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May 29, 1997

Dr. M. Kalin Boojum Research Ltd., 468 Queen St. East Ste 400 Box 19 Toronto. Ont M5A 1T7

Fax: 416-861-0634

Dear Dr. Kalin (Margarete):

Please find enclosed a copy of our final report on the Bacterial Consortia in Acid Mine Groundwater Seepage.

I will be happy to answer any specific question which you may have. I hope this preliminary work sets the stage for further collaboration.

Sincerely,

Peter C.K. Lau. Ph.D

cc. A. Pilon E. Raymond





Institut de recherche en biotechnologie

Biotechnology Research Institute

Canada

National Research Council



Bacterial Consortia in Acid Mine Groundwater Seepage

Final Report (26 pages)

NRCC # 40499

Submitted to:

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Submitted by:

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May 28, 1997



BATERIAL CONSORTIA IN ACID MINE GROUNDWATER SEEPAGE

MAY 28, 1997

Executive Summary

What is expected to be there (bacteria) is there and much more. This study reiterates the versatility and powerfulness of the 16S rDNA sequencing technology in the identification of microbial community in a natural environmental setting without prior cultivation. The challenge at hand is to assess activity and abundance of the culprit and beneficial bacteria in the groundwater seepage path and develop a knowledge-based strategy for the reduction of acidity to combat acid mine drainage problem. The importance of a multidisciplinary approach in this effort cannot be overemphasized.

Background (with notes from M. Kalin):

Ground water seepage originating from pyritic mining wastes (tailing ponds, waste rock piles and mine workings) will likely at one point of time emerge as surface water. It is proposed to treat the seepage while it is contaminated groundwater, resulting in discharge of greatly reduced contaminants to surface waters. The task at hand is to identify the microbial consortia which are present in the groundwater seepage path. This prerequisite information will serve as a base for treatment approaches that can be formulated and a proposal involving a multidisciplinary team developed for 1997 and beyond.

It is envisaged that technology development will take about 3 years. The South Bay site outside Toronto is to serve as a test site. In this site, a seepage path from a tailings pond to an adjacent lake is well defined hydrologically and stratigraphically. Monitoring methods for changes in seepage quality are also in place. Piezometers have been monitored for sometime. The anticipated flow of the groundwater is "clean" water at M28 (Fig. 1), it then flows to the section where it gets contaminated, in the vicinity of M5, M85. M39(A) is the deepest part of the "Kalin canyon" from which the water flows to wards M80 which is in the gravel pit and Kalin canyon. Then the water flows to M60 which is located in the muskeg surrounding Mud Lake. From there it flows towards the outflow M63 passing M62.

The present report deals with a molecular analysis of the bacterial consortia in five water samples collected from sites, M28, M39A, M60A, M63 and M80 as described above. The molecular analysis makes use of 16S rDNA sequencing which is a well established method for identifying bacteria in the characterization of microbial ecology and community structure. This method which uses polymerase chain reaction (PCR) to generate ribosomal DNA (rDNA) of community nucleic acids, has substantial advantages over traditional culture-based methods since it is not dependent on growth and culturing conditions and hence it is generally accepted that environmental diversity is better represented than what cultural diversity is able to portray.

Materials and Methods

Water samples were collected by the Boojum personnel on September 9, 1996. They arrived in Montreal the same day in a cooler containing 40 X 250-ml plastic bottles which were completely filled. The cooler was kept in freezer overnight until processed the following morning. One litre of each water sample was filtered through a Millipore membrane of 0.22 μ m and 5 cm diameter. The filters gave different shades of rusty orange color, a reflection perhaps on the purity of the water sample. The filters were then kept in sterile petri dishes at -20 °C. The pH of the filtrates was 7.5 for M28 and 6.5 for the remaining samples.

Fig. 2 shows a flow chart of the main events leading to the characterization of

microbial communities without cultivation. For extraction of nucleic acids the membranes were thawed. One-half of each membrane was processed as follows; the other halves were kept for further use. The surface of the filterwas washed with 2.5 ml of 0.75 M sucrose, 50 mM Tris-HCl pH 8.3, 40 mM EDTA and 1 mg/ml of lysozyme onto a sterile petri dish. With a razor blade materials from the surface of each membrane were scrapped and the suspension from'each was transferred to individual 15 ml tubes. Incubate for 30 min at 37°C to mix. Add 400 µg of Proteinase K and SDS at a final concentration of 0.5%. Incubate for 2 hr at 37°C while mixing every 30 min. Freeze each mixture in dry ice for 15 min and then thaw at 60°C for 10 min. Adjust the extraction mixture to 0.7MNaCl with a stock solution of 5M. Add CTAB at a final concentration of 1% (w/v) to precipitate polysaccharides and proteins. Incubate at 65°C for 20 min. Add an equal volume of phenol:chloroform:isoamyl alcohol (50:49:1), mix well and spin in a refrigerated centrifuge (4°C) for 10 min at 7000 rpm. Discard lower phase. Extract aqueous phase with a volume of chloroform: isoamyl alcohol (49:1), mix well and centrifuge as before. Precipitate nucleic acids with 0.6 volume of isopropanol at -20°C overnight. Centrifuge at 8400 rpm at 4°C for 10 min. Wash pellet with 70% ethanol and resuspend nucleic acids in 100 µl sterile distilled water.

Amplifications of 16SrDNAs from the various samples were carried out by polymerase chain reactions (PCR) as follows: the eubacteria primers 27F and 1492R (15 pmole/reaction) which would amplify near full length 16S rDNA were used for each genomic preparation (15 μ I DNA/reaction). In each reaction mixture of 100 μ I volume add 100 μ M dNTPs, 10X Taq polymerase buffer (Pharmacia) and 5U of Taq polymerase (Pharmacia). Conditions of amplification are: 1 cycle of 2 min at 94°C and 35 cycles of 1 min at 94°C, 1 min at 50°C and 2 min at 72°C. Keep reactions at 4°C.

Universal 16S small subunit (SSU) rDNA library construction: The PCR amplified products were separated on 0.8% agarose gel in 1X TBE buffer. The desired -1.4 kb bands were excised from the gels and purified with QIAEXII resin which was purchased from QIAGEN. pBlueScriptKSII vector (3.199 kb; Promega) which provides blue-white selection was used for cloning after digestion with *Eco*RV to linearize the plasmid and addition of a thymine to the 3' cohesive ends. Ligation with the PCR amplified fragments was carried out by routine procedures and the mixture was transformed in *Escherichia coli* DH5 cells. Recombinants were selected as white colonies when plated on XGal-containing media. The recombinants were then screened for the presence of 4.6 kb plasmids by direct lysis on gels. Positive candidates containing the expected plasmid size were purified on QIAprep8 columns (QIAGEN).

Using 27F oligomer as primer these DNAs were sequenced with an Applied Biosystems model 373A automated fluorescent sequencer and the AmpliTaq DNA polymerase FS system from Applied Biosystems (Perkin Elmer Canada Ltd.). The 300-500 bp generated sequences were analyzed by the BLASTN program available from the National Center for Biotechnology Information (NCBI). Phylogenetic trees were not constructed since we are only interested in the identity of the bacterial samples.

Results and Discussion

The number of recombinants from each transformation varied from 4 to 135. In descending order these numbers are in the following parentheses according to sampling sites: M28 (135), M63 (85), M39 (47), M60 (27) and M80 (4). The "clean" M28 sample gave the highest number of colonies. Whether this is a reflection of ease of preparation of nucleic acids from this relatively clean sample is not clear.

A total of 105 sequencing reactions were carried out. Forty of these actual 16S rDNA sequences are provided in Appendix I. In each case the five sequences (with their corresponding database and accession numbers) which gave the highest scores and probability according to the BLASTN search program are given. **Table 1** lists the most probable bacterial species in each of the given sample according to their location. Complete files of these searches are available upon request. A list of bacteria with the number of occurences is shown in **Table 2**.

Some general observations:

1. No *apparent Eucarya*. As this represents the eukaryotic arm (fungi, plants, animals) of the life's three domain tree absence of these memebrs may not come as a surprise.

