

1. Project Information

Program	Microbial/CSP 2014
Seq Proj ID	1092600
Sequencing Project Name	Klosterneuburg WWTP active sludge C28.HAv2

2. Read Statistics

Illumina Std PE Statistics

File name	9682.1.140674.TCCTGAG-ATAGCCT.anqpht.fastq
Library	ATOYN
Number of reads	4,006,018
Read type	2x149 bp

3. Read QC Results

The following are the results of reads screened against artifact sequences. Pairs of matching reads ($\geq 95\%$ ID) were removed from the dataset.

Illumina Std PE Read Filter Statistics

Description	Num Reads	Pct Reads
Input	4,006,018	100
Normalized removed	234,730	5.9
Total removed	234,730	5.9
Total remaining	3,771,288	94.1

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	4,006,018	100
Contam identified	7,288	0.2

List of Contaminants Identified

Description	Num Reads	Pct Reads
<i>Escherichia</i>	6,086	0.15
<i>Shigella</i>	1,080	0.03

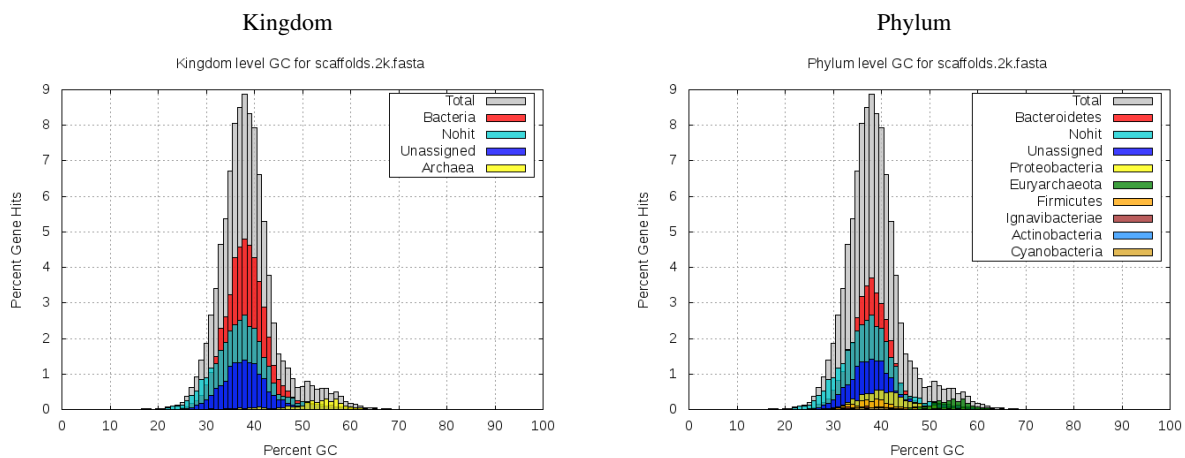
<i>Ralstonia</i>	116	0.00
<i>Cupriavidus</i>	6	0.00

