833	0	70.5	65.5
<i>Mycobacterium fortuitum</i>	12	TCCTTCTTCTATAGGTACCG	100	0	70.8	66.8
<i>Mycobacterium scrofulaceum</i>	3	CCACAAAGTGAGCCTTCG	100	0	71.8	53.1
<i>Mycobacterium simiae</i>	2	CCCGGTTTTTACGAACAAC	100	0	70.6	55.0
<i>Mycobacterium ulcerans</i>	4	GAGAAAACCCGAACCTTCG	100	0	70.5	57.7
<i>Mycobacterium xenopi</i>	3	ACCACGAGAAAACCCGC	100	0	70.2	55.8
<i>Mycoplasma pneumoniae</i>	4	CTCGGTAAACCTCCATTATGTT	100	0	72.4	56.9
<i>Neisseria gonorrhoeae</i>	5	GGATTCCGCACATGTCAAAC	100	0	72.4	60.7
<i>Neisseria meningitidis</i>	900	CGCTTTCGGGCATGAAC	100	0	70.3	65.7
<i>Nocardia brasiliensis</i>	6	CTCCCCTGAAGTACTCAA	100	0	71.4	70.7
<i>Nocardia nova</i>	22	GTACACCAACCACAAAG GTTTTACGAACGACGCGAC	100	0	71.0	68.9
<i>Pasteurella multocida</i>	45	CTCTAGACTCCAGTCTG	100	0	72.7	61.5
<i>Plesiomonas shigelloides</i>	3	CGTCAATGCCACTAGGT	100	0	70.9	57.9
<i>Prevotella</i> sp.	73	AATTAGCCGGTCCTTATTCATGC GGATAACGCCTGGACCTT GCTACACGACGAATTCCGC TTACCGTGCGGACTACC CATCGTTTACCGTGCACT	100	0	72.7	73.0
<i>Proteus mirabilis</i>	17	GGGTATTAACCTTATCACCTTC	100	0	70.9	63.3
<i>Providencia alcalifaciens</i>	3	TAGCATCAACGCCTTCCA GCATCTCTGTAAATTCTCTG	100	0	70.2	69.5
<i>Providencia rettgeri</i>	2	CCTCAAGGGAACAACCTT	100	0	70.2	70.9
<i>Pseudomonas aeruginosa</i>	156	CAAGGTATTAACCTACTGCCCT CGCTACACAGGAAATTCC	100	0	70.7	64.8
<i>Rhodococcus equi</i>	18	GGCTTTCGGGTATATGTCAA	100	0	70.7	58.7
<i>Rickettsia</i> sp.	29	GAAACCGAAAGAGAATCTTCC CCATCATCCCCTACTACA	100	0	71.0	58.0

Species	Targets	Probe sets	Coverage/%	Cross hybridization	Continued Specificity	
					min[$T_m(PM)$]	max[$T_m(MM)$]
<i>Salmonella enterica</i>	137	CCACAACACCTTCCTCC GCTGCGTTATTAACCAACA TGCCAGTTTCGAATGCAGT	98.54	2	71.7	68.6
<i>Serratia liquefaciens</i>	5	GCACTAAGCTATCTCTAGC GGTTATTAACCTTAACGCCTTCC	100	0	73.6	72.3
<i>Serratia marcescens</i>	78	TCTGCGAGTAACGTCAATTGA GAACGTATTAAGTTCACCACC GTATTAAGTTCACCACCTTCC	100	0	70.8	73.4
<i>Staphylococcus aureus</i>	101	TCTCTAGAGTTGTCAAAGGA	99.01	0	70.4	64.7
<i>Staphylococcus epidermidis</i>	56	TACCGTCAAGACGTGCATAG AGGGGAAAACCTCTATCTCTAG	100	1	70.3	66.0
<i>Streptobacillus moniliformis</i>	3	GTTTACAGCTAGGACTACC	100	0	74.0	61.9
<i>Streptococcus pyogenes</i>	75	GTTAGCCGTCCTTTCTG	100	0	70.7	53.5
<i>Treponema pallidum</i>	4	AAGAGGCGTATCGCTACGC	100	0	74.8	59.2
<i>Ureaplasma urealyticum</i>	18	GGAAAGTTCGCGACATGT AGTTCCCCAACTCCCTA	100	0	70.0	59.2
<i>Vibrio cholerae</i>	31	GGTATTAACCTTAACCACCTTCC CTCTAGGGCACAACC	100	0	70.3	64.5
<i>Vibrio vulnificus</i>	20	GAAAGCCACGGCTCAA GCCGCTATTAACGACACC	100	0	70.0	65.2
<i>Yersinia enterocolitica</i>	19	GAGTTCACCACATACGTG AGCATCTCTGCTAAATTCGGT CACCGAAGCATCTCTGC	100	0	71.2	68.9
<i>Yersinia pestis</i>	64	GCATCTCTGCCAAATTCTGT	100	0	73.3	63.5

Group-specific probe sets designed by our method were assessed using the three performance indices of coverage (i.e. the number of sequences in group S_i that we are able to detect), cross-hybridization (i.e. the number of sequences in group S_i^c that probes in P_i are complementary to) and specificity (i.e. the minimum T_m value of PM duplexes formed between group-specific probes for group S_i and target sequences in group S_i , the maximum T_m value of MM duplexes formed between group-specific probes for group S_i and target sequences in group S_i^c).

To achieve high specificity, melting temperatures $T(S_i^c, P_i)$ should be low enough. As shown in Figure 2, the average of $T(S_i, P_i)$ is significantly higher than that

of $T(S_j, P_i)(j=1, \dots, G; j \neq i)$. Distribution of $T_{MM}(S_i^c, P_i)$ and minimum of $T_{PM}(S_i, P_i)$ are shown in Figure 3, for i from 1 to G .

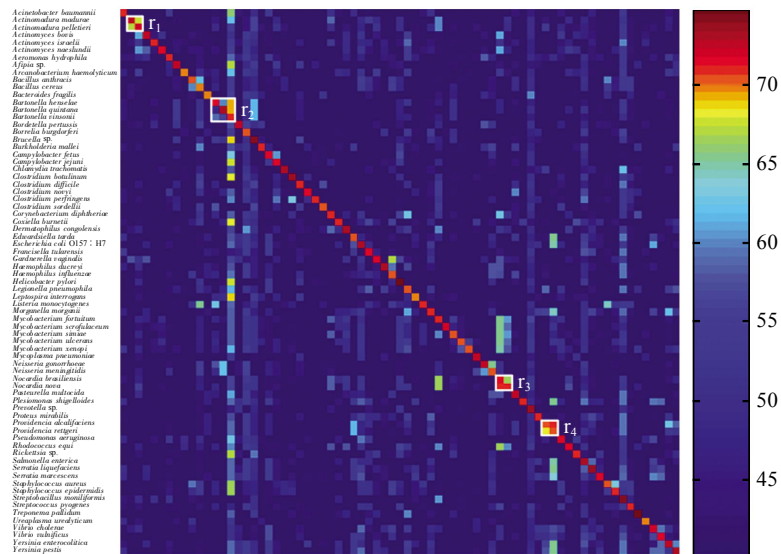


Fig. 2 Thermodynamic calculation of oligo-target hybridization

The X-axis represents 73 group-specific probe sets, the Y-axis 73 groups of target sequences in 73 bacterial species. Probe set i is specific for group i . A 73×73 matrix $H_{73 \times 73}$ is formed based on thermodynamic calculation of oligo-target hybridization. H_{ii} in diagonal is average T_m of PM duplexes formed between probes in probe set i and target sequences in group i . $H_{ij}(i \neq j)$ is average T_m of MM duplexes formed between probes in probe set i and target sequences in group j .

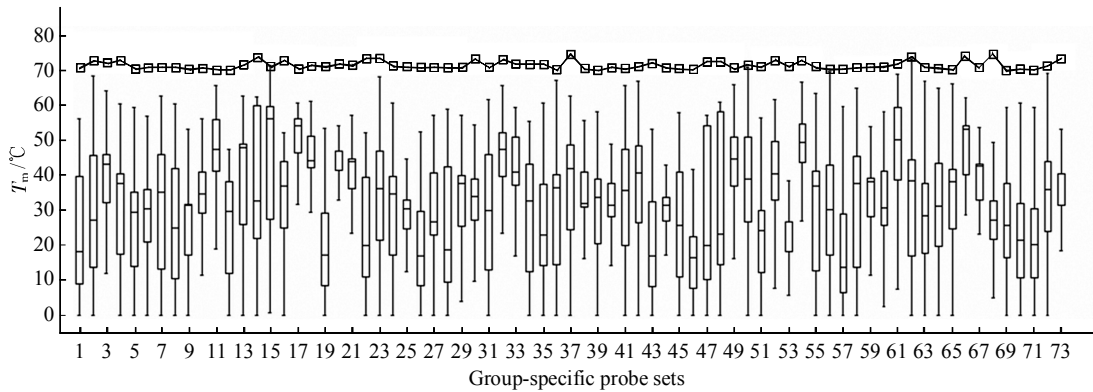


Fig. 3 Discrimination power of group-specific probe sets

The x -axis represents 73 group-specific probe sets, the y -axis the melting temperature. Minimum T_m values of PM duplexes formed between group-specific probes for group S_i and target sequences in group S_i , for i from 1 to 73, are shown as a curve at the top of this plot. Boxes show the distributions of T_m values of MM duplexes formed between group-specific probes for group S_i and target sequences in group S_i^c . T_m values below 0°C were not taken into consideration.

2.2 Probe design for large scale bacteria detection

There are over 600 000 sequences in current RDP release. Among them, over 150 000 sequences in bacteria superkingdom are the specific concern of this paper. Excluding unclassified samples, we obtained a dataset consisting of 48 351 copies of 16 S rRNA gene in 997 bacteria genera. Our method is applied to design group-specific non-unique probes for each genus.

As shown in Figure 4a, our method can provide a solution with high coverage and low cross-

hybridization for the majority of 997 bacteria genera. Group-specific non-unique probes with 100% coverage and no cross-hybridization can be found for 444 of 997 bacteria genera (see Supplementary Table 1S for details). To examine the specificity of designed probe sets, distributions of melting temperatures of non-specific and specific probe-target hybridizations were shown respectively in Figure 4b, c. The majority of melting temperatures of non-specific hybridizations are much lower than that of specific hybridizations. Potential cross-hybridization can be avoided effectively.

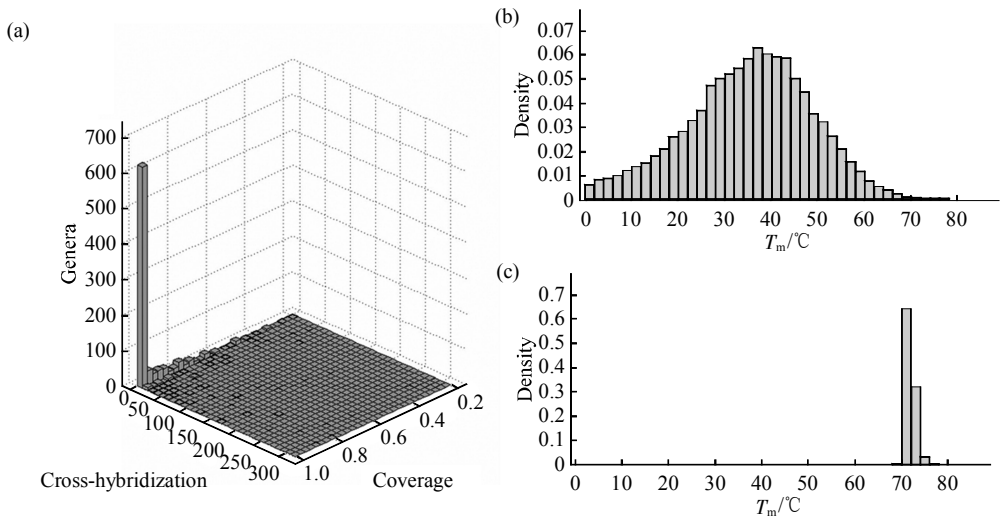


Fig. 4 Performance of group-specific probe sets designed for 997 bacteria genera

Our algorithm is applied to a dataset containing 997 bacteria genera with a total of 48 351 sequences collected by RDP. (a) Coverage (i.e. the number of sequences in group S_i that we are able to detect) and cross-hybridization (i.e. the number of sequences in group S_i^c that probes in P_i are complementary to) of designed group-specific probe sets. (b) Distribution of melting temperatures of non-specific probe-target hybridizations. (c) Distribution of melting temperatures of specific probe-target hybridization.

3 Discussion

We presented a new algorithm using relative entropy and GA to design 16 S rRNA-based group-specific non-unique probes for large scale bacteria detection. Since multiple copies of 16 S rRNA gene have high sequence similarity, probe candidates generation based on relative entropy can effectively reduce the search space. GA can find an optimized combination of probe candidates to specifically detect target sequences in a group. The results demonstrate that the designed 16 S rRNA-based probe sets have high coverage and low cross-hybridization. We think using group-specific non-unique probes to cover all target sequences in a group and evaluating different combinations of probe candidates were the main reasons for the high coverage obtained.

Lower specificity of group-specific probe sets can be observed in regions r_1 (*Actinomadura madurae*, *Actinomadura pelletieri*), r_2 (*Bartonella henselae*, *Bartonella quintana*, *Bartonella vinsonii*), r_3 (*Nocardia brasiliensis*, *Nocardia nova*) and r_4 (*Providencia alcalifaciens*, *Providencia rettgeri*) (Figure 2). In each region all species are in a same genus. 16 S rRNA evolves slowly and target sequences in a same genus are highly similar to each other. Group-specific probe set with high specificity can not be found due to potential cross-hybridization. Several other functional genes, such as *gyrB*, *sod*, *rrs* and *rpoB*, were commonly used to identify closely related species due to higher evolution rate^[27, 28]. Arrays designed based on homologous functional genes can achieve higher specificity. As a result, probe length can be set to 50~70 bases. 16 S rRNA-based probe has relatively lower specificity due to high sequence similarity. Probe length must be short enough (18~25 nt) to achieve single-base discrimination^[29, 30]. In this study, the probe length was 15~30 nt. However, obtaining sequence diversity of homologous functional genes as templates for amplification is a big challenge^[31]. Primer design for 16 S rRNA is a relatively easy task in practice.

The problem of group-specific probe selection is further complicated as all probes must work under the same hybridization condition. In this study probe

lengths are selected based on a predefined melting temperature. By selecting appropriate length for each probe candidate, melting temperatures of all probes can be close enough to work well under the same hybridization condition.

Both number of probe candidates per group and population size of GA affect the time complexity of our algorithm and the performance of designed group-specific probe sets. Since probe candidates generated for each group are only 50. Selecting an optimized set of probes for each group is not a difficult task for GA. Based on the smaller dataset consisting 3 637 copies of 16 S rRNA gene in 73 bacteria species we have examined different population sizes. As shown in Figure 5a, b, 100 individuals are enough to achieve high performance. The performance of group-specific probe sets is measured by fitness function of GA. More individuals can not improve results significantly. However, the amount of probe candidates has a strong impact on the results. The numbers of target sequences in different groups are different. It is possible that sequence variants in a group are much more than that in any other group. More probe candidates need to be generated for this group. It is difficult to estimate how many probe candidates need to be generated for a particular group. We generate same number of probe candidates for each group. As shown in Figure 5c, d, more probe candidates can improve results. However, more probe candidates would broaden the search space. To balance efficiency and performance, we generated 50 probe candidates for each group.

Compared with the total time complexity, the time complexity in stage of GA is negligible (Figure 5a, c). Because it is possible that the probe-target duplex involves more complex secondary structure, combinations containing mismatches and unpaired bases within the duplex. It is a time-consuming task to compute the duplex melting temperature for each probe-target pair in RNAFold^[26]. The computer used for the design tasks described here is HP Proliant ML150 Generation 5 Server with 2 Intel Xeon Quad Core 5 400 series processors and 4GB physical RAM. And it runs the Linux operating system (64 bit).

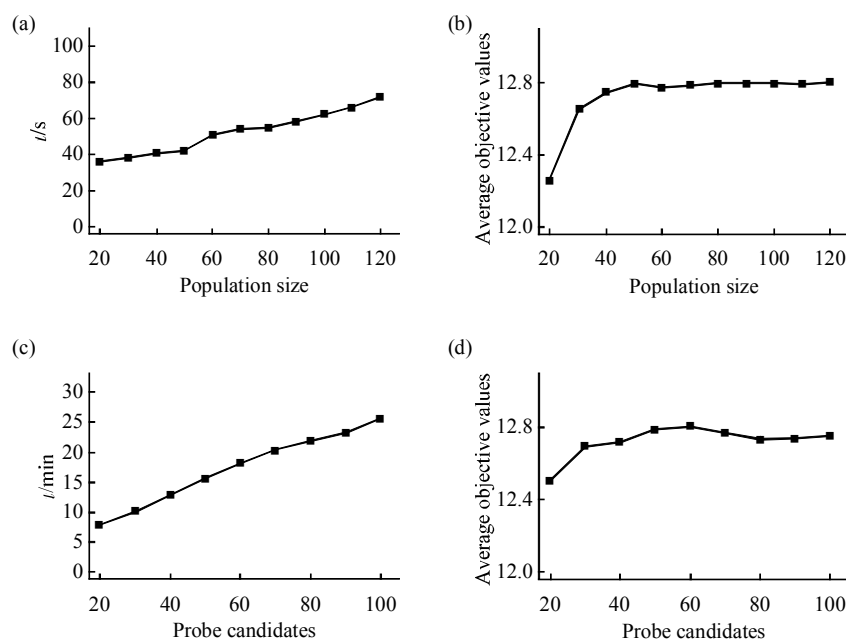


Fig. 5 Effect of the parameters on time and performance

Both number of probe candidates per group and population size of GA affect the time complexity of our algorithm and the performance of designed group-specific probe sets. The performance of group-specific probe sets is measured by fitness function of GA. Population size of GA can only affect the time in stage of GA. (a) Effect of population size of GA on time. (b) Effect of population size of GA on the average best objective value. (c) Effect of number of probe candidates for each group on time. (d) Effect of number of probe candidates for each group on the average best objective value.

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基于组合探针识别的大规模细菌分类 16 S rRNA 基因芯片设计*

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摘要 随着 16 S rRNA 序列资源的不断丰富, 以及寡核苷酸微阵列基因芯片技术的不断进步, 检测复杂微生物菌落中的微生物种群构成成为可能. 现有的序列特异性探针设计算法缺乏足够的覆盖度、灵活性以及效率, 不能满足大规模细菌检测基因芯片的设计要求. 很多组特异性探针设计算法的思路多局限于针对某个目标序列组设计唯一的组特异性探针. 在很多应用场合, 设计单个探针检测组内所有目标序列的目标是很难达到的. 因此, 设计多个探针通过组合方式进行检测是很有必要的. 每个探针能特异性地检测组内一部分目标序列, 通过组合就能提高覆盖率. 然而, 在所有可能的探针组合中找到一个优化的探针组合是很耗时的. 提出了一个可行的基于相对熵和遗传算法的组合探针设计算法.

关键词 16 S rRNA, 基因芯片, 组特异性探针

学科分类号 Q61, Q93

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附录

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Halothece</i>	2	CGCGGACTTGAAAAGCCA	100%	0	71.5363	67.2464
<i>Euhalothece</i>	3	CCAGCCTTTAACAGCAGA CTGCCTAACCGGAGT	100%	0	70.3147	67.5771
<i>Snowella</i>	3	TCCCCTAGAAAAGAGGTTTAC	100%	0	71.6445	71.249
<i>Merismopedia</i>	3	AGACTTACAAGGCCACC AGTTGAGCTCCATTCTTTAACGG	75%	0	71.3062	72.0837
<i>Aphanothece</i>	2	TGCCTATCTGGGGTTAAGC	66.67%	0	75.1184	69.8721
<i>Cyanobacterium</i>	4	CTTTGAAAGCAGACTTGAAGTGC TTCAGTTTCCACACCCTGC GTTTCTATCGCTTTTACGGAGTTG	100%	0	71.1334	71.1584
<i>Cyanobium</i>	55	TACCGTCATGTCTTCTTCC ATCGCTGAAATGGAGTTAAGC	67.86%	31	71.5569	71.34
<i>Gloeocapsa</i>	4	GAATCCCGTACCCCTAC CGACACAGCTTGAGTTGA	100%	0	70.5934	65.2005
<i>Microcystis</i>	130	GCCTTTAGGTCTGTTAAGCAA	96.95%	0	71.7437	55.7914
<i>Gloeotheca</i>	3	CCCTGATTGCGCAGA GAGCTCCAGTCTTTAACAG	100%	1	70.0205	68.3821
<i>Cyanothece</i>	12	AGCCGACTTGAACAACCAC CACGGTCTTTGACAGCAG CTCTAGCACACCCGT CCTTTCCCTTTTCAGGG AGCTCCAGTCTTTAACAGC	76.92%	3	71.0772	74.402
<i>Synechocystis</i>	11	TCCAGGCTTTCACCCAG GCATAACCACCTACGG CCACCTGTCTCTGTGTTT	100%	0	70.0871	73.4202
<i>Synechococcus</i>	216	GAGTGGATACCAGCAAC CAAGTACCGTCAGATCTTCTTC CCAAGAGATTCGCAGGA TTATGGCCAGCAGAGC CAAGATTACGGGATGTCAAATC	83.41%	72	70.2186	76.8232
<i>Planktothricoides</i>	5	TCTTTAACCGCCGACTTGC	100%	0	72.7565	67.582
<i>Halomiconema</i>	2	AGTACTTCTTCCCTGAGAAAAGC	100%	0	74.9915	72.462
<i>Arthrospira</i>	40	CTTTCACCAAGATTCCGGAC	100%	1	71.7418	67.277
<i>Halospirulina</i>	2	AAGCTCCGGTCTTTGACAG	100%	0	70.7524	72.0539
<i>Pseudophormidium</i>	2	CGCATTCTTTAACAGCAGAC CAACTGACTTTCATAACCACC	100%	0	70.6173	70.7312
<i>Geitlerinema</i>	8	CACTCTCAACTTTCGCCGA TTTGACAGCAGACTTGAACGG	88.89%	0	72.3118	69.9089
<i>Pseudanabaena</i>	14	ACACCTAGTACCCATCGTTA CAACAGACTTATCAAACAGCCT AGCAGACTTGATTAGCCAC CAACAGACTTATAAAACCGCC CCCGGTCTTTGACATAAGAC	80%	2	70.3787	72.53
<i>Microcoleus</i>	6	CTTGAATCGCCACCTGC TTCCAGAAGATTGCGGACATG CCTTCCGAGGTTGAGC	85.71%	4	70.5069	70.4731
<i>Symploca</i>	5	TACCCCTCCTGCACT	100%	0	70.7933	70.3255
<i>Planktothrix</i>	77	TTGACAATCCAAGAACCCTTCC	98.72%	2	72.517	68.1153
<i>Lyngbya</i>	11	CCCTCTAGTTCTCTCAGT TCCACTGCCTTTCAGAAGT CAGCTGTTTCCAACCTGGTTC AGCAGACTTGAATTGCCAC	100%	0	70.7076	72.176
<i>Leptolyngbya</i>	46	AGTTGCTCTCCACAGT TTAAGCCTCGCGATTGACAA GTCGATACGGTCAACACCTA CTTCCCAGAGAAAAGAGGTT CCGTCATCGATTCTTCCC TTATCCAGTTTCCACCGC TAAGCTCCGCGCTTTGA	80.85%	8	70.049	71.2841

