

Table S1. Archaeal SSU rRNA gene primer sequences constructed in this study

Name	Sequence (5' - 3')	group coverage	Proportion in mixtures
Archaeal set			
530F mixture (Arch)			
Arch 530F	GTGBCAGCCGCCGCGG	2, 3, 4	24
Arch2 530F	YTGCCAGCCGCCGCGG	5, 6	6
Nano 530F	GTGGCAGTCGCCACGG	15	3
907R mixture (Arch)			
Arch 907R	CCGCCAATTCCTTTAAGTTT	4	10
SAGMEG 907R	CCGCCAATTTCTTTAAGTTT	5	1
Arch2 907R	CCGYCAATTCCTTMAAGTTT	9, 13, 15	1
DSEG SAGMEGB 907R	CCGCCAATTCCTTAAGTTT	10, 30	1
Nano 907R	CCGCCTATTCCTTTAAGTTT	14	1

Archaeal set was not used in this study.

Table S2. Comparison of sequences between consensus sequences in Table 1 and SSU rRNA gene sequence in SILVA SSURef database in 2011. In the database, we identified archaeal 13022, bacterial 282285 and eukaryotic 36054 sequences that harbor both 530F and 907R regions.

(A)

	Number of sequences		Number of sequences without mismatch residues compared to 530F primers													Total sequences	Coverage (%)			
	harbor 530F region		1	2	3	4	5	6	7	8	9	10	11	12	13			14	15	
<i>Archaea</i>	13022	214	6980	4724	358	35	55	0	0	0	0	0	0	0	0	0	0	38	12404	95.2
<i>Bacteria</i>	282285	271887	62	0	1	1	2	557	69	562	120	5	63	474	73	0	0	0	273876	97.0
<i>Eukarya</i>	36054	33165	78	0	0	0	0	496	0	38	1	0	0	6	0	0	0	0	33784	94.0

(B)

	Number of sequences		Number of sequences without mismatch residues compared to the 907R consensus sequences																							Total sequences	Coverage (%)				
	harbor 907R region		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23			24	25	26	
<i>Archaea</i>	12404	1	82	1	11052	155	17	0	1	87	138	0	10	59	63	13	0	0	17	0	0	0	0	0	0	0	0	0	138	11834	90.1
<i>Bacteria</i>	273876	215442	1104	38076	11	0	1739	422	573	0	0	3732	21	2	0	0	7	266	0	103	46	39	63	5983	1339	238	0	269206	95.4		
<i>Eukarya</i>	33784	13	27652	0	48	8	0	0	4	0	0	0	1	363	1	2	0	0	0	0	0	0	0	0	3	0	0	0	28095	77.9	

Table S3. Coverage of 530F and 907R primers targeting SILVA SSURef database at the end of 2011. In the database, we identified archaeal 13022, bacterial 282285 and eukaryotic 36054 sequences that harbor both 530F and 907R regions.

(A) Number of sequences without mismatch residues compared to 530F primers used in archaeal set

	<i>Archaea</i>	<i>Bacteria</i>	<i>Eukarya</i>
Arch_530F	12062	63	78
Arch2_530F	90	3	0
Nano_530F	38	0	0
Total	12190	66	78
Coverage (%) without mismatch residues	93.6	0.0	0.2

(B) Number of sequences with 1 mismatch residues compared to 530F primers used in archaeal set

	<i>Archaea</i>	<i>Bacteria</i>	<i>Eukarya</i>
Arch_530F	298	490	49
Arch2_530F	12	222	16
Nano_530F	9	0	0
Total	319	712	65
Coverage (%) with 1 mismatch residue	2.4	0.3	0.2

(C) Number of sequences without mismatch residues compared to 907R primers used in archaeal set

	<i>Archaea</i>	<i>Bacteria</i>	<i>Eukarya</i>
Arch_907R	11052	11	48
Arch2_907R	159	4	368
SAGMEG_907R	155	0	8
DSEG_SAGMEGB_907R	138	0	0
Nano_907R	63	0	1
Total	11567	15	425
Coverage (%) without mismatch residues	88.8	0.0	1.2

(D) Number of sequences without mismatch residues compared to 907R primers used in archaeal set

	<i>Archaea</i>	<i>Bacteria</i>	<i>Eukarya</i>
Arch_907R	357	15	37
Arch2_907R	13	256	72
SAGMEG_907R	16	1	5
DSEG_SAGMEGB_907R	26	0	0
Nano_907R	27	17	31
Total	439	289	145
Coverage (%) with 1 mismatch residue	3.4	0.1	0.4

Table S4. Tag and OTU abundance of each taxon/division in SSU rRNA gene community structures at 5 and 25 cmbsf in methane seep sediment core 949C3, and abundance of shared tags and OTUs between the two depths. Characterization of each tag-sequence was based on top hit sequences of each tag sequence in Blast analysis. Tags with more than 98% identity were assigned as same OTU.

