

probeBase—an online resource for rRNA-targeted oligonucleotide probes: new features 2007

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ABSTRACT

probeBase is a curated database of annotated rRNA-targeted oligonucleotide probes and supporting information. Rapid access to probe, microarray and reference data is achieved by powerful search tools and via different lists that are based on selected categories such as functional or taxonomic properties of the target organism(s) or the hybridization format (fluorescence *in situ* hybridization or microarray) in which the probes were applied. Additional information on probe coverage and specificity is available through direct submissions of probe sequences from probeBase to RDP-II and GreenGenes, two major rRNA sequence databases. A freely editable user comments field for each probe entry allows any user to add, modify or remove information or to report errors in real-time. probeBase entries increased from 700 to more than 1200 during the past three years. Several options for submission of single probes or entire probe sets, even prior to publication of newly developed probes, should further contribute to keeping probeBase an up-to-date and useful resource. probeBase is freely accessible at <http://www.microbial-ecology.net/probebase>. Email correspondence can be addressed to probebase@microbial-ecology.net.

INTRODUCTION

Numerous ribosomal RNA (rRNA)-targeted oligonucleotide probes have been developed and are widely employed in a variety of hybridization formats for identification (1–3), quantification (4) and physiological characterization (5) of cultivated and un-cultivated organisms in environmental, bioengineered and clinical settings. In order to release researchers from the necessity to tediously survey the literature for already published probes, probeBase, an internet database for rRNA-targeted oligonucleotides [NAR Molecular Biology Database Collection entry number 390 (6)], was established in 2002 to provide a freely accessible,

rapid platform to search for published probes and annotated/pre-calculated information and to facilitate an up-to-date evaluation of probe coverage and specificity (7). Suitable probes in probeBase can be found via the ‘Search’ tool by indicating the name of a probe, a target organism, a certain probe target site or by searching for references. The ‘Match’ service can be used to easily retrieve all probes that are fully complementary to up to 150 query rRNA gene sequences without prior aligning and phylogenetic analysis.

Today, probeBase contains 1258 probes and 8 microarrays from 266 references (status October 2006) and is frequently used by researchers worldwide. For example, probeBase user statistics (accessible on the start page) have recorded an average of 723 hits (corresponding to 554 opened pages) per day in the year 2005.

Here we describe novel features and search services that were implemented into probeBase since 2003, accompanied by significant layout and interface changes, which should collectively enhance data interconnectivity and usability of the database. Furthermore, we present a meta-analysis of all probes deposited at probeBase and have evaluated the *in silico* specificity of frequently used probes targeting major bacterial groups.

NEW DATA AND ANALYSIS TOOLS

Additional probe features

A distinctive accession number that begins with the prefix ‘pB’ is now assigned to each probe, which is essentially defined by its unique sequence. For example, the probes EUB338, EUB338-II and EUB338-III, together targeting most Bacteria (8), have the accession numbers pB-00159, pB-00160 and pB-00161, respectively. This minor but very important new feature of probeBase prevents potential confusion in cases where the same name was incidentally used for more than one probe, especially if a new probe is just a modification of an already existing one.

probeBase now also includes data on rRNA-targeted microarrays [so-called PhyloChips e.g. (9)]. Entries for probes that are used on microarrays contain links to more detailed information on the respective microarray, including a brief description, the technology applied, bibliographic data and

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a list of all probes used on this particular microarray. probeBase will generally host and welcome submission of all microarrays that consist of rRNA-targeted oligonucleotide probes with hierarchically nested and identical specificity for phylogenetically defined groups of organisms. Microarrays with random probes will not be accommodated.

Theoretical thermodynamic probe parameters according to Yilmaz *et al.* (10) have been pre-calculated and added to each probe entry. The standard free energy changes ΔG_1 and ΔG_2 for probe/rRNA duplex formation and intramolecular probe folding (hairpin formation), respectively, and the thermodynamic affinity of a probe to a hypothetical perfectly accessible target site, ΔG_{12} , should aid in the optimization of existing probes, which have so far not been tested for fluorescence *in situ* hybridization (FISH) or which show only weak fluorescence signals [See (10,11) for details].

Furthermore, each probe entry contains a so-called Wiki user comments field, which allows users to instantly add, modify or remove information on the probe and its application without the need for prior registration. The opportunity to easily annotate probes in real-time should promote up-to-dateness of probe information, reporting of bugs and errors in the database or the original publication, and interactions among probe users. This combination of community driven and protected content as realized in the current version of probeBase should generate an optimal resource, less susceptible to abuse than pure Wiki systems.

Each probe entry contains links to directly match the probe sequence against RDP-II and Greengenes, major encompassing, regularly updated 16S rRNA sequence online databases (12,13). probeBase users thereby benefit from the more efficient probe match tool that was recently implemented in RDP-II to directly match probe sequences against *Bacteria* and for the first time are able to match probe sequences against *Bacteria* and *Archaea* at Greengenes. These tools enable researchers to quickly check the current *in silico* specificity and coverage of a probe and thus to validate its originally intended specificity—a step that becomes increasingly important with ever-accumulating rRNA sequence data in public databases.

