uncultured archaeon

Single–cell Assembly QC Report 04/07/2014

1. Project Information

Program	Microbial/CSP 2012
PMO Project	0
Seq Proj ID	1027076
Sequencing Project Name	Geoarchaeota archaeon JGI 000156CP–L10
JGI Project ID	0

2. Read Statistics

Illumina Std PE Statistics

File name	7666.6.80850.ATGTCA.fastq
Library	TGSS
Number of reads	28,857,866
Sequencing depth [†]	866X
Read type	2x150 bp

[†] A genome size of 5.0 Mbp was assumed in this calculation.

3. Read QC Results

The following are the results of reads screened against contaminants. Pairs of matching reads were removed from the dataset.

Illumina Std PE Read Filter Statistics

Description	Num Reads	Pct Reads
Input	28,857,866	100
Contam removed	86	0.0
Artifact removed	311,514	1.1
Total removed	8,857,866	30.7
Total remaining	20,000,000	69.3

List of Contaminants Removed

Description	Num Reads	Pct Reads
gi|357579535|Canis_lupus_familiaris_chr20	44	0.00
human_chr2	34	0.00
gi|357579577|Canis_lupus_familiaris_chr3	32	0.00
gi|357579571|Canis_lupus_familiaris_chr5	24	0.00
human_chr18	2	0.00
human_chr5	2	0.00
human_chr3	2	0.00

human_chr4	2	0.00

The following are the results of reads screened against potential reagent and process contaminants but were not removed from the dataset.

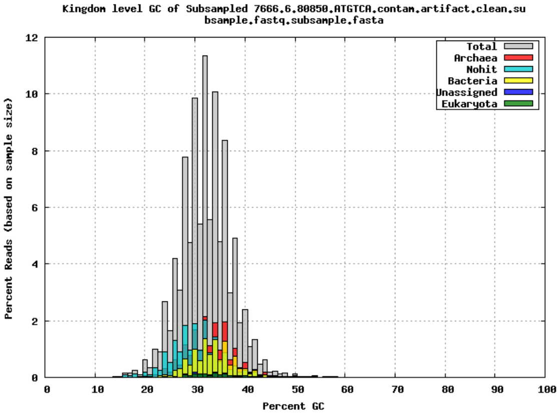
Illumina Std PE Contamination Identification Statistics

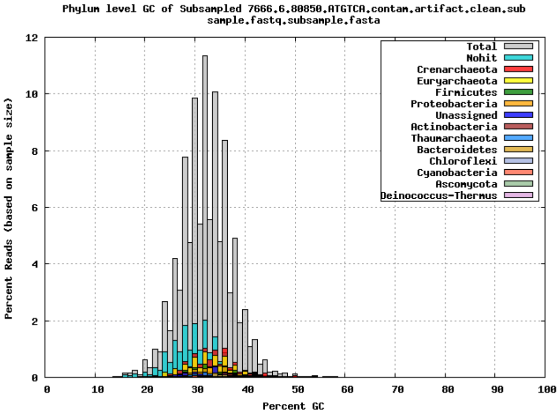
Description	Num Reads	Pct Reads
Input	28,857,866	100
Contam identified	10	0.0

List of Contaminants Identified

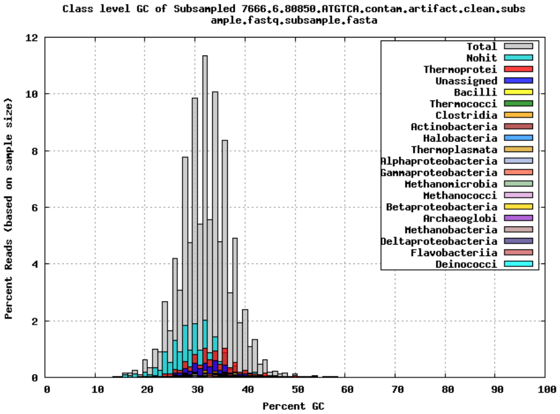
Description	Num Reads	Pct Reads
Escherichia	2	0.00
Delftia	2	0.00
Pseudomonas	2	0.00
Shigella	2	0.00
Ralstonia	2	0.00

GC histogram of the reads subsampled to 10k, overlaid with GC of hits based on BLASTX, shown for different taxonomic levels.