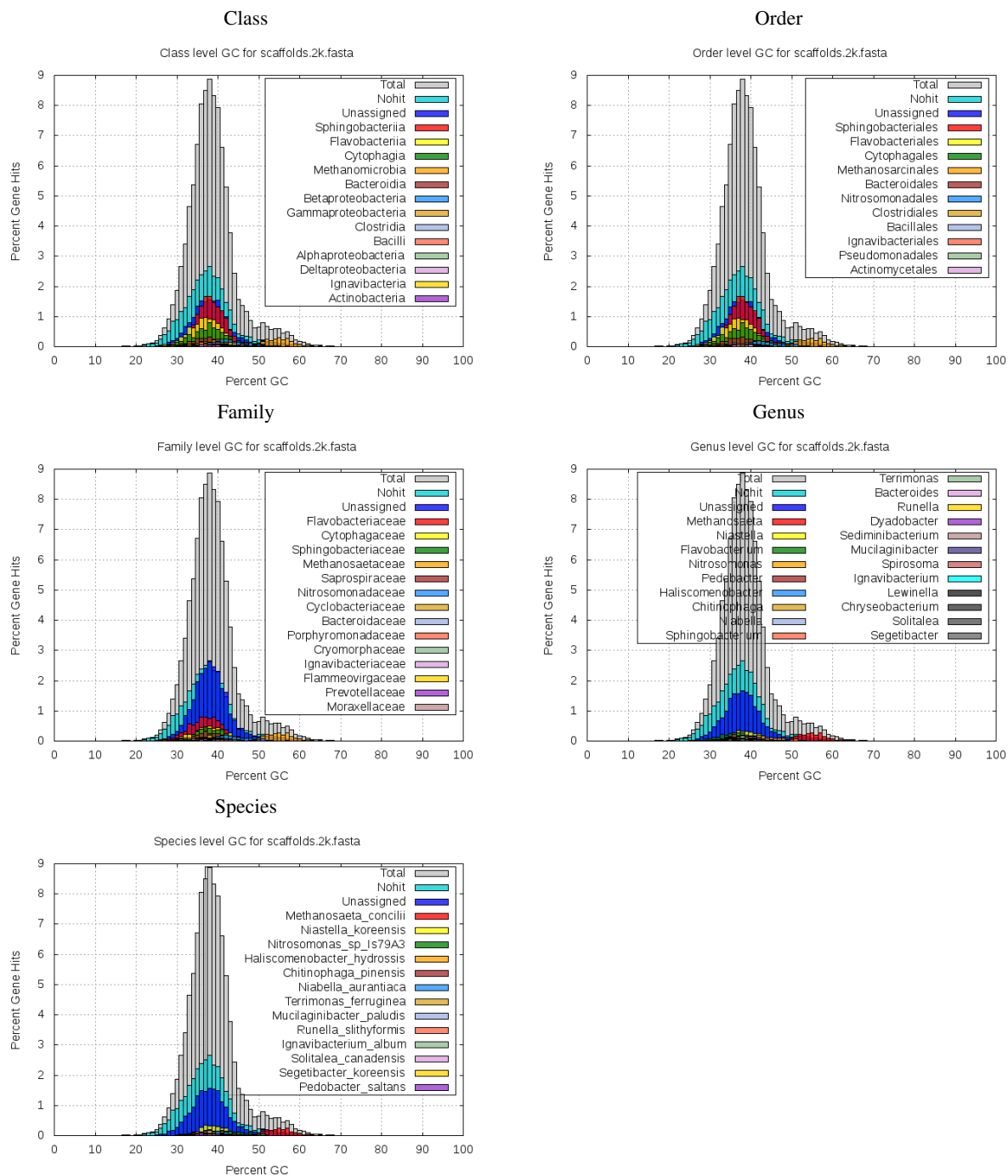
4. Assembly Statistics

Assembly method	SPAdes
Scaffold total	2,469
Contig total	2,845
Scaffold sequence length	18,301 Mb
Contig sequence length	18,287 Mb 0.076% gap
Scaffold N/L50	357/11.56 kb
Contig N/L50	374/10.756 kb
Largest Contig	209.1 kb
Number of scaffolds >50 kb	28
Pct of genome in scaffolds >50 kb	12.67
Pct of reads assembled	66.3

5. Assembly QC Results

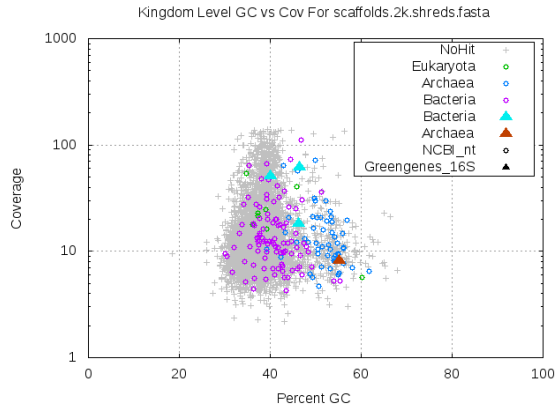
GC histogram of the predicted genes on each scaffold, overlaid with GC of hits based on LAST, shown for different taxonomic levels.



