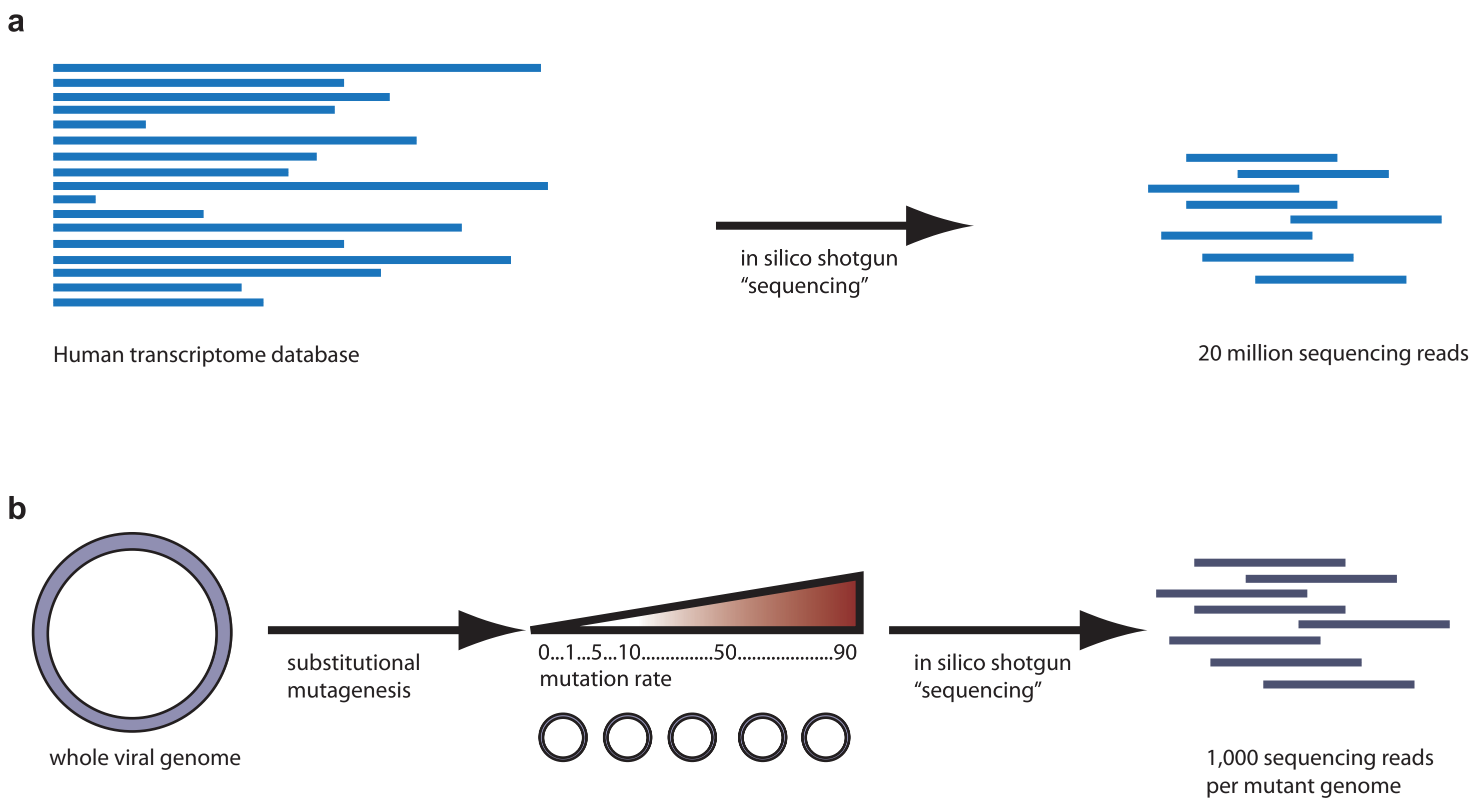