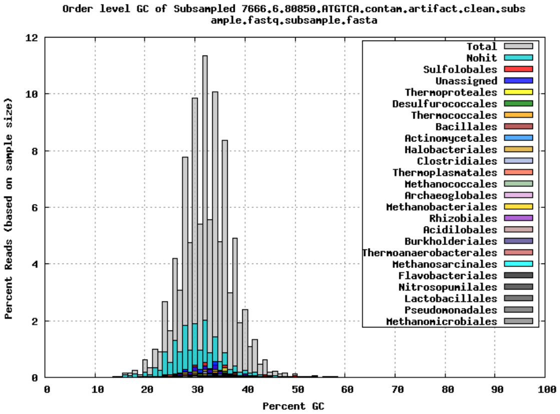

Kingdom


Phylum

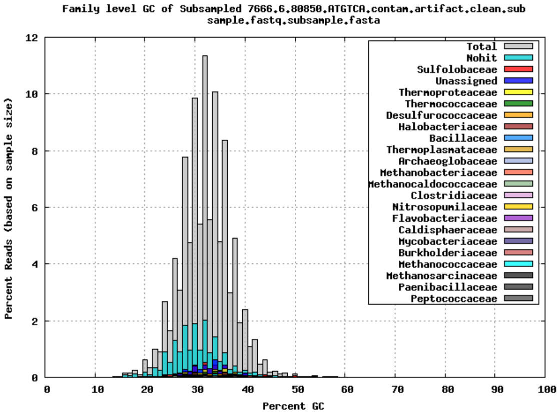
Class



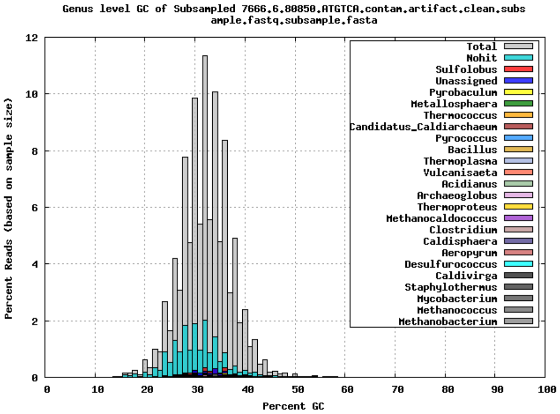
Order



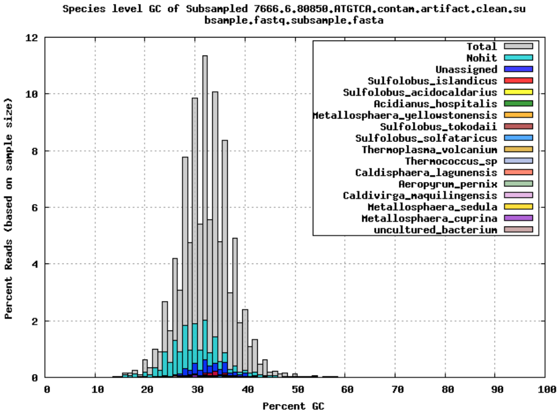
Family



Genus



Species

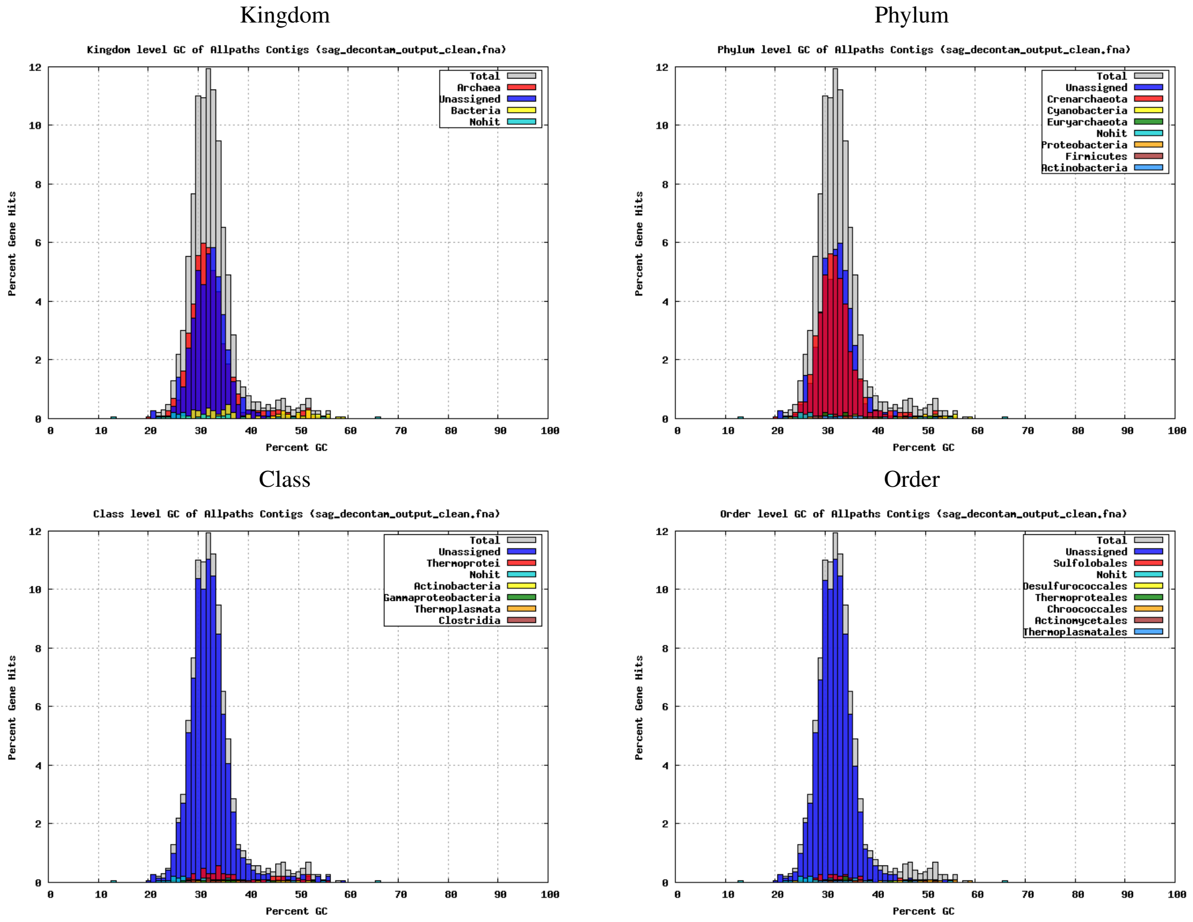


4. Assembly Statistics

Assembly method	SPAdes with auto decontamination
Scaffold total	89
Contig total	89
Scaffold sequence length	1.7 Mb
Contig sequence length	1.7 Mb (0.0% gap)
Scaffold N/L50	18/32.6 kb
Contig N/L50	18/32.6 kb
Largest Contig	75.9 kb
Number of scaffolds >50 kb	6
Pct of genome in scaffolds >50 kb	22.7
Pct of reads asssembled (raw)	83.1
Pct of reads asssembled (decontam)	77.4

5. Assembly QC Results

GC histogram of the predicted genes on each contig, overlaid with GC of hits based on BLASTP, shown for different taxonomic levels.