2. No apparent Archaea. Members of the Archaea include Sulfolobus, Methanococcus, Thermococcus etc. This domain has been referred to as "life's last domain" or "the third form of life" as a result of sequence analysis of the first archaeal complete genome viz. that of Methanococcous jannaschii. This group of supposed bacteria (initially termed archaebacteria) as Carl Woese predicted is neither typically bacteria nor typically eukaryotic, but in between.

In this study, no apparent archaea was detected. This would be expected since one of the two PCR primers used for amplification was not specifically designed to pick up members of this domain. Several archael sequences have been found recently in soil.

3. Bacteria (of the interesting kind) galore.

i) Zooglea/Cytophaga/Flexibacter/Flavobacterium.

[Descriptions of the bacteria listed below are according to Bergey's Manual of Determinative Bacteriology].

The *Zoogloea* genus occurs free-living in organically polluted fresh water and in waste water at all stages of treatment. The type species in *Z. ramigera*. These organisms are actively motile. Although aerobic, growth can occur anaerobically in the presence of nitrate (nitrate respiration). Denitrification occurs with the formation of nitrogen.

The genus Cytophaga is strict aerobe or facultative anaerobe. Some may use nitrite

(NO;) as terminal acceptor. These chemoorganotrophs are commonly found in soil, decomposing organic matter, freshwater and marine habitats.

Flexibacter genus is also chemoorganotrophic. Like cytophaga these species are strictly aerobic or facultatively anaerobic. They are also found widely distributed in soil and freshwater.

Flavobacteria are aerobic but nonmotile. They are chemoorganotrophic and are also widely distributed in soil and water, although also found in raw meats, milk and other food.

ii) **Gallionella ferruginea**. This stands out in the crowd as well. G. ferruginea is a type species of the genus Gallionella. These are strictly aerobic and microaerophilic. Chemolithoautotrophic. Only Fe²⁺ serves as electron donor. It is typically found in oligotrophic ferrous iron-bearing waters; optimally Eh +200 to +300 mV. This is one of the most important iron bacteria, forming large masses of ferrihydrite in bodies of water and water supply systems. This is probably one of the major culprits for the dirty water samples.

iii). **Thiobacillus** ferrooxidansand **Acidophilum**. These are culprits as well. Bad to have but good to see them as an indication that the 16S rDNA technique which negates cultivation of the organisms works. *Acidiphilium* is common in acid mineral environments such as pyritic mine drainage and copper and mine tailings. It may be isolated with *T. ferrooxidans* in Fe²⁺ enrichment cultures, and a common contaminant in these cultures. Acidiphilium is chemoorganotrophic unlike *Thiobacillus* which may be obligately chemolithotrophic.

iv) **Desulfitobacterium**. These species are reported to be most commonly found in anoxic marine or brackish sediments. A number of types have been found in anoxic frshwater sediments. These are strictly anaerobic. Sulfate and in some cases also sulfite and/or thiosulfate are reduced to hydrogen sulfide. Sulfate reducing beateria (SRBs) are good to have since a common strategy of reducing acidity is to promote the growth of SRBs.

v) *Nitrosolubus/Azorcus*. *Nitrosolobus* (type species *N. multiformis*) was reported to be isolated exclusively from soil. This cannot be completely true. These strains utilize urea as ammonia source and can assimilate limited amounts of organic compounds. *Azorcus* is a recently described genus for a special group of denitrifiers capable of metabolizing toluene.

vi). *Paracoccus denitrificans*. This is a type species of *Paracoccus. Paracocci* are nonmotile and aerobic. Nitrate is reduced to nitrous oxide and molecular nitrogen under anaerobic condition. Although not halophilic, this genus is found to occur in soil and presumably in natural and artificial brines.

in groundwater

M28: 28.1-Zooglea ramigera/P. lemoignei 28.2-P. lemoignei/Z. ramigera 28.3-Flavobacterium 28.4-Flavobacterium 28.5-Helicobacter hepaticus 28.6-Helicobacter hepaticus 28.7-R. fermentans? 28.8-Flavobacterium 2M28.4-Zooglea sp. 2M28.5-Zooglea sp. 2M28.6-Zooglea sp. 2M28.9-Zooglea sp. 2M28.12-Zooglea sp. 2M28.16-Zooglea sp. 2M28.20-Zooglea sp. 2M28.11-Cytophaga sp. 2M28.18-Flavobacterium M39: 39.1-Cytophaga 39.2-Cytophaga 39.4-Cytophaga/Flexibacter 39.5-Zoogloea ramigeraa 39.6-Streptococcus gordonii 39.7-Cytophaga 39.10-Flavobacterium 39.11-Flavobacterium/flexibacter 39.12-Cytophaga 39.13-Zoogloea ramigera 39.14-Cytophaga 39.15-Cytophaga 2M39.4-Cytophaga 2M39.5-Zooglea ramigera 3M39.6-Alcaligenes 3M39.9-Gallionella ferruginea 3M39.8-Gallionella ferruginea 3M39.10-Gallionella ferruginea 3M39.15-Flavobacterium 3M39.73-Flavobacterium 3M39.74-Flavobacterium 3M39.75-Flavobacterium 3M39.76-Flavobacterium 3M39.77-Flavobacterium 3M39.82-Flavobacterium 3M39.83-Flavobacterium 3M39.11-poor sequence (not analyzed) 3M39.13-poor sequence (not analyzed) M80: 80.1-Azorcus sp./Nitrosolobus multiformis 80.2-Zooglea ramigera 80.3-M. chitae/Propionibacterium acnes 80.4-Desulfitobacterium dehalogenans M60 : 60.1-Desulfitobacterium dehalogenas 60.2-Gallionella ferruginea 60.3-Proteobacterium (possibly Azorcus denitrificans) 60.4-Gallionella ferruginea 60.5-Thiobacillus ferrooxidans 60.6-Thermus flavus/fiji/thermophilus 60.7-Paracoccus denitrificans 60.8-Bacteria sp. R. fermentans? 60.9-Gallionella ferruginea 60.10-Bacteria sp. R. fermentans? 60.11-Gallionella ferruginea 60.12-Flavobacterium 60.13-Gallionella ferruginea 60.14-unknown/Chlorobium tepidium? 60.18-R. fermentans 60.20-Acidocella sp. or Acidophilum facilis

60.24-R. fermentans 2M60.3-Proteobacterium 2M60.73-Flavobacterium 2M60.80-Flavobacterium 2M60.81--Flavobacterium 3M60.9-poor sequence (not analyzed) 3M60.2--poor sequence (not analyzed) M63_ 63.1-Gallionella ferruginea 63.3-Cytophaga 63.4-Cytophaga/flexibacter 63.6-Cytophaga 63.7-Flavobacterium 63.8-Flavobacterium 63.10-Flavobacterium 63.11-Cytophaga 63.12-Flavobacterium 63.13-Flavobacterium 63.15-Cytophaga 63.16-Cytophaga/flexibacter 63.17-Cytophaga 2M63.71-Flavobacterium 2M63.80-Flavobacterium 2M63.77-Flavobacterium 2M63.76-Flavobacterium 2M63.75-Flavobacterium 2M63.74-Flavobacterium 3M63.6-Flavobacterium 3M63.3-Flavobacterium 2M63.79-Flavobacterium 2M63.82-Flavobacterium 2M63.78-poor sequence (not analyzed) (9 other poor sequences- not listed) Total: 105

Table 2. Candidate bacteria and their abundance

Flavobacterium	32
Cytophaga sp.	16
Zooglea/Z. ramigera	12
Gallionella ferruginea	10
R. fermentans	5
Desulfitobacterium dehalogenans	2
Thiobaciluus ferrooxidans	1
Proteobacterium	2
Acidocella sp./Acidophilum	1
Paracoccus denitrificans	1
Thermus flavus	1
Pseudomonas lemoignei	1
Heliobacter hepaticus	2
unknown/Chorobium tepidium	1
Mycobacterium chitae	1
Azorcus sp.	1
Alcaligenes	1
Streptococcus gordonii	1

Conclusions.

Results of this study reiterate the versatility and power of 16S rDNA sequencing in the characterization of microbial community in their natural settings without the need for prior cultivation of the cells which may be dependent on growth and selective media and in some cases simply unculturable. This study provides a sampling of bacterial diversity present in the groundwater seepage path. Correct identification of the individual strains and assemblage into phylogenetic trees, however, will require near complete sequencing of the 16SrDNAs and additional criteria or polyphasic approaches.