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[T _m (PM)]	max[T _m (MM)]
<i>Spirulina</i>	11	GACCCACAGTTGAGC GAAGTTGAGCTTCCGTCTTT CCCGAGTTGATACAGGC CTTCAGAAAAGAGGTTTACGAC GGCTTATTCCTTAGGTACCG	83.33%	1	70.2915	72.2767
<i>Phormidium</i>	30	GAAAGTTCACGGGATGTCAAAT TCTTCCAGAGAAAAGGG GTCCATCAGCGTCAGTAA GGGTCGATACCTACTAC TCGCCGGCCTTTACAGA CTTTGACAGCAGACTTGAAGTG CTGACTTGTAACACCTACG TCCCCGAGATTCTGGA	54.84%	8	70.078	71.8196
<i>Limnothrix</i>	9	CCATCCCCTTTATGGAGTTA CTTCCTAGAGAAAAGGGG GCACTGATCGGGTCGATA	90%	1	70.8281	71.491
<i>Oscillatoria</i>	36	CAGTTGCAGTCCAGCA CTCTCAGGGATTCTGG ATACAGCACGGGTCGAT GAGTGTGAGTTCTGGTCTAG GTGGTCTTTGACAGCAGA	78.38%	0	70.2648	71.8569
<i>Trichodesmium</i>	9	CCTGACCAGAGTTAAGC CTGACCAGAGTTGAGC	100%	0	70.1756	65.3369
<i>Anabaena</i>	159	TTGAGCCTTGCTCTTTAACAG CAGGAAAATTCGAGGATGTCA CACAGTTGCCTGCGTACT	85.63%	64	70.9854	71.4314
<i>Cylindrospermum</i>	3	CTTTCAAGGAGATTCGCGACAT CCGTCATTGGATTCTTCCT TGACTCTTTGACAGCAGAC	100%	4	71.9262	70.8572
<i>Nodularia</i>	55	CCTAGTAGAGCGCCTTC	100%	5	72.4144	71.963
<i>Nostoc</i>	208	GACTCTTTAACAGCAGACTTAC TTACGGCCTAGCAGAGC CTTTCGAGAAGATTCGCGAC TTTCAGCAGGATTCGCGAC	90.91%	57	70.9746	75.1965
<i>Cylindrospermopsis</i>	55	CCACCGCTTTTATTGGTTAAGC	100%	1	73.3142	74.2642
<i>Trichormus</i>	8	CTCTCTAGAGTTCCTATCTAA TCCACTGCCTTGATCATGT TACTCTAGCTGTGTAGTTTCC	100%	3	72.2467	74.4358
<i>Aphanizomenon</i>	47	GCTTTGATCAGGTTGAGC GAAGGCACTTCCATCTTTC CACACTCTAGCTTTGTAGTTTC	97.92%	28	70.1328	71.8446
<i>Anabaenopsis</i>	4	CTCTGATGAGGTTAAGCC	80%	0	71.6864	65.6013
<i>Raphidiopsis</i>	1	TTCCACCGCTTCTATTGG CCACCGCTTTTATTGGTTGAG	100%	0	70.0993	68.2651
<i>Scytonema</i>	3	ACTACGTGGGTCGATACAC CAGCCGACTTGATAAACCAC	100%	0	71.6328	66.639
<i>Brasilonema</i>	5	AGACTTGAGCAACCACC TCACGACATAGCAACCGAG CCTTCAGTACTTATCCCTGA	100%	0	70.3451	67.8275
<i>Gloeotrichia</i>	2	CCATGTAGTTTCCACTGCTTTT	100%	3	71.4643	73.3625
<i>Rivularia</i>	8	TTCTCGTTTCCAAGAAATCCGG ACTGCCTATATGTAGTTGAGC	100%	2	72.0989	72.4793
<i>Calothrix</i>	34	GTCAGTATTGCTCAGCAG ATAGTTTCCACTGCCTGTATG GTAGACACACAAAACCACC CCTAGCAAGGCGCTTTC	71.43%	0	70.7461	71.5174
<i>Tolypothrix</i>	3	TTCCCGTTTCAAGAGGATTCG GTACCGTCATTCTTCTTCCT	50%	2	70.5897	72.0354
<i>Westiellopsis</i>	6	CCCAGAGTTCCAGACA CCCCAAGATTCCAGACAT	100%	4	70.3812	65.0749
<i>Hapalosiphon</i>	3	ACCGTCATCATCTTCTTGA GACAATAGACTTACACAACCAC	100%	0	70.8688	67.6698
<i>Chlorogloeopsis</i>	6	GACAATAGACATACATACCAC AAGCTTTCACCTTCGGTTCC	100%	0	71.1533	72.6959

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Fischerella</i>	12	TTCTACCGCCTTTATCTGG GGCACCTTCAGCTTTCA	69.23%	2	70.4016	71.3976
<i>Nostochopsis</i>	1	ATAGACACACAGCAACC ATAGACACACACTGCTACC	100%	2	73.4189	71.0553
<i>Symphonema</i>	1	TTCAAGAGGGTTCCGGA TCAAGAGGATTCCGGACAT	100%	0	70.0435	66.5759
<i>Prochloron</i>	25	CAGATTGCGCACCTGT ACGTTTCCGATAGATTGCGCA TTCCGCTAGATTGCGGAC	96.15%	0	70.9033	69.2215
<i>Prochlorococcus</i>	29	CAAGTACCGTCATATCTTCTCC TGCTACTGCGTTCCCAAAG	100%	0	70.7894	70.4646
<i>Prochlorothrix</i>	2	AAGCTGTGTGCTTTAACGACA	100%	0	70.4468	63.8991
<i>Chroococcidiopsis</i>	13	CTGGTGTCTTCCCGATC GAGCCTCGACCTTTAACA CTGTCTCCAAGTTCCTAAG TTCTCCAGATTGCGCG	100%	7	70.4046	71.5164
<i>Myxosarcina</i>	1	CTCCTCAGAAGTTGAGC GAAGGATTCTCGAGATGTCA	100%	1	70.1442	64.2409
<i>Stanieria</i>	2	AGTTAAGCTCCAGTCTTTGAC GAAGGGTCTCGACATGT	100%	0	71.7065	70.3511
<i>Pleurocapsa</i>	5	CCTGTCTTCGAGTTCCT CCTAAGGCACCTGAACCTATT	83.33%	1	72.5736	72.0644
<i>Xenococcus</i>	4	TCTAATCCCTTCGCTCC GTCTTTAACAGCGGACTTGA CCGTACTCTAGTTCACC	100%	1	70.2204	71.0099
<i>Dermocarpa</i>	5	ACCATTACGTCAGATTCTCGA	100%	0	70.3932	55.579
<i>Gloeobacter</i>	3	CCTTTCCGAGTTAGGC	100%	0	72.1971	69.1495
<i>Azorhizophilus</i>	2	TCTGGAACGTTCCGAGG	100%	0	71.5513	62.7261
<i>Azotobacter</i>	28	GCAGGGTATTGCGCTACA TCGCTTACAGCCCTTCC GCAAGGTATTAACCTACAGCC CGACCGAATCGCTGGTAA	96.55%	3	70.4192	72.5227
<i>Azomonas</i>	6	CTGCAGGGTATTAACCGG ACGTCAAAGTACCCACGTATTAG CCTAGCAGTTTGGATGCAATT	100%	0	71.0348	70.198
<i>Cellvibrio</i>	18	TTCTCCCCACTGAAAGTGC CGTCAATCTGCTCAGGTATTAA	100%	0	70.5124	74.0327
<i>Pseudomonas</i>	2921	CCTCCCAACTTAAAGTGCTT CCGCTACACAGGAAATTC AAGTTCATTGGATGTCAAGGC	97.78%	77	71.4003	73.2172
<i>Moraxella</i>	80	CACCTGTATGTGAATCCC CCAACGACTGGTAGACAT CTAGCACCAACAGTATCA	96.30%	1	70.2561	69.094
<i>Acinetobacter</i>	470	GCCTCCTCCTCGCTTAAA GCCACTAAAGCCTCAAAGG GAATTCTACCATCCTCTCC	99.58%	7	72.0967	71.952
<i>Alkanindiges</i>	4	CAGCACCTGTATCAGAATTC GGTAACGTCCACTAGCTG GCTATTAACACCAAGAGCC	100%	0	70.1575	66.3777
<i>Psychrobacter</i>	236	CAAGGGACCCAACGACTA GAGTCTTCTTCACTGCTTAAAG CAAGTTCCCAACGACTAG	99.58%	5	69.7528	69.9484
<i>Methylophaga</i>	25	ACGGATCAAGTCCACAAACG TTATCGCGTTAGCTTCGATACAC GCCAGTCTCAAATGCCAT	100%	0	71.9316	71.468
<i>Thioalkalimicrobium</i>	2	AGTAACGTACAGCAATCAGG AGAAAGTTCTGTGGATGTCAAGG	100%	0	74.1765	74.713
<i>Cycloclasticus</i>	21	CCACTAAGCGGAAACCC	100%	0	72.9626	59.4295
<i>Thiomicrospira</i>	29	CAGTTTTAGGCCAGGAAGT CTAACCTTTCTCTCTACTA CAGTCTCATTAGAGTTCTCAAC	96.67%	3	71.3633	71.639
<i>Piscirickettsia</i>	10	TCCTCAAGGGAATCCAAC	100%	0	70.1396	66.1783
<i>Thioploca</i>	3	CACTAAAAGGTATACCTTCC	100%	0	72.3444	61.0586

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Thiothrix</i>	26	CCTTCCGATCTCTATGCAT TCCCTCTCCCAGATT TAACGTGCATTATCTTCCCAAC	100%	0	70.4129	72.9764
<i>Achromatium</i>	19	GCTTCTTCTTGGAGTAACGT CATCCGACTTACTAAACCGC GAACCTAACGGCTAGTTGA AGGCTGGTATTAGCACC CTGTCTCCAAACGACCCTTTAA	100%	0	71.9512	71.1041
<i>Beggiatoa</i>	21	TCGAATGCAGTTCCCAAGT CGAAGGCACTCCCATATT TCTGAAGAATTCGAGGATGT CTTTGTCTTCCATAGGCTTTC CTTCCTGGGTAACGTCAG CTAAAAGGCAATTCCTTCCAAC CGACTGACTTAACCGCC	77.27%	1	70.1029	71.3171
<i>Francisella</i>	83	CTTCTCTCCCAACTAAAGT CGAATCCAACAGCTAGTACT	100%	0	71.5854	66.8441
<i>Citrobacter</i>	83	CCAAGTTCTGTGGATGTCAA TCTCGAGTTCCCGAAGG CCCCTACAAAGACTCTAGC TAACCACAACGCCTTCCTC ATGACTGCGGTTATTAACCAAC	32.14%	50	70.7547	69.9426
<i>Enterobacter</i>	234	CAAGACTCTAGCCTGC	79.15%	316	70.9236	69.7242
<i>Erwinia</i>	73	TGCGGGTAGCGTCAATC CTCTGAGGTCTTCCGT AGAATTCCGTGGATGTCAAGG GCATCTCTGCCGAATTCC CAATTCCGTGGATGTCAAGG	63.51%	26	70.6109	73.5957
<i>Hafnia</i>	16	GTCAATCGCCACAGCTATTA TAACGTCAATCGTTGCAGCT CTCTAGCTGACCAGTTTCAAAT	82.35%	4	70.3895	71.6967
<i>Klebsiella</i>	168	AACCTTATCGCTTCTCTCC AAGGTTATTAACCTCAACGCC GTAACGTCAATCGACAAGGT	33.14%	65	70.2341	73.5613
<i>Kluyvera</i>	24	ACCTTCAAATAGACATCGTTACGG	24%	0	72.989	71.8624
<i>Morganella</i>	41	CAGATGCAATTCCCGGG	95.24%	2	72.1136	69.132
<i>Proteus</i>	37	CAAGACTCTAGCCAACC TGACTTAATTGCCGCTGC	89.47%	1	71.6164	68.6599
<i>Providencia</i>	17	GACCACTCTTAGATGCCATT CTCTAGCTGACCAGTTTTAG	88.89%	0	70.0014	69.9576
<i>Salmonella</i>	147	TAACGTCAATTAGCTGCGGTAT CACAACCTCCAAGTAGACAT	89.19%	6	71.1402	68.5706
<i>Serratia</i>	214	GTATTAAGTTCACCACTTCCT ATGTGCTATTAACACACTGCC CAACAACTCCAAATCGACATCG CTATTAACACTGAACCCCTCCT GTAACGTCAATTGATGAGCGTA	62.33%	27	70.3631	73.0151
<i>Shigella</i>	104	GGCACATTCTCATCTCTGA	81.90%	128	72.5493	71.6256
<i>Xenorhabdus</i>	84	TTCTCGGGTTATCACC CAATCAACAACGCTATTACGCG TCTGTGGGTAAAGTCAATCA TTAGATGCCGTTCCAGG ATCAACAGCGCTATTACGC AACTTAATTGGCCGCTGC CGAAGCACTTTCGCATCTC CGTCAATCGTAAGCCCTGT CCTCTACGAGACTCTAGT GAAGGCACCTTTGGCATCT	67.06%	4	70.0406	74.6628
<i>Yersinia</i>	155	GTATTAACTCAACCCCTTCC CACCAGCATCTCTGCG	82.69%	3	72.5381	68.8898
<i>Edwardsiella</i>	24	TTGCCAGTCTTGGATGC	100%	0	70.6202	66.7651

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Arsenophomus</i>	38	ACCTTAACACCTTCCTCAC CCCTCTACAAAACCTAGCT CATCCAACCTTAATTAACCGCCT GCCAACTTAATTAACCGCCT ACAGTTTCATGACCACAACC	100%	0	70.3753	71.4376
<i>Plesiomonas</i>	3	CGTCAATGCCACTAGGT	100%	0	70.8933	65.2006
<i>Photorhabdus</i>	88	GCTAAACCTTCCTCC AAGCCACAACCTCATGGC GACTCCAGTCAACCAGT CTTCAGGCCATACCCT GGTCTTCTCTGGATGTCA	92.13%	0	70.5101	71.3639
<i>Buchnera</i>	54	CATCTCTGCTAAATTCGTGG TCACTTCTCTTGGAACAACC CCATTTCTCTATGGATACAACC CTCTCTTGGAACAACCTCT CATTTGAGTTTTAGCCTTGCGA ACCTCTAAGTCGACATCGT GAAACAACCTCCAAGTCGA CGACGAAAGTACTTTACAACC	96.36%	2	69.6232	70.8373
<i>Rahnella</i>	33	AGTAACGTCAATCACCACACG TATCTCTAGCGAATTCCGTGG CAATCGCTGCAGCTATTAACCTA CAATGCACAGTGTATTAACAC	79.41%	15	70.9287	70.7392
<i>Ewingella</i>	4	GCCCACTCAAAATCG AGCCCAACCTCCAAAT	100%	2	70.3974	70.9372
<i>Wigglesworthia</i>	1	CTTCGAAAACCCGAGTAAACT	100%	0	71.5669	61.8824
<i>Pantoea</i>	269	CGAAGGCACCATCTCTG GTCAATGCCGCTGGTTATTA GACTTAACAGACCGCC	69.26%	117	70.5845	70.8423
<i>Candidatus Phlomobacter</i>	2	CTATTAACCCTAACACCTTCC	100%	0	72.0852	71.6169
<i>Aranicola</i>	2	ATTAAGTCACTGGCCTTCC	66.67%	0	73.0689	69.1995
<i>Brenneria</i>	14	GGTTCAAGACCACAGC GTAACGTCAATCAGCGAACGT CTTAAACCTTTCCCTTCTCT AGTCTCTCTGAGGATGTC	93.33%	2	70.2954	66.3138
<i>Buttiauxella</i>	17	CACTGCGGTTATTAACCACAAT	72.22%	25	70.3371	71.2652
<i>Obesumbacterium</i>	1	TCTCTGGCAAATCTCTGG	100%	0	72.1344	68.858
<i>Leclercia</i>	4	GTCAATTGCTGCGGTTATTAAC	80%	205	71.2857	71.7261
<i>Sodalis</i>	15	CGCTGTAAACGCTAACGC	87.50%	0	70.3066	57.7173
<i>Pectobacterium</i>	74	TAATCTCACCGCTTCC GTAACGTCAATCAGCAAGGT TCTAGCCTGTCAGTTTGAATG	93.33%	33	70.3897	70.7265
<i>Cedecea</i>	2	ACTGCGGTTATTAACCACAATCC	33.33%	8	74.4365	74.3531
<i>Trabulsiella</i>	1	GTAACGTCAATTGCTCTGGTTA	100%	0	70.6527	63.9739
<i>Raoultella</i>	32	AGTAACGTCAATCGTAAAGGT CTCAAGCTTGCCAGTTTCAA	45.45%	26	71.3448	72.6859
<i>Dickeya</i>	24	TAACCTTGTCCTTCTCT TCTAGCTTGCCAGTTTGAATG TCAAGACCACAACCTCCAAA GCATCTCTGCAGGCTT	72%	4	70.4111	72.4212
<i>Averyella</i>	3	AGTAACGTCAATGAACAGTGC	25%	10	70.3346	69.7777
<i>Candidatus Riesia</i>	10	GTCATCCGAAGAAGGTGT	100%	0	71.7065	61.057
<i>Candidatus Ishikawaella</i>	12	CCACAAGTCAAGCTTACAGTTT GCTCACAAATCTCAAGTCGACA CACAAGTCAAGCTCACAGTTTT GGAGGGCACTTACACATT	100%	0	71.9222	68.8073
<i>Cronobacter</i>	99	GCAGGATTCCTGGATGT	99%	0	73.6452	70.27
<i>Candidatus Stammerula</i>	20	CGAAGGCACTAAGACATCT CTTTTGAGTTCCCGACCGA CTCTGTCGAATCCGTGG	95.24%	0	70.4664	72.1783
<i>Phytobacter</i>	1	AACCGTATIGCCTTCCTCC ACCGCATTGCCCTTCCTC	100%	0	71.325	74.5033
<i>Escherichia</i>	193	CAAGCTTGCCAGTATCAGAT	89.18%	101	73.7379	73.4255

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Legionella</i>	123	ATCAGTGCTCCCGAAGG GTCCAGTTAATCAGCTCTTAAC CACTACCTCTCCCAT CTGCGCCACTAATCTCATT CAGTATTAGGCCAGGCAG GCTTTCACATCCGACTTAAGTATC CTGTATCAGTGTCCCGAAG	96.77%	7	70.218	73.3488
<i>Fluoribacter</i>	6	CGAAGGCACTATGCATCT CAGCATCCTTAGAGTTCC TGGGTAAACGTCCAGTTAATTAGC	85.71%	8	70.9524	75.8499
<i>Tatlockia</i>	5	GTAGCAGTGCTCCTTAC GCTTCGTCACTAACCTCATTC	100%	0	72.4194	70.5003
<i>Coxiella</i>	20	CCTTGAGAAATTTCTTCCCC TACCCCTTGAGAATTTCTTCCC GACTTAAATATCCACCTACGC GTAACGTCAACGCCCAAGG TTGCACTCTCTGTATTACCGC AGAAAGTGAACCTCCCAACAGC	100%	1	71.5302	74.9602
<i>Aquicella</i>	2	CACTTCTTCCCAACCTAAAGAG	100%	0	72.45	66.2867
<i>Rickettsiella</i>	3	CATCAAACCTAGTCAACCAC	100%	0	70.5433	52.2322
<i>Halotheobacillus</i>	10	TTAGTCCGTCACCGA CTCCCAACGACAAGTCGA GTGCTTATTCAACGGGTAATGT	100%	3	70.0242	70.631
<i>Alkalispirillum</i>	4	TCAAGACTCACGGGTATTAAC AGCCCATAAATGAGCCC	100%	1	71.2147	69.8334
<i>Halorhodospira</i>	5	GTGAAGACGCCAGGTATT TCTAGTCAAGCAGTATCGG GTAACGTGAAGACGCCAGG CTCGCACAGCTATTAACC TTGCGCCCTTCGTATTACC GCTAGTCCTCAGCGTT GCCACTAAGCCTATAAATAGG GTCAACTCGCACAGCTAT	100%	0	70.8246	74.7072
<i>Thioalkalivibrio</i>	9	GGTAGTCCATCTAGAGT CAGTTATTAGCCGACAGCTTTT CTGTAGGTGATGTCAAGAC CACTAAGGGACGAATTCC TAACGTCAAGACCCGGC	80%	0	70.4424	70.3018
<i>Aquasalina</i>	1	TCAAGGCTCACGGGTATTAA	100%	0	71.0512	67.1056
<i>Alkalilimnicola</i>	3	CCACTAAGTCCATAAATGAACC	100%	1	71.9781	66.9588
<i>Ectothiorhodospira</i>	6	ATTAGGCACCCGCTTTTCC GACTGACATAAACACCTACG	100%	0	73.1065	70.0175
<i>Arhodomonas</i>	1	CAAGTCCATAAAGAACCCAACG GCCACGAGCCTCATAAAT	100%	0	71.0171	61.4858
<i>Natronocella</i>	1	ACGTCAAGACTCACAGCT	100%	0	70.052	63.4343
<i>Allochrochromatium</i>	10	TAACGTCAATACCAGCAGG GCCCTTAAATGGACCAAAACG	100%	1	71.3543	67.0286
<i>Thiodictyon</i>	1	GCGACAGTATCAAATGCAGTTC	100%	0	73.8384	71.838
<i>Lamprocystis</i>	5	GCCATAAAATGAGCCCAACG CAGTCTTGCTCCAGG	100%	0	70.0741	62.6166
<i>Thiorhodococcus</i>	5	CTTAAATGAGCCCCACGG CAGTCTTGAACCAGGGG	100%	0	73.2563	68.8038
<i>Thiococcus</i>	3	CCCTTAATTGGATCCAACGG GACTTATCGGTTTTGCGC	100%	0	71.497	69.7074
<i>Marichromatium</i>	10	TCAAACCTCTAGCCAGGC ATCTGACTGACCTTGCC	100%	0	71.902	73.549
<i>Nitrosococcus</i>	16	AACACTGAAAGGCTTACCC CAGTATCAAGTGCCATTCC	100%	0	71.5369	69.7851
<i>Thiorhodovibrio</i>	4	CCAGACTCAAGCTTAGC CAAGACTCAGGGGTATTAAC CAGCGTTTTGGAACCTCAG	100%	0	70.9704	69.6972

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Chromatium</i>	5	TCAAAGCCGATGGGTATTAAC CAGGATTCTCTCGATGTCAA GCAGCCCTAAAACGGA GTGCTTCTCTGTGGGTAA	100%	0	70.0819	64.6229
<i>Thiocystis</i>	7	GCTTTCACAGCGACTGA TTCACAGCGGACTGACC	100%	0	73.8984	72.797
<i>Thiobaca</i>	1	CGCCACCAAAACCTTAAATGG	100%	0	75.2475	71.4852
<i>Rheinheimera</i>	19	TAGCTGCGCTACTCAC ACGGTACAAGACACGAAC TGCCTACTCACAGTAC	100%	0	70.4425	69.3849
<i>Thiocapsa</i>	21	AGGCTTCTTCCCCACT ATCTGACTTACGTTGCCGC	95.45%	0	70.422	72.6649
<i>Halochromatium</i>	5	CTCTGACAGTTCCAAGGA	100%	0	71.7569	61.2316
<i>Stenotrophomonas</i>	279	TCTCTGGAAAGTTCTCGACA GCCAGGCTGGATTTCCTT TCATCCCAACCGGTA ATTAGCCGGCTGGATTCT	86.79%	22	70.7146	70.4942
<i>Luteibacter</i>	8	GTCAGACTGCATGGGTAT AGCCTTGACAGATTTCGCT	88.89%	0	70.0344	69.7284
<i>Ignatzschineria</i>	9	ACAGCTAAGGGTATTAACCTC CCCAACTTAAGATACCACT	100%	0	71.3036	67.8904
<i>Xylella</i>	104	AAAGTTCCGCAGATGTCAAGA CAATTGCTTTTCTTCCCAACA	100%	0	71.1628	72.0604
<i>Frateuria</i>	15	AGTTCGCGCGGTATTAAAC	68.75%	0	71.2322	71.3808
<i>Dokdonella</i>	5	CAAAGTGTGCCAACATCC GTCAGTACTGGTCCAGA	100%	0	72.9516	76.614
<i>Fulvimonas</i>	1	CTAGCTCCCCAGTATCC	100%	0	75.8549	71.0307
<i>Hydrocarboniphaga</i>	1	CGATCTCCAGCTACC	100%	0	70.4914	58.9896
<i>Xanthomonas</i>	181	GGGTATTAACCGACTGCT	78.02%	10	70.895	71.1025
<i>Thermomonas</i>	19	AGCTTCCGTGCATGTCAAG CCAAAGTGCACCCAACC CTTCGATACTGAGCTCCAA CCAGTATCCAGTGCCATT	90%	5	70.4016	69.9528
<i>Lysobacter</i>	37	CTGAGTGTGCCCAACA ACCGTCATAACACCCGAGT GCACTCTAGTAAGCCAGTT GCCTAGTTGCTCCCAA ACCCTAAGCTTTTCTTCCGG GACCTAAGTGTCCCAAC GCCAAGTTGCTCCCAA CTTCGATACTGAGTGCCAAG	76.32%	4	70.3536	76.1535
<i>Luteimonas</i>	5	AGTTCCTGGATGTCAAGG CCTACCGGTATTAACC	83.33%	0	70.524	70.5893
<i>Nevskia</i>	3	GAATCGCTAAGCGACCC GACACCGATTGCTAAGCAA	100%	0	71.5429	70.1537
<i>Rhodanobacter</i>	15	CTCTAGCTAGCCAGTATC TAACCCGTGGTATTTCTGTTCC TCATCCTCCGACGTATTAG GACTGCACAGTATTAAGCATG	87.50%	2	71.3367	73.06
<i>Pseudoxanthomonas</i>	42	ACTGGGTGCCAAGTTGC GCGTTAGCTTCGATACTGG ATCGCTTTTCTTCCCAACAAAAGG CAGCAGGATTCTTCCC	100%	13	71.6197	72.596
<i>Dyella</i>	15	AGTTCGCGCGGTATTAGC TAGCCGGTAGTATTTTCGCT TCAGTGTGATCCAGATGG GCAGACCTGTGTTCTCGATT	93.75%	18	70.3081	77.9743
<i>Aquimonas</i>	4	TCCAAAGTGAGACCAACGT	100%	0	71.1779	59.4481
<i>Siderooxidans</i>	2	AAAGAAGTCTCCTTCTCCAAC ACCTGTGTCCAAGTTCC	100%	0	70.5169	69.5721
<i>Silanimonas</i>	1	ACACTGAACCTCAATTGAGC GATACTGAGCTCCGAAGT	100%	0	71.7569	65.6294
<i>Crenothrix</i>	12	CCAATCTCTCAGGGATTTC CCACTAAACCTTAAAAGGG	100%	0	70.1012	70.4046