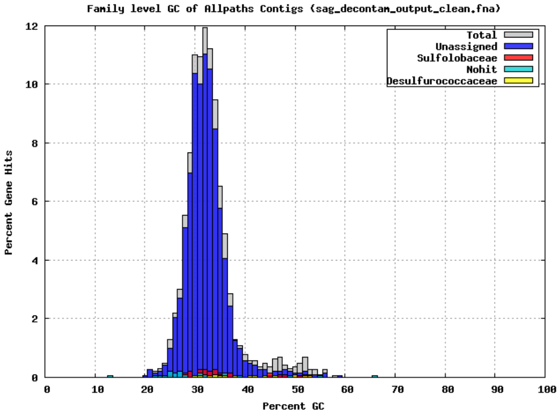

Kingdom

Phylum

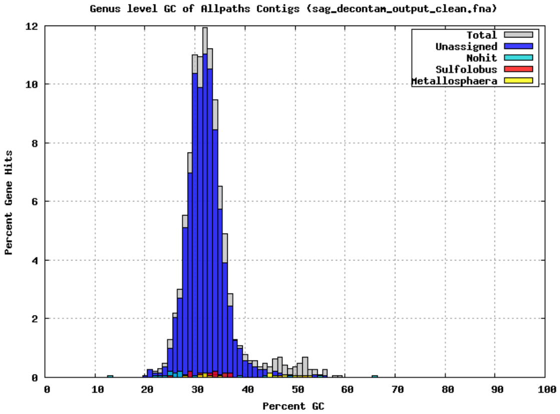
Class

Order

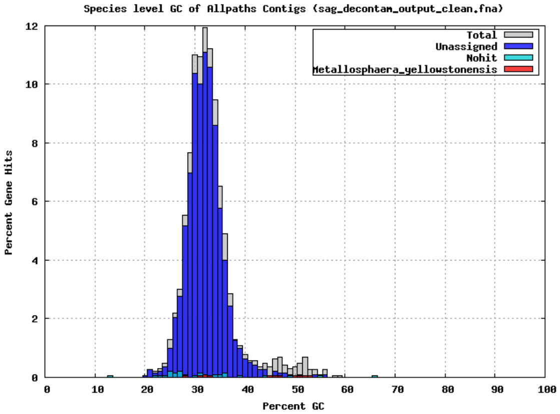
Family



Genus

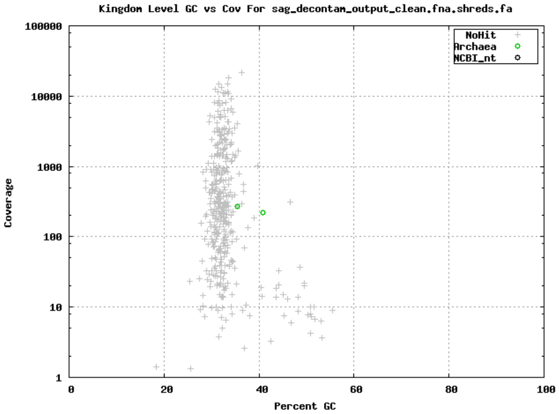


Species