Knowledge and identification of the microbial consortia which are present in the groundwater seepage path will likely provide a sound footage for development of possible treatment approaches for the universally occurring acid mine drainage problems. Future research and technical objectives to be sought include: i) quantification of selective microorganisms present in the groundwater seepage path using probes directed against specific rRNA; ii) further assessment of microbial community present in the groundwater seepage path using denaturing gradient gel electrophoresis (DGGE). DGGE separates different rRNA genes on the basis of their G+C contents. Metabolically active cells contain more rRNA than resting or dormant cells. Hence, the DGGE patterns of PCR-amplified rRNA conceptually represent the metabolically active rRNA-rich bacterial populations.

It is anticipated that promotion of alkalinity and reduction of acidity in groundwater will be solved by microbial solutions through a better understanding of the physiology and biology of the beneficial or culprit organisms, and also through multidisciplinary approaches.

Key References

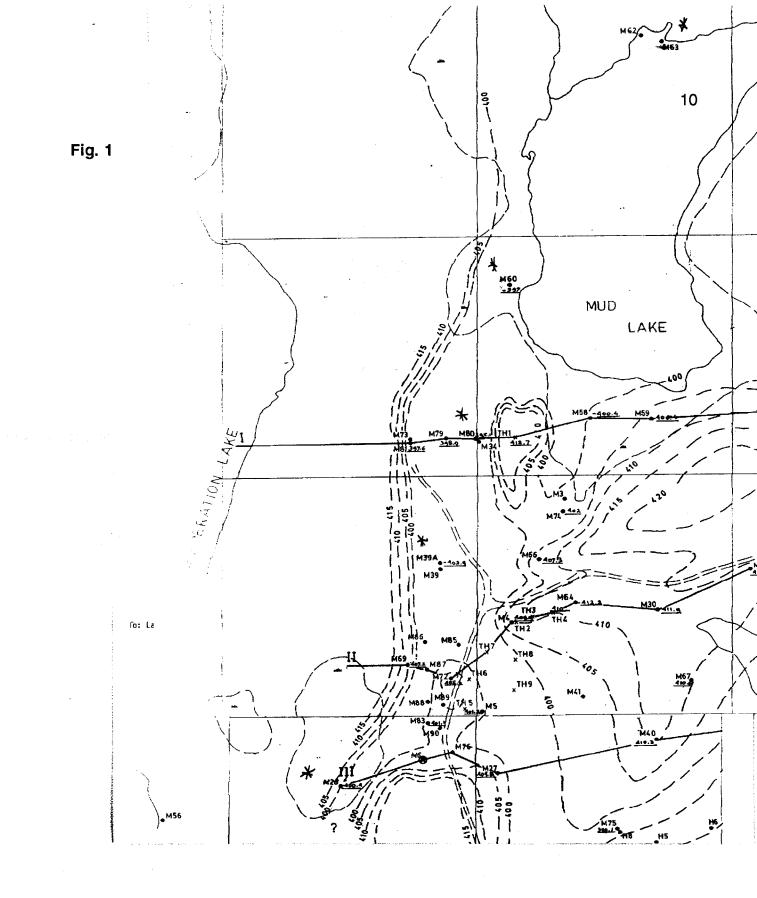
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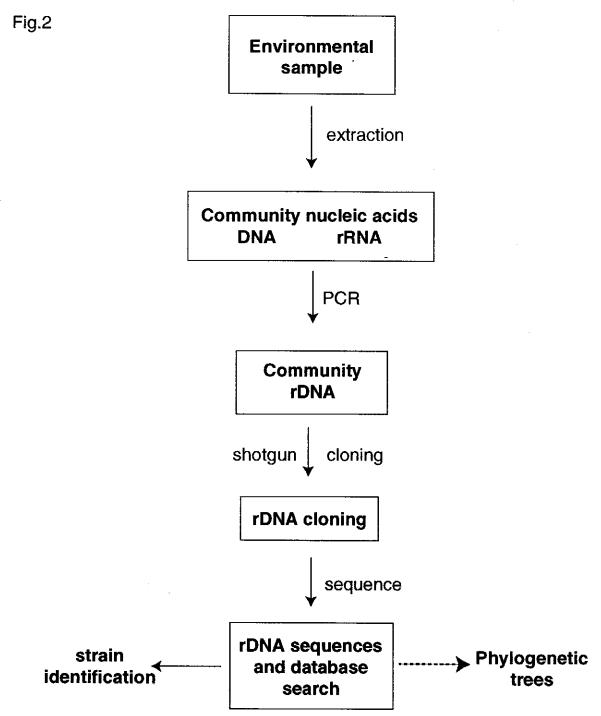
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Appendix I

>SAM281 TTACGGCTACCTTGTTACGACTTCACCCCAGTCACGAATCCCACCGTGGTAAGCGCCCTCCTTACGGTTAAGCTACCT ACTTCTGGTGAAACCCGCTCCCATGGTGTGACGGGCGGGTGTGTACAAGACCCGGGGAACGTATTCACCGCGACATGCTG ATCCGCGATTACTAGCGATTCCAACTTCATGCAGTCGAGTTGCAGACTACTATCCGGACTACGATACACTTTCTGGGA TTAGCTCCCCCTCGCGGGTTGGCGGCCCTCTGTATGTACCATTGTATGACGTGTGAAGCCCTAACCATAAG

			Smallest Sum
Probability		High	
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
emb X74914 ZR16SRRNB 1	Z.ramigera gene for 16S rRNA (ATCC	1466	1.4e-114
emb X92555 PL16SR220 1	P.lemoignei 16S ribosomal RNA (stra	1457	7.9e-114
emb X92554 PL16SRA62 1	P.lemoignei 16S ribosomal RNA (stra	1444	9.6e-113
gb U34035 UBU34035 1	Unidentified beta proteobacterium c	1340	4.6e-104
dbj D14256 PSER16S3 1	Z.ramigera gene for 16S ribosomal RNA.	1333	1.7e-103

>SAM282

TTACGGTTACCTTGTTACGACTTCACCCCAGTCACGAATCCCACCGTGGTAAGCGCCCTCCTTGCGGTTAAGCTACCT ACTTCTGGTGAAACCCGCGCGCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGACATGCTG ATCCGCGATTACTAGCGATTCCAACTTCATGTAGTCGAGTTGCAGACTACAATCCGGACTACGATACACTTTCTGGGA TTAGCTCCCCCTCGCGGGTTGGCGGCCCCTCTGTATGTACCATTGTATGACGTGTGAAGCCCTACCCATAAG

		High	Smallest Sum
Probability		mign	
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
emb X92555 PL16SR220 1	P.lemoignei 165 ribosomal RNA (stra	·1475	2.5e-115
emb X74914 ZR16SRRNB 1	Z.ramigera gene for 16S rRNA (ATCC	1466	1.5e-114
emb X92554 PL16SRA62	P.lemoignei 16S ribosomal RNA (stra	1462	3.1e-114
gb/U34035/UBU34035	Unidentified beta proteobacterium c	1349	8.2e-105
dbj D14256 PSER16S3	Z.ramigera gene for 16S ribosomal RNA.	1342	3.1e-104

>SAM283

TTACGGCTACCTTGTTACGACTTAGCCCTAGTTACCAGTTTTACCCTAGGCAGCTCCTTGCGGTCACCGACTTCAGGC ACCCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGGATCATGGCTGATATCCGA TTACTAGCGATTCCAGCTTCACGGAGTCGAGTTGCAGACTCCGATCCGAACTGTGACCGGTTTTATAGATTCGCTCT GGTCACCCAGTGGCTGCTCTCTGTACCGGCCATTGTAGCACGTGTGTGGCCCAAGGCGTTAAGGGCCGTGATGATTTG ACGTCATCGCCACCTTCCTCTCAGTT

Probability		High	
	High-scoring Segment Pairs:	Score	P (N)
gb L39067 FVBRRDA	Flavobacterium ameridies 165 ribosom	1459	5.9e-127
gb M58781 FBCRR16SB	Flexibacter columnaris 16S ribosomal	1344	2.4e-117
gb M59053 CYTRR16SI	Cytophaga johsonae 16S ribosomal RNA.	1344	2.6e-117
gb M62797 FVBRRD	F.aquatile 16S ribosomal RNA.	1338	8.3e-117
gb M62792 FBCRR16SZ	F.aurantiacus 16S ribosomal RNA.	1337	9.1e-117
-	· · · · · · · · · · · · · · · · · · ·	* ~ ~ ~ ~	1 7. 110