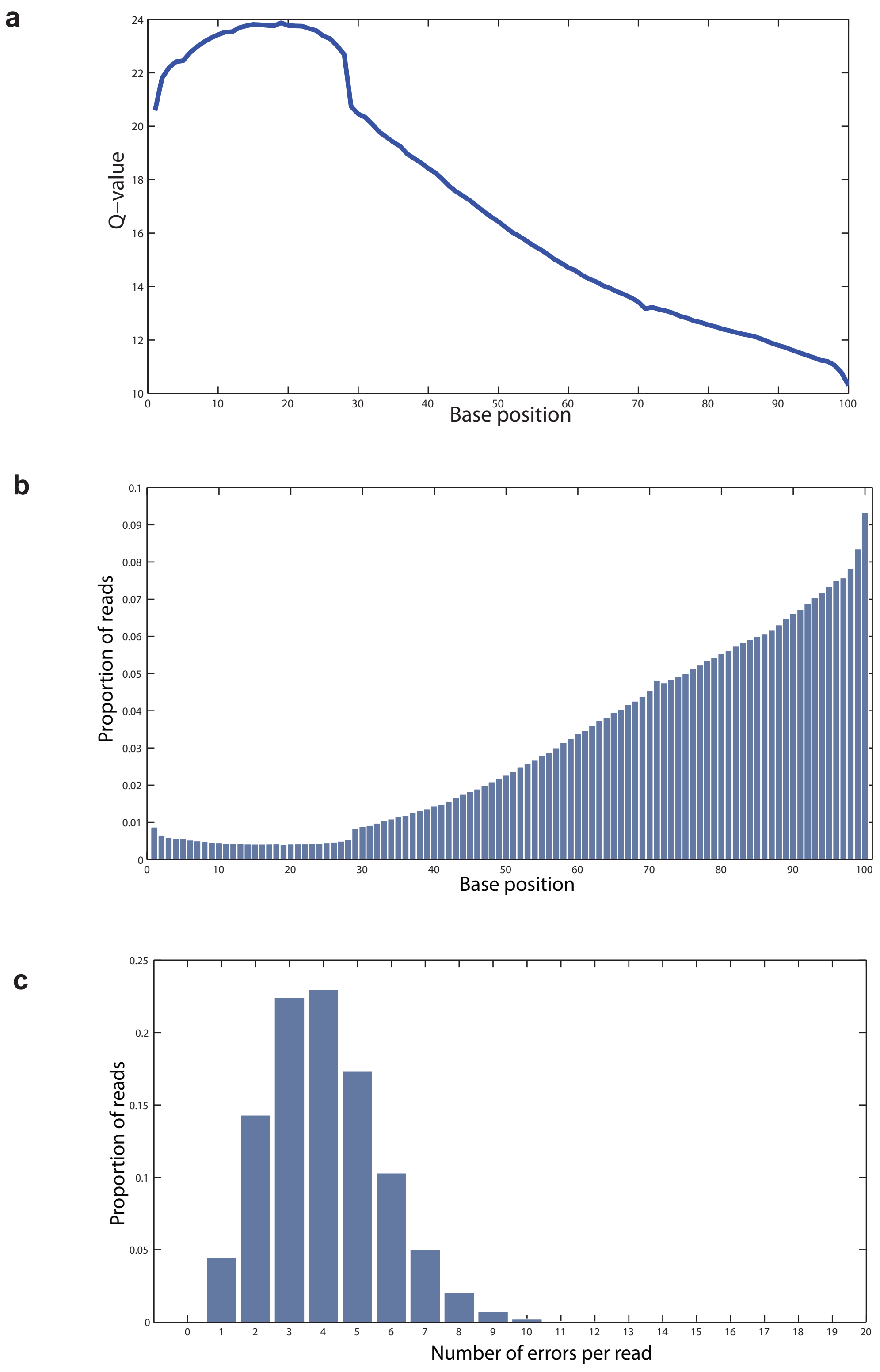