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Methylosphaera</i>	1	CGTAAAGTGACCCAACGG	100%	0	70.9618	59.0074
<i>Methylocaldum</i>	10	CACTGAGAACCTACTCGT GCGCCACTAAACACTCA CAACTGCCATTCCCAGG	100%	0	70.2588	70.8649
<i>Methylococcus</i>	7	CTAAAGGGCTAAACCGGC CGCTCGCCTTTCTTCTC CACACTCGAGCCTGA ATTAACCTCCCGCCCTT	100%	0	69.8266	69.0852
<i>Methylobacter</i>	19	TCAAGCTACCGGGTATTGA AAGCTGCCGGGTATTGA CACTCTCCTCTACTAAACTCTA GTATTAACCGACAGGTTTTCC TACCGGGTATTCAGCC CCTCTCCTGTATTCTAGA TGCGCCACGAATCTTATGA CAAGCTACCGGGGTATTG TCAAGGCATCGGCTATTAAC	75%	2	70.0548	73.1776
<i>Methyломicrobium</i>	15	TCAAGGCATCGGCTATTAAC CGTCAGTTTTAGGCCAGA CAGCATCACGTGTTGGC	81.25%	2	70.1772	70.1093
<i>Methylohalobius</i>	1	TCAGACTGAGCAGGC GGCTATTAACCTGCAAACC	100%	0	71.4036	65.0673
<i>Methylomonas</i>	19	GTCAAGTCTACCGGT GTCAAGACACGGGGTA TAATGTCAGTCTACCGGG	95%	0	71.0516	65.8351
<i>Methylosarcina</i>	2	TTCTATCGGTAATGTCAAGCTGC GCGCCACTAATCTTACGAATAA	100%	1	70.7387	67.7514
<i>Bartonella</i>	99	CACTAACCACGATCGGG CTCGGCGGAATGTTAATGC CTCTGACTTAAATATCCGCC ACCGAGCAGTAAACCGC	92%	1	71.1973	72.1182
<i>Hanschlegelia</i>	2	GAACAGCATGTGTCCAA	100%	0	72.2821	71.4526
<i>Methylosinus</i>	35	TGACGACCATACCGGAC GGAAGGAAGCCATCTCT CTCTAGACCTCCAGTATCAA CTTCTTATCCAGGGTACC GGCGACCATACTGGA	77.78%	6	70.7195	69.0069
<i>Methylopila</i>	1	TACTGCAGCCACTGAC CAAATCTCTCTGGATGTCACAG	100%	0	71.2212	66.2881
<i>Terasakiella</i>	1	GGAACTTCTTTTGACGCTAC	100%	0	70.5932	55.0961
<i>Pleomorphomonas</i>	5	TCGTAICTAAGACACCC	100%	0	70.3255	68.8735
<i>Methylocystis</i>	51	CATCTCTGTGACCATACC CTTGCGGGAAGAAAGCCA GCATGTCAAAAGCTGGTAAGG CCTAGCTGAAGGATACCG ACTTAGAAACCCGCCTACG GAAGAGAAGGCCACATC ACTGAAGGAAGCCATCTCT CATTATCTTCCCGGACAAAAGA	90.38%	14	72.8754	72.8775
<i>Rhodopseudomonas</i>	84	CATCCGAAGAGAAGGGTC GTATCAAAGGCAGTGCC GCCCCACGGTTAACATC	62.35%	6	70.2617	68.5946
<i>Bosea</i>	38	ACTGAAGGAAGCCATCTCT	94.87%	5	71.0629	69.78
<i>Blastobacter</i>	5	CATCCGAAGAGAAGGGTC GTATCAAAGGCAGTGCC GCCCCACGGTTAACATC	66.67%	0	70.609	70.0936
<i>Rhodoblastus</i>	1	AGTCCGGCATTTACAC	100%	0	73.5966	71.9085
<i>Nitrobacter</i>	67	TGTGCTCCATGCTCC	91.18%	0	71.4733	69.5762
<i>Afipia</i>	50	TGCTCTATGCCCGGA GCGCCACTGAAGAGTAAACT GCATCTCTGCCGATCAAAC	64.71%	19	71.5001	73.8138
<i>Oligotropha</i>	1	CGCCACTGAAAAGTAAACTTTCC	100%	9	73.6692	72.666
<i>Bradyrhizobium</i>	738	GCGCCACTAGTGAGTA CCGAACCTAAGATCTTCAGTAT	98.24%	162	71.0929	72.9047
<i>Rhodoplanes</i>	9	TCTCCGGAACCGTCGT GTCATTATCTTCCCCACC	100%	2	72.0593	68.7988

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued	
					Specificity	
					min[T _m (PM)]	max[T _m (MM)]
<i>Blastochloris</i>	13	CTTCACCATCGAAAGAGCTT ATCTCTGGGAACCGTCG GAGGCTTAAAGCGTTAGC	92.86%	1	70.3092	70.0104
<i>Devosia</i>	24	GCCACTGAAGGGTAAAC TACCTCTCCGGACTCTA GCCACTGAAGTGTAACAC CACCGAAGTGAAGGAAACCA	80%	2	70.3858	71.7191
<i>Hyphomicrobium</i>	57	CACCGACAAGCAAGCTTG CACTGTCCATTGCTGG CTCTACCGGAATCAGTG CGGGACTTATTCTCCGG	89.66%	2	70.6983	66.4989
<i>Angulomicrobium</i>	1	CATCTCTGATCCACTTCC	100%	0	72.197	63.1088
<i>Rhodomicrobium</i>	4	CACGTAGCACGACATGTC GAGGACCATCGATCTCTC	100%	0	71.2788	65.7587
<i>Pedomicrobium</i>	6	GTGTCCCGACCTATTGC	100%	0	73.278	62.9229
<i>Methylocella</i>	7	CTGGAAACCAACCGGACAT CTCTGAAACCATAACGAGA CTCTGAAACCACAAACGAGAC	100%	0	70.9327	67.8584
<i>Beijerinckia</i>	22	CTCTGGTAACCAACGAGAC AGCCAAACTGAAGGAAACC	100%	0	71.0772	71.869
<i>Chelatococcus</i>	8	AGCCGAAGTGAAGGAGA TCTCCGGTATCCAAACGA TCGAGATCCTCAGTATCGA CTCCGGTAACCAACGAGA TCGTCCCGGACGAAAGA	100%	3	70.1203	69.0145
<i>Deffluviobacter</i>	1	GAAGTTCGAGATCGACAGT	100%	0	71.0171	68.6643
<i>Hoeflea</i>	2	AGGCAGTACCAGGGT	33.33%	0	72.1045	68.9369
<i>Pseudaminobacter</i>	5	CTCGAACTCTAGATCAGC CCTAACTGAAGGATACCGTC GTAAACCTGCCGACAGC	100%	1	70.285	70.5241
<i>Chelatovorus</i>	1	CTAGCCTCCATCCGT	50%	0	70.2682	62.5759
<i>Mesorhizobium</i>	336	ACAAGTAACTTGCCAACGGC TCTTACCGTTGAAAGAGCT	91.39%	26	70.956	70.5774
<i>Phyllobacterium</i>	45	ATCTCTGGTAACCCGCG CCTAACTGAAGGAAACCATCT GCCTAACTGAAGGATACCAT TGAAGGGTCCCATCTCT CCTAACTGAAGGATCCA	97.83%	21	70.1594	71.715
<i>Aminobacter</i>	19	CTCTAGACCGGAGT CTCTCTCGGACTCTAGATTG CACTAAGGGCGAAGGTT	95%	9	70.8649	73.6957
<i>Parvibaculum</i>	5	TCCTCTCCAGATCTCTAG GACTCTAGCCATCCAGTTTT TGCACTAGGTCCAGC	100%	0	70.021	72.3366
<i>Rhizobium</i>	610	TCCATACTCCAGATCGACA TCTCCGATACCGTCATTATC GTAGCTAGCCGGACAT	86.91%	174	70.5003	73.1288
<i>Agrobacterium</i>	266	AAGTGGACCCCATATCTC AATGCCCATACCCCGAATG CTTCCATACTCAAGATAACCAG CAGCCGAAGTGAAGGTCA GTCTCCACTGCCCAA	86.89%	52	70.2139	73.6546
<i>Sinorhizobium</i>	241	TCTCTCGTATCCGCGATC CCTTTCCTACTCTAGAC CCGAAGTGAAGGATAGGA GACTCTAGATTGCCAGTATG	78.93%	39	70.1778	72.1182
<i>Ensifer</i>	19	ATACATCTCTGTAATCCGCGA AACGTCTCCGTAATCCGC	80%	37	71.1123	71.6146
<i>Amorhomonas</i>	2	ACTGAAGATTACCGTCTCC	100%	1	70.6872	67.5105
<i>Kaistia</i>	6	TCTTCATCGCGGAAAGTGC	100%	0	72.4366	72.2988
<i>Candidatus Liberibacter</i>	4	TGGTAAACCACCAACAGC	100%	0	74.3687	69.7087
<i>Brucella</i>	106	CAAAGTACCCCAACGGC CCTAACTGAAGGATAGTGTG	92.52%	5	70.3703	70.1588

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[T _m (PM)]	max[T _m (MM)]
<i>Pseudochrobactrum</i>	2	CAAAGTGTCTCCACTAAGATTC GAAAGGCTGAAAGTGTCTC	100%	0	70.7973	66.885
<i>Ochrobactrum</i>	225	CACCGAAGAGTAACTCC CGAAGAGTAACTCCCC AGCCGAAGTGAAGACACAT	97.79%	9	69.1767	69.1524
<i>Mycoplana</i>	7	CGAAGAGTAACTCCCCG AGAGAAAGCCAGATCTCTC GCCTAACTGAAGGAAAACATCT	62.50%	36	70.6121	71.0672
<i>Rhodobium</i>	12	GGAAACCGTCTCCGTAA CCTAGCTGAAGGATACCG	76.92%	8	70.6703	71.8829
<i>Meganema</i>	3	TCTGGAAGTCGTCTCGG	100%	0	71.748	70.1976
<i>Roseomonas</i>	33	ACACTGAGGTCTAGGAAC GTATCAAGCGCAGTCC GCGTTAGCTACAACACTGA GTCTTAAGCGCAGTCC	91.18%	7	70.1986	71.7369
<i>Methylobacterium</i>	259	GTTCCGCTGTCTCTTTC GTTGAGCCACAGGCT AGGATCCCCGATCTCTC GTCAAAGGATGGTAAGGTT	98.46%	10	70.3379	69.0069
<i>Marteella</i>	2	GAGGTAGCACCAAATGTCAA	100%	0	72.6113	72.1962
<i>Aurantimonas</i>	10	AGGATAAATCCTCCGACGG CACCGAGTGATAAATCACC	100%	1	70.8173	68.7274
<i>Fulvimarina</i>	3	CACCGACGAGTAAACCC	75%	0	72.1356	60.4983
<i>Labrys</i>	9	ACACTAGCTGTCCACC	90%	0	71.5975	67.8202
<i>Azorhizobium</i>	11	CCATTGCTGGAAGAAATCC CCATTGCTGGAAGAAATCC	100%	12	71.2176	66.6127
<i>Xanthobacter</i>	24	CTGAGAGGTAAACCCC CATTGCTGGAAGAGATCCAT GTGCAGGCTCATTGCT	88%	0	70.6072	69.5168
<i>Ancylobacter</i>	9	TCCGAAGAGAAGAAACCATCT TCCGAAGAGAAGAACCATCT GCATGTCAAAGGCTGGTA	100%	1	70.5475	70.2475
<i>Starkeya</i>	2	AAGAGCTTGGTCCATCTC	100%	2	70.9479	65.2015
<i>Orientia</i>	13	CCAGATGACAGCTTTCGC	100%	0	73.0052	68.0568
<i>Rickettsia</i>	104	TGCGAAACCGAAAGAGAACTTC AATTCCATCATCCCCACTAC CAGCATTACCTGATAGCAACTA GGTATGATTTCCATGTCAAGGA	100%	0	71.6058	69.3379
<i>incompatibility symbionts</i>	6	CGACCCTATTCTTCGAATAG CCTATCCCTTCGAATAGGTATA GGAATTCCTCTATCTCTTTC CGACCCTATTCTTCGAATAG	100%	4	72.3958	72.3074
<i>Wolbachia</i>	70	AGGGATACGACCTTCATGT ACCTCTAGTCTAGCAGTATT GGGACTTCTTCTGCAGG	100%	5	70.6527	72.3166
<i>Ehrlichia</i>	74	ATTAAGAGCAGTCCAGGG CCCACATTCAGCACTCA CTCTCTCACGTTCGAGT	84%	0	70.1775	69.0029
<i>Neoehrlichia</i>	6	ATTAAGAGCAGTCCAGGG	100%	0	75.2808	70.0769
<i>Anaplasma</i>	85	CCCACATTCAGCACTCA CTCTCTCACGTTCGAGT	100%	9	70.7892	70.3999
<i>Neorickettsia</i>	14	ATTAAGAGCAGTCCAGGG CCAGGCAGTTTGAAGCAA TGGCAACTAGCGACGAG AACCGACAGAAATGATCTCTC CCGTTACCGTCATTATCTTCAC	100%	0	70.3893	66.8886
<i>Holosporaceae</i>	9	TCTCATCTCCTTAATCACCATG CCAGGCAGTTTGAAGCAA TGGCAACTAGCGACGAG AACCGACAGAAATGATCTCTC CCGTTACCGTCATTATCTTCAC	100%	0	70.0548	68.9116
<i>Candidatus Pelagibacter</i>	1	TAATCTCTTAGAGTCGCGACG	100%	0	71.249	58.4158
<i>Caedibacter</i>	2	TCCTCTCCATAATCCCC	100%	0	71.5644	60.3427
<i>Thalassospira</i>	8	GTCAGATCTAGTCCAGG	100%	0	72.3492	66.9321
<i>Rhodocista</i>	6	CAAGCTCACCAGTCTCA GAAAGGAAAACCCCATCTCT CCTCAAGCTTAACAGTCTCA	85.71%	0	70.6559	68.1068
<i>Rhodovibrio</i>	7	GTTTCAAGGGCACTTCC CGGTCATCATCTTCGGG	87.50%	0	70.6326	68.5687
<i>Roseospira</i>	6	ATTCCATACCCCTCTCC GAATTCATGCCCTCTCT	100%	0	70.4174	69.7761
<i>Skermanella</i>	1	GACTTGAAGTACCGCC	100%	0	70.5845	66.0988

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Dechlorospirillum</i>	3	GGCAGTATCAAAAGCAGT	100%	0	70.4153	65.6669
<i>Caenimicrobium</i>	1	CCGAAGTGAAGTCTCATCTC	100%	1	71.9599	71.2511
<i>Inquilinus</i>	5	CAACTGAAGAGGAGGGTT	100%	0	72.3927	68.559
<i>Magnetospirillum</i>	17	CTGACTGATCGAACCGC GGCTTTCACCTTCTGACTATGA	100%	0	71.0698	70.8933
<i>Azospirillum</i>	94	CGTGCGAAAGAGCTTTACAAC CTTCTTCTCACGCTACCG GCTTTCACCTTCTGACTAAACAG CTAAGAGCCCCAACGT ACTGAAAAGTCTCATCTCTGAG	93.68%	17	70.1969	70.3981
<i>Rhodospirillum</i>	15	TACCGTCATCATCTTCACAC GGGGATGCTTTTCTTCTCTG CTCTAAGAGCCCCAACGTC	87.50%	0	70.2711	68.726
<i>Telmatospirillum</i>	3	TCGTCCCACCCGAAAAGA	100%	0	72.2907	71.3891
<i>Rhodospira</i>	1	CTCTGACTGATCAGACC	100%	0	72.4018	67.5632
<i>Tistrella</i>	4	CGATACTAAGTATCCCAACGT	100%	0	70.8574	59.0477
<i>Phaeospirillum</i>	7	CTCTTCCAACCTCCAGC AATTCCCTTACCCTCTC	87.50%	0	70.2415	68.033
<i>Roseococcus</i>	1	TGTATCAAGCGCAGTCC	100%	0	71.4072	71.2457
<i>Stella</i>	1	GACACTGAAACCCCTAGG	100%	0	71.559	61.0287
<i>Gluconobacter</i>	71	TGCCCCACCTGAAAGTGC	100%	0	75.0404	70.6376
<i>Oleomonas</i>	1	GCGTTAACTTCGACACTGA	100%	0	70.6202	69.6975
<i>Paracraurococcus</i>	4	TGAAGTTCTAGGAACCTCAAC AATTCCGCGAGCCTCTC	100%	0	71.5386	70.3108
<i>Acidisphaera</i>	4	ACTGAGCGACTAGGTGC GACACTGAACGGCTAAGC GAATTTACGCGTGACTGTATC	80%	0	70.7076	69.6406
<i>Gluconacetobacter</i>	73	AGGTTGCCTAACATCTAGC GGTGTGCTTATCGCGTTAAC GTGCTGGAGGTCTCT	100%	5	71.4642	71.8024
<i>Swaminathania</i>	1	CCATCTCTGAATACAAGCC	100%	0	72.8217	62.967
<i>Muricoccus</i>	1	TCTTCCATCCTCAAGCC	100%	0	71.6539	71.4864
<i>Kozakia</i>	3	CTTGCGGGAATAACCATCT	100%	0	70.5182	66.6498
<i>Asaia</i>	34	TGACTGTGTAACCGCC TGACTGTATGAACCGCC	97.14%	2	70.0894	70.7757
<i>Granulibacter</i>	3	GAGTTACTAGGTAACCCAACAT	100%	0	73.0789	64.1068
<i>Acidiphilium</i>	68	GCTAAGCACCCCAAC	100%	1	70.5471	65.5182
<i>Acidocella</i>	12	TCGTCCCTGCCAAAAGTG	100%	0	70.8509	69.8788
<i>Acidomonas</i>	16	CTCGAACTCCAGTCAACAC	100%	0	72.5041	61.4783
<i>Acetobacter</i>	73	GAATACAGCCTCTCCATACA CTCTGAATACAGCCTCTACA CTCTAGTCTGCACGTATCAAAAT ACCTGTGCGGTAGGT TCTCTGCCTACAGCC	93.24%	0	70.3314	72.8442
<i>Acidicaldus</i>	1	GAATTTACGCCCCGACGA	100%	1	70.7441	69.9089
<i>Rhodopila</i>	1	CACTCAAGTCCATACGTATCA	100%	0	71.2136	69.8987
<i>Phaeobacter</i>	8	GTGTGACACCAACAAGCA CTAGGTACCCGAGT GCTGACGACTGGCATT	88.89%	19	70.2426	69.243
<i>Roseobacter</i>	110	CCGAACAACGCTAACCC CTAACTTTCTGATCCGCC TACAGTCATTATCTTCCCCAG	90.99%	225	70.9109	69.7812
<i>Loktanelia</i>	23	TTAGGTGTGACACCAAAGG CACTTGGTCTCTTACGAGA	66.67%	9	71.1584	70.8701
<i>Rhodobaca</i>	3	CTGAAAGAACCATCTCTGG	100%	0	70.8433	66.5332
<i>Rubellimicrobium</i>	3	GGCGAAAGGGCTTTACG	100%	0	70.7573	67.4091
<i>Methylarcula</i>	9	TTTACCCCTAACTTTCTCTGT CTCCTGGTCCCTTACAAA TGATAGTTTTGGAGGCCGT TGGTAGTTTTGGAGGCAGT	70%	0	70.1217	73.3961
<i>Roseivivax</i>	4	GTTAAGTGTGTACCGAACA GGGTACGTCATTATCTTCC	60%	1	70.4774	69.6782
<i>Amaricoccus</i>	5	CGCGACGTCCAAGTCAA CGAACTGAAAGACCGGTC TTCCGTTAGAGTGCCC GCCGCGATATCCAAGTC GACGTCCAAGTCAAGGG	100%	1	70.3318	71.0353

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Rhodothalassium</i>	1	ACCGAAACTCTAAGAGCC	100%	0	70.4476	69.3
<i>Thalassobacter</i>	2	TCTCCAGAAATCTCGATGAGG	100%	1	74.8435	63.4803
<i>Thalassobius</i>	4	GAAAGGAACTATCCATCTCTG	100%	1	70.6177	72.1321
		CTCTAACTTAATAATCCGCCTACG				
		TCTAACTGACTAATCCGCC				
<i>Sulfitobacter</i>	94	CTGCGTCACCGAAGTGAA	85.26%	21	70.1554	73.3995
		AGGAGTTTATGAGGCAGTT				
		GGCGAAAGTGCTTTACGATC				
		CCACAAACAGCTAGTAGAC				
<i>Pseudorhodobacter</i>	2	CCTGACAACTAGCCC	100%	0	70.1406	69.1049
		CAAAGGCCGTTCCAAGG				
		TTCACCTCTAACTTCCAGT				
<i>Staleyia</i>	6	CGATCTCTCGGTTTGCA	42.86%	2	71.715	68.3902
<i>Pannonibacter</i>	2	CCCGTGGTAGCACCAAAT	100%	4	72.5547	63.2267
<i>Tetracoccus</i>	1	CCGAACTGAAAGCTCGATCT	100%	1	71.8872	70.5059
<i>Jannaschia</i>	14	ATTTCACCTCTGACTGATCGG	66.67%	0	71.6306	71.8446
		CCCTAACTTAATAATCCGCC				
<i>Paracoccus</i>	156	TACCGTCATTATCTTCACAGC	98.09%	20	70.7222	69.2779
		GCTGTCTCGCATGT				
		CCCAGCTGAAAGAGCTTT				
		GACCGATAGTTTGAAGGCAG				
<i>Antarctobacter</i>	2	GCCTAACTGAAAGCTCCATC	100%	0	74.0447	69.8438
<i>Octadecabacter</i>	24	CCTAAGGGAATATCCATC	92%	0	70.763	64.2999
<i>Ahrensia</i>	6	AGCCTAACTGAAGGTATCTG	100%	3	70.049	69.0631
		GCCGAACTGAAAGTCTCCAT				
		ATCTTCACCGTTGAAAGTGC				
<i>Ruegeria</i>	33	GGTTTCTTTACCTGCTACAGT	88.24%	9	70.5241	69.2579
		CTTTACCTGCTACCGTCAT				
<i>Pseudovibrio</i>	6	ACCCCTAACTTATCAATCCGC	100%	1	72.6758	72.1959
		TAGCAAGCTACCTGACAAAC				
<i>Oceanicola</i>	6	GGGAACCATGGATCTCTC	100%	0	72.6302	73.6957
		GGCGAAAGAGTTTACGACC				
		AGGGAACTGGATCTCTCC				
<i>Haematobacter</i>	13	AACGATCTCTCGTAACCGC	92.86%	16	71.12	71.5969
		ACCTGTGTGGGATCCAG				
<i>Stappia</i>	19	TGCTACCTGACGGCT	100%	3	70.2437	72.7426
		CTGCCTGACAACTAGCTT				
		GCGCCACCAAATAGCATG				
		GCCACCAAATTGCATGCA				
<i>Roseovarius</i>	9	CCGAACAGTATACTACCC	80%	1	70.0936	70.8499
		CTCGAACTCTAGACTGGA				
		CCAGACCAGAAGTTTTGG				
		ATATCCAGCCGAACAGAC				
<i>Catellibacterium</i>	1	GTGCAGTGTCTCTTGC	100%	0	71.442	59.9279
<i>Hirschia</i>	1	CCTCTTACTTACTAATCCGC	100%	0	70.6874	65.2623
<i>Maricaulis</i>	20	CTAACAGCATGCTGCCAG	100%	2	72.9508	69.4874
		GATCCGCGATCGGTATGT				
<i>Hyphomonas</i>	21	GCATGCCTGCCAACAA	100%	0	70.6745	65.2024
<i>Oceanicaulis</i>	3	CTCTCTAATCCGCGATAGG	100%	0	71.8494	66.7454
		GAAGGTAAGGGTCTCC				
<i>Porphyrobacter</i>	26	CCAAGATTCTAGCTTCCC	77.78%	1	71.8354	70.7295
		CTCTCCAAGATTCTAGCTCT				
<i>Erythromicrobium</i>	1	GACAGCTAGTTATCAGCGTTTA	50%	0	71.7418	64.7534
<i>Erythrobacter</i>	77	GTACATAGGTCCCGAAG	91.03%	4	70.4988	68.6506
		CTCCAGAAACCGTCTTAG				
		CGAAGGGAAGGAATCTGT				
		CCAAGCTCCATGAGC				
<i>Citromicrobium</i>	4	GGATGACTTAATGCGTTAACTGC	80%	1	74.8846	72.1021
<i>Kaistobacter</i>	5	CCAGGCGGATGACTTAAC	83.33%	1	73.0197	72.9969
<i>Sphingosinicella</i>	3	CAAAGCCTAAAAGGCC	100%	2	71.6419	61.5883
<i>Sphingobium</i>	53	ATCCGCGATAGGGATGT	81.48%	46	70.8785	70.3346
		TGATTCAAGCAATCCAGTCTC				
<i>Zymomonas</i>	14	CACCCAGATACCATGTAC	93.33%	0	71.217	68.9765