>SAM286

		Smallest Sum
Probability	High	
Sequences producing High-scoring Segment Pairs: N	Score	P (N)
gb L39122 HECRGD Helicobacter hepaticus 16S ribosom	mal 389	7.2e-93
gb M88144 HECRR16SBE Helicobacter sp. (strain B52D Seym	nou 385	3.8e-92
gb M88141 HECRR16SBC Helicobacter sp. (strain B13A Seym	10u 389	3.9e-92
gb L36143 HECFRC Helicobacter pullorum (strain NCTC 4	: 1 380	9.4e-92
gb L36146 HECFRF Helicobacter pullorum (strain UB 3	16 380	9.6e-92

>M287

		High	Smallest Sum
Probability Sequences producing N	High-scoring Segment Pairs:	Score	P (N)
dbj D84619 D84619 5	Unidentified beta proteobacterium D	616	1.7e-120
dbj D16211 RHYFR2D 3	R.fermentans gene for 16S ribosomal	833	1.9e-119
dbj D16212 RHYFR3E 3	R.fermentans gene for 16S ribosomal	833	1.9e-119
emb X95837 BS16ST3	Bacteria species 16S rRNA gene, str	847	2.9e-118
dbj D84645 D84645	Unidentified beta proteobacterium D	620	2.0e-117

>2M28.4

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Sequences p	roducing High-se	coring Segmer	t Pairs:		High Score	Probabilj P(N)	ity N
gb U63941 Z dbj D84625		ea sp. 16S r	ibosomal R	NA gene,	1542	1.7e-145	
		tified beta	proteopact	erium DN	1295	1.0e-144	
dbj D84620 1		tified beta	proteobact	erium DN		2.0e-142	•
dbj1D8456411		bea sp. DNA f	or 16S rib	osomal R	1287	3.0e-142	4
dbj D84572 I	084572 Zoog10	bea sp. DNA f	or 16S rib	posomal R	1269	5.1e-140	4

GCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCAGCACGGAGCTTGCTCTGGTGGCGAGTGGCGAACGGGTGAGT AATATATCGGAACGTACCCTGGAGTGGGGGGATAACGCAGCGGCGAAAGTTGCGCTAATACCGCATACGATCTACGGATGAA AGTGGGGGGATCGCAAGACCTCATGCTCGTGGAGCGGCCGATATCTGATTAGCTAGTTGGTAGGGTAAAAGCCTACCAA GGCATCGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTGGGGAATTTTGGACAATGGGGGGCAACCCTGATCCAGCAATGCCGCGTTGAGTGAAGAAGGCCTTCGGGTTGT TAAAGCTCTTTTGTCAGGGGAAGAAACGGTGATTCCTTAATACCGATTGCTAATGACNGTACCTGAAGAATAAGCCCCG T

	·		Smalle Sum	st
Sequences producing	High-scoring Segment Pairs:	High Score	Probabil P(N)	ity N
gb U63941 ZSU63941 dbj D84564 D84564 dbj D84625 D84625 dbj D84572 D84572 dbj D84574 D84574	Zoogloea sp. 16S ribosomal RNA gene, Zoogloea sp. DNA for 16S ribosomal R Unidentified beta proteobacterium DN Zoogloea sp. DNA for 16S ribosomal R Zoogloea sp. DNA for 16S ribosomal R	1489 1507 1462	7.3e-164 2.6e-161 1.8e-160 2.5e-158 2.5e-158	4 4 4

>2M28.11

GACGCCTAACACATGCAAGTCAAACGGGATTGAGGGCGAGCTGTCATGACAGTGGCGCACGGGTGAGTAGACGCGTAT GCAACCTACCGTTTGGCTGAGGGATAGCCCATATAAATGGGGGATTAATACCTCATAGGTTCTTTTGATCCGCATGGAT TGGAAAGATAAAGCTCCTGCGGTTAGAAGATGGGCATGCGTGACATTAGTTTGTTGGTGAGGTAACGGCTCACCAAGA CTACGATGTCTAGGGGGTTCTGAAAGGATGATCCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAG CAGTGAGGAATATTGGTCAATGGGCGCAAGCCTGAACCAGCCATCCCCCGTGCAGGATGAAGGCCCTATGGGTCGTAA ACTGCTTTTATACACCAAGAA

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Sequences producing H	igh-scoring Segment Pairs:	High Score	Probabil	ity N
emb X84553 UNSBR2008 gb U63940 CSU63940 gb U64015 UEU64015 gb U63938 FSU63938 4	Unknown organism, partial 16S rRNA Cytophaga sp. 16S ribosomal RNA gen Unidentified eubacterium SCB38 16S Flavobacterium sp. 16S ribosomal RN	911 972	1.2e-101 1.2e-100 1.6e-100 2.0e-99	4 5
gb1U639431CSU63943 4	Cytophaga sp. 16S ribosomal RNA gen	920	2.4e-98	

>2M28.18

Sequences producing	High-scoring Segment Pairs:	High Score	Smalle Sum Probabil P(N)	
gb1U639401CSU63940 gb1U858891FSU85889 dbj1D845811D84581 gb1U858901FSU85890 gb1U639431CSU63943	Cytophaga sp. 16S ribosomal RNA gen Flavobacterium sp. IC001 16S riboso Flexibacter sp. DNA for 16S ribosom Flavobacterium sp. ACAM123 16S ribo Cytophaga sp. 16S ribosomal RNA gen	1313 1386 1463 1377	1.1e-129 2.2e-128 2.6e-128 5.4e-127 1.2e-124	3 3 2 3

TACGGCTACCTTGTTACGACTTAGCCCTAGTTACCAGTTTTACCCTÁGGCAGCTCCTTGCGGTCACCGACTTCAGGCA CCCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGGATCATGGCTGATATCCGAT TACTAGCGATTCCAGCTTCACGGAGTCGAGTTGCAGACTCCGATCCGAACTGTGACCGGTTTTATAGATTCGCTCCTG GTCACCCAGTGGCTGCTCTCTGTACCGGCCATTGTAGCACGTGTGTTACCCCAAGGCGTAAGGGCCGTGATGATTTGAC GTCATCCCCACCTTCCTCTCAGTTTGCACTGGCATCTCGTTAAATTCCCGACATGATCGCTGGCACTAACACACA

			Smalle: Sum	st	
Sequences producing	High-scoring Segment Pairs:	High Score	Probabil	ity N	
gb L39067 FVBRRDA gb M58781 FBCRR16SB gb M59053 CYTR16SI gb M62797 FVBRRD gb M62792 FBCRR16SZ	Flavobacterium ameridies 165 ribosom Flexibacter columnaris 165 ribosomal Cytophaga johsonae 165 ribosomal RNA. F.aquatile 165 ribosomal RNA. F.aurantiacus 165 ribosomal RNA.	1579 1579 1573	1.3e-133 5.5e-124 5.8e-124 1.8e-123 2.1e-123	1 1 1	-

1396

				Smallest Sum
Probability			High	Dam
Sequences proc N	ucing High-scoring Segment Pa	irs:	Score	P(N)
gb M58835 STRF 3	R16SB Streptococcus bovis 16	S ribosomal RNA.	632	6.3e-104
-	16SRR2 Streptococcus gordonii		• 884	2.2e-101
-	PPDACA S.pyogenes DNA for opp	A, oppB, oppC,	614	5.9e-101
emb X68418 ST1 3	Function device for	r 165 rRNA	586	4.0e-97
gb M58839 STRR 3	R16SE Streptococcus salivariu	us 16S ribosom	566	6.1e-97

M3913

AGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGGCAACC GNTGGCGAGTGGCGAACGGGTGAGTAATATATCGGAATGTACCCTAGAGTGGGGGGATAACGTAGCGAAAGTTACGCT ATACCGCATACGATCTTCGGATGAAAGTGGGGGGATCGCAAGACCTCATGCTCATGGAGCAGCCGATATCTGATTAGC AGTTGGTAGGGTAAAAGCCTACCAAGGCGACGATCAGTAGCTGGTTTGAGAGAACGACCAGCCACACTGGAACTGAG CACGGTCCAGACTCCTACG