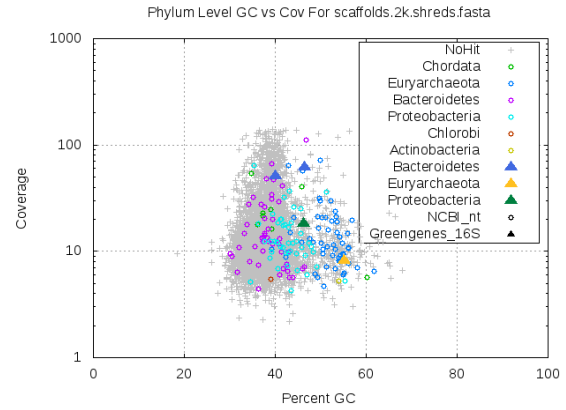


GC vs coverage based on GC of NCBI nt and Greengenes 16S rRNA gene hits to the assembly using megablast, shown for different taxonomic levels.

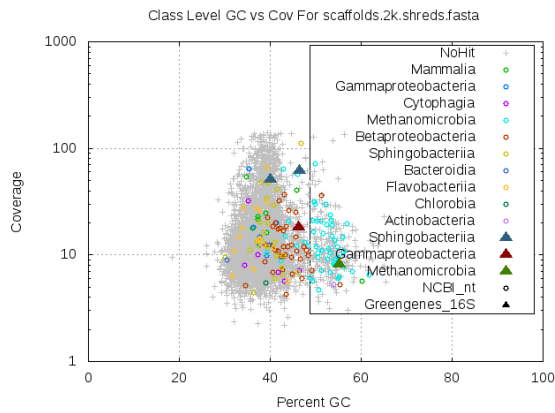
Kingdom



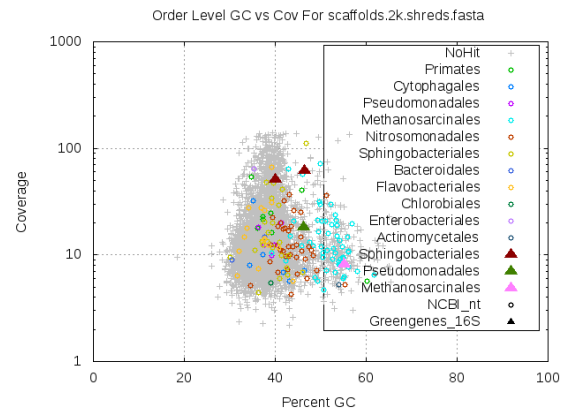
Phylum