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Blastomonas</i>	4	GCGTCAATAATTGTCCAGCGAG CACCCAAGTCCCATGAAC	80%	0	73.4075	74.5849
<i>Sphingopyxis</i>	47	TGAGTCCGGACAGCTAG GCAACTAGGGATGAGG CTGACCTCTCCAAGATTCAA GTAGCAACTAAAGATAGGGG	91.67%	22	70.8028	71.8829
<i>Lutibacterium</i>	4	TCAATAATTGTCCAGCAAGTCG CCAGAAACCGTCGAAGG	100%	0	70.4514	66.8389
<i>Novosphingobium</i>	46	CAAGTACCCGGACAGCTA GATATAGCGCGGGATGT CAAGTGACCCGGACAGCTA TAAAGTCATTATCTTCCCGGG GGGATGTCAAACGCTGGTAA	80.85%	20	71.3965	73.1583
<i>Sphingomonas</i>	405	CTACCTCTCTCTGGATTCT CAGCCGAAGTGAAGGAAATC CTCCCGGTACAGTCATTATC GCTTATCTCTCCGGTACT GGAAATCGCGATGAGGAT CTATCCAGCCGAAGTGA CTCACCTCTCCAAGATTCT	88.67%	141	70.2915	73.8253
<i>Nitrobacteria</i>	2	GAAGTAAACTCCCGACAAC	33.33%	3	72.424	65.6254
<i>Phenylobacterium</i>	12	CGAAGGCAATTCGAGG TCTGACTAACTGTCCGCC CGAAGGGAAAACCGGATCT	92.31%	2	70.885	71.8168
<i>Woodsholea</i>	1	CAATACCAGTCCAGTGTGT	100%	0	72.6278	70.5674
<i>Asticcacaulis</i>	10	TACTCAAGGCTGACAGT TCAGTCATGATCCAGTAAGT	81.82%	0	70.221	66.563
<i>Brevundimonas</i>	133	GAAGAGAAAGCCAGATCTC GCAGTCCCATTAGAGTTC GTTCCACACACCTCTCT GAGTTCCACATACTCTCTC	99.25%	16	70.0104	70.7973
<i>Caulobacter</i>	72	CTTCTCCGGATTAAGTCC ACCTCTGACTAACTATCCGC AGCGTCAGTACCAGTCC GTCAGTAACGGACCAAGT	78.08%	5	71.8295	71.4664
<i>Kordiimonas</i>	4	GGCACTGAATAGCAAGC TCAGCAGTTTTGAGGGC CTCCAGGATTCTAGCTC TCTCTGGAAACCGCGATTG	100%	0	70.1045	71.397
<i>Arcobacter</i>	63	GTCTAGCCTCACAAACAATA GTTTACGCACCGAAATGTGTCA	100%	0	71.8732	69.6392
<i>Sulfurospirillum</i>	25	TTACTGCCCTGACGTGC TACCGTCATGTTCTTGACCT CCTGTCACTTCATTCTAGC AGCTACCGTCATTTCTTGAC ATCGCAACAAGTGTGAC CGCAATCGGTATTCCTAGTG	100%	2	70.2349	70.6687
<i>Campylobacter</i>	253	CAAGCAGTTCTATGGTTAAGC TTCACGCATTAGCGTCAGT GCTCCGAAAAGTGTATCCTT TGCTTATTCAAGAGGTACC	99.21%	0	71.1171	70.8509
<i>Sulfuricurvum</i>	3	TTAAGTGCATCCAGAGATGA	100%	0	70.0242	64.4633
<i>Sulfurimonas</i>	4	TCAAGCTCCAGTAAACTGG	100%	0	72.7331	62.2488
<i>Wolinella</i>	2	GGTCAGTTTCAAATGCAGTT	100%	0	71.9927	66.9774
<i>Flexispira</i>	8	CTGTTTTAGGGTCTAGC CCTCTTGATCTCTACGGA CGCAATGAGTATTCCTTTGA	100%	20	70.5101	66.7331
<i>Helicobacter</i>	416	CTGTTTTAGGGTCTAGC CCTCTTGATCTCTACGGA CGCAATGAGTATTCCTTTGA	99.52%	9	71.0402	70.8513
<i>Caminiibacter</i>	3	CTTCAAAGAAGGCACCC CCGCGCTCCTTCAAAGA CAGTTTTGGAAGCAGTTCCA	100%	0	70.2341	67.8502
<i>Nautilia</i>	2	CTACCGAAGTAGCACC	100%	0	70.2217	64.7661
<i>Nannocystis</i>	4	GTTCAGTGGATTTAACCCA	100%	0	70.5758	65.8654

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Haliangium</i>	3	CTTAACCTGGCAGGTTTCG GGCAACTGACGACAGA	100%	0	70.8716	69.4592
<i>Myxococcus</i>	114	TCAAGCACGACAGTTTCGG TCTCTGAAGGATTCTGAGG CACTCAAGCACGACAGTTTC	95.65%	5	71.7065	71.34
<i>Anaeromyxobacter</i>	9	TCCTCTCGAGGTACC	100%	0	70.1074	68.381
<i>Corallococcus</i>	46	CAAGGCAAAGCGTATTAGG AAGGATGTTAGCCTTCGG	74.47%	2	70.281	63.4742
<i>Archangium</i>	4	GAAGGATTCCAAGGATGTCA CGTCAAGCCTGAAGATGT	60%	1	71.4721	68.0635
<i>Stigmatella</i>	9	CTCCTTGCGGAGCAACTA	90%	0	73.1363	68.7007
<i>Cystobacter</i>	27	GCCCTGGAGTATTAGC TCAACACCCACGACACC	92.86%	9	70.9934	70.4841
<i>Melittangium</i>	3	CAAGCCGGAGCGTATTAG AGCCCCTGAGTATTAGC	100%	1	70.0406	65.4695
<i>Sorangium</i>	16	ATGTTAGCCAACCCTTATTCG	100%	0	70.1785	61.9626
<i>Chondromyces</i>	8	GACCTTTCGACCAGGTTC CGCGCTGGTTATTCACC	100%	0	71.8946	69.2958
<i>Polyangium</i>	2	CAGCCCTTATTCGTCC AACTCGAGCTCGGAAGT	100%	0	70.1302	66.132
<i>Desulfuromusa</i>	3	ACCGTCAGCACTCTTGC	100%	1	72.7838	67.1515
<i>Desulfuromonas</i>	19	CTGACCCTATGTTTCCA CCCCTACACTCGAAATTC CCGATTGGAGGTCTTCC ACTCTCTGTCTCCAGG CAGACCGATGGGTATTAT	95%	2	70.0879	72.8217
<i>Geopsychrobacter</i>	1	GACTCCCAAGTTTCCAAGG	100%	0	74.4974	73.064
<i>Geobacter</i>	40	CCACTTTCATGGAGGTTC CACCCCTGTGTTTCCAC CGGATTTCACTCCTACACTA GCATCTACCTGTACTCAAGT	92.68%	3	69.7898	73.2554
<i>Trichlorobacter</i>	2	GTCAAGAAAACCGGTATTAAC	100%	2	70.613	63.2187
<i>Pelobacter</i>	13	CAAGTACCATGGGTATTAACC CTGACCTAACTGTTTCCAGAAA ACCAATCCAGCCGAATG GCAAGTACGATCGGGATGT	92.86%	0	70.1594	70.2365
<i>Desulfomicrobium</i>	37	CCCGCAGTTTCAAGTGCA CCCTGACTTACAAGGC GAAGATCAAGTCCCCGAC	97.37%	0	69.7801	71.3146
<i>Desulfothermus</i>	1	GGCTATTAACCCATACCCTT TACTACAGGACCCACATC	100%	0	70.4326	72.0871
<i>Desulfohalobium</i>	2	AACTCTAGCTCCGCAGT CTGAAGTCCTACGACCC	100%	0	73.4305	73.6848
<i>Desulfonatronum</i>	3	GAAGATTGCTCCCAAC TCGAATCCATGCAGTTCTTCC	100%	0	70.5934	66.1033
<i>Desulfovibrio</i>	219	TATTCGACCCAAGGCGG TTTCAAACGCAATTCCTCGGT CTATGAACAGGGGTTGCG CTACAGCACCGAAGGT GGATTTCACTCCTACACC	95.45%	53	70.7435	71.0615
<i>Bilophila</i>	2	GGCACCGAGACATACATC	100%	0	72.8826	68.6581
<i>Lawsonia</i>	2	GCACCGAAGATAACTCC	100%	1	70.1315	61.2295
<i>Desulfobotulus</i>	1	TCTGCAACCAACACCTAGT AACCACACAACCTTCTCCC	100%	0	72.1319	66.6778
<i>Desulfonema</i>	3	AGTCCCAACCGAATTGAT TCCGTCTCCGAAAATTCC GGATCACTTAATGCGTTAACGC	100%	0	70.4522	69.7327
<i>Desulfocella</i>	1	GCACTGAATGCTTTATGCAC	100%	0	71.7179	59.9708
<i>Desulfobacterium</i>	19	GGTACCGTCAATAAAATGCACT TACCGTCAATAGACTATCCTG GCTGGCAACTGAAGACAA GCAGATTTTAATACCGCTACA TCTCTCTCCCATCTC CTTTCACATCTGACTGGCAA	80%	0	70.2683	71.7292

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Desulfosarcina</i>	6	CCAGTCTTTCGATAGGATTCC GTACCGTCAACACATGGC TCACCATGTGGGCTCT CCTACTTTCATAGGAATTCCCG	100%	1	71.1549	67.3005
<i>Desulfococcus</i>	5	TACCGTCAATACGCACACTACT TCAACATACGAAGGTTCTTCC CCACATAAACTTTCTTCCCACT	100%	0	71.0116	61.4628
<i>Desulfatibacillum</i>	1	CTCCTGAGTATTAATCAGAAGC	100%	0	72.517	61.1212
<i>Desulfofaba</i>	2	GGGCACTCATAAGTTTCCAAAT GCACAGCAGGAGTCAATA CAAAAGTTTCCAAATGATTCCAGG	100%	0	71.3463	68.4052
<i>Desulfobulbus</i>	19	AAGGGGTCAACACCC ACACAGAGGGGATCAACA AGAAGGGATCAACACCC TACTCAAGCCTGACAGTTTC	95%	1	70.3895	71.02
<i>Desulfotalea</i>	10	GTCATCGGATTCTCAAGAG ACTCAAGCTTCCCAGTATC TGTCACCGAGTTCCTCAAG	90.91%	0	70.3346	71.2825
<i>Desulfocapsa</i>	7	CTCCGGCAATGAGACGAT CTCCTCAATGAGGCACT CGATTAACCGCCCAAC TACACACTTCTTCCCATCTGA ACGATCAACCGTCCCAAC	87.50%	2	70.0776	69.504
<i>Desulforhopalus</i>	6	AGCACCTGTCACCGAATTC CCGTCAAGCTATACAGGT TTTCATCGGGATTCTCGGG	100%	1	71.4692	67.767
<i>Nitrospina</i>	2	GGTATCACGGGAGTCC CAAGCCGAATAGTTTCAAACGC	100%	0	72.0712	67.8846
<i>Syntrophobacter</i>	8	AGGAATGATTACCCACTACAC CTTCCTCTCCAGTACTCAAG CTCACAGAGCATATTCCTC	88.89%	0	70.531	72.1552
<i>Desulfoglaeba</i>	1	GTCAAACACAGAGGCTC	100%	0	70.2415	58.6799
<i>Thermodesulforhabdus</i>	1	CCTAGGTCCCATCGT TAAAGAGGGCACCCC	100%	0	70.0427	69.8944
<i>Desulfacinum</i>	3	ACTCAAGCAGAGCAGTTTC ATACCCGCTGCACCTA	100%	0	70.3435	69.9565
<i>Desulforhabdus</i>	1	CCCATCCTCTCCAGT	100%	0	72.175	69.384
<i>Desulfomonile</i>	13	CCTGACAGGGCTTTACA ATTGACAGGGCTTTACAACC	100%	0	69.9936	72.0056
<i>Syntrophus</i>	12	AGCGTCAGTATAGGACCA TTTCTTCCCCTCGACAGA CCAACAGGCATTTCTTCC CCCGCATTCGTTTCTTTCCA GTCAAAGTACAAAGGCTGTTAAC	100%	0	70.216	71.6169
<i>Bdellovibrio</i>	34	CACTGAAGAGGTCAAAGACT CTAGGATCCCTCGTCTTAC GCCTATTAGACCCGTGA AGTTAGCCGATCCTTTCTTAC CTGAAGGGGTCAATACT	65.71%	1	70.6043	70.8509
<i>Bacteriovorax</i>	129	ATCAGTTTTGGCCGATCTTCT CATCTCTACGGATTTTACCC GGACCCTATACCATTTATCC	96.92%	15	70.428	68.5526
<i>Desulfurella</i>	12	AGGCAAGAATCGTTACCC	100%	0	70.0703	58.0481
<i>Gallionella</i>	20	CTTTCGGGCACAATCACATC CCGTCATCTACTCCTAGTATTA GAAGTCTCCTTCCCC	100%	0	70.2892	68.6643
<i>Spirillum</i>	3	TCTGGCTTATCTAACCGC GAGCAATGCTTTTCTTTCTC	75%	0	70.4801	68.6103
<i>Nitrosomonas</i>	81	GAGCGTCAGTGTTAATCCAG GTTACTAAGCCTAGTAAGG CTGCTACACATGGAATTCC GGTACCGTCATAAATCATAGCT CTGTAGTTCAACGCATTCC TTCAAACGCAATTCAGGTTA CTTGGTTCCCTTTCGGG	98.78%	0	70.1299	73.8896

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Nitrosovibrio</i>	6	CTGTGTTACCGTCTCTT TCTTCTCCGTACCGTCAG CCCGTTAAGGTCCGACAA	71.43%	0	70.1978	71.0889
<i>Nitrosospira</i>	77	CCGTCAGTTGTACGGAT CAGTTTCATTAAAGTGCCCAAC CCGTCATTAGTCACGGGTATTA CGTCAGTGATCACAGGTATT GTCAGTAGTCACAGGTATTAG GGTATTAACCGTGACCGTTTC TAGCTGCGTTACCAAGC	100%	4	70.4709	74.502
<i>Malikia</i>	2	CAGCTAAGGACCAACAAC	100%	1	71.0307	60.6449
<i>Acidovorax</i>	97	TACTGAGTCAGTCAAGACC GCTATACAGTCACAAATGCAG CTATCTCTAAAGGATTCCGTAC	68.37%	28	70.4801	68.9901
<i>Hylemonella</i>	9	TGTATTAGAGAAGACCGTTTCGC GTATTAGTAGGCGCCTTTTCG	100%	0	71.8979	68.6213
<i>Roseateles</i>	5	CATCCCTCCGAAGTATTAGT GCTCAAAGGATTCTTCCC	100%	0	71.397	69.8776
<i>Caldimonas</i>	1	ATCACTCCGAGGTATTAGC	100%	0	71.5097	65.9912
<i>Pelomonas</i>	5	TCATGAGTCCCGGT ACCAGAACCTTTTCTTCCC GCACTCTAGCTATGCAGT	83.33%	3	70.9372	74.5004
<i>Curvibacter</i>	2	CCAAACCTTTTCGTTCCGTACAAA CCGTCATGAGTCCAACGTAT	66.67%	0	71.0291	68.4936
<i>Lampropedia</i>	5	CGTCATATGACCAGGGTATT	100%	0	71.0359	62.2757
<i>Diaphorobacter</i>	8	GTCATGACCCCTCTTTATTAG	100%	5	70.6103	69.3004
<i>Polaromonas</i>	16	GAGTACTAATGCACCAAC	100%	2	72.0006	62.7014
<i>Variovorax</i>	108	GGTTCCTTTTCGAGCACTAA CCGTCATTAGCCTTCTGTAT GAAAAGGCCGTTTCGTTCCG	88.99%	14	70.763	72.3338
<i>Ramlibacter</i>	5	CGTCTTACATACCCGC GTCATTACGCCGAGGTATTA CCCAGGCCTTTTCGTT GTACCGTCATTAGCAACC	83.33%	0	70.0822	71.8256
<i>Simplicispira</i>	5	CGTCTCTGCGATCTTCC	100%	3	71.9751	70.4366
<i>Hydrogenophaga</i>	53	GACCGTTTCGTTCCGTACC GTATTAACCCGGACCGTTTC TTAACCCAAGACCGTTCGT TGAGTCAGAAGAGACCC GTTACTGAATCGATCAAGATCC	74.07%	0	70.2526	69.4952
<i>Giesbergeria</i>	4	GGATTCTGCCATGTCAAAGG AACCTTTTCGCTCCGTACAAAAG	100%	0	71.2308	73.7774
<i>Comamonas</i>	127	CTTTCGAGCACCCCTCTA GTTACTGAGTCAGTTAAGACC CCCGAAGCGTTTTCGTTCC CCATGCAGTCACAAATGCAG CACAATGGCAGTCCAG	90.63%	10	71.4126	74.139
<i>Alicyclophilus</i>	4	GCACCTTTTCGTTCCGTACAAAAG	100%	3	73.0875	70.6963
<i>Ralstonia</i>	251	CTAGAGTGCCCTTTCGTA CAGTCACCAATGCAATTCC CTAGAGTGCCCTTTCGTA CCACTCCAGGTATTAACC	82.94%	54	70.7933	71.9163
<i>Polynucleobacter</i>	79	ACTTCGTTAGTGACATGTCAAGG CGGACTGTGTTAGAGCC GTTAGCTACGTACTCAGGAAT	100%	0	72.6269	71.5753
<i>Burkholderia</i>	918	TGCGCCGGTCTCTTTTC CGGTCTTAGCAAACCGC CCCCTCTGCCATACT TGTATCGGTTCTCTTTCGAGC	93.91%	89	69.8872	71.241
<i>Thermothrix</i>	1	CAGGCAGTCCCTTAAAG GATTCCGTCACAGGAC	100%	0	71.4714	70.3092
<i>Pandoraea</i>	45	CTTGCAAGTCACGAATGCAG	97.83%	3	71.7168	69.8872
<i>Limnobacter</i>	5	CAAGGAAGTAAATCCCCAACAA	100%	0	71.8401	66.0855
<i>Lautropia</i>	2	TTTCACATCGGTCTTGCAAAAC	100%	0	70.2325	67.7385

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Paucimonas</i>	2	GTCATTAGAGCCAGGTA	100%	0	70.6169	66.1466
<i>Cupriavidus</i>	119	GACATACTCTAGCCTTGC TGACAGTCACAAGCGCC GTATTAGCCAGGGCCATT GTGTCCACTTTCCCTTTTCG	98.33%	82	71.6569	73.175
<i>Candidatus Glomeribacter</i>	4	CCGATTATTTACCGGAGCC	100%	0	72.4227	58.4134
<i>Collimonas</i>	23	CAGTCACAAATGCCATTCC	95.83%	2	70.7933	71.2193
<i>Oxalobacter</i>	14	CCCCTCACACTCT TAGCCTCAGCCGTTTCTT GCCCAATCAATTTCTTCCC GCATTTCACATCTGTCTTACAC	86.67%	1	70.4588	69.2541
<i>Janthinobacterium</i>	35	GGATTCCAGCCATGTCAA GGCTTCCGTACATGTCAA CCTGTGTACTGGTTCTCTTT ATTAACCCAGCCGTTTCT GATATTAGCTCGAGCCGT CTCTCTGGGATTCCGTAC	77.78%	7	70.1769	70.5744
<i>Herminiimonas</i>	8	ATCTGTATTAGAGTCCACC	88.89%	0	70.0894	71.6775
<i>Massilia</i>	27	CGATCTCTCGTGGCTTC GGCACGGATATTAGCC GGATTTACGACAGACTTACA GTCTACTTCACGCGTTAGTG TTAGCCGTGGATATTAGCC TCTCCGGGATTCTTCC	78.57%	10	70.221	72.4212
<i>Duganella</i>	8	AGCCACGACCTTTTCTTCC CTCTCGAGGATTCTCTGA GCTGTGGATATTAGCCAC	44.44%	1	70.4424	70.3644
<i>Naxibacter</i>	5	ATTAGCTGGAGCCGTTTCT TCTCCAGGATTCTTCC GACTTACAAACCGCCTGC CGGATATTAGCCGGAGC	83.33%	2	71.3431	69.903
<i>Herbaspirillum</i>	63	CTCTTCAGGATTCCATCC GCTGCGTTACCAAGTCAATTA	100%	62	70.6448	71.5114
<i>Telluria</i>	2	CTCATTGCCATGTAGCACTAA TCTCGGAGGTTGCTGA CAGATATTAGCTGAGGCC	100%	0	70.4957	66.2008
<i>Derxia</i>	1	GTTACCAATCAGTATCCTGA	100%	0	70.3637	58.7249
<i>Pusillimonas</i>	4	ACATACTCTAGCCCGC TCAGTGACGCCAGGTATT	80%	1	71.1768	71.5699
<i>Azohydromonas</i>	3	GAGTAGAAACCCACCCAA	100%	1	71.6198	60.0369
<i>Kerstesia</i>	1	GGTATTATCCCGGGCGA TCCACTCGGGGATTATCC	100%	1	72.3259	69.2958
<i>Bordetella</i>	100	CAGTTGCCCCAGGTATTA CTGCGCCGTTTCTTCC CAGGGCCGTTTCTTCC TTAGCCAGGCCTATTCTTTC CGTCAGTTGCACAGGATATT CAGGTATTAGCTGGCGC ATTAGCCTGCGCCTTTTCT	80.20%	0	70.5893	75.6825
<i>Oligella</i>	10	TTCACACCGTTCTTTCCAAGC	100%	0	73.0172	62.8405
<i>Advenella</i>	5	TACTAAGCCCCGAAGGG	100%	6	73.3961	69.3379
<i>Alcaligenes</i>	120	GTTCTCTTGGGAGCACTT GACATACTCTAGTCCGGG GATACCTTTTCTTCTGTGCAA CTCCTGTCAATTCTTCCC GCTTATTCTGCAGATACCGT GTTCCGGTTCTCTTACGAG GTCCAGATTCCCTTGC	61.16%	41	70.1978	73.941
<i>Sutterella</i>	4	GCTCTAAACTTTTCTTCCCTG GTCCAGATTCCCTTGC	100%	0	70.4289	62.3315
<i>Achromobacter</i>	102	CTCTTGCGAGCACTTCCAAA GCAGTTCCAAAGTTAAGCTC	91.26%	71	71.3538	73.6858
<i>Tetrathiodibacter</i>	4	TTATCCGAACCTTTTCTTCCC CAGTTCCGGGATTATCC	100%	7	70.0776	67.3858

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Castellaniella</i>	11	ACGTACTCTAGCTCGGT TTAGCACAGGTATTAGCC ATCCCAGGCTGTTTCTTC	100%	1	70.198	70.2682
<i>Taylorella</i>	35	AAGGCCTTCAATCGCACA	100%	0	69.1208	64.1781
<i>Pigmentiphaga</i>	4	TACCAAGCTCCGAAGAACC GTATTATCCGCGCCGTTTCTTT	100%	0	71.696	68.0499
<i>Pelistega</i>	1	CTAAAGCATTCCGAACATGTCA	100%	0	70.4507	61.1612
<i>Methylophilus</i>	13	CTCATGGATTTTACTCCACC	100%	1	71.6282	64.2729
<i>Methylovorus</i>	1	CCTGTGTCTACTTTCCCT	100%	1	70.8638	69.7744
<i>Methylobacillus</i>	7	AGCGTGC GTTACTAATGGA CTCCACCAACAAGTGTGA CGTCATCCTCATCCAGTATT	75%	0	70.1973	69.4936
<i>Chromobacterium</i>	38	TTAACCAGCGGGATTTC CTTTCACATCAGACTTGCAC GTACGCTACCAAGGATTCAA	100%	0	70.4832	70.3435
<i>Iodobacter</i>	3	ATTCCTCCCGAACAAAAGC ATTAGCTCTCAGGATTTCCTC	100%	0	70.0014	66.1044
<i>Vitreoscilla</i>	1	CTACCAAGAGCTTTTAATCTCC	100%	0	72.7245	60.336
<i>Chitinibacter</i>	1	AGCCACAAGGATTGTCTCC CCTCCAGAACAAAAGCGC	100%	0	72.4676	66.859
<i>Vogesella</i>	2	ATTAACACCAGGCATTGCTC	100%	0	71.5106	64.2262
<i>Aquitalea</i>	3	TAGCGCTAGGGATTTC GCGGTATTAACGCTAGGG	100%	0	71.2627	67.8322
<i>Stenoxybacter</i>	10	CTACTAATGTGGCAAGCACA	100%	0	71.5813	57.5305
<i>Aquaspirillum</i>	11	ATCCTCTCACAGACTCAAG GCGTTACTTCGTTACTGAGTCA CATTTCTGCCATGTCAAAGGTG CACTCCAGCTATACAGTC CAGGCGGACAACCTTCTC	75%	3	70.2792	70.763
<i>Alysiella</i>	7	GAACTGGTATTATCAATCCGC	100%	0	71.2693	57.8177
<i>Conchiformibius</i>	8	GTATTAGCACGCCTCTTTTCT CATCAGTCATGGGTATTAGC GGCACAAGCCTATCTCT CTATCTCTAAGACCTTCCGT CAAAGACCGAGGTCTCC	100%	0	71.3783	68.2819
<i>Microvirgula</i>	5	GATTTCTCCCTGACAAAAGAG	100%	0	72.3291	71.2857
<i>Simonsiella</i>	4	CCGTCATCAGCATCTGATAT GGTATTAACACCCGGCTT	100%	0	70.5366	67.8585
<i>Eikenella</i>	4	ATCCAGTTCAGAACGCAGT	100%	3	72.6342	71.2571
<i>Kingella</i>	12	GGTACCGTCATCAGTTAATGA	53.85%	0	71.5066	65.7878
<i>Laribacter</i>	29	CACCAGAAACCGAAATCTCCAA	100%	0	73.5287	58.3497
<i>Bergeriella</i>	2	CATGAGGATTTCGTTATTAGC	100%	0	70.3703	60.2257
<i>Neisseria</i>	975	CTTCCCTGACAAAAGTCCTT ATCCTGCTTAAGTAACCGTC	99.80%	6	70.4129	70.6695
<i>Zoogloea</i>	20	CTGCGTTACTCAATGAGTCT	80.95%	1	70.428	65.753
<i>Sterolibacterium</i>	1	GTAATAAGGAAGTCACCTTC CGTCAGTAAACCAGGGTATT	100%	0	70.7441	66.1381
<i>Thauera</i>	39	GTCACCTAGTGCAATTGCT GGCCTATGTTAGAGACC TCATCCACGCAGAGTATTAGC	100%	2	72.426	71.6256
<i>Azoarcus</i>	61	AGAGTTACCTCTACGAACAAC GATTCCAGGCATGTCAAGG AGCCGTGCAGTCACAAG	93.55%	1	70.4646	72.1739
<i>Propionivibrio</i>	4	TTAGAGCGTGCCATTCTTTC CCCTTCCAACATCCAGTT GCGACACTAAAGGTTTAACC CGTCATCCACATCCTGTA CCTCTAGTTGCACAGTCT	100%	0	70.3574	71.7625
<i>Azovibrio</i>	6	GTCACAAGCGCATTCCC CCGTTACTAATAGGTTTACCC	100%	1	71.4376	69.5679
<i>Dechloromonas</i>	20	ACCCAAATCTCTCCGGG CTTCCAGTCACAAGCGCA GGTACTAAATGGTTTACCCAC GTACCGTCATCCACACAG	100%	1	70.6586	72.6456