Search probeBase

In order to extend the search options of probeBase, it is now also possible to search for a certain probe sequence, the accession number of a probe and references. Regarding the latter option, users can survey all references (including titles, authors and abstracts) that are available in the database via a simple key word search. probeBase then returns a list of references and all probes originally described therein, allowing a quick overview of all probes that were published by a certain author. Finally, all search options can now also be restricted to only those probes that were already applied for FISH or microarray hybridization.

Lists and probe categories

A new option to view and retrieve probes, references and microarray layouts from probeBase is the 'Lists' service. The following dynamic lists, (pre)compiled according to

different criteria, are currently available (suggestions of new probe categories by users are welcome).

- (i) Lists of probes that target a certain functional or taxonomic category of organisms (e.g. sulphate-reducing prokaryotes or organisms of medical/hygienical relevance)
- (ii) A list of all probes
- (iii) A list of all references
- (iv) A list of all probes that were successfully used for FISH
- (v) A list of all rRNA-targeted oligonucleotide microarrays
- (vi) A pre-calculated, but regularly updated list showing the coverage of selected probes targeting major taxonomic groups of bacteria (exemplified by Table 1)

Because probes targeting major taxonomic groups of bacteria are those most frequently applied in ecological, biotechnological and clinical studies, we have re-evaluated their *in silico* coverage against the RDP-II database [release 9.41, (12)]. A few selected probes with a good coverage of >70% for the respective target taxon are exemplarily shown in Table 1. Knowing the exact target range of a given probe is vital for meaningful interpretation of the hybridization results.

Distribution of probes along the 16S/18S rRNA sequence

Using the roughly 1200 probes currently available at probeBase we have analyzed the distribution of their respective binding sites on the 16S/18S rRNA sequence (Figure 1). The number of all probes and FISH probes covering a certain target position on the 16S/18S rRNA displayed a weak but significant positive correlation with the variability of the respective sequence position (the non-parametric Spearman-Rho correlation coefficients were 0.404, $P < 0.001$ and 0.410, $P < 0.001$, respectively). Most probes in the database target highly variable sequence regions, e.g. around *Escherichia coli* position 450 and 650, while conserved regions, e.g. around position 350 and 1400 are typically occupied by only few probes that are specific for higher taxa, such as the bacterial probe EUB338 or the universal probe UNIV1390. This analysis may provide guidance for the development of novel probes and the selection of suitable target sites.

SUBMISSION OF PROBES

Several options are provided on the probeBase website for submission of single probes and whole probe sets, including microarray (PhyloChip) probes. It is also possible to deposit newly developed probes in probeBase already prior to their publication in a scientific journal, thereby apprising probeBase users of the new probes and the upcoming publication. In this case, the information on the actual probe sequence might be hidden until the day of publication, and only the short name, the specificity, and the target molecule of the probe are displayed. Additionally, the contact details of the person who has deposited the probe are shown.

AVAILABILITY, ERROR REPORTING AND CONTACT

probeBase is maintained by the Department of Microbial Ecology, University of Vienna, Wien, Austria. Data and

Table 1. Selected *in silico* coverages of published group-specific probes in probeBase, as analyzed with the RDP II probe match tool