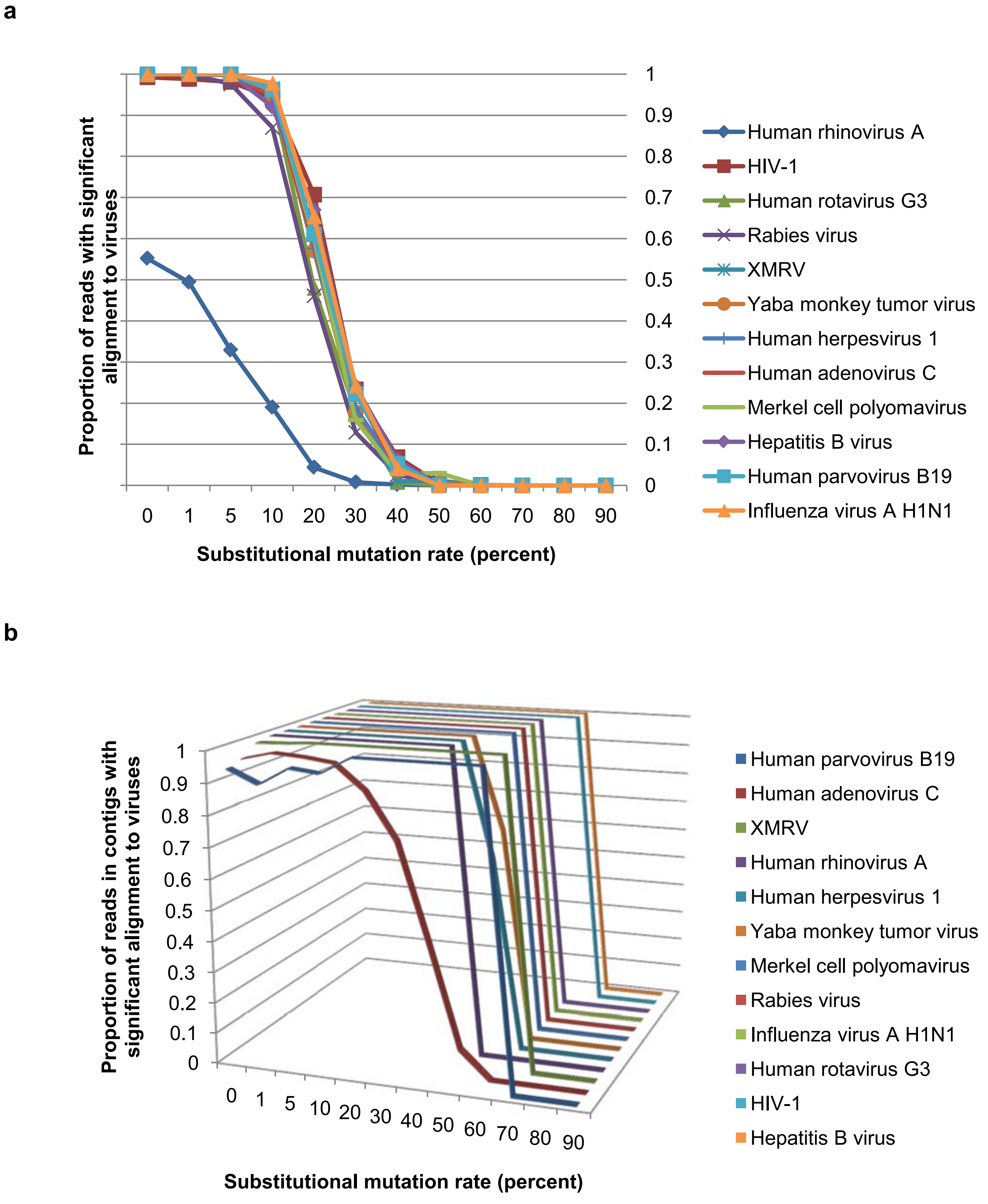