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Hydrogenophilus</i>	7	CAGTCACAAGCGCAATTCCT	100%	0	73.9549	73.2714
<i>Thiobacillus</i>	28	TACCGTCATTATCTTCGTCGT GCTTTCACATCTATCTTTCCCA ATGGTTTACCACCACCC GCTTCGTTACTAAGGATTTC GTCAACAGCACGGACTG GTTAGAGCGTACCGTTTCG CTCTGACAAGTTCTCTGG CAACGCAGTTCCCAGGTTAA GGCAAGTTCCGGACATG	79.31%	1	70.1449	73.2178
<i>Procabacter</i>	6	GCATGTCAAAGGCAGGTA TTTCTCCCAGACAAAAGAGC	100%	0	73.2423	70.2475
<i>Spiroplasma</i>	68	TCCCTTACAACAGACCTTTAC CCCGGGATTTAACCTAAAC ATTCCATCTACCTCTCCTG ACCGAACTTAGTCCGACAC CACTAACCTCTCTATACTCTA	98.55%	1	70.2954	70.9277
<i>Entomoplasma</i>	6	ATGTAGTTTTCAAGCGGAACC TCAATAACAAGCCAGTAAACCG TCTCTGCATTCTAGTCAG	71.43%	1	70.1778	71.3699
<i>Mesoplasma</i>	11	GTAGCTTTGCACTGTATGTC CCTCTCCTATGTTTCTTCC AATGTTAGCCTCCAGCATATC GCTTTAACCTCAAACCTGCGAA TTGTCACCTATGTATTACCGC GAGCATTTCCTCTCAAGTGT CTGCAGTTTTCAAGGCGA	91.67%	0	70.2404	71.7654
<i>Candidatus Phytoplasma</i>	587	GCGGAGTACTTAATGTGTTAAC TTCAGCACTGGTTTAACCC GCCTCTATCTAACTCAAGT CAGTCTTGCTAAAGTCCC GTCAGTAAAGACCCAGC GCTAAAGTCCCCACCA	99.66%	10	70.6202	70.7146
<i>Acholeplasma</i>	17	CCTTAGGCGCAACACTTA TTACACCCAGACTTAAATGG GAATCCACTTGCTCTGT TGTACATCTGTTAGCCTCC GTCAGTATAGACCCAGC	94.44%	1	71.1774	73.3523
<i>Candidatus Hepatoplasma</i>	1	CAATTACAGTTCAGACAGCC	100%	0	72.2988	67.9804
<i>Ureaplasma</i>	27	TCCCCAACTCCCTACT CCTTGCGGTAGCAGTA	96.43%	0	70.7658	67.2304
<i>Mycoplasma</i>	412	AGTATCCAAAGCGTGCTGG GAACGTACTACTCAGGCG TTAAGTTTCACTCTTGCGAGC CCGAAACACAAATCCCACAA CCATCTGTACCTTGTTAAC GCAGGACTTGACCAACA CTTCCTCCCGATTACTCG GAGTTCCAGAACTCCCTA TACCGTCAAGACAAAGGCTT TAGCTTTGCAAGGGATGT GCTTACCTCTCTTGCACTCT GCCTGCGCTTGCTTTA	84.02%	18	70.1763	74.1765
<i>Erysipelothrix</i>	23	CCGCCATAACTATTACACATCT ATTCCCTCTTCTATCGTTCT TCTATGGCATCACGGGG TCTATGGCATCACGGGG AACATAATCCACCGCTTGTC CAGTTTCTATGGCATCACGG CCCTCTTCTATCGTTCTTC CTATGGCATCACGGGGTTAA CTATGGCATCACGGGGTTAA	100%	0	71.4334	74.3303
<i>Solobacterium</i>	6	CAGTTTAAAGTGGCTGGATTGG	100%	0	75.1951	61.2014
<i>Bulleidia</i>	2	TCTCTAGAACTTCGCAAGG	100%	0	71.3848	57.3341

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Anaeroplasm</i>	2	CAGCACTGATTATCACCAACA GCTCATCAGTATCTCTTGCT	100%	0	71.6617	65.5187
<i>Asteroleplasma</i>	1	CGAACGTAGTACCCAGG	100%	0	71.1348	67.3328
<i>Abiotrophia</i>	7	TGGCAACTAGTTATAGGGG	75%	1	72.2946	71.2738
<i>Aerococcus</i>	34	ATACCGTCAAGACTGTAGC CAGTTTCCAATGCACTTCCA	91.43%	0	71.1603	71.2361
<i>Globicatella</i>	1	CCTCTAGTAGCCGTTCT GCCGTTCTTCTCTAACAACA	100%	0	70.1433	69.5186
<i>Facklamia</i>	8	TCCCGAAGGATTTCAACC CCTATCTCTAGGTTATGCATC CTCTAGGCTATGCAAGAG CCGTAGGATTTACCTATCT	100%	0	70.0703	72.6863
<i>Carnobacterium</i>	48	AGTAGTTACGCTCATCCTTGT TTCTGGTTAAATACCGTCAGG CAGGGGATGAGCAGT	91.84%	1	70.7859	67.7252
<i>Desemzia</i>	3	CAAGGGACGAGCAGT CAAGGGACGAGCAGTTAC TCCAATGACCCTCCCT	100%	1	70.1985	70.9172
<i>Marinilactibacillus</i>	12	TAGATACCGTCAAGGCC	100%	0	70.9635	71.2695
<i>Alloiococcus</i>	1	GCAGTGACTCTATCC	100%	0	71.8882	61.0965
<i>Alkalibacterium</i>	18	CCGTCATGTATCTTCCCC CTTTGGGTGGTCAGA AGGAGGTGCCAGTTAC	100%	0	70.062	69.8575
<i>Trichococcus</i>	10	CAAGGCTGGAGCAGT TACTCTCCGACTTGTTCTTCT	100%	0	71.3515	69.4133
<i>Allofustis</i>	1	GGAGTTCTTGGCTAACAAC	100%	0	70.7773	58.5023
<i>Granulicatella</i>	19	GTTACTCTATGCGCTGTTCT CTCTAGCACTTGTTCTTCTC	85%	11	70.9116	71.1171
<i>Streptococcus</i>	1003	ACAGTTTCCAAAGCGTACTATGG CTCCCCTTCTGCACT CACTGTGTGAACCTTCCACT AGCCACAGCCTTTAACTTC	98.41%	1	70.0871	69.7744
<i>Lactococcus</i>	156	CGTCCCTTTCTGGTTAGA TGTATCCCGTGTCCCGA CAGAGAACTTATAGTCCCT	98.73%	4	70.9277	70.7658
<i>Oenococcus</i>	12	CACTAGGAGGCGGAAAC	100%	0	72.3599	67.9382
<i>Leuconostoc</i>	93	GAAGAACAGCACTATCTTAG GGAAACCTCCTAACACCTAG CCTTTCTGGTATGGTAC	100%	0	70.3933	72.2637
<i>Weissella</i>	64	CCCCGAAGAAAGCTTCATC GCCGTTCTTCTGGTAAGATA CCCCACATTTAACAGTTACTCT GATAACGCTTGAACATACGTA TACCGTCACACATTGAACAGT	95.38%	1	71.4985	72.9371
<i>Pediococcus</i>	43	AGTTACTCTTACCCACGTTCT ACCCTCCAACACTTAGTAATC CTTTCTGGTTAAATACCGTCACTG	75%	0	70.6695	71.1961
<i>Paralactobacillus</i>	1	GAGATAGCAGAAGATGTCAAG	100%	0	70.4747	66.5759
<i>Lactobacillus</i>	783	CTTTCTGATTGAATACCGTCACTG TGCATGACTTCGGTTAAGC GAACTCCTAATCTCTTAGGC ATCCATGTCCCCGAAGG GCCGACAACAGTTACTCT CCGATGCACTTCTTCGGT AGCCGAAGGCTTTCACATC TGTCTCTTCTGCACTC GCTACCCATGCTTTTCA CTACCTCTATCCTTCTTAC GTCAGTACGTGAACAGTTAC	96.81%	47	69.9373	72.6278
<i>Melissococcus</i>	3	CACGAGGAAAACAGTTACTCT	100%	0	70.4949	58.847
<i>Vagococcus</i>	18	GAGAACAGTAACCTCTCTCACTT AACAGTTACTCTCCCACTTG CGTCAAGGTGAGAACAGTTA CCGTCAAGGTGAGAACAG	94.74%	1	70.0993	68.5782

Continued						
Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Enterococcus</i>	274	CATCTACTCTCATCCTTGTC ATACCGTCAAGGATGAAC GAAGGGGAAGCTCTATCT	70.91%	4	70.1582	71.0431
<i>Tetragenococcus</i>	18	GCATTTCTCTTTCTCTGT CCTGTTCTTTGCTGACAAC GAAGGGGAAACGCGATC TAGGGCGGTCAAAGGA	100%	0	70.2588	70.5238
<i>Jeotgalicoccus</i>	3	CGTCATCTCAAGGCCAG CCGTCACGTAGTAAACAGTTAC	100%	0	70.9524	67.8584
<i>Gemella</i>	23	GGTCAGAACACAATCTCTT CTCTCCTGCACTCAAGTTTA CTCTTGTCCTCACAGT TCTACGGCAATTTACCGG	100%	0	71.4322	75.1331
<i>Macroccoccus</i>	12	CAAGGTACGTGCAGTTACT TACCGTCAAGGTACGTTACAG	100%	14	70.4471	73.7058
<i>Salinicoccus</i>	18	CCCGATGGACAGTTACTC CTCAAGTCCGCCAGTTTC TCGGTGAGCAGTTACTCT	84.21%	1	70.7265	71.217
<i>Staphylococcus</i>	473	GCTTTCGCACATCAGCG CGTCAAGATGTGCACAGT CCGAAGGGGAAAACCTCTATC ACGTGCACAGTTACTTACAC	99.58%	30	70.2775	72.2623
<i>Amphibacillus</i>	9	CCTATTCGAATGGTACGTGTTT TCTCTAGGATTGTACGCG GCCTATTCGACTCATACTGT CAATGACCGGTTGTGGTTGA	40%	0	71.8126	72.162
<i>Terribacillus</i>	3	CCAAGATTGTACGCGGAT	75%	2	70.7295	65.2487
<i>Ureibacillus</i>	34	GTATTTGTACGGCAGTCTC	100%	3	70.0797	61.5627
<i>Virgibacillus</i>	64	CGCTATCAGAGGATGTCA GTATCTCTACCGATTGCAGA CTGTTTGAACGGCACTTGT GCCAACCTATTGCAATGG GAAGGGAAACATCCTATCTCT CTGTGTTCTCTACATCTCT	61.54%	23	70.0993	70.5573
<i>Marinibacillus</i>	3	ATCTCTAGACCGGGCA	100%	4	70.3468	66.3196
<i>Halobacillus</i>	108	AGCGTCAGACACAGACC AAGGTACCGCTCTATTTCGC CAGCGTCAGAAACAGACC	90.83%	1	72.331	70.8701
<i>Filobacillus</i>	6	CCTATTCGAACGGAACCTGTTC GGAAACCCCTAACACC	100%	1	71.0698	69.9478
<i>Exiguobacterium</i>	94	GAAGGGGAAGGTACATCT GGAAGGCACATCTCTGT GTACGAGCATTACCTCTCG CACCTGTCACCCCTTGT TCAGTTATAGGCCAAAGAGT	95.79%	2	71.497	73.2618
<i>Gracilibacillus</i>	19	CTAGGGACATCCGAAGA	80%	1	70.4374	62.9641
<i>Lentibacillus</i>	7	GTAACTTCAGCACAAAGGG CCGCCATCAGAGGATGT	100%	0	72.1574	68.4565
<i>Caldaterra</i>	1	CTTCGAACCGCGCATGTT TTTCCAGTGGCCTCC	100%	0	71.4908	67.5347
<i>Geobacillus</i>	261	GTGACCCCTCTAACACTT AAACGGCGCTCCTTCGT CCTATTGCAACGGTACTTCTTC TCTCCAGGGTTGTCAGG TCTCTTGGGTTGTCAGG	93.51%	51	71.0889	73.53
<i>Thalassobacillus</i>	1	TGTCTCCAGGGGTGT	50%	1	71.6756	69.2408
<i>Halolactibacillus</i>	2	CAAGATACCGCCCTATTTCG	66.67%	0	70.8635	67.1782
<i>Bacillus</i>	3603	GGATTGTCAGAGGATGTCAA CTAGGGTTGTCAGAGG TGGTCAACGGGATGTCAA CTTCTGGTCAGGTACC GAAACCCTCTAACACTTAGC GTGGCTTCTGGTTAGG GTACTCAAGTCTCCAGTTT	87.15%	309	69.6377	72.6082

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Oceanobacillus</i>	34	CCGTCAAGGTACTATCAGT GGTCAGTTACTACCAACTT CGTCAAGGTACGATCAGTTAC	80%	8	71.76	70.8972
<i>Lysinibacillus</i>	84	TACAGTGGTCAACGGGA TCAAGTCAAGCCAGTTACTAC	96.47%	76	70.3738	66.4887
<i>Jeotgalibacillus</i>	1	GTCACTTTGCCATAAGG	100%	0	71.6554	66.9271
<i>Anoxybacillus</i>	44	CGATCTCTCGGGTTGTC TACCGCCAGTTACTGC TGACTGCGGTACTTGTCT	86.67%	8	70.4174	70.8572
<i>Saccharococcus</i>	2	GGTTGACCCCTTAACACT	66.67%	0	70.9662	64.8662
<i>Pontibacillus</i>	7	CTAGGGATAGCGCAAGA CAAGGTACCAGCATTTCCTC	100%	2	71.4739	68.4782
<i>Alkalibacillus</i>	8	AACAGTACGTGTCTTCCCT CTGTCCGAAGATGTCAAGA	77.78%	0	70.315	70.7818
<i>Salinibacillus</i>	2	AAGGTACAAGCAGTTCTC	100%	0	70.0797	60.7936
<i>Filibacter</i>	1	TGTATCTCTACACCGGG	100%	1	70.9151	70.3936
<i>Planomicrobium</i>	19	GGAAAGCCTTATCTTAAGG CCCGAAGAGAAAACCTTAT	60%	6	70.6687	68.4086
<i>Kurthia</i>	18	CATTACCTAACGCATTGTCT TATCTCTAGGACGGTCATTGG AGAACGGTCATTGGGATGT GTCAAGGTGCGTTCATTAC	89.47%	0	70.217	76.1361
<i>Sporosarcina</i>	36	AGTTACTCCCGTACGTGT TCAAGGTACAGGCAGTT GGGAAAGGCTTATCTCTAAAC GTTACTCTCGTACGTGTCTTC GTACCGTCACTCTCGTTTC	72.97%	15	70.0627	71.9556
<i>Planococcus</i>	57	GAAGGGAAAGCCTAGTC GAAAACCTTCGTCACTCACG CGTCAAGGTACCAGTAGTTA CAAGGTACCAGTAGTTAGT ACCAGTAGTTAGTTGGTACTTG GAATGCTTAATGGGTTAGCTG CCGTCAAGGTACCAATAGTT	94.83%	24	70.086	72.275
<i>Caryophanon</i>	7	GCGTCAGTTACAGACCG CAGTTACTACGCTACTTGTT GCCAGTTACTACGCTAC	100%	7	70.2996	71.7931
<i>Listeria</i>	63	CAGTACTTTACGATCCGAAAACC GTCAAGGGACAAGCAGT ACCGTCAAGGGACAAGC	100%	1	71.9112	70.8395
<i>Brochothrix</i>	19	TCCAGAGTGGTCAAGG TCTCCAGAATGGTCAAAGG CCCCGAAGGGAAAGTTCT CCGAAGGACAGTTCTGTC	100%	5	70.4647	75.215
<i>Sporolactobacillus</i>	17	GCATTTCTCTCATACTCATT ACTGCAAGAGCATTTCCTC CGTTCTTCTCCGGCAACA CGTCACAGCACTAGCATT CGTCAAGGTACCAGCTTA	100%	1	70.0956	70.4743
<i>Tuberibacillus</i>	1	GCGCCATCATTTCTATGAC	100%	0	73.3092	62.2018
<i>Marinococcus</i>	18	CTGTCACATCGGACTGA CTGGAAGTAGCCAAGG AGGGGAACCTTCTGTCT TCGACTGACGTGGGTTTC GCAACTAGGGATAGAGGTTG TTTCGCACGTCAGAAACAGAC	100%	1	70.0993	68.6981
<i>Brevibacillus</i>	124	GTGGAATACCGCTTTCCT CGTCCCTGACAACAGAA ACTTGGTCGTCCCTGAC GGAATACCACTTTCCTCTC CACTCAAGCTACACAGTTTC	94.40%	5	70.1364	70.285
<i>Cohnella</i>	8	AGTTTCCAGTGCCTACGG GTCATCGGACTAGCAGTT ACACGTCCTTATTCTTCCC TACCGTCATCGAATGAGTAGT	100%	2	70.2174	73.2962

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	
					max[Tm(MM)]	
<i>Paenibacillus</i>	612	GGCTTTCTTCTCAGGTAC GCACCAAGGGTATCGA AACTTCGGCACCAAGGG	94.13%	52	70.0277	73.2401
<i>Oxalophagus</i>	1	CATTCCGGGATCCTCTC GTCATTCCGGGATTCTC	100%	0	71.001	65.8594
<i>Aneurinibacillus</i>	24	GAAGAGAGCTCTATCTC GCACTGAGGATTGGAGTC TGACTTAAGAAGCCGCC	100%	2	70.6424	71.5075
<i>Ammoniphilus</i>	2	CGTCAAACCGAGATCCTC TCTCTAAATCGGTCAGAGG	100%	0	70.5625	71.2806
<i>Thermobacillus</i>	1	GCCTACACCATCTCTGG	100%	0	74.9567	64.8673
<i>Alicyclobacillus</i>	90	GTCACCTGTGAGTCC CTCACAAAGGAGCTTTCC GGAATTCCTTGCCT	100%	1	70.5318	70.014
<i>Pasteuria</i>	16	CTTCCCGATGAACAGGG	100%	0	72.0539	60.4259
<i>Sulfolobacillus</i>	41	ACGTAGTTTGAGGGGC GGCTGAAAACCGCTACG GGATACTTAGTGCCTAGC	100%	0	70.8912	70.6976
<i>Thermoactinomyces</i>	10	AAAACCGTCTCACTACG CAACCTTCCAGGCTGTTT ACGCAATCGGGCTATTAC	90.91%	2	70.9972	68.916
<i>Planifilum</i>	4	AGAGTTGTTGCGCCCTCC	100%	0	73.4139	64.8075
<i>Laceyella</i>	5	TCATCCAGTTTCAGGTGC	100%	0	71.567	71.1106
<i>Catonella</i>	5	GAAGAAAAGGCTCCATTACGAA CTGCTTCCACTCTCAACTTG	100%	0	72.8031	72.8031
<i>Butyrivibrio</i>	56	ACCGACTCCCTATGGAA TCTTGTCCTGAAGGAAGA GGTACCGTCGTTTCTTCC GTCTCAGATGTCCCGA GATCTCAAGATCTGGTAAGG GCCTTCTTCCCTGCT TGACTCCCTATGGAAGC	94.74%	15	70.4957	74.0829
<i>Pseudobutyrvibrio</i>	10	TACTTTGTCTAGTGGATGTC	45.45%	4	70.895	67.2417
<i>Coproccoccus</i>	3	TACTGAACATAGGGGTTC CCGAAGAGAAGTTCCGGT	75%	0	71.5497	71.9147
<i>Catenibacterium</i>	6	CTTCTCTATAGCTATGAGGATG	100%	0	71.4821	58.4689
<i>Lachnobacterium</i>	4	GTTTCCAATGCAATCCCATGG CCCCTCCGATACTCTAG TCCGAAGAGAGACTACATTAC CCCTCCGATACTCTAGC TCCCTGCTGATAGAACTTTAC	100%	7	70.8833	74.3627
<i>Ruminococcus</i>	80	TTCAAATGCAGTCTATGGGT CCCAGGTGGATTACTTATTGT GAGCTTCCTCCTAAAGTA ACATACCGAGATACTTCTTCC CTCTAAGCTATGCTCACGA CGTCCCTGTCAACAGAG GTTCATTCACTCGATGTCAAGA ATCAGACTTGCCACACC GCACCGAAGAGCAATGC	82.72%	20	70.0776	75.9948
<i>Roseburia</i>	6	CATGTTCTGTATCGGGAT TACTGCTGGCTACTAACTAT	100%	1	70.7076	70.531
<i>Oribacterium</i>	1	TACTGCTGGCTACTAACTAT	100%	0	70.7854	64.1521
<i>Lachnospira</i>	8	TTGTTCGAAGAAAAGGTCACA CTTGATGCCACCTAC CAGTTTCAATAGCAGTTCCGA	100%	1	70.019	67.073
<i>Anaerostipes</i>	4	GAATACTTACTGCGTTGGC GTCTCCAATGTCTCGAAAGA	100%	1	70.1978	66.8507
<i>Anaerofilum</i>	1	GTAGATTAAACACCCAACTTGC	100%	0	72.4282	60.2437
<i>Moryella</i>	2	ATCCTTTGGATGCCAACAC	100%	0	71.136	61.0201
<i>Peptoniphilus</i>	27	AGTTTCATGCTTGCGCACG ATCGGACTTGCCATGCC ACATCTGACTTGCAATTCCGC CGGTCAATATTATGCAAGCC CTTTAGAGTTCCCATCCGAAAT	96.43%	6	70.4964	76.3174