	Tags				OTUs			
	5 cmbsf		25 cmbsf		5 cmbsf		25 cmbsf	
	Total	Shared	Total	Shared	Total	Shared	Total	Shared
Archaea								
Ancient Archaeal Group (AAG)	0	0	15	0	0	0	6	0
Deep Sea Archaeal Group (DSAG)	17	17	90	78	4	4	12	4
Marine Group 1 (MG1)	535	376	81	77	50	8	12	8
Miscellaneous Crenarchaeotic Group (MCG)	2	2	44	14	2	2	16	2
Deep Sea Hydrothermal Vent Euryarchaeotic Group 6 (DHVEG 6)	74	17	162	2	47	2	120	2
Deep Sea Euryarchaeotic Group (DSEG)	0	0	5	0	0	0	5	0
Marine Hydrothermal Vent Group (MHVG)	0	0	10	0	0	0	8	0
Miscellaneous Euryarchaeotic Group (MEG)	0	0	4	0	0	0	4	0
ANMEs	5	3	51	21	3	1	7	1
uncultured Thermoplasmatales	0	0	116	0	0	0	46	0
other archaea	46	14	140	22	26	2	81	2
Total (Archaea)	679	429	718	214	132	19	317	19
Bacteria								
Acidobacteria	311	167	290	158	161	49	125	49
<i>Armatimonadetes</i> (OP10)	1	0	6	0	1	0	6	0
<i>Bacteroidetes</i>								
<i>Bacteroidia</i>	143	124	9	4	18	3	8	3
<i>Cytophagia</i>	130	100	99	92	33	9	15	9
<i>Flavobacteria</i>	214	112	57	45	50	9	18	9
<i>Sphingobacteria</i>	68	11	23	12	29	4	13	4
other <i>Bacteroidetes</i>	127	59	107	71	50	12	36	12
<i>Chlamydiae</i>	2	0	3	0	2	0	3	0
<i>Chlorobi</i>	45	29	105	84	18	7	17	7
<i>Chloroflexi</i>	94	48	538	212	52	17	178	17
<i>Deferribacteres</i>	28	14	108	69	23	11	38	11
<i>Elastimicrobia</i> (TM1)	4	0	8	0	4	0	6	0
<i>Fibrobacteres</i>	6	1	9	1	4	1	7	1
<i>Firmicutes</i>	115	19	20	2	24	2	14	2
<i>Fusobacteria</i>	5	4	36	25	5	4	8	4
<i>Gemmatimonadetes</i>	104	48	98	55	46	12	36	12
<i>Lentisphaerae</i>	66	13	24	12	28	5	17	5
<i>Nitrospirae</i>	47	20	105	45	31	12	55	12
<i>Planctomycetes</i>	453	168	370	156	258	64	214	64
<i>Proteobacteria</i>								
<i>Alphaproteobacteria</i>								
<i>Rhizobiales</i>	34	12	22	8	18	5	13	5
<i>Rhodobacteriales</i>	62	33	64	55	26	5	14	5
<i>Rhodospirillales</i>	206	66	26	19	90	8	14	8
other <i>Alphaproteobacteria</i>	81	27	20	16	36	2	6	2
<i>Betaproteobacteria</i>	41	30	37	33	11	3	7	3
<i>Deltaproteobacteria</i>								
SAR324	62	26	31	21	17	4	10	4
<i>Bdellovibrionales</i> <i>Bacteriovoraceae</i>	34	6	22	15	29	3	9	3
<i>Desulfurales</i> <i>Desulfuraceae</i>	16	11	67	40	7	4	20	4
<i>Desulfobacteriales</i> <i>Desulfobacteraceae</i>	1255	1074	906	772	130	46	116	46
<i>Desulfobacteriales</i> <i>Desulfobulbaceae</i>	34	11	52	46	10	3	9	3
<i>Desulfobacteriales</i> <i>Nitrospiraceae</i>	75	51	45	14	21	6	22	6
other <i>Desulfobacteriales</i>	6	4	22	8	3	1	12	1
<i>Desulfosomnoidales</i> <i>Desulfosomnoidaceae</i>	60	0	3	0	5	0	3	0
<i>Desulfosomnoidales</i> <i>Geebacteriaceae</i>	16	5	10	4	10	2	6	2
other <i>Desulfosomnoidales</i>	45	27	14	12	18	4	6	4
<i>Desulfosporimoniales</i>	1	0	1	0	1	0	1	0
<i>Mycoscoziales</i>	59	21	75	39	35	4	34	4
<i>Syntrophobacteriales</i>	32	24	87	77	9	1	7	1
other <i>Deltaproteobacteria</i>	241	109	155	92	122	31	76	31
<i>Epsilonproteobacteria</i>								
<i>Campylobacteriales</i> <i>Campylobacteraceae</i>	159	1	2	1	25	1	2	1
<i>Campylobacteriales</i> <i>Helicobacteraceae</i>	539	443	260	238	47	8	29	8
<i>Nastelliales</i>	5	0	1	0	3	0	1	0
other <i>Epsilonproteobacteria</i>	0	0	1	0	0	0	1	0
<i>Gammaproteobacteria</i>								
<i>Acidithiobacterales</i> <i>Acidithiobacillaceae</i>	265	241	525	486	28	15	40	15
<i>Alteromonadales</i>	57	33	45	43	20	6	8	6
B47.8	82	81	101	101	2	1	1	1
<i>Chromatiales</i> <i>Chromatiaceae</i>	47	39	20	17	11	5	8	5
<i>Chromatiales</i> <i>Ectothiorhodospiraceae</i>	7	3	9	2	4	2	6	2
<i>Chromatiales</i> <i>Grandisociococcaceae</i>	6	1	6	6	5	1	1	1
<i>Chromatiales</i> <i>Nitrosooccus</i>	1	1	4	4	1	1	1	1
<i>Methylocoziales</i>	277	246	72	63	32	8	13	8
<i>Oceanospirillales</i>	57	20	40	35	25	4	9	4
other sulfur-oxidizing group	179	173	271	264	13	9	13	9
<i>Thiotrichales</i> <i>Psycricetiaaceae</i>	97	57	68	54	21	6	13	6
other <i>Thiotrichales</i>	138	123	122	104	25	14	30	14
<i>Xanthomonadales</i>	189	124	119	103	58	19	24	19
other <i>Gammaproteobacteria</i>	301	166	415	312	115	25	98	25
<i>Zetaproteobacteria</i>	1	1	10	6	1	1	4	1
other <i>Proteobacteria</i>	52	23	70	58	21	5	17	5
<i>Spirochaetes</i>	160	103	57	24	30	7	25	7
<i>Tenericutes</i>	39	0	2	0	14	0	1	0
<i>Verrucomicrobia</i>	6	0	1	0	4	0	1	0
BRC1	19	2	14	2	16	2	13	2
OD1	3	0	12	0	2	0	12	0
OP3	27	8	44	9	18	4	29	4
OP9	2	2	32	5	1	1	3	1
OP11	0	0	6	0	0	0	5	0
SR1	56	0	4	0	35	0	3	0
TM7	1	0	1	0	1	0	1	0
WS3	60	30	112	51	29	15	56	15
TA06	24	13	59	12	14	6	38	6
TM6	38	14	34	7	23	3	28	3
other <i>Bacteria</i>	813	443	759	528	256	27	230	27
Total (Bacteria)	8030	4865	6972	4849	2335	549	1973	549