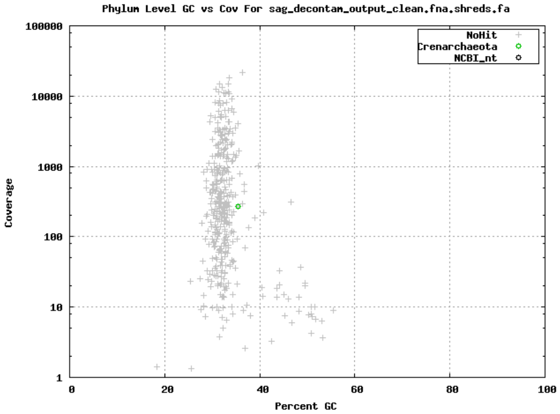


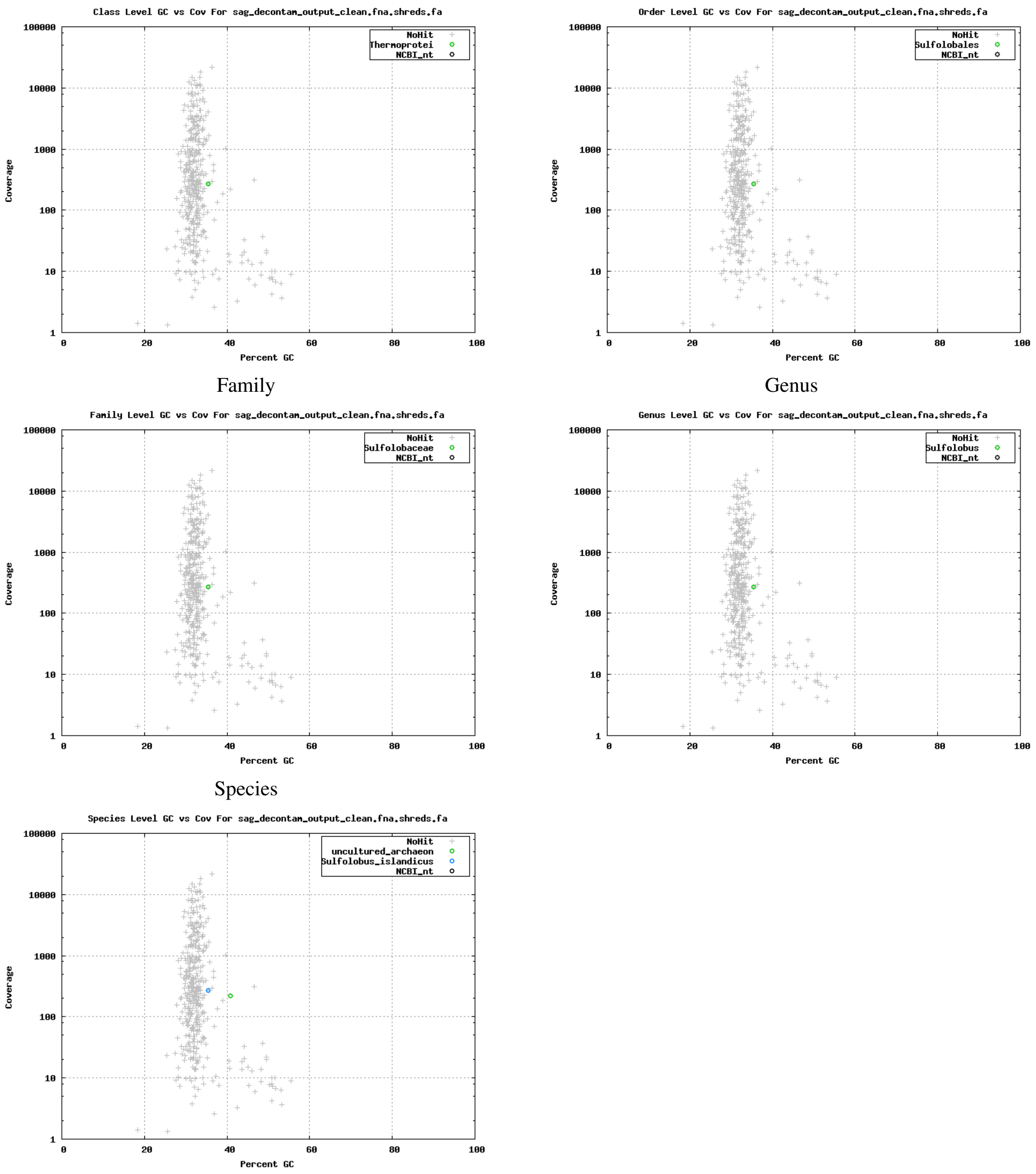
GC vs coverage based on GC of NCBI nt and Greengenes 16S rRNA gene hits to the assembly using megablast, shown for different taxonomic levels.