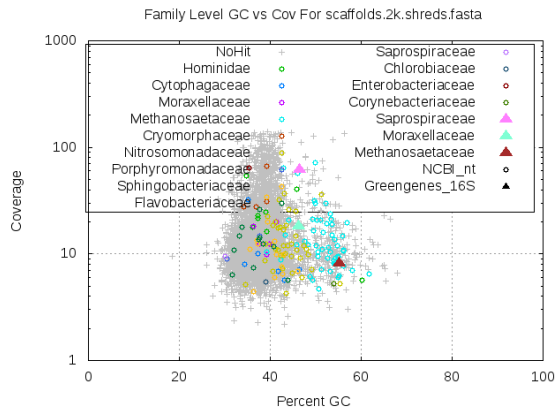
Class



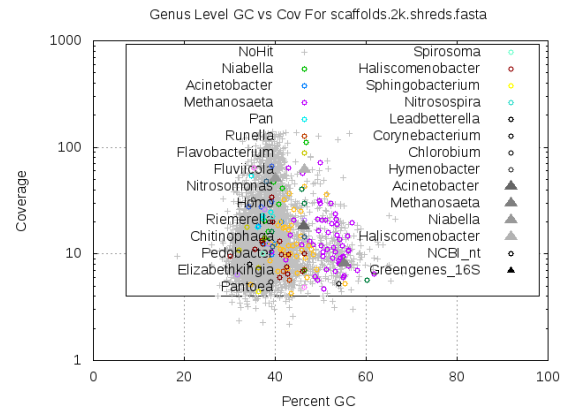
Order

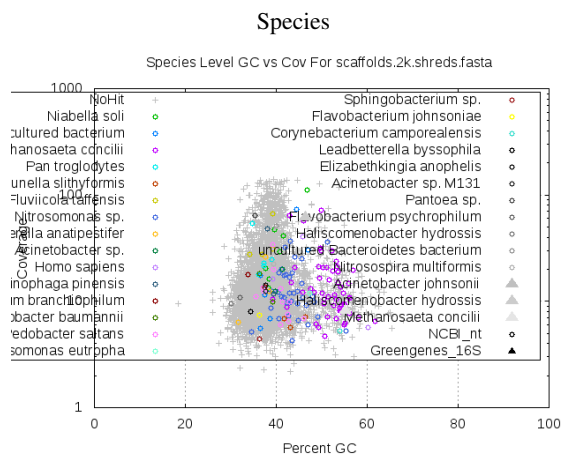


Family

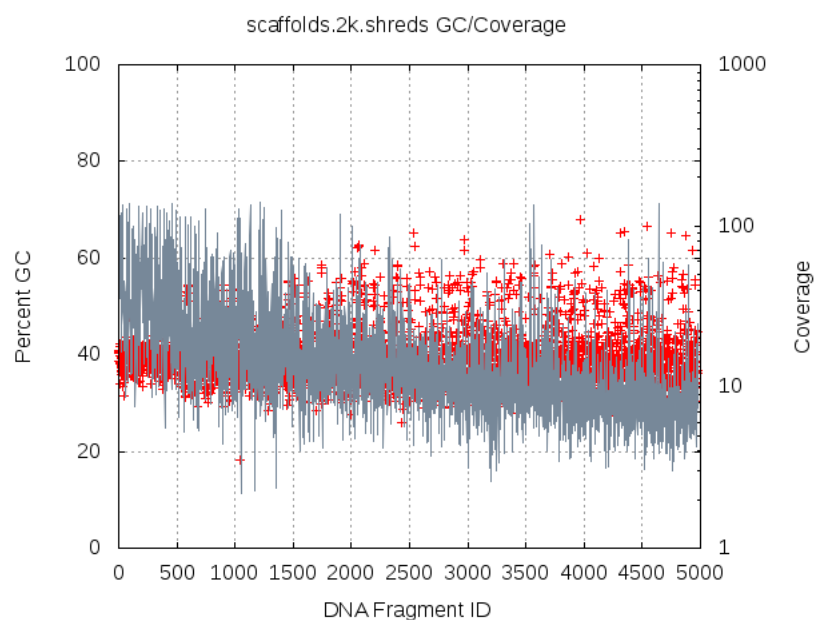


Genus

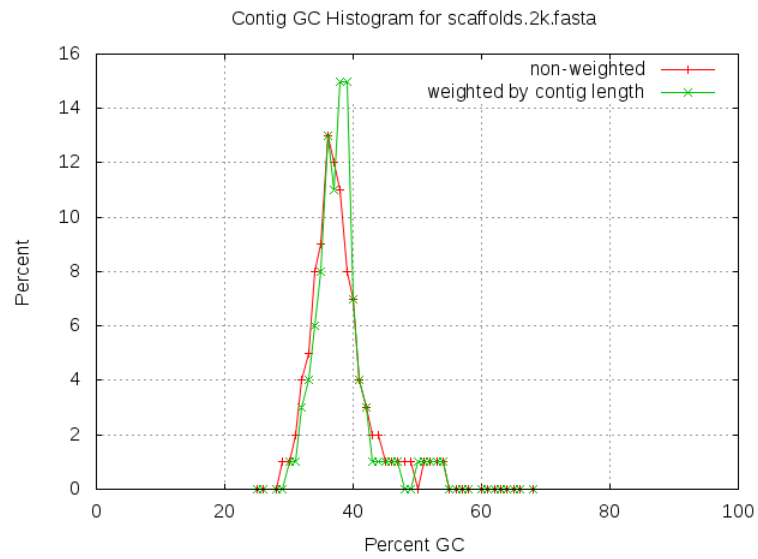




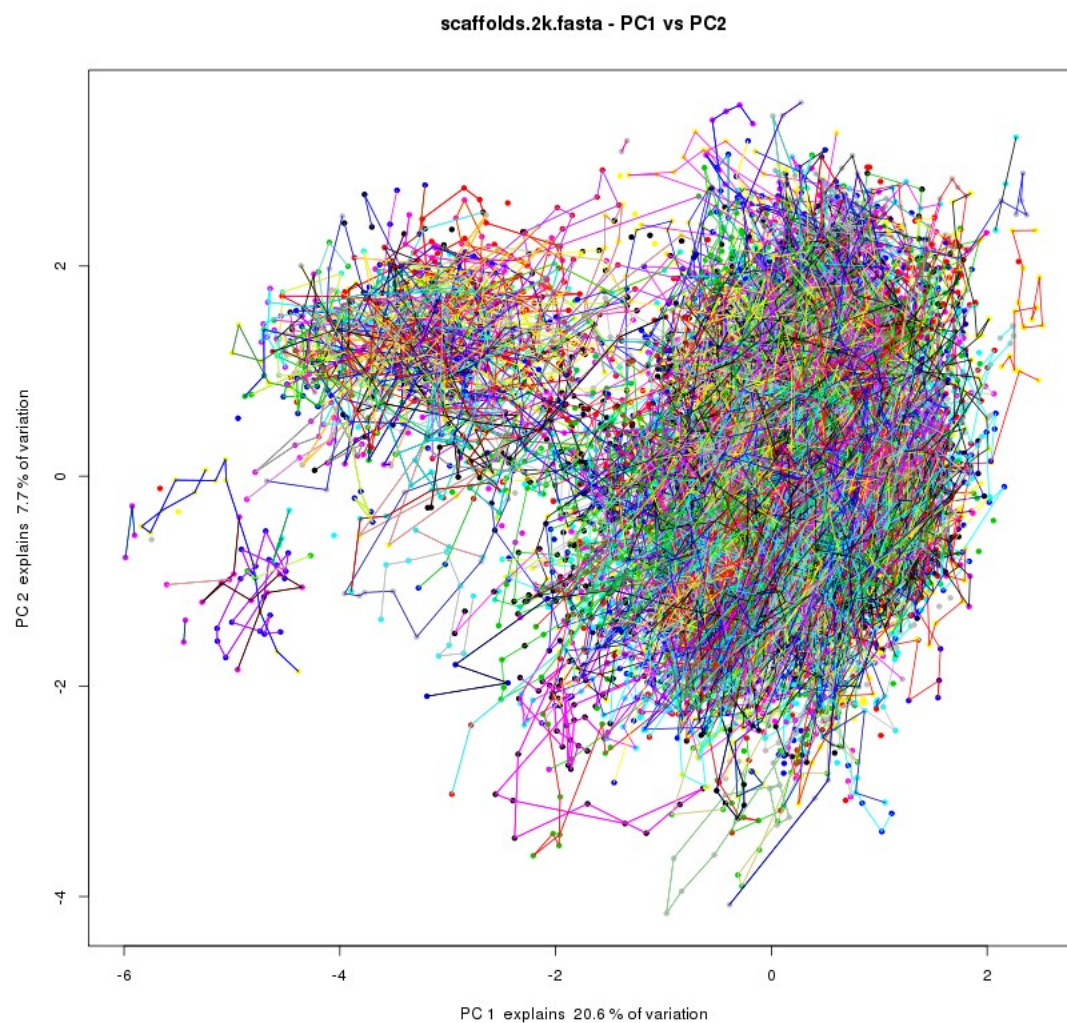
Coverage vs GC. Scaffolds were shredded into non-overlapping 5 kbp fragments and the GC content of each shred was plotted as a point, colored by scaffold id. Coverage was calculated by mapping the fragment library to the final assembly and plotted as connected points.



GC histogram of the scaffolds, including scaffold length weighted distribution.