Table S5. Diversity and richness indexes of whole microbial community structures and each taxon/division at 5 and 25 cmbsf in methane seep sediment core 949C3. Tags with more than 98% identity were assigned as same OTU.

	Tags		OTUs		Chao1		ACE		Shannon		Shannon evenness		Simpson	
	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf	5 cmbsf	25 cmbsf
Total	8709	7690	2473	2296	6558.0	6374.0	11117.8	11269.3	6.49	6.54	0.83	0.84	0.0063	0.0055
DHVEG6	74	162	47	120	130.3	405.0	151.5	436.8	3.45	4.65	0.90	0.97	0.0504	0.0054
MGI	535	81	50	12	65.5	(22.5)	71.7	(23.5)	2.22	(1.10)	0.57	(0.44)	0.2776	(0.5701)
<i>Acidobacteria</i>	311	290	161	125	426.4	200.9	607.3	339.4	4.64	4.44	0.91	0.92	0.0173	0.0149
<i>Bacteroidetes</i>	682	295	182	90	431.8	192.2	696.2	306.8	4.21	3.77	0.81	0.84	0.0388	0.0400
<i>Chloroflexi</i>	94	538	51	179	100.6	456.9	156.0	812.7	3.68	4.39	0.94	0.85	0.0229	0.0244
<i>Planctomycetes</i>	453	370	257	214	568.5	582.2	984.5	1125.6	5.21	5.06	0.94	0.94	0.0067	0.0068
<i>Alphaproteobacteria</i>	383	132	170	47	400.2	117.9	732.3	193.1	4.59	2.96	0.89	0.77	0.0172	0.1194
<i>Gammaproteobacteria</i>	1703	1817	362	278	880.0	616.9	1214.8	986.3	4.68	4.08	0.79	0.72	0.0226	0.0544
<i>Deltaproteobacteria</i>	1936	1490	415	331	1103.0	767.2	1649.2	1260.9	4.39	4.61	0.73	0.79	0.0443	0.0227
<i>Epsilonproteobacteria</i>	703	264	75	33	123.2	222.0	195.6	439.0	2.37	1.68	0.55	0.48	0.2091	0.3396

Tag population of the MGI at 25cmbsf was smaller than 100, and excluded from the discussion.