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Finegoldia</i>	8	CTTCCCATAGGACAGAAGCTT GAGCTTTCTCCTATGGTA	100%	1	70.0847	69.1572
<i>Tissierella</i>	4	GAAGGTAAAGCCTATCTC GAAGGTAAAGTCTATCTCTAG TTTCAAGTGCTTACCACAGT	100%	0	70.7892	67.1056
<i>Sedimentibacter</i>	10	GCACTCAAGTCATACAGTTTCA CTGAATATCGTACCCAACA GGCTACCGTCATTTCTTTCT CACATCTCTGCATCTGTCAA CCAAAGGCTTACATTGGTTGA CTAGAGGGTCCAGCTTTA	100%	1	70.2696	72.1615
<i>Fusibacter</i>	1	GTTTAGCAGTCTCCGAAGA TCTCATAGGACAGAGCTTTAC AGCCGTAGCCTTTAACCAC TTTCTCTCCTCACTCAAGC TTAAACACCTGTCCGAGGG TCGAAAACCTTCTCGCTCAC CGAAGGCTTCTAAATCAC ACCTGTAATACAGTAGACCGA CCATACCTCAAGATGAGC	100%	0	71.2971	73.0753
<i>Peptostreptococcus</i>	36	GTTCAGTCTCCGAAGA TCTCATAGGACAGAGCTTTAC AGCCGTAGCCTTTAACCAC TTTCTCTCCTCACTCAAGC TTAAACACCTGTCCGAGGG TCGAAAACCTTCTCGCTCAC CGAAGGCTTCTAAATCAC ACCTGTAATACAGTAGACCGA CCATACCTCAAGATGAGC	86.49%	3	72.0382	74.0473
<i>Anaerococcus</i>	39	CCGAAGGTAAAGTCTCTCGA CTAAGTTGTCTTAGCTGTC TCCCCGAAGACACTGATAT TAGAGTGCACAGCCTGA CTTCCCCTCATCTACTCAA CACAGTTCGCAAGGCTTACA CCCTGTAAAGGTCTGTCA GGCGAACAATAGTTAAGCC	92.50%	2	70.0014	62.2014
<i>Filifactor</i>	4	CACTGAGTCTCCCCAAC GCATTGAGTCTCCCC CACTGAGTCTCAACGGAC GCACCGAAGTCTATCGAC CAAGGCAAGTAGAGGTTGAG	100%	0	70.5605	71.6169
<i>Sporanaerobacter</i>	1	ACCGAGCTTTCAGTGGG TACCGTCATCTTTCTTCCCTT CCACCTGTCTCTTGTC ACTTCAGACTTGCATCGCC GATATATAGCTATCTCCCCA GCAGTTTCCAATGCAGTCC CAAGCTCTGCAGTTCAC AAGCAATGCTTCCCAACAC CCGACACCAAGTATTCATCG GAAATCCACTTACCTCTCC CCGTCATCTTCTTCGGCTTT GGGTACGTCATTATCGTGC GGCCCAACAGTAGTTGA GGTACCGTCACTTCTTCTTC GGAGAGGCTCTAATCTC	100%	0	73.327	67.6391
<i>Helcococcus</i>	4	TACGGGATTAGTTGGATGTCA CAATGCTTTACGGAGTTAAGC	100%	0	71.6871	69.5437
<i>Anaerovorax</i>	6	CACTGAGTCTCCCCAAC GCATTGAGTCTCCCC CACTGAGTCTCAACGGAC GCACCGAAGTCTATCGAC CAAGGCAAGTAGAGGTTGAG	100%	0	70.5488	71.417
<i>Acetobacterium</i>	30	ACCGAGCTTTCAGTGGG TACCGTCATCTTTCTTCCCTT CCACCTGTCTCTTGTC ACTTCAGACTTGCATCGCC GATATATAGCTATCTCCCCA GCAGTTTCCAATGCAGTCC CAAGCTCTGCAGTTCAC AAGCAATGCTTCCCAACAC CCGACACCAAGTATTCATCG GAAATCCACTTACCTCTCC CCGTCATCTTCTTCGGCTTT GGGTACGTCATTATCGTGC GGCCCAACAGTAGTTGA GGTACCGTCACTTCTTCTTC GGAGAGGCTCTAATCTC	100%	2	70.6132	71.9231
<i>Mogibacterium</i>	7	TACGGGATTAGTTGGATGTCA CAATGCTTTACGGAGTTAAGC	100%	0	71.7369	66.3434
<i>Eubacterium</i>	104	ACCGAGCTTTCAGTGGG TACCGTCATCTTTCTTCCCTT CCACCTGTCTCTTGTC ACTTCAGACTTGCATCGCC GATATATAGCTATCTCCCCA GCAGTTTCCAATGCAGTCC CAAGCTCTGCAGTTCAC AAGCAATGCTTCCCAACAC CCGACACCAAGTATTCATCG GAAATCCACTTACCTCTCC CCGTCATCTTCTTCGGCTTT GGGTACGTCATTATCGTGC GGCCCAACAGTAGTTGA GGTACCGTCACTTCTTCTTC GGAGAGGCTCTAATCTC	39.05%	17	70.5182	75.7371
<i>Anaerospira</i>	2	TACGGGATTAGTTGGATGTCA CAATGCTTTACGGAGTTAAGC	100%	0	70.3255	61.3696
<i>Dehalobacter</i>	11	CCAGTTTCTCAGGCACG CCTTCGTCCCTGAAGAC GGCAGTTCGTCTAGAGT CGTCATCCTTCTTCCCC CTCAAGCCCAATGGTTTCA CCCCTCTACTGCACT CTCAAGTCCACCAGTATCA AACTAACCGTAGGGGTTGC CCTCTATGTGCTGGC	100%	0	70.6553	65.0125
<i>Pelotomaculum</i>	9	CAAGTTCGCTCTAGAGT CGTCATCCTTCTTCCCC CTCAAGCCCAATGGTTTCA CCCCTCTACTGCACT CTCAAGTCCACCAGTATCA AACTAACCGTAGGGGTTGC CCTCTATGTGCTGGC	100%	0	70.9635	75.0533
<i>Desulfotomaculum</i>	77	CAAGTTCGCTCTAGAGT CGTCATCCTTCTTCCCC CTCAAGCCCAATGGTTTCA CCCCTCTACTGCACT CTCAAGTCCACCAGTATCA AACTAACCGTAGGGGTTGC CCTCTATGTGCTGGC	85.90%	1	70.1479	72.3052
<i>Thermincola</i>	2	CAAGTTCGCTCTAGAGT CGTCATCCTTCTTCCCC CTCAAGCCCAATGGTTTCA CCCCTCTACTGCACT CTCAAGTCCACCAGTATCA AACTAACCGTAGGGGTTGC CCTCTATGTGCTGGC	100%	0	70.4805	63.5567
<i>Peptococcus</i>	2	TCTCTGCGGAGTTCTAAC CAGATGCACGTTTCAGGG TCCGAGGAAAAGGATACATTAC CAATGCACCCCTGGA	100%	0	70.7463	68.9489

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	
					max[Tm(MM)]	
<i>Carboxydotherrhus</i>	4	AATAAGAGAACTTCGTCCCGG CACTCACCCCTTTTCGAATGA	100%	0	70.9603	66.6765
<i>Desulfosporosinus</i>	29	TAAGGATTCGTAGGATGTCAAGC ACGCCGGACTTATTCATCC GTGGCCTCAATATTTCAAAGC GCACTCTCAGGGTTGA	96.67%	0	72.2326	74.668
<i>Desulfotobacterium</i>	29	GTACCGTCATGTATCTGCAT CTTTCACACCAGACTTAAACGT GTCCCTGAAGACAGTAC CAGTTTCCGATGCAGGC	96.67%	0	71.0836	69.966
<i>Oscillospira</i>	4	CTGACTTGCAGACCC GTACCGTCTTCTGCTCTT CCGAAAGGAAAACTGTATCTCT	100%	0	70.4424	68.3431
<i>Syntrophomonas</i>	21	GGCTTCGCCAGATGT CCTCCTGATTCTACGCAT TACCCAGTTTCAGGTGC GCCAGATAAACATTCGTCCCTT	100%	0	70.3126	72.1182
<i>Aminobacterium</i>	1	TGAGCCCATGACTTTAACAGC	100%	0	75.3063	66.1556
<i>Thermaerobacter</i>	4	GGTTCGCGGTGATGTCAA	100%	0	71.466	70.2954
<i>Dethiosulfobrevibrio</i>	5	CAGCAAGACAGTTTCGAGT	100%	0	72.156	66.0102
<i>Anaerobaculum</i>	1	TGAGCCGTAACCTTACACC	100%	0	71.217	60.9253
<i>Caldicellulosiruptor</i>	15	CCGTCCAAAGAGGTTTACAC	100%	0	72.8209	60.3463
<i>Anaerobranca</i>	5	AGCCCCGCATTTTAACACC TCACATCTGACTTTGTATAACCG	100%	0	70.2656	69.3071
<i>Carboxydocella</i>	1	TATGGATGTCTTGACCGAAGC	100%	0	74.5843	58.733
<i>Sporotalea</i>	1	CTCTAGAGTCATCAGACGAT CTATCTCTAGAGTCATCAGA	100%	0	70.6874	65.7743
<i>Megasphaera</i>	36	CACCTGTTTTGCGTCTTC TTATGCTGTTCCGACAAAAACC CTACCCGTTGCGAGCAAT GCAGTCTCTCCTGAGT AGTTTCGGTCCCCTCAC	100%	1	70.7163	71.3141
<i>Zymophilus</i>	1	GTGGCTTTCTAGTTTGATACC	100%	0	73.5355	67.4436
<i>Centipeda</i>	1	GGTACCGTCATTACAAAGGA	100%	0	70.9859	67.4291
<i>Veillonella</i>	70	GACCTGTCCATCAATGTCA GTGGCTTTCTATTCCGG	100%	0	70.0915	66.2411
<i>Dialister</i>	27	ACCTGTTTTCTCGTCTCCC TACCGTCACTTCGCCATAT CTATGGATTGCGATCAATGTCAAG CAGTTTCCTTCCCATCAC	96.43%	1	70.2933	72.1344
<i>Selenomonas</i>	85	AGAGAGGGAGCTATCTC TGTTTTCGTGTCTCCGAAGAG ACTTACCGTCCCACCTG TTCTCAAACCCGGTTTTCGTC CTGTCTCCGAAGAGAGG CCCGCACTTTTAAGACTGAC	86.05%	4	70.1582	73.1224
<i>Dendrosporobacter</i>	1	CTAGACCTTTCACTCAATGTC	100%	0	70.6784	67.2601
<i>Pectinatus</i>	4	CCGCACTTTTAAGATCCGC	100%	0	72.1574	61.0208
<i>Sporomusa</i>	15	TCCCGAAGGATAGGAATAA CAAACGACAGAGCTTTACGA GCACTTAGACACACGACTTAAG GTTGAGCTCCGCACTTAG GACTTAAGAATCCACCTACAC	93.75%	0	70.5366	72.1598
<i>Anaerovibrio</i>	1	ATCAAGTCTCTCGTCCC CTGTTTTCGTGTTCCGAAGAAG	100%	0	70.2798	70.6373
<i>Mitsuokella</i>	2	TTATTACCATCTCCACATTCTG TTATTACCATCTCCACATTCTG ATTACCATCTCCACATTCTG	100%	0	70.2318	62.4302
<i>Propionispora</i>	2	TAGTTTTCTGACCCCTCAC ATTCACCTACAGCACATTCTG	100%	0	71.3507	59.7616
<i>Pelosinus</i>	1	CATATCGCACATTCTGCCCT CTTCCGAAGAAGAACTTCCTA	100%	0	70.1266	68.7085
<i>Desulfosporomusa</i>	4	CATTATTCACATGCTACGTTTCG TCCGAAGATAGGAATAATCTC	100%	0	70.9206	67.5434

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Phascolarctobacterium</i>	2	GTTACACACTGCACGT	100%	0	71.6104	60.8133
<i>Allisonella</i>	1	CGTCACTTCCTTAGGCTATTAA TGCAGATTGCGATCAATGTCA	100%	1	70.7146	72.487
<i>Acidaminococcus</i>	16	GTTTGCTACCCTGGC	100%	0	70.7698	71.3071
<i>Alkaliphilus</i>	15	AAGGGAAGACTCCGGTTAA TGGTCCGAGGGATGT GCACTCAAGCTCTACAGTTT	100%	1	70.1588	72.2926
<i>Acetivibrio</i>	1	GTTGAGCCCCGAATTTCAC	100%	0	72.0455	67.3656
<i>Anaerotruncus</i>	1	TACTTCACTCAAGAACGGCA	100%	0	70.5539	67.4845
<i>Clostridium</i>	854	TCCTCAAGTACCGTCATTATC CGTGGCTTGTTCCTTGG CTTCCTCCACGTAAACC GATGTTCTTCTAATCTCTACG GCTCAACTTAATGGTAGCAACT AGATGTCAAGTCTAGGTAAGG GTCACCTCCTTCGTCC GGCTTCCTCCATTACAGA GTACCGTCACTCGGATAC CTTCGCCTATCTCTAGGTTA GGCTTCTTAGTCAGGTAC CAGTTACAGTCCAGAGAGT CCTGTACTCAAGCTAAGC CCTGACTTATTTAGCCGC TCCCAGTAAGTCGCC	79.65%	120	70.0039	74.0159
<i>Tindallia</i>	2	AGAGCCTTTAGAGGTATGT CGTCAATCCTTCTTCCCTT ATTAAGAAGCGGTCTGAGG TCACCTCAGTCCAAAAGG	100%	3	70.4531	71.5887
<i>Coprobacillus</i>	2	GAAGGGAAGGACACGT	100%	0	70.3175	60.1216
<i>Tepidibacter</i>	4	TCTACAGGATTCGGATGTCA TCATCGTTACAGCGTGACTAC CTCCTCCCTTACCGT ACCGTCATTATCGTCAGGG ACCCTCATTATCGTCAGGG	100%	1	70.0675	67.3387
<i>Bryantella</i>	1	GAATGCAGTCCCAAGT CCGAAGGAACTTCCCACATT	100%	0	72.6415	68.8581
<i>Caloramator</i>	11	CACTGTCCTCTCCATCC GGCCGTTTCGGAGTTGA CTGGCAACTGGGGATAAG TCTCCATGCCTCTAGAC CAGTTTCTGTGCAATCC	100%	0	71.4714	70.9474
<i>Sarcina</i>	3	TCAGTTGACGTCCAGACA CATCTCTCAAGACATCCAGTTT	100%	0	70.2954	60.7834
<i>Heliobacterium</i>	9	ATCCCACACCTAGTACC CAGTTTCATGTGCATCCC CACACTATCGAGCTTTACGA CGTCATCCCTTCTTCCC CACACTACTGGGGTTTACAA	100%	1	72.5293	71.0537
<i>Heliorestis</i>	3	GCACCGAAGGGTCTAAC CTGAAGGGTCTTTACCC	96.92%	2	70.6424	73.8784
<i>Heliophilum</i>	1	GTACCGTCCCTTTCGTG	100%	0	70.763	65.0916
<i>Heliobacillus</i>	2	GTACCGTCCCTTTCGTG	100%	0	70.2575	69.5335
<i>Thermoanaerobacter</i>	64	GATGTCAAACCCCGGTAAAG ACCTTAAAGGCTATTGCGC CTCGAATGACCGCTAC GCGATACCCCTTGGGT	100%	1	70.3753	67.624
<i>Coprothermobacter</i>	7	AGCCGATAGGTTTCACGT CTTCCTGTTTCATCCGAAGA	100%	0	70.1763	67.6215
<i>Thermosediminibacter</i>	1	AGCCGATAGGTTTCACGT CTTCCTGTTTCATCCGAAGA	100%	0	70.0344	67.8207
<i>Moorella</i>	19	CTTTCGGTTCCTACTACC CCGTCACTCTTCTTCCC GGTCACTTATTGCGTTTGCT	100%	0	70.3858	74.7048
<i>Thermacetogenium</i>	1	CAGAGTATAGGGTTGCT	100%	0	70.7534	67.688
<i>Sporotomaculum</i>	1	CGGATAGCTTCCGCTAC	100%	0	71.8142	65.7281
<i>Thermovenabulum</i>	3	ATGATTTTACTACCCACACC GTCAGITCAAGTCCAGAGA	100%	1	72.85	67.8947

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Halanaerobacter</i>	6	CACAGATGGTCATAGGATGT	100%	0	73.1394	66.5332
<i>Halobacteroides</i>	3	AACGATCACAGGGATGTCAA TTTCCTGTAGCAGTCGC	100%	0	70.4588	64.4212
<i>Acetohalobium</i>	1	GTGTGATCCAAAGGACCAAT	100%	0	71.8459	58.6285
<i>Orenia</i>	2	GTCGAACCTCCAACACC CTGTGTCCTCAAAGAGG	100%	0	70.4203	69.9373
<i>Halanaerobium</i>	18	GGGATGTCAATTCTCGGTAA	100%	0	69.5589	64.0883
<i>Thermolithobacter</i>	3	GTACTCAAGACGACCAGT	100%	0	72.3566	71.9418
<i>Meiothermus</i>	48	GCGTCACAAATCGTCCAGG GCACCCAACAGATGTCG TTCCTTCCGGTATCTGC	100%	3	70.1328	70.3812
<i>Oceanithermus</i>	2	CTTGACACCGGACTTAACGG GGTACCGTCATTCCCTT	100%	0	72.5234	75.0355
<i>Thermus</i>	155	GCGCTATTACCTGGGT TAGCCTGAGCGTATCC GTTCTCCCGGTATCTG	96.79%	2	71.0647	70.6373
<i>Deinococcus</i>	117	TGATTCCGGGTAACGCTTG CACGTTTATCGCGTTGGCT GAAATCCAGTATCCAGTCCA TCTCCAGAGGTCAAGAC CACCTTCTGTCCAGTAAC AGCGTCACCTTCTGTCC GCTATTAGCGAGGTACC	95.76%	0	70.2537	70.6969
<i>Truepera</i>	1	GTGCTATTAGCTGTCTAC	100%	0	72.5082	65.6027
<i>Ilyobacter</i>	3	ATCAACGTTCCACCGAAGC AGCACTTTACGATCCGAAGA	75%	1	70.1972	67.1229
<i>Fusobacterium</i>	74	TTCCAACGCCATACGGAGT GAGCCCCGCAATTTTACA CCAACGCAATACAGAGTTGA	100%	8	71.0836	73.196
<i>Streptobacillus</i>	2	TGCTTCCATCTTATCCGAGT	100%	0	72.905	68.8654
<i>Leptotrichia</i>	30	TACTCGTGCAGTTCGT ACACTCTTCATGCCAC CTTCTCCTGCTCTTCG CACTCTTCATGCCACA	96.77%	0	70.0312	68.4636
<i>Sneathia</i>	6	TAGGCAAGCCTATGGT	100%	1	70.4603	63.9923
<i>Propionigenium</i>	3	TCAGGCTCAGTATTCATCC GAAGTGGCACCAAGC	100%	0	70.6634	64.2039
<i>Cetobacterium</i>	1	CTGACTTCTCCATCGGC	100%	0	72.1754	71.2643
<i>Thermodesulfovibrio</i>	7	GTGCTGGCTCCTTAGA CTGGCTCCTTAGGGTT	87.50%	0	72.5308	72.1739
<i>Nitrospira</i>	39	TCCTCCCCGTTATCCTG TGAATAGTTCCTCTCGACC GTTACCCAGGGTCAT CCTTCTCCCGATCTCTA GTTCTCCTGGGCAGT AGTATCAAAGGCAATACACAG CTGTGTTAGCTTCCCGAT	90%	0	70.0375	72.5748
<i>Leptospirillum</i>	43	TGGCAACAAAGGGCGA GCACGGGAGGTTTAACC AAGCCTTCTCCACAC	100%	0	70.2197	71.0353
<i>Candidatus Xiphinematobacter</i>	2	CATACAAACGTGCACC	100%	0	70.0205	70.2648
<i>Verrucomicrobia subdivision</i>	2	TATTCACCTCCAGGAATTGT GCGTTCGTTGGTGGACTTAA	100%	0	72.0409	69.7692
<i>Rubritalea</i>	4	TCGAACCCACCTACACC CTTACTCTCATATGACAGGA	100%	0	70.5817	67.7903
<i>Prostheobacter</i>	4	GTTGATCGAGGCAGTCT	100%	0	70.5444	70.3644
<i>Verrucomicrobium</i>	2	CCTGTGCAAAGCGTGATTG CCATTACTCCCTGATGACA GCTTCTCTTGCGATGA	100%	0	70.0967	67.6032
<i>Pelagicoccus</i>	2	TTAGCCACTCCTTCCTC CTTCCCACTCTCAGAGA	100%	0	70.8716	67.2696
<i>Puniceicoccaceae</i>	1	AGTTAGCCACCTCTTCC	100%	0	71.8339	66.0545
<i>Opitutus</i>	6	CCACCTTCCCACTCTT TCAAAGAGGGTTTGCTCC	100%	0	70.531	71.3038

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Lentisphaera</i>	2	CAGTATTGTGCCAGTAAGC	100%	0	71.7078	66.8431
<i>Victivallales</i>	1	ACCTCCTACAGACCC	100%	0	70.8649	67.781
<i>Candidatus Fritschea</i>	1	CCAACTACACCAAGTATGC	100%	0	71.1564	61.4035
<i>Parachlamydia</i>	4	CCGAATATTAGTCGGCTTCTC CAGCTTCTCTTGCTCC	100%	0	71.3215	61.8518
<i>Candidatus Protochlamydia</i>	4	TTGAGTCCAGTTACACCAAG	100%	0	70.1217	67.5122
<i>Waddlia</i>	3	AATTCCGTTTTCTCCGCTTG	100%	0	70.3787	71.6169
<i>Chlamydomydia</i>	82	TAACCTTCTCTTCCGCCTAC CTGCCGCGGTCAAATACA GACTATCCACATCAAGTATGC	100%	0	70.2151	72.765
<i>Chlamydia</i>	46	GTGAAATTCCCTTTTCTCCATC GACTTGCTTTCCACC AATCGGGACGTTATTAATGCC GCGGGTATTAATCACTATCTC CCTGTGTACCTGTCCT	95.74%	0	70.2325	71.1703
<i>Dictyoglomus</i>	1	TGACCTCCCTGAGTTGA	100%	0	72.258	61.9339
<i>Chlorobium</i>	44	GCTGCGACACTGATCAACAT TGCCGGTAAACCGACATG CTGTTACAGCTTCCCGAA CTTCCTCCACAAAAGGACT CAGTACTCGACCAGAAGA CAGGGAGTTTAGCTGCAATA CAGTTCTCGACCAGAAGA	93.33%	17	70.0243	70.3468
<i>Pelodictyon</i>	7	GCACCTCATCTTCAATGAGTT GCAAGCCGGGATGTT	50%	0	70.6237	67.5993
<i>Chloroherpeton</i>	3	GTACATCAACGACCAGAAGA CATAGCGAGACTTTCTTCTC	100%	0	70.7064	61.4524
<i>Chlorobaculum</i>	28	GATGGTAACTAATTGTAGGGG CCAGCAAAAGGATTTTACATTCCG GCTGCGACACTGAACAATAT GTCAGTACTCGACCAGAA GGCAAGCCGACCTAT	96.55%	20	70.0649	72.4514
<i>Prosthecochloris</i>	40	GTCAGTTGTCGACCAGAA GAAGCATGTCATGTGTTAAGC	90.24%	2	71.8012	68.827
<i>Owenweeksia</i>	1	CTCAGCCTGTCAATCTAC TAGGCACTAGTCAGACTAAT	100%	0	70.3126	66.3592
<i>Brumimicrobium</i>	1	ACACCAACTGTATATCGCTGA	100%	0	72.1408	61.6697
<i>Blattabacterium</i>	102	GTTTGCCGACACTTATTCGT	100%	0	70.2341	62.2408
<i>Aquimarina</i>	7	ACAAAACCGCTACGGAC CAAGGTGTACACGTAGC CTGACTTACAAAACCCGCC	100%	1	70.5697	72.947
<i>Ulvibacter</i>	2	CCACTCAATCCGAAGATCGA	100%	0	72.3032	68.5794
<i>Maribacter</i>	12	GGTACCGTCAGTCAACTA CAAGACCAACAGTATCAAAGG	53.85%	6	70.4471	66.9491
<i>Coenonia</i>	1	CACATTCCGGCCACTTC	100%	0	70.6559	67.7237
<i>Yeosuana</i>	1	CCACTCAGGTAAACCC	100%	0	70.0166	61.5017
<i>Mesononia</i>	4	TCAGTCTCAATCGACCAACA TCAGACTCAATCGTCCAAACA	100%	1	70.2644	61.389
<i>Robiginitalea</i>	1	GGGCATTATTCTCCCGTAAAA	100%	0	70.7284	64.0921
<i>Psychroserpens</i>	12	TCTTACAGTACCGTCAAGATC TACCGTCAGCTTTTCACAC TCACACTACATTTAAGCCCTG CAGTATCAGAGGCAATTCCA	69.23%	10	70.2861	73.7194
<i>Bizionia</i>	6	GTCTTATTAGTGACCTGCCT ACTCAGATCGAAATCCGAACAG TTGGTTTAGCCACTCAGTC CCGAAATCCGAACAACACTAGTA CAAGCCTCTACTCGTAGA TCAGTCCGAAAACCGAACAAAC	85.71%	0	70.2784	70.1299
<i>Riemerella</i>	101	CTGACTTACTAGCCCCGC CTCTGAACCATATAGCCCAAAA CATTCAGCTACCTCAAC	100%	1	70.6559	72.3884