Supplementary Figure 1. Generation of artificial shotgun sequencing reads. (**a**) 20 million reads were generated from a human transcriptome database by randomly selecting 100-mer sequences. (**b**) A set of twelve virus genomes were selected (see **Methods**). For each genome, substitutional mutations were introduced at twelve distinct mutation rates, and for each of these mutated genomes 100-mer sequences were chosen at random to produce the read set.

Supplementary Figure 1

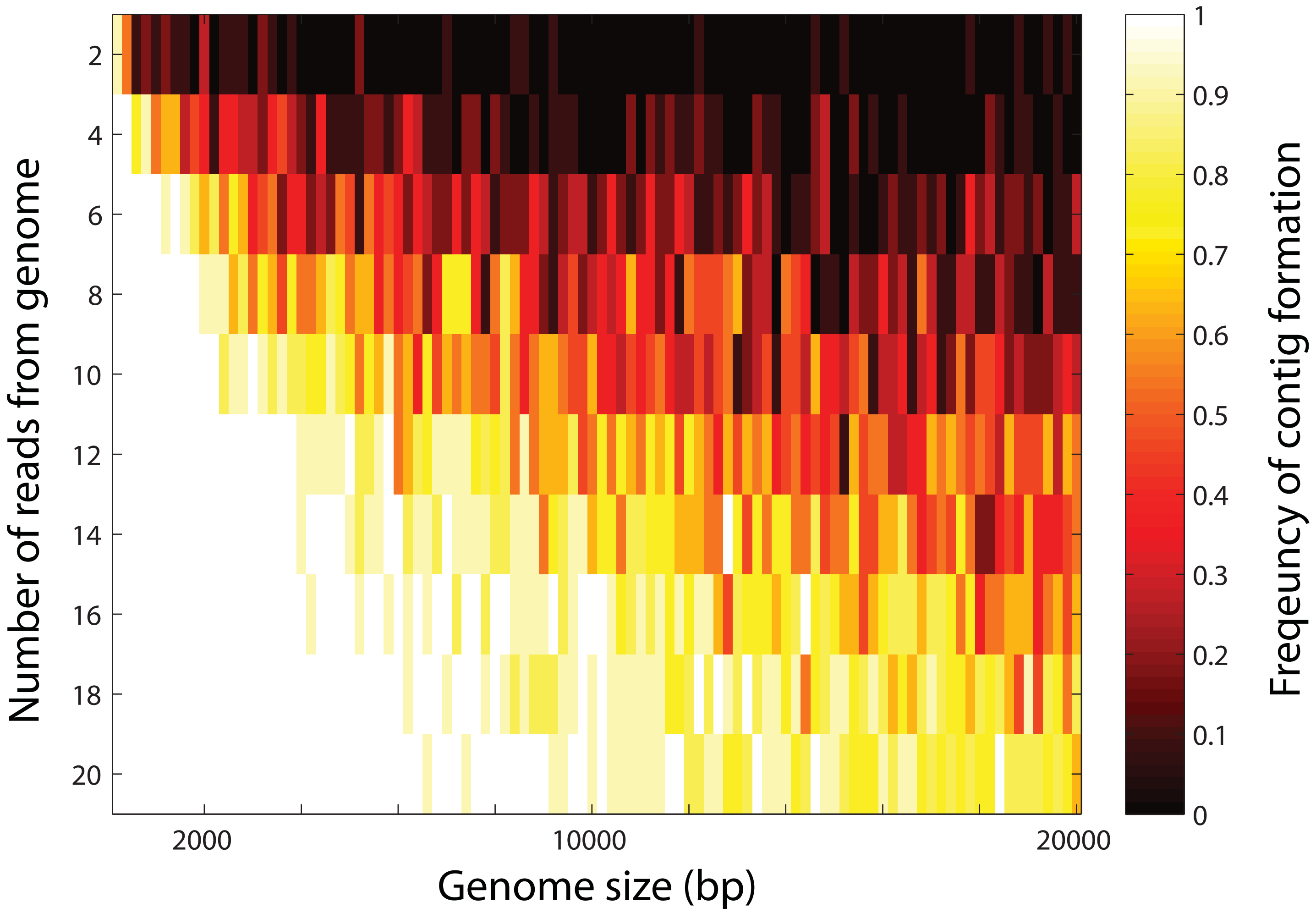


Supplementary Figure 2. Applying a sequencing error distribution model to artificially generated reads. (**a**) Average quality score plot for a typical set of reads generated by the Illumina GAII Sequencer. The Q-value is defined as Q=-10*log10(*p*/(1-*p*)), where *p* is the probability that the corresponding base call is incorrect. (**b**) The error distribution was applied to the complete set of reads generated as shown in **Supplementary Figure 1**. The plot shows the proportion of the read set containing a substituted base at the indicated position. (**c**) The proportion of the read set is shown as a function of the number of sequencing errors per read.



Supplementary Figure 3. Identification of virus-derived reads by sequence similarity. (**a**) Reads were generated as indicated in **Supplementary Figure 1b**. The proportion of reads identified as a virus sequence (MegaBlast alignment, E-value < 10e-10) is shown as a function of the substitutional mutation rate of the mutated genome. (**b**) Independent assemblies were performed on all reads from each of the 144 genomes. The proportion of reads incorporated into contigs that were identified as a virus sequence (MegaBlast alignment, E-value < 10e-10) is shown.

Supplementary Figure 3



Supplementary Figure 4. Probability of contig formation for sequences randomly generated from a genome of varying size. This figure shows the probability of forming contigs from a set of simulated, virus-derived reads. Reads were generated by selecting random 100-mers from a series of genomes ranging in size from 100bp to 20,000bp, producing between 2 and 20 reads per genome. The heatmap indicates the frequency among 11 replicates with which a contig of at least 175bp was formed from these reads by *de novo* assembly.