		Smalle: Sum	s t	
Sequences producing High-scoring Segment Pairs:	High Score	Probabil: P(N)	ity N	
gb1U639411ZSU63941 Zoogloea sp. 16S ribosomal RNA gene, emb1X749141ZR16SRRNB Z.ramigera gene for 16S rRNA (ATCC 2 dbj1D846251D84625 Unidentified beta proteobacterium DN	1187 1526 1200	5.4e-120 1.6e-119 5.3e-115	2 1 2	
dbj D84564 D84564 dbj D84572 D84572 Zoogloea sp. DNA for 16S ribosomal R	1440 1422	9.9e-115 3.1e-113	1 1	

AACACGAACACGTCGAGGGGTCAGAGTTCGTCAGGAAGTATAGACCGGCGCACGGGTGCGTAACGCGTATGCAATCTA CCTTTCACAAAGGGGATAGCCCAGAGAAATTTGGATTAATACCTTATAGTAATACGACTTGGCATCAAGATGTATTTA AAGATTTATCGGTGAAAGATGAGCATGCGTCCCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGGGT AGGGGTCCTGAGAGGGAGGTCCCCCCACACTGGTACCGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAAT ATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTAT ACAGGAAGAAACACTCGCGACGTGTCCGAGCTTGACGGTACTGTAGGATAGGATCGGCTAACTCCGTGCCAGCAGC CGCGGTAATACGGCAGGATC

			Smalle	st	
			Sum		
		High	Probabil	ity	
Sequences producing	High-scoring Segment Pairs:	Score	P(N)	ท้	
gb U63940 CSU63940	Cytophaga sp. 16S ribosomal RNA gen	1432	1.5e-148	3	
	Flexibacter sp. DNA for 16S ribosom	1382			
		1358	1.6e-144	3	
	F.aquatile 165 ribosomal RNA.	1338	4.2e-144	3	
gb1U639361FSU63936	Flavobacterium sp. 16S ribosomal RN	1387	3.2e-142	3	
	gb U63940 CSU63940 dbj D84581 D84581	dbj D84581 D84581Flexibacter sp. DNA for 165 ribosomgb M59053 CYTRR16SICytophaga johsonae 165 ribosomal RNAgb M62797 FVBRRDF.aquatile 165 ribosomal RNA.	Sequences producing High-scoring Segment Pairs:Scoregb U63940 CSU63940Cytophaga sp. 16S ribosomal RNA gen 1432dbj D84581 D84581Flexibacter sp. DNA for 16S ribosom 1382gb M59053 CYTRR16SICytophaga johsonae 16S ribosomal RNA 1358gb M62797 FVBRRDF.aquatile 16S ribosomal RNA 1338	Sum Sequences producing High-scoring Segment Pairs: gb U63940 CSU63940 dbj D84581 D84581 gb M59053 CYTRR16SI gb M62797 FVBRRD Sum Cytophaga sp. 16S ribosomal RNA gen 1432 1.5e-148 Flexibacter sp. DNA for 16S ribosom 1382 4.6e-145 Cytophaga johsonae 16S ribosomal RNA 1358 1.6e-144 F.aquatile 16S ribosomal RNA. 1338 4.2e-144	Sequences producing High-scoring Segment Pairs:High Probability Score P(N) Ngb U63940 CSU63940Cytophaga sp. 16S ribosomal RNA gen 1432 1.5e-148 3dbj D84581 D84581Flexibacter sp. DNA for 16S ribosom 1382 4.6e-145 3gb M59053 CYTRR16SICytophaga johsonae 16S ribosomal RNA 1358 1.6e-144 3gb M62797 FVBRRDF.aquatile 16S ribosomal RNA 1338 4.2e-144 3

>2M395

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Sequences producing H	igh-scoring Segment Pairs:	High Score	Sum Probabil P(N)	ity N
emb X74914 ZR16SRRNB dbj D14256 PSER16S3 gb U34035 UBU34035 emb X92555 PL16SR220 emb X92554 PL16SRA62	Z.ramigera gene for 16S rRNA (ATCC Z.ramigera gene for 16S ribosomal RNA. Unidentified beta proteobacterium c P.lemoignei 16S ribosomal RNA (stra P.lemoignei 16S ribosomal RNA (stra	2281 776 2167	5.8e-190 8.7e-183 5.0e-178 7.7e-178 2.1e-175	1 5 2

>3M965 39-6

CGAACGCGAAAGTAGGGGCCAACCCTGCGAGTAGAGTGGCGAACGGGTGAGTAATACATCGGAACGTACCCAGGCAGTG GGGGACAACCTCGAGAAATCGAGGCTAACACCGCATACGTCCTGAGGGAGAAAGCGGGGGGATCCGTAAGGACCTCGCG CTCATGGAGCGGCCGATGTCGGATTAGCTAGTTGGCGGGGGTAAAAGCCCACCAAGGCTACGATCCGTAGCTGGTTTGA GAGAACGACCAGCCACACTGGGACTGATACACCGCCCACACTCCTACGGGAGGCAGCAGTGGGGGAATTTTGGACAATG GGGGAAACCCTGATCCAGCC

Sequences producing H	igh-scoring Segment Pairs:	High Score	Sum Probability P(N) N	
emb X84466 UNSBR1022 2	Unknown organism, partial 16S rRNA	761	1.9e-92	
emb X84518 UNSBR1090 3	Unknown organism, partial 16S rRNA	773	1.9e-91	
dbj D84625 D84625 3	Unidentified beta proteobacterium D	795	7.0e-91	
emb X84493 UNSBR1058 2	Unknown organism, partial 16S rRNA	982	2.3e-90	
emb X84610 UNSBR2080 3	Unknown organism, partial 16S rRNA	773	4.8e-90	
		· · · •		

AACCGGCTTTGCGTGCTTGCACCGGAGTTAGTGGCGAACGGGTGAGTAATATATCGGAACATATCCGGAAGTGGGGGG FAACGTGACGAAAGTTTCGCTAATACCGCATATGCCCTGAGGGGGGAAAGGGGGGGATCGCAAGACCGTCTCGCTTTCG GAGTGGCCGATACCGGATTAGCTACATGGTGAGGTAAAGGCTCACCATGGCGACGATCCGTAGCTGGTCTGAGAGGAC GACCAGCCACTCTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC

Sequences producing H	igh-scoring Segment Pairs:	High Score	Smalles t Sum Probability P(N) N
gb L07897 GLLRGDA 2	Gallionella ferruginea 16S ribosoma	641	3.4e-87
emb X84620 UNSBR2090 2	Unknown organism, partial 16S rRNA	544	5.5e-73
emb X84544 UNSBR1119 2	Unknown organism, partial 16S rRNA	524	2.0e-71
~emb X84465 UNSBR1021 2	Unknown organism, partial 16S rRNA	587	3.0e-71
emb X84518 UNSBR1090 2	Unknown organism, partial 16S rRNA	542	1.7e-70

>SAM601

TAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGGCCTTTGCGAGGTTCG AAGCTCGAGGTTCGAAGTTCGAATTAAATTGACTTCTGAGGTATTAAAAGGCCACAGAGAAATTCTTAATTCGCACAT CGTGCATCGCACTTCGAACTTCGCGGGTAGTGGCGGACGGGTGAGTAACGCGAGGGTAACCTACCCATAAACCCGGGA CAACCCTTGGAAACGAGGGCTAATACCGGATAATCTTTTAGCTTGGCATCAAGCAATAAGAAAAGGTGGCCTCTGAAA ATGCTACCGATTATGGATGGACCCGCGTCTGATTAGCTAGTTGGTG

			Smallest Sum
Probability		High	
Sequences producing N	High-scoring Segment Pairs:	Score	P (N)
gb M34417 DSORR1618 3	Desulfotomaculum orientis 16S ribos	388	2.7e-71
gb L28946 DTORRD 2	Desulfitobacterium dehalogenans 165	•681	1.1e-70
gb1U685281DCU68528 2	Desulfitobacterium chlororespirans	668	2.7e-61
emb X94975 DH16SRRNA 2	D.hafniense 16S ribosomal RNA	625	1.0e-58
emb X99706 SG16SRR1	S.glycolicus 16S rRNA gene	375	1.3e-52