pB accession number	Probe name	Specificity ^b	Reference	Probe evaluation with RDP II probe match ^a	Coverage ^c (%)	Hits in target taxon ^d	Total non-target hits ^e	Major non-target taxa ^f
				RDP II target taxon				
pB-00327	UNIV1390	all organisms	(16)	domain <i>Bacteria</i>	92	75934	NA ^g	—
pB-00159	EUB338	<i>Bacteria</i>	(17)	domain <i>Bacteria</i>	90	169389	NA ^g	—
pB-00021	ALF968	<i>Alphaproteobacteria</i> , except of <i>Rickettsiales</i>	(18)	class <i>Alphaproteobacteria</i>	79	9750	1876	class <i>Deinococci</i> (38/364), phylum <i>Proteobacteria</i> (11138/51072), class <i>Fibrobacteres</i> (48/53)
pB-00432	DELTA495a	<i>Deltaproteobacteria</i>	(19)	class <i>Deltaproteobacteria</i>	73	4236	729	phylum <i>Gemmatimonadetes</i> (343/391), phylum <i>Genera_incertae_sedis_W53</i> (23/68)
pB-00050	CIV/V/1342	heterocystous strains of cyanobacteria (sections IV and V)	(20)	<i>Cyanobacteria</i> subsection 4	96	491	36	—
pB-00044	CFB1082	class <i>Bacteroidetes</i>	(21)	class <i>Bacteroidetes</i>	85	8485	3	—
pB-00046	CFB563	<i>Flavobacteriales</i>	(21)	order <i>Flavobacteriales</i>	84	3301	302	—
pB-00718	GNSB-941	<i>Chloroflexi</i> (green nonsulfur bacteria)	(22)	phylum <i>Chloroflexi</i>	94	1240	816	phylum <i>Thermomicrobia</i> (8/11), phylum <i>Genera_incertae_sedis_Dehalococcoides</i> (81/89), unclassified <i>Bacteria</i> (706/6563)
pB-00645	GSB-532	<i>Chlorobiaceae</i> (green sulfur bacteria)	(23)	family <i>Chlorobiaceae</i>	91	221	0	—
pB-00283	PLA46	<i>Planctomycetales</i>	(24)	family <i>Planctomycetales</i>	75	599	310	phylum <i>Leptisphaerae</i> (38/54), phylum <i>Genera_incertae_sedis_BRC1</i> (10/11), phylum <i>Genera_incertae_sedis_W53</i> (21/25), unclassified <i>Bacteria</i> (232/6286)
pB-00049	ChIs-0523	<i>Chlamydiales</i>	(25)	order <i>Chlamydiales</i>	93	189	21	—
pB-00183	HoAc1402	phylum <i>Acidobacteria</i>	(26)	phylum <i>Acidobacteria</i>	95	693	718	unclassified <i>Bacteria</i> (602/3249)
pB-00056	CYA361	phylum <i>Cyanobacteria</i>	(20)	phylum <i>Cyanobacteria</i>	91	4043	438	phylum <i>Firmicutes</i> (143/49033), unclassified <i>Bacteria</i> (254/10078)
pB-00435	NTSPA714	phylum <i>Nitrospira</i> , not <i>Thermodesulfobrio islandicus</i>	(19)	phylum <i>Nitrospira</i>	71	373	4	—
pB-00535	TDSBM1282	genera <i>Thermodesulfobacterium</i> and <i>Geothermobacterium</i>	(19)	phylum <i>Thermodesulfobacteria</i>	84	36	0	—
pB-00601	TM7522	candidate phylum TM7	(27)	phylum <i>Genera_incertae_sedis_TM7</i>	92	264	12	—

Note: The RDP II probe match only includes bacterial but no archaeal or eukaryal small subunit rRNA sequences in the analysis.

^aRDP II probe match was performed with database release 9.41 (July 28, 2006) containing 253 813 bacterial 16S rRNA sequences. The search for each probe was restricted to sequences of good quality with data in the respective probe binding region.

^bThe intended specificity of the probe.

^cThe percentage of sequences within the RDP II target taxon that show a full match to the probe sequence.

^dThe number of sequences within the RDP II target taxon that show a full match to the probe sequence.

^eThe number of sequences outside the RDP II target taxon that show a full match to the probe sequence.

^fIf applicable, additional information on the major non-target taxa are provided. The number of sequences that show a full match to the probe versus the total number of sequences within a non-target taxon are shown in brackets.

^gNA, not applicable because RDP II only contains bacterial 16S rRNA sequences.

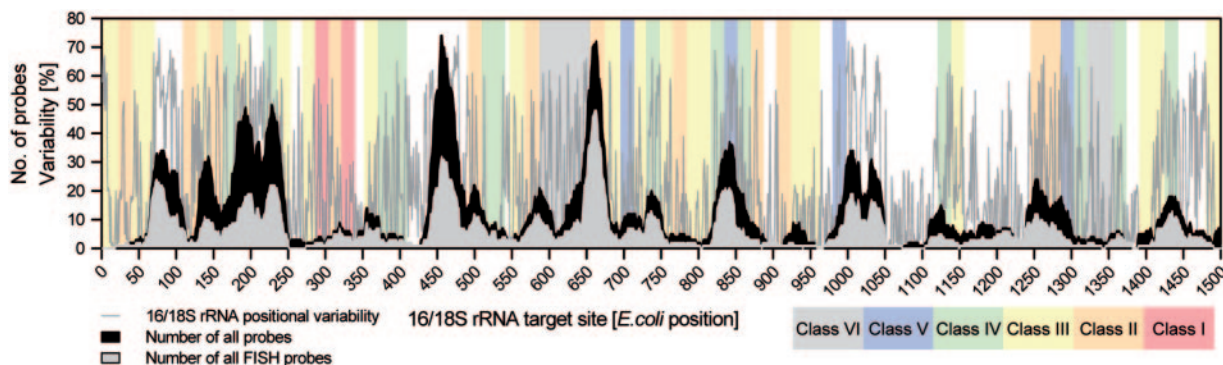


Figure 1. Number of probes per target site and positional variability of the 16S rRNA molecule (status July 2006). Positional variability profile of the 16S/18S rRNA in percentage was created using the ARB database “ssu_jan04_corr_opt.arb” (14). Background colors indicate the different fluorescence brightness classes (I, bright to VI, dim) according to the consensus accessibility map of bacterial and archaeal 16S rRNA for FISH probes, established by Behrens *et al.* (15); uncolored regions were not analyzed.

analysis tools are available at <http://www.microbial-ecology.net/probebase/>. We welcome questions or comments concerning probeBase and highly appreciate reports of bugs, errors or missing probes. You may contact us by email to probebase@microbial-ecology.net.

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