Kingdom



Phylum





Class

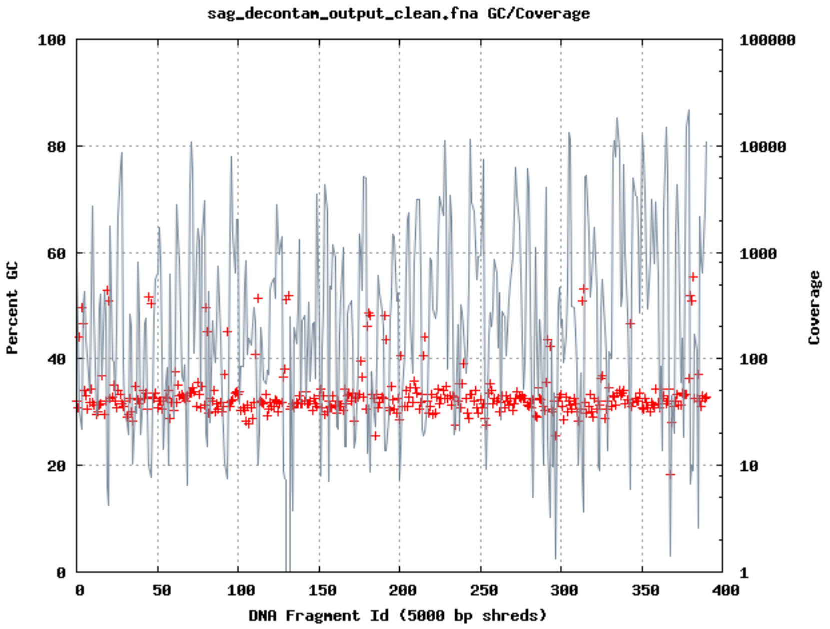
Order

Family

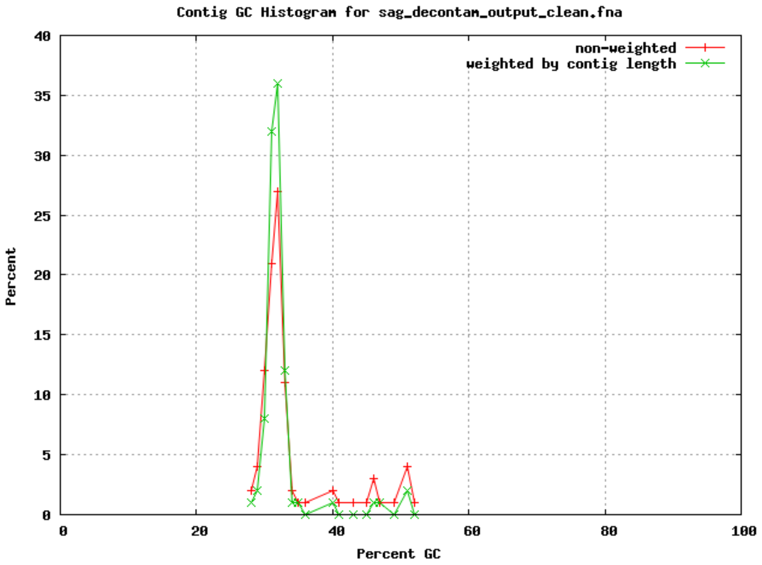
Genus

Species

Coverage vs GC. Contigs were shredded into non-overlapping 5kbp and the GC of each shred was plotted as a point, colored by scaffold id. Coverage was calculated by mapping the fragment library to the final asssembly and plotted as connected points.



GC histogram of the contigs, including contig length weighted distribution.

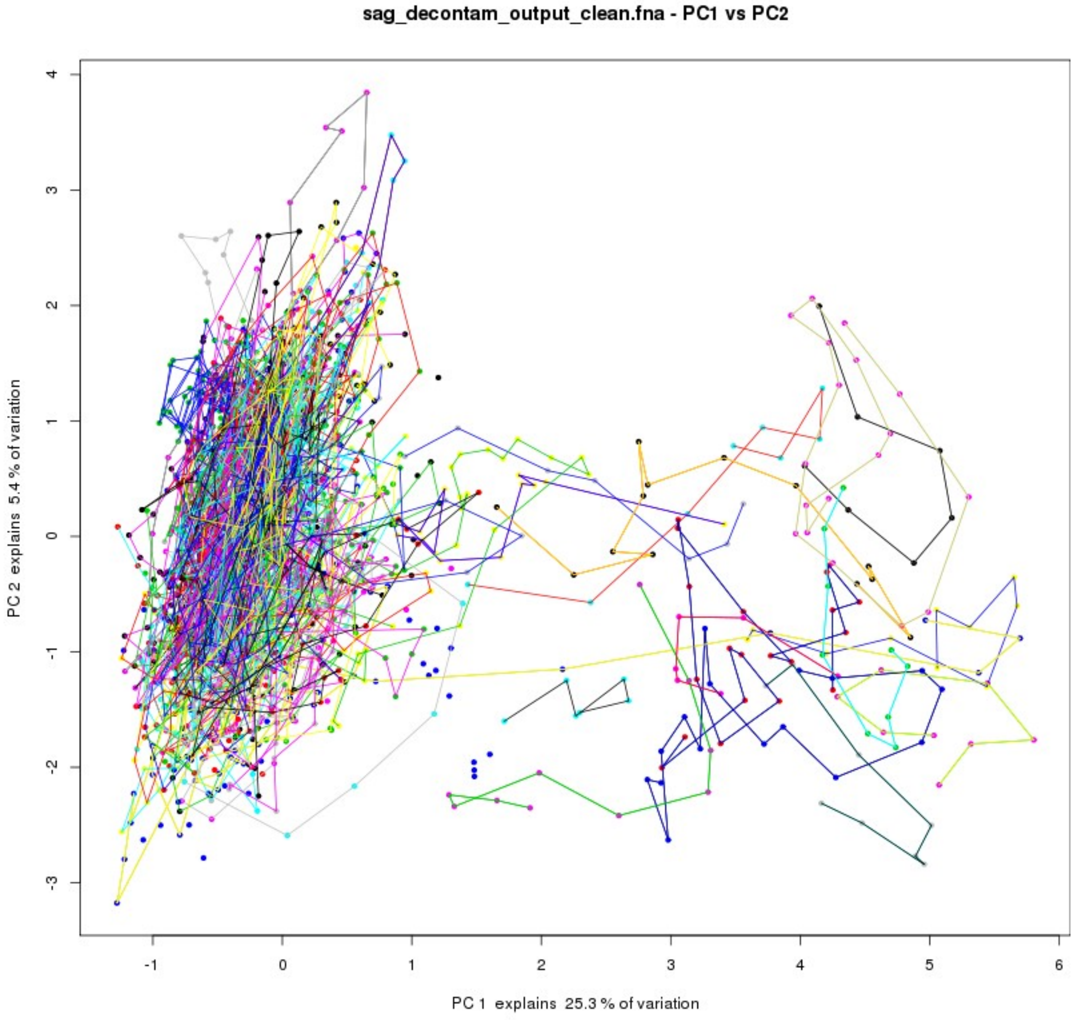


List of contigs and average percent GC, grouped in bins of 5:

Pct GC Bin	Contig Name
25	NODE_50_length_11236_cov_95.8108_ID_103, NODE_71_length_6503_cov_192.994_ID_149, NODE_73_length_6314_cov_26.4531_ID_153, NODE_76_length_6056_cov_18.8212_ID_159, NODE_80_length_5604_cov_281.579_ID_165, NODE_96_length_4878_cov_281.327_ID_211
30	NODE_1_length_75851_cov_758.926_ID_1, NODE_2_length_73144_cov_398.274_ID_3, NODE_3_length_66427_cov_1778.35_ID_5, NODE_4_length_61123_cov_824.336_ID_7, NODE_5_length_55719_cov_1079.9_ID_9, NODE_6_length_52032_cov_282.999_ID_11, NODE_7_length_48417_cov_185.684_ID_13, NODE_8_length_44711_cov_2190.23_ID_15,

	NODE_9_length_41378_cov_144.777_ID_17, NODE_10_length_41321_cov_2356.69_ID_19, NODE_11_length_38327_cov_1375.49_ID_21, NODE_12_length_38195_cov_2169.82_ID_23, NODE_13_length_37190_cov_490.381_ID_25, NODE_14_length_36550_cov_255.024_ID_27, NODE_15_length_36476_cov_407.507_ID_29, NODE_16_length_35874_cov_346.141_ID_31, NODE_17_length_34702_cov_1520.27_ID_33, NODE_18_length_32580_cov_2522.65_ID_35, NODE_19_length_32126_cov_5783.81_ID_37, NODE_20_length_31303_cov_124.723_ID_39, NODE_21_length_28042_cov_1703.95_ID_41, NODE_22_length_25252_cov_163.011_ID_43, NODE_24_length_23569_cov_218.285_ID_47, NODE_25_length_22378_cov_163.706_ID_49, NODE_26_length_21957_cov_136.72_ID_51, NODE_27_length_21189_cov_1689.07_ID_53, NODE_28_length_20919_cov_1375.65_ID_55, NODE_29_length_20293_cov_249.362_ID_57, NODE_30_length_18835_cov_153.268_ID_59, NODE_31_length_18776_cov_294.389_ID_61, NODE_33_length_17009_cov_1556.15_ID_65, NODE_34_length_16095_cov_261.278_ID_67, NODE_35_length_15495_cov_85.551_ID_69, NODE_36_length_15479_cov_111.284_ID_71, NODE_37_length_15322_cov_156.297_ID_73, NODE_38_length_15090_cov_374.476_ID_75, NODE_39_length_14457_cov_1554.54_ID_77, NODE_40_length_14420_cov_225.012_ID_79, NODE_41_length_14090_cov_5240.78_ID_81, NODE_42_length_13252_cov_137.758_ID_83, NODE_43_length_13007_cov_940.997_ID_89, NODE_44_length_12715_cov_836.495_ID_91, NODE_45_length_12273_cov_292.523_ID_93, NODE_46_length_12220_cov_732.621_ID_95, NODE_47_length_12113_cov_502.159_ID_97, NODE_48_length_12048_cov_219.225_ID_99, NODE_51_length_10961_cov_88.4575_ID_105, NODE_53_length_10542_cov_35.6054_ID_109, NODE_54_length_10338_cov_1806.05_ID_111, NODE_55_length_10227_cov_102.843_ID_85, NODE_56_length_10187_cov_232.742_ID_113, NODE_57_length_9837_cov_145.557_ID_115, NODE_59_length_9010_cov_3456.74_ID_119, NODE_61_length_8339_cov_26.154_ID_131, NODE_63_length_8142_cov_63.013_ID_135, NODE_64_length_7869_cov_378.122_ID_137, NODE_66_length_7395_cov_103.374_ID_141, NODE_70_length_6527_cov_322.241_ID_147, NODE_74_length_6248_cov_761.281_ID_155, NODE_75_length_6082_cov_18.5444_ID_157, NODE_78_length_5704_cov_8931.69_ID_163, NODE_79_length_5608_cov_15.6231_ID_123, NODE_83_length_5497_cov_281.99_ID_171, NODE_87_length_5223_cov_30.1418_ID_187, NODE_89_length_5142_cov_29.3668_ID_195, NODE_92_length_4960_cov_48.8377_ID_203
35	NODE_23_length_24734_cov_413.486_ID_45, NODE_65_length_7397_cov_5.7088_ID_139
40	NODE_60_length_8601_cov_6.30084_ID_129, NODE_68_length_6826_cov_14.0418_ID_145, NODE_85_length_5278_cov_11.7722_ID_179, NODE_90_length_4979_cov_9.48457_ID_185
45	NODE_49_length_11800_cov_15.229_ID_101, NODE_58_length_9826_cov_18.4541_ID_117, NODE_62_length_8262_cov_9.36847_ID_133, NODE_77_length_5722_cov_14.1288_ID_161, NODE_81_length_5574_cov_229.352_ID_167, NODE_91_length_4971_cov_3.97803_ID_201
50	NODE_52_length_10744_cov_5.75208_ID_107, NODE_69_length_6798_cov_6.3365_ID_127, NODE_72_length_6418_cov_4.84441_ID_151, NODE_82_length_5527_cov_4.11056_ID_169 NODE_86_length_5258_cov_4.90698_ID_181