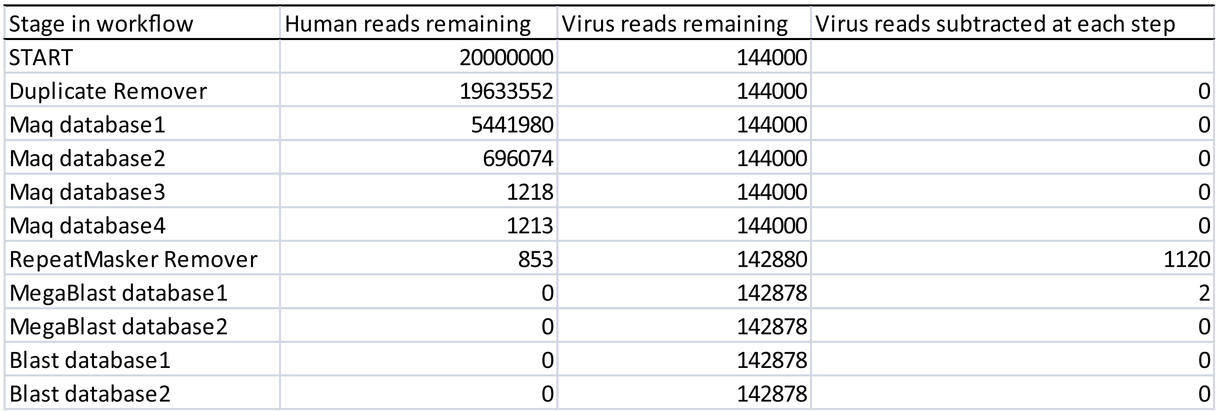
Supplementary Table 1. Bacterial genomes used to construct an artificial sequence dataset to test the metagenomics module of PathSeq. Shown is the set of bacterial genomes that were each used to create a set of 10,000 random 100-mers. The species were chosen to represent both evolutionary relatedness and divergence to test the ability of the metagenomics module to properly assess the microbial representation of a mixed sample.

Accession	Definition
NC_003228.3	Bacteroides fragilis NCTC 9343, complete genome.
NC_009614.1	Bacteroides vulgatus ATCC 8482, complete genome.
NC_013316.1	Clostridium difficile R20291, complete genome.
NC_009615.1	Parabacteroides distasonis ATCC 8503, complete genome.
NC_004663.1	Bacteroides thetaiotaomicron VPI-5482, complete genome.
NC_010816.1	Bifidobacterium longum DJO10A, complete genome.
NC_008618.1	Bifidobacterium adolescentis ATCC 15703, complete genome.
NC_012781.1	Eubacterium rectale ATCC 33656, complete genome.
NC_012778.1	Eubacterium eligens ATCC 27750, complete genome.
NC_010655.1	Akkermansia muciniphila ATCC BAA-835, complete genome.
NC_011353.1	Escherichia coli O157:H7 str. EC4115, complete genome.
NC_004668.1	Enterococcus faecalis V583, complete genome.

Supplementary Table 2. Metagenomic analysis on a sequence dataset constructed from a set of twelve bacterial genomes. Shown is the number of reads that were identified as matching to the indicated bacterial genome (actual number of reads is 10,000 for each species).

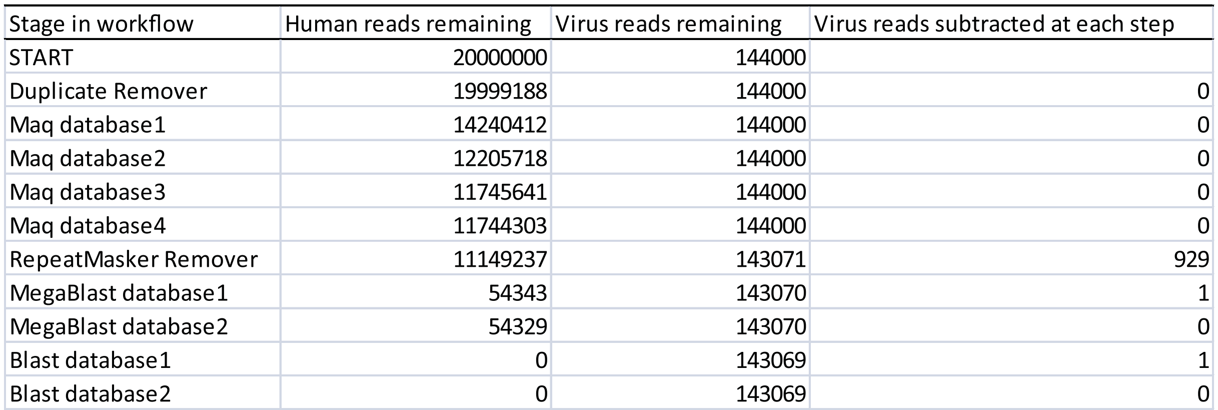
Genus	Species	Number of Reads	Fraction Genome Coverage
Bifidobacterium	adolescentis	9877	0.376091154
Eubacterium	eligens	9903	0.368491132
Bifidobacterium	longum	9933	0.333497209
Akkermansia	muciniphila	9954	0.310035802
Enterococcus	faecalis	9900	0.264391797
Eubacterium	rectale	9953	0.249987753
Clostridium	difficile	9829	0.208571056
Parabacteroides	distasonis	9929	0.186090932
Bacteroides	vulgatus	9943	0.173829585
Bacteroides	fragilis	9929	0.169107843
Bacteroides	thetaiotaomicron	9933	0.146473822
Escherichia	coli	5133	0.089648584
Staphylococcus	aureus	3	1.04E-04
Bifidobacterium	dentium	1	3.79E-05
Shigella	dysenteriae	1	2.29E-05
Shigella	sonnei	1	2.07E-05