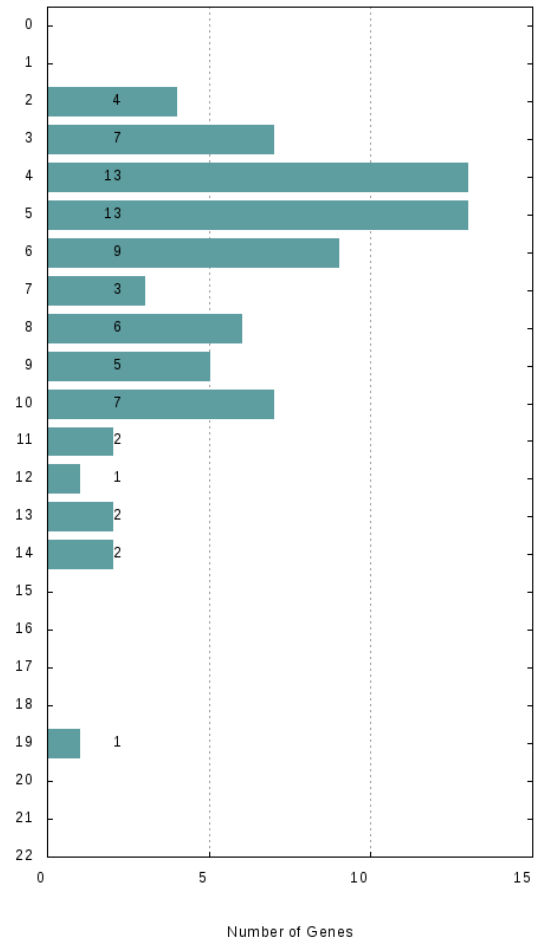
Principal component analysis of tetramer frequencies of scaffolds. Detectable variations are highlighted in color.



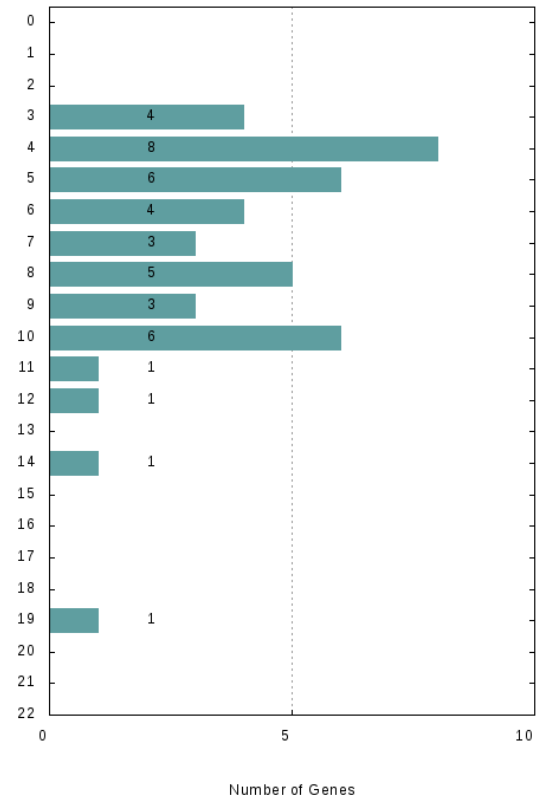
Estimated genome recovery derived from analysis of universal single-copy genes detected in final assembly.