Principal component analysis of tetramer frequencies of contigs. Detectable variations are highlighted in color.



Estimated genome recovery derived from analysis of universal single-copy genes detected in final assembly.

HMM	Pct Recovered
bacteria	49.56 %
archaea	100 %

6. Sequence Data Availability

The following sequence fasta files can be downloaded from our JGI portal website.
http://www.jgi.doe.gov/genome-projects

Filename	Description
sag_decontam_output_clean.fna	SPAdes with auto decontamination

7. Annotation Data Availiability

The annotation of the assembled contigs can be found within IMG.
http://img.jgi.doe.gov

8. Methods

Single Cell Minimal Draft

Genome sequencing and assembly
The draft genome of was generated at the DOE Joint genome Institute (JGI) using the Illumina technology [1]. An Illumina std shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform which generated 28,857,866 reads totaling 4,328.7 Mb. All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts [2]. Following steps were then performed for assembly: (1) artifact filtered Illumina reads were assembled using SPAdes [3] (version 3.0.0), (3) Parameters for assembly steps were –t 16 –m 120 —sc —careful —12. The final draft assembly contained 89 contigs in 89 scaffolds, totalling 1.7 Mb in size. The final assembly was based on 3,000.0 Mb of Illumina data. Based on a presumed genome size of 5.0 Mb, the average input read coverage used for the assembly was 600.0X.

Genome annotation
Genes were identified using Prodigal [4], followed by a round of manual curation using GenePRIMP [5] for finished genomes and Draft genomes in fewer than 20 scaffolds. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, KEGG, COG, and InterPro databases. The tRNAScanSE tool [6] was used to find tRNA genes, whereas ribosomal RNA genes were found by searches against models of the ribosomal RNA genes built from SILVA [7]. Other non–coding RNAs such as the RNA components of the protein secretion complex and the RNase P were identified by searching the genome for the corresponding Rfam profiles using INFERNAL [8]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes (IMG) platform [9] developed by the Joint Genome Institute, Walnut Creek, CA, USA [10].

1. Bennett S. Solexa Ltd. Pharmacogenomics. 2004;5(4):433–8.
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4. Hyatt D, Chen GL, Lacascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 2010; 11:119.
5. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 2010; 7:455–457.
6. Lowe TM, Eddy SR. tRNAscan–SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997; 25:955–964.
7. Pruesse E, Quast C, Knittel, Fuchs B, Ludwig W, Peplies J, Glckner FO. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nuc Acids Res 2007; 35: 2188–7196.
8. INFERNAL. Inference of RNA alignments. http://infernal.janelia.org.
9. The Integrated Microbial Genomes (IMG) platform. http://www.ncbi.nlm.nih.gov/pubmed/24165883
10. Markowitz VM, Mavromatis K, Ivanova NN, Chen IMA, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 2009; 25:2271–2278.