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Muricauda</i>	9	AAGAAAGGGTGTCTCC CCGAAGAAAGGGACATTTCTG GTCGATGGTTGGTCACC TCAGACACCTACACGTAGG	100%	2	70.6541	70.4846
<i>Sandarakinobacter</i>	1	GTGACTGTATTTCTTCCGT	100%	0	72.3052	64.5823
<i>Olleya</i>	1	GCCACTCAAACCAAAGTCC	100%	0	74.529	69.9214
<i>Kaistella</i>	3	GTCTCTGAACCCGAAGA GTCTCTGAACCCGAAGTC	75%	0	70.1772	66.306
<i>Subsaxibacter</i>	1	GTGCGGATGTTTCTTCC	100%	0	70.052	65.5465
<i>Wautersiella</i>	22	GTGAGTCCGAAAACCCAACA CACATTCCAGCAACTTCAACAT	100%	1	71.4511	70.1012
<i>Candidatus Amoebinatus</i>	1	GCACCTTGGAATTGCC	100%	4	74.3388	71.1008
<i>Capnocytophaga</i>	66	GCAGTTACTCAGTTGAGC GCACCTTGAAAACCTGTCC CCAACTACTTCACAACCATICA	98.51%	0	71.0273	66.4009
<i>Dokdonia</i>	3	ATCGATCTACACGTAGACC AAGGCCAAACAACCTAGTATCC	100%	1	71.0099	73.0963
<i>Formosa</i>	8	GTAGAGATGTTTCTTCCTGT CCGTCAAGCTGCTACAC GCTTTCGTCCATCAGCT	88.89%	13	70.5726	71.1556
<i>Algibacter</i>	4	CACTCAGAAACAAGTCCGA CCACTCAGAACAAAGTTCC GAAGCAAGTTCGAACAGC GAGCGGCAGGATTTAC	100%	0	70.6109	70.0281
<i>Arenibacter</i>	6	GCCCTTGTCTTCCC CAGTAGAGCACACGTGC TCAGAAGGGCACACGT CAGAATATGGTTAGTAACCTGC	85.71%	2	71.1311	70.7979
<i>Lacinutrix</i>	4	TCACACTACATTTAAGCCCTG AGCACCTGTAGATTGTCC	80%	5	70.9375	68.5861
<i>Chryseobacterium</i>	174	CCTTCAGCTCTCTACACG CTCTCACGAGAGTAGGTT AGCTGTGAGATTTACCAC CTTAGTCTCTGAATCCGAAAACC ACACGTATCTAGGTTATCCC CTTGAAAATTGCCGAAGGA CTACTTATCCAGCCACTTCTA CAACTGAATGGTAGCAACTAG TCAGTTGTTGCTTAGTAACC	96%	10	69.8438	73.5476
<i>Leeuwenhoekiella</i>	7	GTCCGAAGAAAAGAATGTTTCC GACCGGACAGCTAGTAAC GTAGTCCTTATTCTTCCGT	87.50%	3	71.1262	71.9997
<i>Vitellibacter</i>	1	CTTCACACTAATTCAAGGCGA	100%	0	71.3077	59.0899
<i>Winogradskyella</i>	12	GTCAGCTCAGAACACGTT GTAGATGGGTTTCTTCCC CTCAAACCGAAATTCGAACAGC CTCAGGCAATTCTACAGTTGA	76.92%	1	71.5178	68.6869
<i>Ornithobacterium</i>	7	CTCAACTTGC GTTGGCAG	100%	0	70.5036	67.3328
<i>Salegentibacter</i>	10	CACTCAGCTCTCAATCGA TTGAGCGGAAGACTTTCAC CCGTCAAACACCCCTCAC	90.91%	1	70.32	70.1352
<i>Gaetbulibacter</i>	2	CGTCAAGTTCTATCTCGTA	100%	0	71.6282	67.2679
<i>Krokinobacter</i>	4	TAGTCACTACGCCGA CACGTAGATCTTATTCTTCCC	100%	4	71.0911	71.12
<i>Gramella</i>	4	GAGTGGTTCTTCCGTATAAAA TCTACACGTAGATGTGGTTC	100%	1	70.216	67.3381
<i>Myroides</i>	3	CATCAGCGTCAATAAGTACGTA CCATACTATTCAAGTCAGACAG TGGCAGTGTCTAGTTAAG	100%	0	70.3542	62.4953
<i>Elizabethkingia</i>	26	AGCCACTTCAACCTTACTC GGACTTGAACAACAGCCT	100%	1	70.0797	65.0937
<i>Sediminicola</i>	3	TCCGAAGAAGGCTCTATC	100%	1	70.0312	69.0069

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Flavobacterium</i>	404	TTACTTCCCGCAATTCAAGC TAGTAGTAACCTGCCTTCG CCGTTTGATCGTTGAGCGA CCTTGTAATCTGCTTGCGAAA GCGTCAATAACGGCTTAGAC CAGTATCAATGGCCGTCCA TGCTTGCGAAAAGTCTGTTCC CGCTTTCGTCCATTAGCGT CCTTGTAACCTGCTTGCGA	81.48%	14	69.9665	71.9135
<i>Niabella</i>	2	GCTTTCGCTCAGACACTTAT	100%	1	71.8889	72.5232
<i>Psychroflexus</i>	12	CCACTTCACAAGCACTCAAG	92.31%	0	72.2156	61.5433
<i>Aquorivita</i>	7	ACCCAGCACACCAATGT	100%	0	71.6346	72.0694
<i>Gelidibacter</i>	15	GCCACTCAGATAAATCCGA GCCACTCAGATAAATCCGAA CTACCCCATGCAACTTACAT GATATCTATGCATTTACCGG GCTACACCACATATTCTAACC TTGCCCGAAGAAAAGTCTATCT GACCTGTCAATCCACATTTAAGC TTGCCCGAAGAAAAGTCTATCT	87.50%	5	71.1402	72.4184
<i>Zobellia</i>	14	TCAAGAACCCGGACAGC CCCTGTCAAAATGCATTTAAGC GCCCGAAGGAAAAGGTATCT	86.67%	5	71.8816	71.3733
<i>Tenacibaculum</i>	38	CCGTACACGTACGACTGT TCACTGAGTCGAAACCCA CAGTACCGTCAAATACCTACTC TCACTCTACATTTAAGCCCTG GCTTAGTCACTGAACCGAAG	92.31%	12	70.0819	71.6509
<i>Empedobacter</i>	5	CTTGCACTCTGTCCGAAGAAAT TAGCCACTGAAGACGAAAC	66.67%	0	70.1386	72.8744
<i>Polaribacter</i>	30	CTACTTCCATATGACTCAAGTC GCGTCAGTACATACGTAGT	100%	2	71.4285	66.5256
<i>Cellulophaga</i>	38	TGACTTAACAGCCCGCC CCATCAGTATCAAAGGCAATTC GTGTCCTTATTCTTCTGT	64.10%	6	70.2954	74.5295
<i>Pibocella</i>	4	GCTACACTACATATTCCAGC AGTACCGTCAGTAAGCTAC	80%	2	70.6482	73.776
<i>Bergeyella</i>	6	CCATAGGTCCTTAGTCCT	85.71%	0	71.3122	68.6981
<i>Subsaximicrobium</i>	1	CAGTGCATATTAGTAATCTGC	100%	0	73.1384	60.6789
<i>Haloanella</i>	2	TTGGTCTCTGAATCCGAAAATCC	100%	4	73.9493	71.2841
<i>Gillisia</i>	5	TCTTGCGACCGAACAGC GCACCACCTTGTAATTGTCTT	83.33%	0	72.4194	65.2609
<i>Stanierella</i>	1	GTCAGAGTTCCACACGT	100%	0	71.6138	62.6309
<i>Sejongia</i>	2	GCTACCTCTAATTCACCTAAG GCTAACTTATCGCTTTCGCTTG TACAGTACCTTCAGCCAG	100%	0	70.4696	69.161
<i>Flexithrix</i>	2	GTTCTCCCGTATAAAAGAAC	100%	6	70.6448	61.3934
<i>Thermonema</i>	3	TGCCTTACTACGGTTAAGC	100%	0	71.3196	64.5873
<i>Flammeovirga</i>	12	GTCAAAAAGCAATTCGAGTCCAGG	92.31%	0	74.8998	61.6796
<i>Persicobacter</i>	7	TTCTGGAACCGTCGCTC	100%	4	70.3574	67.5177
<i>Balneola</i>	1	CGTCACACCAGATCGAAATC	100%	0	71.033	58.7411
<i>Salinibacter</i>	18	GACTCAAGAGCAACAGTTTC	100%	0	70.3175	65.3963
<i>Chitinophaga</i>	7	CAATAGTTGTGTAGCCAGCT GCTACAAAAGACCCTTTCAG CAATATTGTGTAGCCTGCTG ACAGGCTGTCCGGTA CACGGACTTACTGACC	87.50%	1	70.0119	68.4052
<i>Rhodothermus</i>	12	ATCAGAGGCAATTCTCCGG	100%	0	74.3795	72.8292
<i>Sphingobacterium</i>	56	CGCAATCGGGTCTGA TCGCAACATCGAGTTATCATCG TACTTGCTTATTCCGCCC GACAGTTAAGCTGCCGTC GCAATCGGTGTCTGAGACAT	63.16%	3	71.1186	73.33
<i>Parapedobacter</i>	1	GCCGTGTATCGCGAACAT	100%	0	71.6006	65.5669

Continued						
Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Pedobacter</i>	52	GCTACTTGTACATTCCGC CTTCCGGTACATTACGCTAA CGTGTGATTGCTCACTGA TCAGCGTCAATAGGACCA CGGTACATTCAGCTACTTAC TAACTGTATATCGCTAACAGCG GTTTATTCCTCGCAAAAGC	100%	24	70.0651	73.5161
<i>Sphingoterrabacterium</i>	3	CTACACGTAGGTGGGTTTATTC TTACCGTACATTACAGTCTTC CAATCAAACCATAGTAAGCTGC CTCCCTATACATAGAAAGGT	100%	1	70.1396	68.4853
<i>Mucilaginibacter</i>	1	TCCGTCTCTGAATCATTAC	100%	0	70.3669	65.5122
<i>Pocheonia</i>	2	TACGGTACATTACAGCCC GCCTACCTCAGGTATATTC	100%	0	70.7083	70.0822
<i>Aureispira</i>	4	CTCAGTTACTTACCTATTC CTCTCCAAGGATTAATCCC	100%	1	70.0776	62.9405
<i>Lewinella</i>	6	GGCCCAAATAAATTCAGGC CTACATGCCACATTCCGC	100%	0	70.4522	67.3626
<i>Haliscomenobacter</i>	2	GCGCACGTCGAGTAATCA CCGTCAACTGCACATAAATGTC	100%	0	71.5174	67.3213
<i>Saprospira</i>	13	TCAGACACTCACTCATTACAAG AATGGTTTCCCTCAGTCAC CTTATTCGATCATAACCCAGT CGTACCGTGGTTTAGCC AATCAAAGCGTAGTGTGCTGC CAGCACCTTACATTCTGTGTAT CTGACTTACCAGCCCA	100%	2	70.0086	67.2946
<i>Niastella</i>	2	TACTCATCTTGCCACGC CCTTGTTTCGGGTCATTGC CACCTTGTTTCGGGTCCTTG CTACACCATAAAGGCATAACAG CATATAGGCATAACAGCGAGT GCCGCTACATCATATACACATA CGGTGTTCTGATGATATCT CACCTAAAGGCATAACAGC	100%	1	70.1128	69.7188
<i>Sporocytophaga</i>	4	TACTCATCTTGCCACGC	40%	0	74.7999	64.3305
<i>Algoriphagus</i>	21	CCTTGTTTCGGGTCATTGC CACCTTGTTTCGGGTCCTTG CTACACCATAAAGGCATAACAG CATATAGGCATAACAGCGAGT GCCGCTACATCATATACACATA CGGTGTTCTGATGATATCT CACCTAAAGGCATAACAGC	90.91%	2	70.054	71.5299
<i>Hongiella</i>	6	CATATAGGCATAACAGCGAGT GCCGCTACATCATATACACATA CGGTGTTCTGATGATATCT CACCTAAAGGCATAACAGC	85.71%	0	70.3753	71.4107
<i>Spirosoma</i>	10	GCTACATGACCCATTC	100%	0	72.3492	62.9138
<i>Adhaeribacter</i>	1	TTCGCTAAGACGCTGACAG	100%	0	70.9085	68.4582
<i>Cyclobacterium</i>	7	TACCGGCAATTTCTCCC AAATTCATGTACCCCTCC	75%	2	70.676	72.1367
<i>Kaistomonas</i>	1	TCAACGGCATGTAAAACAGTTAAGC	100%	0	74.7195	58.5112
<i>Belliella</i>	1	GCGTCAGTTATCGATAAGTACA	100%	0	70.3964	60.6551
<i>Echinicola</i>	4	CAGTTGCCGATCAGTACAAT	100%	0	70.4423	56.9808
<i>Taxeobacter</i>	9	CTCTAACTGTGTATCGCTAGC GCTAAGCCAGTAACTATGT CAGGTGTAGCCTAGTCAG TAGTATCCATGGCAGTTCTG CGCCGACATCGAGTAATCAT GGACTTAACGGTTCCCTAAG	80%	1	71.0431	70.83
<i>Dyadobacter</i>	7	GGACTTAACGGTTCCCTAAG	100%	1	71.1797	66.9486
<i>Chimaereicella</i>	1	CACTCTACAGCATAACAGC GCTACACCATCAAGGCATAA	100%	0	72.1306	64.4399
<i>Pontibacter</i>	2	GCCAACATCGAGTCATCA	100%	0	70.2357	66.7227
<i>Flectobacillus</i>	8	GATTTCTCTGTACCACTCAC GCTACCTCAATCCAC TACCTCAATCACACTCAAGT ACTCAGAAAGCCTTCATCC AGACGCTGACGGTGTATGC TACTTACCGGTTTCCCTAAGC GCTTTCGCTAAGTCACCAAC ACCGCTACACCTTCCATT TGCCAGCGAGTATCC	88.89%	0	70.0459	66.0578
<i>Hymenobacter</i>	22	CCAAGACTTACCGCTG GCTACACCATCAATCCGC TCCGCAGAAAACTTTCTTCC GGCAATTGATCTCGCAAGAC	86.96%	1	70.2963	67.2587
<i>Microscilla</i>	9	CCAAGACTTACCGCTG GCTACACCATCAATCCGC TCCGCAGAAAACTTTCTTCC GGCAATTGATCTCGCAAGAC	80%	0	70.8079	73.8959

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[T _m (PM)]	max[T _m (MM)]
<i>Flexibacter</i>	83	TCAAGCTGCTACGCATAGC CCGCTTACCTTAACTAACTCA CACCCTTAAACCCAGTGAA CACGCACTGTATGTCGC CCTTGCTAAAGCAGTTTACAAG CCTACGGTACCGTCAACT GGAGGCTCTATCTCTAAAG GCATACTACAGTTAAGCTG CTTACAGTACCGTCAAGGC AATGGCAGTTCGCGAGTTAAG CGTACCCTTTAAACCAATAATTCC CACCGCTACATCATAAATTCC TTTCTGATGCCCTTCCCTC	69.05%	41	70.1973	73.5435
<i>Rhodonellum</i>	1	TGTACCCCATCAACACTC	100%	0	70.1575	65.0699
<i>Cytophaga</i>	128	CGCTACACAACACATTCC TAGAGCGGTTTCTTCTGT CTACACCACGAATTCTGGCA GCCTACCTCAACCATATTC CTTGTTTCGCGTCCGAAGAA CTACAGCTAGTATCCATCGT GATACCGTCATCAAGGTAC CTAGTAATAGGGTTGCGC GTCAATATGCATTTAAGCCCTG TAAGCCCCGATCTTTAACGA GGTACCGTCATTATTCGACGC GAAGAAGTGTTTCCACCTCA TTTCCAGTCCGTCGTC	41.86%	36	70.4774	74.0159
<i>Roseivirga</i>	3	TCGCTCACATTCTAGCC ATGATTCACTCGCATTCTAGC	100%	0	71.2887	69.7242
<i>Arcicella</i>	4	GCACTGCAATCGTTAAGCGA ACCACTGACCCGAAAGC	100%	3	71.9112	68.8717
<i>Runella</i>	4	CTCCACTTCTCTCAAGTC	100%	0	70.3852	61.7063
<i>Rikenella</i>	2	GGATGATATTACCCTCGTAC AGATGTAGATTAGAGGGCTG AGAGAAGCCCTCTTTCAAAGG	100%	0	70.2711	63.6085
<i>Alistipes</i>	8	ATCGTCGCTAACTTTCAAGCC TGTGTATCGCCGACAGC CCGAAGGGAAAATCCTGTTTC	100%	3	71.6524	73.8492
<i>Marinilabilia</i>	2	CGGCAAATAACTACTCGTAGC	100%	0	73.6217	64.9197
<i>Dysgonomonas</i>	3	CTGCTATTGCTAGAAAGTCC GCCGATGCTTATTCGTA CTG	100%	0	70.724	71.0698
<i>Porphyromonas</i>	62	AGCATACTGTATATCGCATACG TCAATGCAATACTCGTATCGC GGAATACTTATCGCTTTTCGCTT CCTTACGACGGCAGTCT	93.65%	0	70.086	70.198
<i>Tannerella</i>	18	TAAGCGACAACTTTCAACCGC	100%	0	72.4325	70.5645
<i>Parabacteroides</i>	18	CCGAATGCGTTCAAACCC CCTACCTCAAACACACTCA	94.74%	0	70.4989	64.9397
<i>Barnesiella</i>	1	GGATACTTGCAATACGCTAC	100%	0	71.7351	59.5082
<i>Prevotella</i>	187	CGCTCGTTATGGCACTTGA AACATCTCTGTATCGTTCTCC CCGGACCTTCCGTATTAC CAGTTCGCGCTGCAAGT TCGATACCCGCACTTTCGA GCCTGGACCTTCCGTATT CAGCCTACGCTCCCTTTAAA ATACCCGCACCTTCGAG	100%	0	70.1772	73.6959
<i>Bacteroides</i>	205	TTAAGCCCGGTAAGGT GCACCTACACATCTGC CAGCATGACCTGTTAGTAAC CACATCTTACGACGGCAGT ACTGCAATTTACGGTTGAGC TCACCTGTTAGTAACCGG	87.86%	3	70.0602	72.0167

Continued						
Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Anaerophaga</i>	1	ATCCACTACTCGTAATGGC CAGATCTGTCACCTCC	100%	0	71.2598	60.5297
<i>unclassified Acidobacteria</i>	3	CCTCCACAGCAGTTCTTG ACAGCTTGTCAGAAGCATTTT CTCGACTCTACTCCCTTG GGGGCTAAGATAAGGTAC	100%	1	70.6394	65.783
<i>Holophaga</i>	43	CACCTCTTCTGGAGTCC CGTCAAGCTTAAGCGCTAT TGACTTAGTTGGCCGCC GGCAGTTCTCAGTTGAG ACAGTTCTTGCGAAAAGGCAC CCGCCAAAAGAGCTTTACAC GTAATTCCGAACAACGCTGG CTTCTTCCACCGGTACC ACTTTCATCTCCTGTCCTAG GATGGTTTAGGGCTAGGA TACCGTCAACCCCTTGC ACGAAATGAATCGCTCACACC CAGCTCCAAGTTGAGC TGATGGCGTATTCGACCAC CCAGTATTCATCGTTAGGG	72.73%	3	70.049	74.5295
<i>Edaphobacter</i>	1	CACGACAGGATTGGGTAC	100%	0	71.7654	66.8664
<i>Acidobacterium</i>	14	GCGGGGAAGGGATATTCTA GTGTTCTCCAGATATTACGC CGAACAACGCTTGCTCC TTCTTCCGGCTTTTCGC TCATCTGCATTCGAGCC TCCGCAGCAGCCTTTAAG CGTCAGAAATGGTCCAGTAAG TCCACCGGAATTTAAACCCAG TGTTTCCACTCCGGTCC ATTCTTCCACTCGACAGG CATACTTACCGGTTAGCTTCC	93.33%	1	70.8983	72.176
<i>Geothrix</i>	1	GCAGCAGTTCTTGCGAAAAG	100%	0	72.2349	67.7641
<i>Terriglobus</i>	3	AGATTTACACGAACTTGCCA	100%	0	70.0416	58.9166
<i>Solibacter</i>	2	GTATTTCTACCGCGTTGAC	100%	0	71.8872	61.8409
<i>Fibrobacter</i>	56	GTGGCAACTGGAACGTG GTGGTTTACACACCGAAATGC	100%	0	70.0014	69.0179
<i>Gemmatimonadaceae</i>	1	CCGAGCTTTCACCACAC CAGAAGGTTTCGACATGATGT	100%	0	72.0144	61.1469
<i>Caldilinea</i>	1	GACAGATTGACTCCACC	100%	0	71.2825	66.629
<i>Dehalococcoides</i>	73	CGGTTAAGCCGGGAAATTC CAAGCTCCGAGTATCC	100%	0	70.6352	72.597
<i>Herpetosiphon</i>	2	ACATCATCGACCACCTACC	100%	0	73.6109	59.2842
<i>Oscillochloris</i>	4	AGTCGTCTCGTATCGGC	100%	0	70.218	61.203
<i>Roseiflexus</i>	4	TGCACCGTCATGTCCAG	100%	0	70.5173	62.9527
<i>Chloroflexus</i>	5	GACACCGTAACCTACC GGTAGACACGGGTAAC TAGACACCTGTAACCTACC	100%	0	70.0627	62.224
<i>Thermomicrobium</i>	4	GTGCCAGACATGTAAC TACCGTCGCTCGATCGT GTCTGGCCAGAAAATCACAA GACCTCTCCAAGTTAAGC	100%	0	70.989	66.8441
<i>Thermodesulfobacterium</i>	15	GCCTGGCTTAGCTATTCA AGTAAGGGTCCCTCC CAGAGCGCGTAGAGTAAAC TTCCACCAGGGTACAC	43.75%	0	70.0575	70.2938
<i>Geothermobacterium</i>	47	CAGAGCGCGTGAGAAAC CTGACTGGGCTCTTAAC GAGCGCGTGAGAAAACC	100%	5	71.1541	69.0875
<i>Gemmata</i>	4	CGGGGTACTTAGCATTTTAACT	100%	0	70.688	58.8411

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Planctomyces</i>	20	AGCCCAGTACACTGTCT AACGTTTCGCACAGTTCGTATTAC CTACGTCCGTGAGAACC CTGACAGTGGTTTACAACC GACAAGGCCAGTGT TTTCATCCCTCTGACAGC	95.24%	0	71.2616	71.1768
<i>Pirellula</i>	39	AACTAGAGATAAGGGTTTCGC CCACCATCAGTTCCGCTT TTTCCTCCCACTAACAGC GGTGTTCTGTATGATATCAAC TGTAAGGCTCCCGAAAAG CATCTCACCTGTAGAGC CAGGCGGAGACTTAATGG	80%	1	70.615	73.4314
<i>Isosphaera</i>	4	GCTAATCCCTGCATGTCAAG GCGCGTAGCCTCGAATTA	100%	0	71.785	67.6245
<i>Rhodopirellula</i>	12	GATCGGTCAAACATGATGGA CCATGCATTTCCTAACAACTGA TTCTCTGAAGGTCGGT	100%	2	70.5896	67.6147
<i>Blastopirellula</i>	1	CCTGTGAAAAGCTCTCC CCTCCACAGATAGGTCAA	100%	0	71.0818	60.7146
<i>Borrelia</i>	358	TGATATCAACAGATTCACCC TTTCCAACATAGGTCCACAGT CCGGGGCTTATTATATAAATTAACG	100%	1	71.2428	68.6304
<i>Spirochaeta</i>	49	TCATACCGCATTTCCC CTTATTGCTACCTGACGT CTCCTGTATTCAAGCCC TTCCAATGCGTGTCCACAG CCTGATAGGCTTATTCCTC CACCCCTTCACTATCATGTCAAA CACCGGTCTTTCAAACAAGAC ATGCGTGTCCGCAGTTAAG CCAATGCGTCTCTCCAG CTTTCACGAGAGACTTGGA CCCCAAGAAAAGAACTTTACAACG CCCCTCTGTGATTCAAGT AGCGTCAGTAAGAAGCCA TACCGTCATCACCCAGG GTCAGTATTCAGCCAGG TCCTCTGCTAACGTCATCG CAGTCATTGGCCAGAAGT GTTCAACCGATGGCAACA CAGCACTAGTAGTATCTAC	60%	1	70.0014	73.4616
<i>Treponema</i>	1049	GATTTACCCCTACACGC CCTCGCGTACCATCGAAT TCCTCAGCGTTACCTGT CAACCTTTCGGCCTTCC TTCCCTCCGTGATTCAAG GATATCTACAGATTTACCC CCCCTCCATGACTCT TGGTAACTGGCAGTAGG	96.57%	32	69.237	73.2618
<i>Brevinema</i>	2	CTCGCGCAACTCTAAGT	100%	0	70.287	56.733
<i>Leptosira</i>	224	TCCCGTTCACTACCCAC CCAACAACCTGGTAGACAAC	100%	0	70.8833	69.4592
<i>Turneriella</i>	1	ACGCCCTCAACGAACGT	100%	0	71.0597	60.3892
<i>Leptonema</i>	4	GGTGCTTCAGACAGGT	100%	0	73.1301	59.268
<i>Brachyspira</i>	197	GCCTATTTTAATAGGAGCACC CCGAGGCTTACATTATCTAC	100%	0	70.2933	59.5356
<i>Hydrogenobacter</i>	25	TGGGCAGTTCCGTATGC CCTTACGGACGACGTCT TACCGTCACCCACAGA	96.15%	1	70.6687	73.9789
<i>Hydrogenivirga</i>	4	CTCCAGAGGAACGAAAACC GAGTGCTCCCCAAAAGG GTTACCCGTGGCATGTCAA TTACCCCGCATGTCAA	100%	0	70.281	72.3246