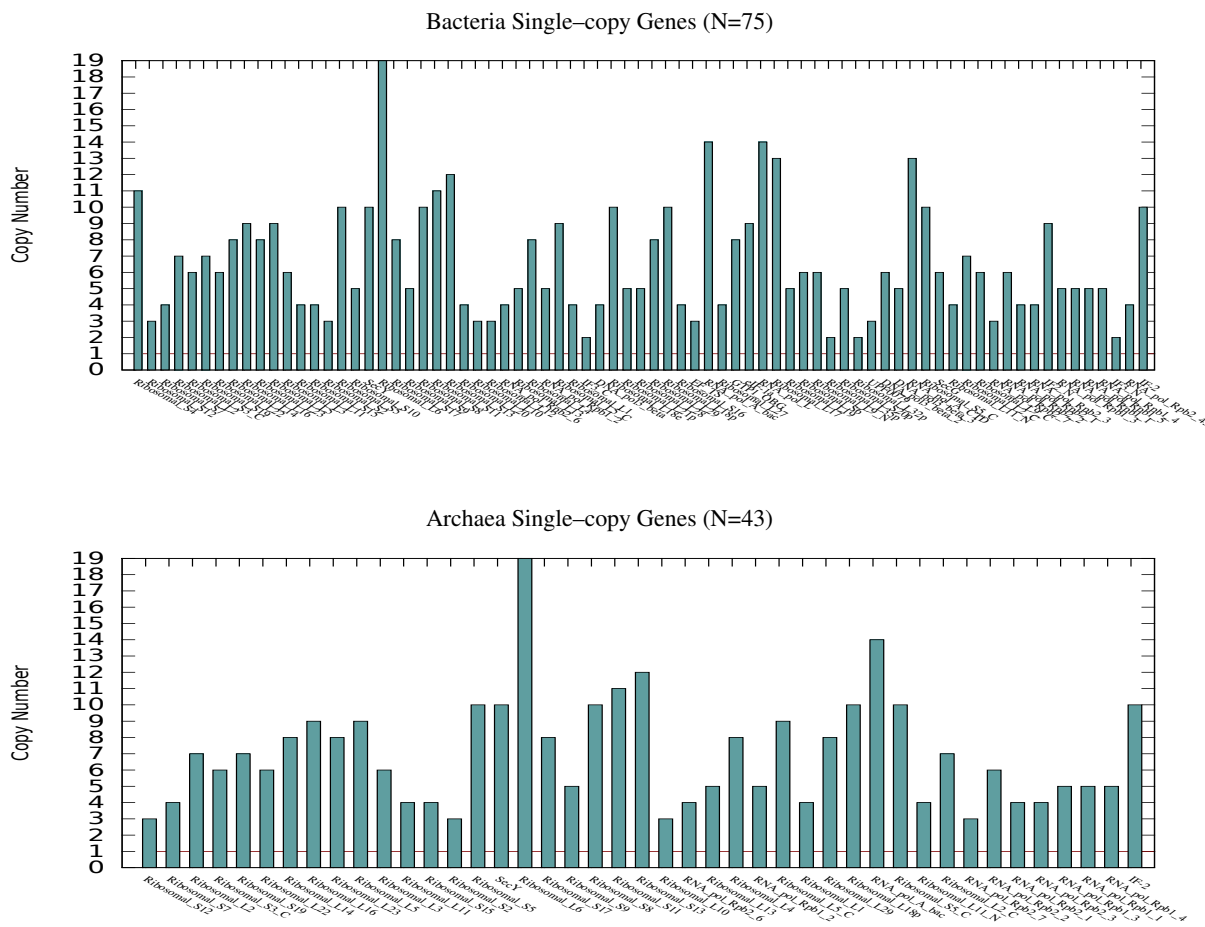
HMM	Pct Recovered
archaea	100 %
bacteria	100 %

Bacteria Single-copy Gene Histogram



Archaea Single-copy Gene Histogram





6. Sequence Data Availability

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

7. Annotation Data Availability

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

8. Methods

Cell-Enrichment Minimal Draft

Genome sequencing and assembly

The draft genome of *No Tax ID assigned* was generated at the DOE Joint Genome Institute (JGI) using the Illumina

technology [1]. An Illumina standard shotgun library was constructed and sequenced using the Illumina NextSeq platform which generated 4,006,018 reads totaling 596.9 Mbp. 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 [2], a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts. Furthermore, the reads were screened for human, dog and cat contaminant sequences. Reads with high k-mer coverage ($>100\times$ average k-mer depth) were normalized and error corrected to an average depth of $100\times$. Reads with an average k-mer depth of less than $2\times$ were removed. Normalization was performed using BBNorm [3] and error correction was performed using Tadpole [3]. (1) Artifact filtered and normalized Illumina reads were assembled using SPAdes (version 3.6.0) [4]; (2) assembly contigs were trimmed 200 bp at the ends and discarded if length is <2 kbp or read coverage is $<2\times$ (BBMap: `nodisk ambig=all maxindel=100 covstats=covstats.txt minhits=2 and filterbycoverage.sh: cov=covstats.txt mincov=2 minr=6 minp=95 minl=2000 trim=200 overwrite=true`). Parameters for the SPAdes assembly were `-t 16 -m 120 -sc -careful -k 25,55,95 -12`. The final draft assembly contained 2,845 contigs in 2,469 scaffolds, totalling 18.287 Mbp in size and was based on 561.9 Mbp of Illumina data.

1. Bennett S. Solexa Ltd. *Pharmacogenomics*. 2004;5(4):433–8.
 2. Mingkun L, Copeland A, Han J. DUK, unpublished, 2011.
 3. B. Bushnell: BBTools software package, URL <http://sourceforge.net/projects/bbmap>.
 4. Bankevich A, et.al, SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; 19:455–77.
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