Supplementary Table 3. PathSeq performance on artificially generated sequence data. Reads were generated by sampling random 100-mer sequences from a human transcriptome database, generating 20 million reads, or from a set of twelve virus genomes each substitutionally mutated at twelve distinct rates, generating 144,000 reads. The rows represent the number of reads remaining at each step in the PathSeq workflow.



Stage in workflow	Human reads remaining	Virus reads remaining	Virus reads subtracted at each step
START	20000000	144000	
Duplicate Remover	19633552	144000	0
Maq database1	5441980	144000	0
Maq database2	696074	144000	0
Maq database3	1218	144000	0
Maq database4	1213	144000	0
RepeatMasker Remover	853	142880	1120
MegaBlast database1	0	142878	2
MegaBlast database2	0	142878	0
Blast database1	0	142878	0
Blast database2	0	142878	0

Supplementary Table 4. PathSeq performance on artificially generated sequence data with introduced sequencing errors. Reads are the same as in Table 1 except that “sequence errors” were introduced into the reads according to a sequencing error distribution model.



Stage in workflow	Human reads remaining	Virus reads remaining	Virus reads subtracted at each step
START	20000000	144000	
Duplicate Remover	19999188	144000	0
Maq database1	14240412	144000	0
Maq database2	12205718	144000	0
Maq database3	11745641	144000	0
Maq database4	11744303	144000	0
RepeatMasker Remover	11149237	143071	929
MegaBlast database1	54343	143070	1
MegaBlast database2	54329	143070	0
Blast database1	0	143069	1
Blast database2	0	143069	0

SUPPLEMENTARY METHODS

Modifications made to the Illumina RNA-Seq Protocol. Total RNA (500 ng) was heated at 98ºC for 100 min in THE RNA Storage Solution (1 mM sodium citrate, pH 6.4; Ambion/ABI, AM7000) to fragment the RNA to a mean size of ~500 nucleotides. Quality of RNA fragmentations was assessed on a Bioanalyzer 2100 (Agilent). First-stand cDNA synthesis was performed by adding random hexamers (Invitrogen, 48190-011) to the RNA and heating at 70ºC for 10 min, and then immediately incubating at 50 ºC for 1 h upon addition of Superscript III reverse transcriptase (Invitrogen). Second-strand synthesis was carried-out with *E. coli* DNA ligase and *E. coli* DNA polymerase I (Invitrogen) for 2.5 h at 16 ºC. cDNA was purified using the MiniElute PCR Purification Kit (Qiagen) and evaluated using Bioanalyzer. End-repair, addition of adenine to the 3' end of the DNA fragments, and adapter ligation was performed as described in Guttman *et al.*, except a 2:1 molar ratio of adapter to DNA fragment was used during adapter-ligation. The resulting adapter-ligated fragments were purified on a 4% SeaKem LE agarose gel (Lonza) and a 400-500 base pair band was cut out of the gel and purified using the MiniElute kit. PCR was performed with Phusion DNA polymerase (Finnzymes) and adapter-specific primers using the following conditions: 2 min at 98 ºC; [10 s at 98 ºC, 30 s at 65 ºC, 30 s at 72 ºC] for 13 cycles; 5 min at 72 ºC. Following PCR, a second round of gel extraction was performed as described above, and the product was submitted for Illumina sequencing.