>SAM602

			Smallest
Probability		High	Sum
	ligh-scoring Segment Pairs:	Score	P (N)
gb L07897 GLLRGDA 1	Gallionella ferruginea 165 ribosoma	1436	4.6e-112
gb U63941 ZSU63941 2	Zoogloea sp. 16S ribosomal RNA gene	958	9.5e-96
emb X65589 TM16SR 2	T.mixta 16S rRNA	996	1.4e-95
gb U63953 ASU63953 2	Alcaligenes sp. 16S ribosomal RNA g	958	4.4e-94
gb L35509 NTL16SR 2	Nitrosolobus multiformis (ATCC 2519	958	5.3e-93

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGTATGCTTTACACATGCAAGTCGAACGGGCTTTGGTGCTTGCA CCGGAGTTAGTGGCGAACGGGTGAGTAATATATCGGAACATATCCGGAAGTGGGGGGATAACGTAGCGAAAGTTACGCT AATACCGCATATGCCCTGAGGGGGGAAAGGGGGGGGATCGCAAGACCTCTCGCTTTCGGAGTGGCCGATACCGGATTAGC TAGATGGTGAGGTAAAGGCTCACCATGGCGACGATCCGTAGCTGGTCTGAAAAGACGACCAGCCACACTGGAACTGAG ACACGGTCCAGACTCCTACGGAAGCACCAGTG

		High	Smallest Sum
Probability		mign	
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
gb L07897 GLLRGDA 1	Gallionella ferruginea 16S ribosoma	1382	1.5e-107
gb U63941 ZSU63941 2	Zoogloea sp. 16S ribosomal RNA gene	913	5.0e-92
emb X65589 TM16SR 2	T.mixta 16S rRNA	951	7.7e-92
gb U63953 ASU63953 2	Alcaligenes sp. 16S ribosomal RNA g	913	2.3e-90
gb L35509 NTL16SR	Nitrosolobus multiformis (ATCC 2519	913	3.0e-89

>SAM605

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGGTAGCAGGCCTTCGGG TGCTGACGAGTGGCGGGACGGGGGGGGAATGCGGGGAATCTGTCTTTTAGTGGGGGGACAACCCACGGAAACTTGGGCT AATACCGCATGAGCCCTGAGGGGGGAAAGCGGGGGGATCTTCGGACCTCGCGCTAAGAGGAGAGCCTACGTCCTGATTAC CTAGTTGGTACGGTAAAGGCCTACCAAGGCGACAATCACTAGCTGGTCTGAGAGGAC

			Smallest Sum
Probability		High	
Sequences producing N	High-scoring Segment Pairs:	Score	P (N)
emb X75266 TF16SRRN 2	T.ferrooxidans (N-Fe2) gene for 16S	•921	4. 9e-89
emb X75267 TF16SRNA 2	T.ferrooxidans (N-Fe4) gene for 165	912	2.8e-88
emb X98210 TS16SRDNA 2	Thiobacillus sp. 16S ribosomal DNA	•921	3.6e-88
emb X93478 PBDSM2111 2	Phototrophic bacterium 16S rRNA gene	•952	2.4e-87
emb X75268 TFN16SRRN	T.ferrooxidans (N-Fe3) gene for 16S	•926	1.1e-85

>SAM606

TTACGGTTACCTTGTTACGACTTAGCGCCAGTCACCAGTTTTACCTTCAGCCCCGATTACTCGGAGATTTCAGGTACC ACCAGCTCCCATCGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACGGCGGCGTAGCTGATCCGCCATTA CTAGTGATTCCGTCTTCATGGAGTCGAATTGCAGACTCCAATCTGAACTGAGCCCACTTTTTTGCGATTAGCTCCACC TCGCGGTTTGGCAACGCTTTGGAGTGGGCCATTGTAACACGTGTGTAGCCCCAAGGCATAAAGGCCGTACTGACTTGACG TCATCTCCATCTTCCTCCCGCCTTATCGCG

			Smallest Sum
Probability		High	oum
	igh-scoring Segment Pairs:	Score	P(N)
gb L09660 TTHAT62	Thermus flavus AT-62 16S ribosomal R	975	2.0e-82
gb L10067 TTHDNAFRA	Thermus Fiji 3A DNA fragment.	975	2.0e-82
gb/M26923/TTH16SRAA	T.thermophilus 16S rRNA gene, comple	975	4.5e-82
gb/L09670/TTHHS	Thermus sp. HS A.1 16S ribosomal RNA	966	1.1e-81
gb L10069 TTHDNAFRC	Thermus Tok3 A.1 DNA fragment.	957	1.1e-81

>SAM607

TTACGGCTACCTTGTTACGACTTCACCCCAGTCACTGAGCCTACCGTGGTTGGCTGCCTCCATTGCTGGTTGGCGCAC CACCTTCGGGTAGACCCAATTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGTCATGC TGTTACGCGATTACTAGCGATTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAACTGAGATGGCTTTTTGGG ATTAACCCATTGTCACCACCATTGTAGCACGTGTGTAGCCCCAACCCGTAAGGGCCATGAAGACTTGACGTCATCCACA CCTTCCTCCGGCTTATCACCGGCAGTTTTTCCTA

Probability		High	Smallest Sum
	igh-scoring Segment Pairs:	Score	P (N)
emb X69159 PD16SRRNA 1	P.denitrificans 16S rRNA	1538	1.3e-120
emb X53855 RSRRNC 2	R. sphaeroides ribosomal RNA operon	1302	5.6e-119
emb X53853 RSRRNA 2	R. sphaeroides ribosomal RNA operon	1302	5.9e-119
emb X53854 RSRRNB 2	R. sphaeroides ribosomal RNA operon	1302	6.0e-119
dbj D16427 RCAC5J 1	R. capsulatus 16S rRNA gene.	1506	6.0e-118

>SAM608

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCGGCACGGGAGCAAT CCAGGTGGCGAGTGGCGAACGGGTGAGTAATATATCGGAACGTGCCCAGTCGTGGGGGGATAACGCAGCGAAAGCTGTG CTAATACCGCATACGATCTCTGGATGAAAGCGGGGGGGACTCGCAAGGGCCTCGCGCGGATTGGAGCGGCCGATATCAGAT TAGCTAGTTGGTGGGGTAAAAAGCCCACCAAGGCGACGATCTGTAGCTGGTCTGAGAAGACGACCAGCCACACTGGAAC TGAGACCCGTCCACACTCCTACGGGAAGCACAGTG

		** 4 - 3	Smallest Sum
Probability		High	
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
emb X95837 BS16ST3 3	Bacteria species 165 rRNA gene, str	870	1.9e-109
dbj D84619 D84619 5	Unidentified beta proteobacterium D	598	1.9e-107
dbj D16211 RHYFR2D 3	R.fermentans gene for 16S ribosomal	815	1.0e-105
dbj D16212 RHYFR3E 3	R.fermentans gene for 16S ribosomal	815	1.0e-105
gb L33981 LTTRRDC 3	Leptothrix sp. (strain NC-1) 16S ri	953	8.0e-105
the second second	A H A H A H A H A H A H A H A H A H A H		

>SAM609

TTACGGCTACCTTGTTACGACTTCACCCCAGTCATGAAACCCACCGTGGTAAGCGCCCTCCTTACGGTTAGGCTACCT ACTTCTGGCAGATTCCACTCCCATGGTGTGACGGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGACATGCTG ATCCGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTCGAGACTCCAATCCGGACTACGATCGGCTTTCTGAGA TTAGCTCCCCCTCGCGGGTTTGGCAACCCTCTGTACCGACCATTGTATTACGTGTGAAGCCCTACCCATAAG

Probability		High	Smallest Sum
Sequences producing H N	ligh-scoring Segment Pairs:	Score	P(N)
gb L07897 GLLRGDA 2	Gallionella ferruginea 16S ribosoma	1242	6.6e-112
gb/U22536/ZSSSURNAS2 2	Zoogloea sp. small subunit ribosoma	1129	1.8e-109
emb X92415 ASP16SRRN 2	Alcaligenes sp. 165 ribosomal RNA	1179	1.2e-106
emb X74913 ZR16SRRNA 2	Z.ramigera gene for 16S rRNA (ATCC	1116	7.1e-103
gb \$55008 \$55000\$2 2	16S rRNA (Burkholderia mallei, ATCC	1062	3.6e-102