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Thermocrinis</i>	57	CAAGACAGGCAGTTCGG AAGCGCCCATGGTTTACGG CCGTCATGCCCTGAGATT	96.55%	1	72.6751	75.9793
<i>Hydrogenobaculum</i>	296	CTCATACGAGGAAGCAAATAAGG GCCTACCTGAACAGGA	100%	0	72.6224	62.4697
<i>Aquifex</i>	5	TTCCACAACCTCCGCTTTCG	83.33%	0	70.3822	62.4812
<i>EX-H1 group</i>	4	AGGATAATACCTAGCCACATC	100%	0	71.4464	58.8174
<i>Thermovibrio</i>	1	TGCTTTCGGCAGGCTAC CTCAACCCTGACTTTCGC	100%	0	73.0127	66.226
<i>Desulfurobacterium</i>	7	CGTCACAGAAGTAGGCTG CCTAGACCAGCAGTATC GTAGAGGCTATTAACCCC	100%	1	71.002	69.6869
<i>Balnearium</i>	2	CACTTATGTGGCCAAACGC	100%	0	73.3214	67.0218
<i>Sulfurihydrogenibium</i>	84	TTACGACACAGGCTACTTTCG GACTACATTCGTAGCCCA GTGTTTACACCCCGAAGG	97.65%	119	70.0819	72.1182
<i>Persephonella</i>	10	CAGCCTAGCGACCTTTC CTTTCACCTTCCACTTACC	100%	0	70.9932	70.7435
<i>Hydrogenothermus</i>	138	AGTAGATTGCGCAACGTTTCG GTTAGCTTACGGCACGG	97.12%	58	71.9547	73.4943
<i>Thermosipho</i>	15	GTCATCACCGAGGCTTTC GCTTAGCGTCCAATTAACC	100%	0	71.0971	69.3684
<i>Thermotoga</i>	30	GCGTTCCTGCCGATATC CTCAGTACGGACGTCCA GGCTTAGCATCCAATTAACCA	100%	0	70.5518	68.5011
<i>Fervidobacterium</i>	11	TAGCGTCCTCACCTT AGTTTCCAGCGCATACC	100%	0	70.0427	67.2247
<i>Petrotoga</i>	5	CTTCAGCACAGAGAGTCT	100%	0	71.3676	60.4415
<i>Geotoga</i>	2	TTCTCCGTCTCGTCGA	100%	0	71.2156	69.2541
<i>Thermopallium</i>	1	CCTAAGTGAAGAGTCCC	100%	0	71.2865	59.7205
<i>Marinitoga</i>	3	GCACGAACGGTACTACC	100%	0	72.4885	56.5424
<i>Geovibrio</i>	1	TAAGGTTCTGCGGTTAGC	100%	0	71.0672	62.1742
<i>Deferribacter</i>	4	GGCCTTACTACGGTTGA	100%	0	70.6373	65.7593
<i>Flexistipes</i>	2	GTTATCAGCCAGGTAGC AGCCTTTAACCTCCGACTT GTGTAAGGCGTACTACC	100%	0	70.086	66.3829
<i>Mucispirillum</i>	8	GACTAATGACTTACAACCCG	100%	0	70.1433	55.2265
<i>Acidimicrobium</i>	8	GTACCGTCAGTTTCGTCC	100%	0	72.0967	73.8553
<i>Rubrobacter</i>	10	AGTTTCCACTGCACGCC TTTCCGTGCGCTTGCAG	100%	0	70.1111	70.7187
<i>Conexibacter</i>	2	CTATTCATCGCCGAAGCTTC TAGGCTATTAACTGTGAAGC	100%	0	71.3122	65.4226
<i>Thermoleophilum</i>	2	ACCGCTATTACGGACC	100%	0	72.1757	64.7149
<i>Solirubrobacter</i>	3	TCAGTAACGTCCAGCG	100%	0	71.3222	66.3166
<i>Olsenella</i>	11	TGTTTCAGACTCCTTTCGGC CCGGATTTGACTCCCGA TTCACCTAGATGTCAAGCC CCCTACCAAATCAAGC	100%	1	70.7847	72.7081
<i>Atopobium</i>	18	CGGAAAGTATAATCTCCCCAC TACCAAACTCAAGTCTGCC	94.74%	0	72.3395	71.544
<i>Slackia</i>	2	CAGTGAAGGCCAGA GGCTCACGGTGATGTCAA	100%	1	70.9252	65.1766
<i>Eggerthella</i>	9	CGAGGTTAGAGTTTC GCCCTCGGATTAGAGA	100%	0	70.1093	63.6202
<i>Coriobacterium</i>	3	GTCTCGTCCCTGCTGAAA CCGTCTGATCTCTCCC	50%	0	73.1965	72.5736
<i>Denitrobacterium</i>	3	ACCGTCAACTTCTTCCCTG GGATTTGAGATCCCGCT	100%	0	70.0993	65.9834
<i>Collinsella</i>	14	TTCCACCCGATATCTGC	100%	2	70.1328	64.1774
<i>Bifidobacterium</i>	141	AGCGATGGACTTTCACACC CATCCAGCATCCACC	99.30%	6	70.174	71.9121
<i>Gardnerella</i>	2	CACCCAAAAGGCTTGCTC	66.67%	0	72.6456	64.5752
<i>Alloscardovia</i>	3	ATCTCTGAGGCCATCGT	100%	5	72.4638	68.2009

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Metascardovia</i>	1	GCTTGCTCATGAACAAAAGC GAGGCAATCGTTAACATGTCA	100%	1	70.9895	66.7169
<i>Scardovia</i>	3	CGTTAAGCAGTAAGCTTTCACA	100%	1	71.5997	60.4301
<i>Aeriscardovia</i>	1	CTTGCTCCTTACCAAAAAGC	100%	0	70.5381	57.3642
<i>Actinomyces</i>	123	GCGGCTTGACCATGA CCTGTGATCTTGCCC AAAGAGGTTTACAACCCGA GATTTCACGACAGACGCGA CGCTTCTTTACCCACTACC TCCAGCTACCGTCAACC	89.52%	5	70.174	70.2938
<i>Arcanobacterium</i>	15	GGCACATCGCAGTGT CATGGAAAAGGCCCC CTACGGTACAGAACCCATG GAACCCATGGAAAGGCC	87.50%	0	70.245	68.2557
<i>Actinobaculum</i>	11	GTCCCAGCGTATGTCAAG CAGAACCCCGGAAAAGG CTGTACACCACCAACCAA	100%	0	71.1564	65.9334
<i>Varibaculum</i>	4	TAATCAACCCCTACACTCAAC	100%	0	71.5647	61.3496
<i>Mobiluncus</i>	24	CAGGATAGCATCCCCA	100%	0	70.1352	59.5045
<i>Actinoplanes</i>	111	ACGAGTTTTCGCGCATGT AGTCTCCGATGAGTCCC GACTGCAATGTCAGGGTT CTGAATGCTGGCAACATGG	44.64%	11	71.5055	71.8078
<i>Micromonospora</i>	267	CAAGTTTTGCGGCCATGTCAAA GGCAACATCGAACGAGG	95.90%	94	71.9262	70.3318
<i>Catenuloplanes</i>	6	GAGGATTGCGGTGATGTCA GGATTTACATTTCGACGCGA	100%	1	71.4107	70.1973
<i>Dactylosporangium</i>	16	CCCGTATCAGTGCAGG	94.12%	0	70.4105	67.9494
<i>Catellatospora</i>	12	GCATCTCTACGGCTTAAACG CAGTTAAGCCACGGGTTTTTC GTTTTACGGACGACGCAA TCTCTGACGAATTCCGGT	92.31%	1	70.2726	71.6224
<i>Pilimelia</i>	2	CAATATCGGCCAGAGAC	100%	1	72.667	70.2682
<i>Couchioplanes</i>	2	GCATCTCTGCGAGTTTTCGA	100%	7	74.2141	73.0108
<i>Virgisporangium</i>	3	CAAGCCCGCAGTTAAGCT	100%	0	73.2371	73.4616
<i>Verrucosipora</i>	6	ATCTCTGTGAGATTCCGG CTCTGCAGGATTTCGG	100%	6	70.8224	71.2182
<i>Salinispora</i>	33	TACCGTCACAAACGCTTCG	100%	0	70.178	65.7913
<i>Asanoa</i>	5	CTGGAAGTTTTGCGGTGATG	100%	0	70.5423	67.375
<i>Solwaraspora</i>	18	CAGTTGAGCTGTGGGTTT	100%	8	70.5726	71.0307
<i>Polymorphospora</i>	1	GATTTCCGCCCATGTCAAAC	100%	2	72.1235	69.6381
<i>Luedemannella</i>	2	AGTCGACGCAACAAGCC	100%	0	72.0233	71.9651
<i>Actinocatenispora</i>	3	AAACCGTGAGTGGTCC	100%	0	73.8631	71.0353
<i>Lechevalieria</i>	9	CTACCGGTTTCCAGTGC CCCCTGCCAAACTCAAG	90%	8	71.0571	69.4809
<i>Actinokineospira</i>	8	GATATCTCTACCGGTTTCCA AGTTTCCAGTGCATGTCAAGC	88.89%	11	71.0946	74.6756
<i>Lentzea</i>	20	CTCTAGAGCTTTCTAGTCC GCAAGCTTGGAGTTAAGC GAAGTCCCCACCTAG	80.95%	10	71.3796	71.5574
<i>Saccharothrix</i>	38	AGGCTCCACGTTAAGCG CTGTGAACAGCCACA	87.18%	14	70.2588	66.6885
<i>Actinosynnema</i>	7	CAATATCTCTACTGTTTCCAG	100%	11	70.7309	67.0418
<i>Amycolatopsis</i>	147	GCACGCTCCACGTTAAG ACAACGTGGATGTCGCC GTCTGGCGCATGTCAA GACAACGTGGAAGTCGC	90.54%	5	70.1982	66.5259
<i>Saccharopolyspora</i>	26	TGCTTCTTCTACACCTACC ACATGGGGCAAGGGT GTGGAAACAGTCCCC GGCTGTCTAGTGCATGT	92.59%	0	70.7435	71.4769

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Pseudonocardia</i>	113	GCACAGAGACCGTGGAAT CGTCAATTGCTTTCGTCC TCAAGTGATGCCCCTATCG CGGTCGAAAGAGGTTTACAAC GATGTCTGGTGCATGTCAAA	99.12%	1	70.8384	71.5097
<i>Actinopolyspora</i>	10	TCACCTTTACGCTTCGTCC GCTTCTTTAGCCCCCTAC	100%	1	70.2186	67.2799
<i>Saccharomonospora</i>	25	TCAAGTGATGCCCCTATCC CACGTGAACAGCATCCCA CTCAAGTGTGCCCGTATC GGACACGTGAACAGCATC	92.31%	7	71.6256	73.383
<i>Streptoalloteichus</i>	1	CATCTCTAGGGGTTTCC	100%	2	71.7929	68.4945
<i>Kibdelosporangium</i>	4	GAACCCGTGGAAGAGC	80%	0	72.0303	66.9044
<i>Thermocrispum</i>	2	GCGCTTAATGAGTTAGCTAC TACGTGCTGGCAACGTA	100%	0	70.7208	71.5574
<i>Kutzneria</i>	4	CATCTCTGCAGGTTTCCA CACAAGGGAGCCTACA CAGGCGGGGAATTAATGC CAAGGGAGCCTACATCT	80%	0	70.4374	74.1144
<i>Prauseria</i>	2	CCAACTAAATGACCCTGTATC	100%	52	70.3423	69.0902
<i>Actinoalloteichus</i>	2	TTCTTCGCGCTGAAAGAG	100%	2	70.1594	65.4037
<i>Crossiella</i>	1	GACTGCAAGCTCAGAGTTAA	100%	0	72.8116	71.8889
<i>Prauserella</i>	4	GTCAGTATCACCTTCGT	100%	0	72.1574	64.896
<i>Thermobispora</i>	5	GGCAACACAGGATGG GATCACAAAAGATCCCCAC	100%	0	70.0651	66.2321
<i>Jiangella</i>	1	GTACACGACCCTAAAAGG	100%	0	70.3974	57.2785
<i>Goodfellowia</i>	1	GAGGGTTTCCGGTGT	100%	0	70.2829	66.3806
<i>Streptomyces</i>	3012	CATCCGACGTGACAAGC GGAATTCGATCTCCCC CCTGTCTCCAGAGTTTCC GCTGGCAACACAGAACAA GTATCGACTGCAGACC	97.71%	117	70.1986	75.8029
<i>Kitasatospora</i>	61	CGTTTCCGGCGTATGTCAA GCCAGTTTCCGGTATATGTC	19.35%	1	70.3858	70.1575
<i>Streptomycoideis</i>	1	GTTACCGTCACGTTAGCTTC	50%	0	71.3448	68.865
<i>Streptacidiphilus</i>	49	TAGGGTTTTCCAGTGTATGT CTGGCAACACAGAACGAG	96%	3	70.049	66.6888
<i>Spirillospora</i>	2	GGTTCGTCCCTGTGTAAG	33.33%	0	72.0112	70.9423
<i>Thermomonospora</i>	12	CACCGCAAACCCGAGT GGTCTTCCATGAGTCC	84.62%	0	71.6864	72.9047
<i>Actinomadura</i>	92	GAATTCATGCTCCCTA CAGACCCACGGTTAAGC CTACCTGCCTCTAGTC GCGGAATCCGTGGAAGA	93.55%	16	70.5893	71.9085
<i>Actinocorallia</i>	12	GGTACCGTCTTGATTCTGTC TCAGATTCTGCTCCCTGCT CCGTCTGTTTTCTGTC	100%	3	70.5241	67.1515
<i>Streptomonospora</i>	6	GCCTGCTCAGTGGTAAA CTGCGTCGGTGGTAAAAGAG	100%	1	72.8238	71.0671
<i>Nocardiopsis</i>	126	GTTAGCCGGTCCTTATTCC GCGTTGGTGGTAAAAGAGGTTT	97.64%	8	73.8509	73.4866
<i>Thermobifida</i>	12	CTACCTCAAGCCTGC	100%	2	71.0735	64.6752
<i>Microtetraspora</i>	7	AAGCTTTCGGGTGTATGTCAA CATCTCTGAGGCTTTCC CAACGTTTACAGCGCGGA	75%	1	71.0149	71.1524
<i>Planomonospora</i>	4	TAAGCCCCAAGCTTTCAC AAGAGGCACCCATCTC	100%	55	70.0525	71.1751
<i>Streptosporangium</i>	55	ATCCAAAGGAGGCC AGCCCCAAGCTTTCACA	91.07%	4	70.1978	66.6159
<i>Microbispora</i>	22	CCGTAGTTAAGCCACGG AAAGGAGGCACCCATCT	91.30%	0	70.315	71.7888
<i>Herbidospira</i>	2	GAAGAGGCGACCATCT	100%	1	70.1561	66.1277
<i>Planobispora</i>	1	GCAGGTACACGTCACTTT GGTACACGTCAATTTCTGTC	100%	1	70.7573	73.1959

Continued

Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[T _m (PM)]	max[T _m (MM)]
<i>Planotetraspora</i>	2	TGTTTCCGGGTGATGTCAAAC	66.67%	0	72.6786	72.5818
<i>Nonomuraea</i>	43	AGCTGCAGCTTTCATGG CAACATGGAGCAAGGGTT GCTTTCCGGGTGATGTCAAA	88.64%	4	71.3734	73.2301
<i>Acrocarpospora</i>	5	CGTTTCCGGGTGATGTCA	83.33%	10	70.3605	70.1396
<i>Sphaerosporangium</i>	4	GTTAAGCCATGGGCTTCA TGTTACCGATGAATGTCAAGC	100%	10	70.8573	71.3122
<i>Tropheryma</i>	8	CTCTACGCCTTTCGGTATA	100%	0	72.424	71.0516
<i>Actinotalea</i>	2	GTCTCCATGAGTTTTCGGTATA TCTCCACGAGTTTTCGGTATAT	100%	1	70.8743	71.5601
<i>Oerskovia</i>	6	GCAGTCTCTTATGAGTCC	100%	3	72.6415	73.1288
<i>Cellulomonas</i>	74	CTCGAGTTTTACAGCAGAC GACCTTGCGGAACAACCA GAGCCTGAGGTTTTTACA CACTTTTCCGGTGATGTCAAG	66.67%	11	70.8066	72.6578
<i>Promicromonospora</i>	13	GTGTACGAGTGTCCAAAGAG GTCCAAAGAGAACCCC	92.86%	2	71.0889	71.6699
<i>Xylanimicrobium</i>	1	GCACTTTTCCGGTTCATGT	100%	0	71.5621	66.7389
<i>Isoptericola</i>	6	AAGGCCTTCTTCCCTGC CTGAGTGTTTCCGGTGC	100%	6	72.0694	73.7782
<i>Cellulosimicrobium</i>	24	CTGTGCACGAGTGTCCAA	96%	6	73.8492	71.9121
<i>Dermatophilus</i>	5	TACATCTCTGCACTTTTCCG	50%	0	70.1582	69.629
<i>Brevibacterium</i>	69	CCCGGAATTCACAGCAG TCCAGACTCCCCTAC CGTTAAGCGTTGCGTTTCCA TTCCAGAACGGTCCAGT	95.71%	1	70.2197	68.5683
<i>Nostocoida type II</i>	5	AACTCGTGAAAAGAGTCCCA ATACGAGTGTCCAAAGAGC	66.67%	1	70.4989	69.0655
<i>Oryzihumus</i>	2	GCGCTTCTTCGGTACTGAAA	100%	0	72.7495	68.5248
<i>Janibacter</i>	31	CCAAAGGGAGCATCC CTAAAAGGGGCACCC CTGTATACCGACCAAAAGGG TGGAATGAGGCCAC CTGTATACCGACCCAAAGG	87.50%	12	70.1752	73.8684
<i>Terrabacter</i>	23	CGACAAACCACCTACAAGC GGAAGTTTCCGGTATATGTCA	95.83%	33	70.9503	70.1763
<i>Knoellia</i>	7	CCATCTCTGAATGTTTCCGG	50%	0	73.307	73.7241
<i>Kribbia</i>	2	CCCATCTCTGGAAGTTCC AGTTGAGCCCCGGAATTTTC	100%	2	71.3477	74.9069
<i>Tetrasphaera</i>	13	CCATAACGCGCTGCAAC GTGAAAAGGGTCCCACA GAGCCCCGGATTTTACA	100%	59	70.3103	73.1958
<i>Phycococcus</i>	2	GCGTTCTCGCATATGTCAAG	100%	0	70.5118	63.0986
<i>Ornithinococcus</i>	6	TCGCTTCTTCAGAGCTGAAA	71.43%	1	70.6121	65.5261
<i>Serinococcus</i>	4	GATCGTCCGGTGTATGTC	80%	0	71.2156	71.0623
<i>Ornithinimicrobium</i>	5	TCTGGGAGTTTCCGGT GTCACAAAAGCTTCGTCAGA	100%	1	70.32	70.2008
<i>Jonesia</i>	5	TACAGGAATCGTGGAACAATC	100%	0	70.0334	61.2612
<i>Georgenia</i>	9	TTAAGCCTCGCGTTTTCACAG CTTGCGTTTTCACAGCAGA CAGGCGCAAGGTTAAGC	90%	0	71.3238	70.635
<i>Beutenbergia</i>	1	GGCTTCCCGTACATGTCA GCACCAAGTGTCCAAAGA	100%	0	72.2385	68.5182
<i>Kytococcus</i>	7	TGGAATGAGACCCACGC	100%	0	73.3242	70.3284
<i>Dermacoccus</i>	13	GCATGATTCGGGGT TCTGGCACATTCGGGT CTGCATGATTCGGGTGT	100%	6	70.5381	71.2187
<i>Rarobacter</i>	3	GCAGAACTCGTGGAAGA	100%	0	71.437	68.6182
<i>Sanguibacter</i>	9	ATCACTCCCTATCTCTAGG GATGCAAGCTCAAGGTT TATGAGTCCCCACCC	90%	8	70.3695	71.2643
<i>Yania</i>	1	CATAGAATAGGGGTTCCGC	100%	0	72.9846	65.4837

Genera	Targets	Probe sets	Coverage	Cross hybridization	Continued Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Brachybacterium</i>	42	CTTCCCTACTGAAAGGG GCACCAGTCCGAAGA ACCTGTGAACCAGTCC CAGTCCAAAGAAAAGCCACAT	97.67%	4	70.3536	69.9741
<i>Dermabacter</i>	6	GCAAGCCCAACGTTAAGC CAGTCCAAAGAAAACCACATCT	100%	0	71.7384	68.858
<i>Micrococcus</i>	108	ACTTTCACGACAGACGCGA	70.64%	9	71.639	71.1578
<i>Citricoccus</i>	12	CTGTGGCGATCGAGAAC CGGTCGGGAACATGTC AA	100%	9	70.9266	68.0964
<i>Kocuria</i>	86	AGGAGTTCAGTTCCTC TACCGTCACTTGTGGCT GTACTGTCACTGTGGCT GTTTGCTGTTAACAACCATGC GCTTCTTCCGTGCTGAAA	59.77%	4	70.0375	70.5036
<i>Arthrobacter</i>	580	GTAAACCGACCGCAAGC CCCTACATCACTCTAGT GTACCCACTGCAGAAC ACCTGTAAACCGGCCAC CAGTTAATGCCAGAGAC	92.25%	30	70.0393	70.8933
<i>Renibacterium</i>	10	CAACTAAGGATCAAGGGTTGC AGTCCCCACCATAACGG CACCTGTGAACCAACCCAAA	100%	0	72.3599	72.5779
<i>Nesterenkonia</i>	23	CAGCAGACGTGGCAAAC GCGGTCCAGTGTATGTCA CATAGGATAGGGGTTGC GTACCGTCACAAATAGCTTCT	66.67%	19	70.5022	71.0459
<i>Rothia</i>	28	TCGATGCTAACAGAGGTTTAC GTCAATCTCTCTTCTCC GTACCCACTGCAAACC TTCGATGCTAGCAGAGG	89.66%	2	70.6754	70.6299
<i>Frigoribacterium</i>	17	ACCATCTCTGGACGTTTCC CTAGTTCCCAACGTTTCACG	50%	0	70.3218	73.9606
<i>Leifsonia</i>	44	GAGTGTCCAAAGAGTTGAC AAAGCACATCGCTTCTTCC	80%	32	71.295	71.5753
<i>Agreia</i>	7	CATTTCTGGCCCATCTCG	25%	0	71.8946	67.2804
<i>Subtercola</i>	5	GTACCGTCAAACCGAAGTTC AAACCGAGGAATGGCCC	83.33%	0	70.4423	70.7405
<i>Plantibacter</i>	8	CCCAATTACGTGCTGGC TCTTCCCTGCTAAAAGAGG	88.89%	14	71.9768	71.8637
<i>Rhodoglobus</i>	1	CCGTCATGATGTGACAAGC	50%	2	72.6456	72.2452
<i>Okibacterium</i>	2	GAGTGTCAAAGAGTTGACCATT	66.67%	0	72.489	68.3274
<i>Clavibacter</i>	39	GCACGTTTCCGGTATATGTCAA CAGGCGGGGAATTAATGG	35%	2	72.9806	73.662
<i>Rathayibacter</i>	12	TGCACCCCTACGTAATTAC TCAAGCCGAAGCCCTT AAAGGTCCCACATCTAGT	92.31%	0	70.1759	74.5631
<i>Cryobacterium</i>	18	GTTTCCTCTATATGTCAAAGCC ACAGGAGACGCAACAAACC CAGGAAAACCCAGTCGCT	89.47%	9	73.064	70.4709
<i>Curtobacterium</i>	70	TTTCACAGCAGAGCGACA GAAACCGTGGAAGGTCC	88.73%	1	70.4832	68.2194
<i>Leucobacter</i>	21	AACAGGCCCTACATCTAG GTGTCCAAAGAGTTCTGTATC	100%	0	70.437	70.2861
<i>Rhodococcus</i>	484	GTATATGTCAAACCCAGGTAAG AGGAACCCACACCTAGC TCTACGGTTTTCCGGTGTATG CACATCTCTGCAGTCGT	84.54%	45	70.1799	73.8703
<i>Nocardia</i>	483	CGTGTTTCTGGTGTATGTCAA CGCTTTCGCTTCTCAGC ATCCCCTGCAAGCTTAAGG GACGAACCGCCTACAAG CAAGTTTTCACAAACGACGCGA GTCCCTGTCAAAGCGG	97.31%	36	70.0014	72.8388

Continued						
Genera	Targets	Probe sets	Coverage	Cross hybridization	Specificity	
					min[Tm(PM)]	max[Tm(MM)]
<i>Gordonia</i>	127	GCACGCCTACAATTGAGTTG	98.44%	12	71.9079	74.5073
		AACCACAAGGGAACGACTATC				
		TACGGCACGGAATCTCGT				
		AAGTCCCCGGCATAACC				
<i>Millisia</i>	1	GATTCCACAGACGACGC	100%	0	71.0486	67.781
<i>Skermania</i>	12	TCCCAACCGAAAGAGGT	100%	0	71.238	66.3365
<i>Tsukamurella</i>	36	CACCACCTGTATAGAAGG	100%	0	70.8785	69.4897
		GGCACAAGGCAAACCAC				
		GGCAACATGAGACAGG				
<i>Dietzia</i>	61	GGATTTCACATCTGGCTTAACA	100%	1	71.1747	71.0777
		GGCAACTATGAGACAGG				
		CTGTATCTCTACAGCTTTCTG				
<i>Williamsia</i>	11	ACGCTTGAGTTAAGCC	100%	0	70.0427	68.9434
		CACGCCCGATGTTAAGCA				
		CTTTCCAGTCCATGTCAAACC				
<i>Segniliparus</i>	3	ACCCACAAGGGAACGATCT	100%	0	74.2141	70.1287
<i>Corynebacterium</i>	401	AGACGACGCGACAAACCAC	95.52%	7	70.0933	72.8885
		TACTCAAGTTATGCCCGTATC				
		CACAAGGGAACTACATCTCTG				
		CACTCAAGTTATGCCCGTAT				
		CCCCTACAGCACTCAA				
<i>Mycobacterium</i>	634	GTTAAGCTGTGAGTTTCACGA	95.27%	2	70.221	71.8628
		CCAAGGAAGGAAACCCAC				
		GTCGATGGTGAAAGAGGT				
		CCAAGGAAGGAACCC				