PathSeq runtime and performance. PathSeq analysis was performed using a cluster of 19 worker nodes and 1 master node, which were EC2 Large CPU instances (7GB of memory and 2 processor cores). Full analysis of HeLa cell RNA-Seq data described in this report was performed in approximately 13 hours (wall clock time) for a total price of $89 USD. The CPU time for this analysis was approximately 270 hours. Actual runtime and cost may vary depending on congestion on the Amazon EC2, Internet traffic, and the method of data upload. Because of its parallel architecture, PathSeq can analyze substantially larger datasets in a similar timeframe simply by increasing the cluster size.

Metagenomic analysis. The metagenomic analysis module of PathSeq reports the relative abundance of bacteria and archaea. This analysis begins with a MegaBlast alignment of the readset against the complete set of fully sequenced bacterial and archaeal genomes (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/, downloaded 2010-03-30), reporting all hits with >90% sequence identity and >90% query coverage. The top 30 hits for each read are reported. Using these alignment results, classifications of each read are attempted at the phylum, then genus, then species level. If a given read cannot be classified uniquely at a given classification level (i.e. it has multiple hits to different reference sequences with equivalent E-values), then it is considered ambiguous and discarded from analysis at that level. Using species-level classifications, the fraction-genome-coverage is calculated for each species that received a hit, and this metric is used to quantify the relative abundance of a given species, normalized by the genome size.

Introduction of "sequencing errors" into simulated sequence data. Sequencing errors were introduced into the simulated reads based on quality scores seen in a whole-genome sequence dataset of a glioblastoma multiforme (GBM) primary tumor (sequenced as part of The Cancer Genome Atlas; data available via the NCBI Short Read Archive (SRA) identifier SRX010704). The average quality score for each base along the length of the reads was calculated across the dataset and offset by -5 (**Supplementary Fig. 3a**). This was converted into a probability value and used to “mutate” our simulated reads (i.e. for a sequence error probability of 0.001, there is a 0.1% chance that the base will be converted to a different base).

Human whole-genome ovarian tumor sequencing data. The human ovarian tumor whole-genome sequencing dataset was sequenced as part of The Cancer Genome Atlas, and the data is available via the NCBI SRA identifier SRX010747. This is a 101 base pair, paired-end sequence dataset with a nominal fragment length of 264 base pairs.

Supplementary Data Set 1. Microbial sequences identified among the unmapped clone end sequences from *Wheeler et al.* We performed a MegaBlast alignment of all contigs against bacterial, viral, and fungal nucleotide databases. The most significant alignments are reported.

Query name	Query length	BLAST Database	Hit ID	Bit score	E-value
contig freeze2_11177	914	Bacteria	gi|191636824|ref|NC_010999.1|	1431.17	0
contig freeze2_11275	999	Bacteria	gi|191636824|ref|NC_010999.1|	1594.6	0
contig freeze2_10679	1167	Bacteria	gi|191636824|ref|NC_010999.1|	2057.96	0
contig freeze2_30701	816	Bacteria	gi|198282148|ref|NC_011206.1|	712.082	0
contig freeze2_31723	737	Bacteria	gi|198282148|ref|NC_011206.1|	533.272	8.09E-149
contig freeze2_14303	866	Bacteria	gi|91774356|ref|NC_007947.1|	838.979	0
contig freeze2_10972	976	Bacteria	gi|116493574|ref|NC_008526.1|	1636.89	0
contig freeze2_11051	1117	Bacteria	gi|116493574|ref|NC_008526.1|	1792.63	0
contig freeze2_30191	850	Bacteria	gi|198282148|ref|NC_011206.1|	658.247	0
contig freeze2_10883	1429	Bacteria	gi|191636824|ref|NC_010999.1|	2400.2	0
contig freeze2_10866	1012	Bacteria	gi|116493574|ref|NC_008526.1|	1461.93	0
contig freeze2_10893	1018	Bacteria	gi|191636824|ref|NC_010999.1|	1236.98	0
contig freeze2_10847	1718	Bacteria	gi|191636824|ref|NC_010999.1|	2798.2	0
contig freeze2_29354	953	Bacteria	gi|198282148|ref|NC_011206.1|	633.252	8.43E-179
contig freeze2_10443	1376	Bacteria	gi|191636824|ref|NC_010999.1|	2223.31	0
contig freeze2_10302	1610	Bacteria	gi|116493574|ref|NC_008526.1|	2342.52	0
contig freeze2_10881	859	Bacteria	gi|258506995|ref|NC_013198.1|	1286.97	0
contig freeze2_10536	1560	Bacteria	gi|191636824|ref|NC_010999.1|	2384.82	0
contig freeze2_10792	1135	Bacteria	gi|191636824|ref|NC_010999.1|	1658.04	0
contig freeze2_10806	1223	Bacteria	gi|191636824|ref|NC_010999.1|	1909.92	0
contig freeze2_11056	1045	Bacteria	gi|116493574|ref|NC_008526.1|	1542.68	0
contig freeze2_10841	1408	Bacteria	gi|191636824|ref|NC_010999.1|	1992.59	0
contig freeze2_18067	798	Bacteria	gi|190572091|ref|NC_010943.1|	783.221	0
contig freeze2_10478	938	Bacteria	gi|191636824|ref|NC_010999.1|	863.974	0
contig freeze2_12874	596	Fungi	gi|162949218|ref|NC_001139.8|	346.771	4.33E-93
contig freeze2_12205	809	Fungi	gi|162949218|ref|NC_001139.8|	419.833	6.02E-115
contig freeze2_12696	727	Fungi	gi|162949218|ref|NC_001139.8|	398.684	1.26E-108