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			Sum	
Semionaca producine litel		High	Probabil:	itv
Sequences producing High	1-scoring Segment Pairs:		P(N)	N
dbj D16212 RHYFR2D R. dbj D16212 RHYFR3E R. dbj D84619 D84619 Ur	teteria species 16S rRNA gene, str fermentans gene for 16S ribosomal fermentans gene for 16S ribosomal didentified beta proteobacterium D wkholderia sp. isolate N3P2 16S r	833 833 616	3.6e-115 2.1e-111 2.1e-111 5.5e-109 6.2e-107	2 2 4

>M6011

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			Smalle	sι	
			Sum		
		High	Probabil	ity	
Sequences producing H	igh-scoring Segment Pairs:	Score	P(N)	N	
gb L07897 GLLRGDA	Gallionella ferruginea 16S ribosoma	1515	6.9e-127	3	
emb X92415 ASP16SRRN	Alcaligenes sp. 165 ribosomal RNA	1470	4.7e-123	3	
gb U22536 ZSSSURNAS2	Zoogloea sp. small subunit ribosoma	1420	8.7e-120	2	
emb X74913 ZR16SRRNA	Z.ramigera gene for 16S rRNA (ATCC	1407	8.1e-118	3	
emb X57026 BB16SRNA	B.bronchiseptica 16S ribosomal RNA	1407	9.8e-118	3	
			-		

>M6014

TGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCTACGAGAAAAGAGTANCNATACTCTGAG TAAAGTGGCGCAAGGGTGCGTAACCCGTAAGCAATCTACCTTTAGGCGGGGGAATAATCCAGCGAAAGCCGGACTAATA CCCCATAATGCAGCGGCACCGCATGGTGATGTTGTTAAAGTCTATATGACGTCTANAGATGAGCTTGCGTCTGATTAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCTACGATCAGTAGCTGGTCTGAGAAGATGATCAGCCACACTGGAACTGA GACACGGTCCAGACTCCTACGGGAA

Sequences producing High-scoring Segment Pairs:	Smalles t Sum High Probability Score P(N) N
emb[X84451]UNSBR1003 Unknown organism, partial 16S rRNA g	468 5.6e-71
gb/M58468/CHBSSRIBRR Chlorobium tepidum small subunit rib	448 1.9e-70
emb X85131 SBRRNA16S S.buswellii 16S rRNA gene	493 1.1e-69
gb/M31769/CHBS&RNA C.limicola ribosomal RNA small subunit.	444 2.1e-69
emb/Y07607/BS16SRM13 Bacterial species 16S rRNA gene (par 3	426 3.8e-69

>M6020

TAGAGTTTGATCCTGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCGCGGTCAGTAATGGCAGCG GCGGACGGGTGAGTAACACGTAGGAATCTATCCCAGGGTGGGGGACAACAGCGGGAAACTGCTGCTAATACCGCATGA TACCTGAGGGTCAAAGGCGCAAGTCGCCTTGGGAGGAGCCTGCGTCTGATTAGCTTGTTGGTGGGGGTAAAGGCCTACC AAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCAAACTCCTACGGGAAG CAGCAGTG

Sequences producing H	igh-scoring Segment Pairs:	High Score	Sum Probabil P(N)	
dbj D30771 ACD16SRNAG dbj D30771 ACD16SRNAD emb Y07647 BS16SR3C	Acidocella sp. DNA for 16S rRNA Acidiphilium facilis gene for 16S r Acidiphilium aminolytica gene for 1 Bacterial species 16S rRNA gene (pa Unknown Proteobacterium, alpha-1 su	1354 1293 525	1.3e-112 4.2e-111 5.1e-106 3.1e-102 7.3e-99	2 2 4

>2M603

CGCTGGCGGCATGCCTTACACATGCAAGTCGAACGCGCAAAGTAGGGGCAACCCTGCGAGTAGAGTGGCGAACGGGTG AGTAATACATCGGAACGTACCCAGGAGTGGGGGGGACAACCTCGAGAAATCGAGGCTAACACCGCATACGTCCTGAGGGA GAAAGCGGGGGATCCGTAAGGACCTCGCGCTCATGGAGCGGCCGATGTCGGATTAGCTAGTTGGCGGGGGTAAAAGCCC ACCAAGGCTACGATCCGTAGCTGGTTTGAGAGAACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG CGGCTAACTACGTGCCAGCAGCCGCGGGGTAATACGTTAGGTGCGAACGTTAATCGGAATTACTGGGCCTTAAAGCGTG CGTTAGCGGTTCT

			Smalle: Sum	st	
Sequences producing High-sco	ring Segment Pairs:		Probabil: P(N)	ity N	
emb/X86577/AS002516s Alcali	denes 00-19 165 rPNA dono som	929 929 929 929	6.2e-159 3.9e-158 4.0e-158 4.1e-158 4.3e-158	5 5 5	,

>2M6080

CGCTTAACACATGCAAGTCGAGGGGTATAGTTCTTCGGAACTAGAGACCGGCGCACGGGTGCGTAACGCGTATGCAAT CTACCTTTCACAAAGGGATAGCCCAGAGAAATTTGGATTAATACCTTATAGTAATACGACTTGGCATCAAGATGTATT TAAAGATTTACCGGTGAAAGATGAGCATGCGTCCCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGG GTAGGGGTCCTGAGAGGGGAGATCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGA ATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGCAGGATGACGGTACTATGGATTGTAAACTGCTTTT ATACAGGAAGAAACACTAGGACGTGTCCGAGCTTGACGGTACTGTTAGAATAAGGATCGGCTAACTCCGTGCCAACCA G

			Smalle Sum	s t
Sequences producing	High-scoring Segment Pairs:	High Score	Probabil P(N)	ity N
gb U85889 FSU85889 gb U85890 FSU85890 dbj D84581 D84581 gb M59053 CYTRR16SI gb U63940 CSU63940	Flavobacterium sp. IC001 165 riboso Flavobacterium sp. ACAM123 165 ribo Flexibacter sp. DNA for 165 ribosom Cytophaga johsonae 165 ribosomal RNA. Cytophaga sp. 165 ribosomal RNA gen	1936 1981 1982	1.1e-160 2.3e-160 1.0e-159 1.2e-157 2.3e-157	2 1 1

Smalles t

TACGGCTACCTTGTTACGACTTAGCCCTAGTTACCAGCTTTACCCTAGGCAGCTCCTTGCGGTCACTGACTTCAGGCA CCCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGGATCATGGCTGATATCCGAT TACTAGCGATTCCAGCTTCACGGAGTCGAGTTGCGGACTCCGATCCGAACTGTGACCGGTTTTATAGATTCGCTCCTG GTCACCCAGTGGCTGCTCTCTGTACCGGCCATTGTAACACGTGTGTAGCCCCAAGGCGTAAGGGCCCGTGATGATTTGAC GTCATCCCCACCTTCCTCTCAGTTTGCACTGGCAGTCTCGTTAGATCCCGAGATGACTCGCTG

			Smalles Sum	t
		High	Probabil:	ity
Sequences producing	High-scoring Segment Pairs:	Score	P(N)	N
gb L39067 FVBRRDA	Flavobacterium ameridies 16S ribosom	1722	6.1e-136	1
gb M62797 FVBRRD	F.aquatile 16S ribosomal RNA.	1610	1.9e-129	2
gb M58781 FBCRR16SB	Flexibacter columnaris 16S ribosomal	1607	3.0e-129	2
gb M59053 CYTRR16SI	Cytophaga johsonae 165 ribosomal RNA.	1607	3.3e-129	2
gb1M587641CYTRR16SA	Cytophaga aquatile 16S ribosomal RNA.	1581	4.4e-127	2

M631

Probability		High	Smallest Sum
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
gb L07897 GLLRGDA 2	Gallionella ferruginea 16S ribosoma	1138	4.5e-95
gb U63941 ZSU63941	Zoogloea sp. 16S ribosomal RNA gene	696	9.4e-84
gb1U225341ZSSSURNAS1 3	Zoogloea sp. small subunit ribosoma	743	1.4e-83
gb L33976 SHLRRD 3	Sphaerotilus natans 16S ribosomal R	682	2.5e-83
gb L33978 SHLRRDB 3	Sphaerotilus natans 16S ribosomal R	676	3.0e-83

>M634

Drobabilt			Smallest Sum
Probability		High	
N .	High-scoring Segment Pairs:	Score	P(N)
gb1U639401CSU63940 3 -	•Cytophaga sp. 16S ribosomal RNA gen	1078	5.1e-118
b M59053 CYTRR16SI	Cytophaga johsonae 16S ribosomal RNA.		
			2.2e-116
lbj D84581 D84581 2	- Flexibacter sp. DNA for 16S ribosom	1306	1.1e-115
b M93151 SORRR16S 2	• Sporocytophaga cauliformis 16S ribo	1360	6.2e-115
ID1003338[E2063938	Flavobacterium sp. 16S ribosomal RN		115
2	sp: 165 Fibosomal RN	1297	2.3e-114

TTACGGCTACCTTGTTACGACTTAGCCCTAGTTACCAGTTTTACCCTAGGCAGCTCCTTGCGGTCACCGACTTCAGGC ACCCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGGATCATGGCTGATATCCGA TTACTAGCGATTCCAGGCTTCACGGAGTCGAGTTGCAGACTCCGATCCGAACTGTGACCGGTTTTATAGATTCGCTCCT GGTCACCCAGTGGCTGCTCTCTGTACCGGCCATTGTAACACGTGTGTAACCCCAAGGCGTAAGGGCCGTGATGATTTGA CGTCATCC

			Smallest Sum
Probability		High	buin
Sequences producing High- N	scoring Segment Pairs:	Score	P(N)
gb11.39067 FVBRRDA •Flav	obacterium ameridies 16S ribosom	. 1568	4.2e-123
gb1M58781 FBCRR16SB Flex	ibacter columnaris 165 ribosomal	. 1453	1.7e-113
gb1Mb9053[CYTRR16SI Cyto] 1	phaga johsonae 165 ribosomal RNA.	1453	1.8e-113
gblN62797 FVBRRD F.aq	uatile 16S ribosomal RNA.	1447	5.6e-113
gblNo2792 FBCRR16SZ F.au	rantiacus 16S ribosomal RNA.	1446	6.6e-113

>M638

 $\label{eq:tacct} TTACGGCTACCTTGTTAGCCCTAGTTACCAGTTTTACCCTAGGCAGCTCCTTGCGGTCACTGACTTCAGGC ACCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGGATCATGGCTGATATCCGA TTACTAGCGATTCCAGGCTTCACGGAGTCGAGTTGCAGACTCCGATCCGAACTGTGACCGGGTTTTATAGATTCGCTCCT GGTCACCCAGTGGCTGCTCTATGTACCGGCCATTGTAGCACGTGTGTAGCCCCAAGGCGTAAGGGCCGTGATGATTTGA CGTCATCCCCACCTTCCTCTCAGTTTGCACTGGCAGTCTCGTTAGATTCCCGACATGANTCGCTGGCAACTAACAACA G \\ \end{tabular}$

			Smallest Sum
Probability		High	
Sequences producing N	High-scoring Segment Pairs:	Score	P(N)
gb L39067 FVBRRDA 2	Flavobacterium ameridies 16S riboso	1747	3.5e-146
gb1M627971FVBRRD 2	•F.aquatile 16S ribosomal RNA.	1635	1.7e-137•
gb1M587811FBCRR16SB	Flexibacter columnaris 16S ribosoma	1632	2.6e-137
gb M59053 CYTRR16SI 2	Cytophaga johsonae 165 ribosomal RNA.	1632	3.0e-137
gb/M58764/CYTRR16SA 2	Cytophaga aquatile 16S ribosomal RNA.	1606	3.9e-135
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>M6316

			Smalle Sum	
Sequences producing H	ligh-scoring Segment Pairs:	Score	Probabil P(N)	ity N
gb U63940 CSU63940 gb M59053 CYTRR16SI dbj D84581 D84581 gb U63938 FSU63938 gb U63943 CSU63943	Cytophaga sp. 16S ribosomal RNA gen Cytophaga johsonae 16S ribosomal RNA. Flexibacter sp. DNA for 16S ribosom Flavobacterium sp. 16S ribosomal RN Cytophaga sp. 16S ribosomal RNA gen	1424 1352 1341	2.9e-124 4.2e-122 5.8e-122 4.7e-121 6.0e-121	2 2 2

>SAM801

IAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGAGTGCTTTACACATGCAAGTCGAACGGCAGCACGGGGGCTTG CTCCTGGTGGCGAGTGGCGAACGGGTGAGTAATGCGTCGGAACGTACCGAGTAATGGGGGGATAACGCACCGAAAGGTG IGCTAATACCGCCTACGCCCTGAGGGGGGAAAGCANGGGATCATTAGACCTTGCGTTATTCGAGCGGCCGACCTTCTGA ITAGCTAGTTGGTGGGGTAAAG

Probabilitar		High	Smallest Sum
Probability Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
gb L35508 NTL16SRA 3	Nitrosolobus multiformis (ATCC 2519	552	1.3e-78
gb L15532 AZORR16SC 2	Azoarcus sp. (strain S5b2) 16S ribo	893	2.8e-71
emb X97599 BP16SRT65 3	Beta-proteobacterium 16S rRNA gene,	480	2.4e-70
emb X97592 BP16SRT67 3	Beta-proteobacterium 16S rRNA gene,	478	5.0e-70
gb L06170 NGORR16SD 3	Neisseria canis 16S ribosomal RNA.	562	1.5e-69

>SAM802

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGGGCAACC TGGTGGCGAGTGGCGAACGGGTGAGTAATATATCGGAATGTACCCTAGAGTGGGGGGATAACGTAGCGAAAGTTACGCT AATACCGCATACGATCTACGGATGAAAGTGGGGGGATCGCAAGACCTCATGCTCATGGAGCAGCCGATATCTGATTAGC TAGTTGGTAGGGTAAAAGCCTACCAAGGCGACGATCAGTAGCTGGTTTGAGAGAACGACCAGCCACACTGGAACTGAG ACACGGTCCAGACTCCTACGGGAAGCAGCAGTGGGGAATTCTTGGACAATG

			Smallest Sum
Probability		High	
Sequences producing H N	igh-scoring Segment Pairs:	Score	P(N)
gb U63941 ZSU63941 2	Zoogloea sp. 16S ribosomal RNA gene	1317	8.2e-131
emb X74914 ZR16SRRNB 1	Z.ramigera gene for 16S rRNA (ATCC	1656	1.9e-130
dbj D84625 D84625 2	Unidentified beta proteobacterium D	1330	8.0e-126
dbj D84564 D84564 1	Zoogloea sp. DNA for 16S ribosomal	1570	1.4e-125
dbj D84572 D84572 1	Zoogloea sp. DNA for 16S ribosomal	1552	4.3e-124

>803

			Smallest Sum
Probability		High	bala
	High-scoring Segment Pairs:	Score	P (N)
gb M29560 MSGRR16SF 4	M.chitae 16S ribosomal RNA.	1365	8.1e-129 '
gb M61903 PRSRROA 4	Propionibacterium acnes small subuni	1361	9.3e-128
gb1M586821FUSSRRNAD 4	Fusobacterium prausnitzii small subu	1022	1.9e-115
dbj D84616 D84616 6	Unidentified Actinomycetes bacterium	424	1.6e-112
dbj D84587 D84587 6	Unidentified Actinomycetes bacterium	424	3.4e-108
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			Smallest Sum
Probability		High	
	ligh-scoring Segment Pairs:	Score	P(N)
gb M34417 DSORR1618 4	•Desulfotomaculum orientis 16S ribos	788	7.1e-116
gb L28946 DTORRD 3	Desulfitobacterium dehalögenans 165	1119	8.4e-112
gb U68528 DCU68528 3	Desulfitobacterium chlororespirans	1115	3.7e-103
emb X94975 DH16SRRNA 3	D.hafniense 16S ribosomal RNA	1081	8.5e-101
emb X99706 SG16SRR1	S.glycolicus 16S rRNA gene	580	2.3e-95