Supplementary Data Set 2. Microbial sequences identified among the novel sequences from the Asian and African genomes from *Li et al.* The set of novel sequences from the Asian and African genome were downloaded (http://yh.genomics.org.cn/download.jsp#pd) and aligned by MegaBlast against bacterial, viral and fungal nucleotide databases. The most significant alignments are reported.

Query name	Query length	BLAST Database	Hit ID	Bit score	E-value
C119303880_1_334.0:1-332	332	Viruses	gi|82503188|ref|NC_007605.1|	639.02	0
C119524968_1_364.0:1-362	362	Viruses	gi|82503188|ref|NC_007605.1|	696.7	0
C119600616_1_375.0:1-373	373	Viruses	gi|82503188|ref|NC_007605.1|	717.85	0
C119674423_1_386.0:1-384	384	Viruses	gi|82503188|ref|NC_007605.1|	739	0
C119705567_1_391.0:1-389	389	Viruses	gi|139424470|ref|NC_009334.1|	748.613	0
C119726695_1_395.0:1-393	393	Viruses	gi|139424470|ref|NC_009334.1|	756.304	0
C119731793_1_395.0:1-393	393	Viruses	gi|82503188|ref|NC_007605.1|	756.304	0
C119744189_1_397.0:1-395	395	Viruses	gi|82503188|ref|NC_007605.1|	760.149	0
C119748653_1_398.0:1-396	396	Viruses	gi|139424470|ref|NC_009334.1|	762.072	0
C119896026_1_423.0:1-421	421	Viruses	gi|82503188|ref|NC_007605.1|	806.294	0
C119902298_1_424.0:41-422	382	Viruses	gi|82503188|ref|NC_007605.1|	735.154	0
C119921199_1_427.0:1-425	425	Viruses	gi|82503188|ref|NC_007605.1|	817.83	0
C119940413_1_431.0:1-429	429	Viruses	gi|139424470|ref|NC_009334.1|	825.521	0
C120002643_1_442.0:1-440	440	Viruses	gi|139424470|ref|NC_009334.1|	846.67	0
C120149024_1_469.0:1-467	467	Viruses	gi|82503188|ref|NC_007605.1|	898.583	0
C120191480_1_478.0:1-476	476	Viruses	gi|139424470|ref|NC_009334.1|	915.887	0
C120258135_1_491.0:1-489	489	Viruses	gi|82503188|ref|NC_007605.1|	940.882	0
C120330447_1_507.0:1-505	505	Viruses	gi|82503188|ref|NC_007605.1|	971.645	0
C120346593_1_510.0:1-508	508	Viruses	gi|82503188|ref|NC_007605.1|	977.413	0
C120492119_1_544.0:1-542	542	Viruses	gi|139424470|ref|NC_009334.1|	719.773	0
C120522603_1_551.0:1-549	549	Viruses	gi|139424470|ref|NC_009334.1|	1056.24	0
C120531769_1_554.0:1-552	552	Viruses	gi|139424470|ref|NC_009334.1|	1040.86	0
C120703811_1_598.0:1-596	596	Viruses	gi|82503188|ref|NC_007605.1|	1135.07	0
C120935232_1_665.0:1-663	663	Viruses	gi|82503188|ref|NC_007605.1|	1275.43	0
C120951592_1_671.0:1-669	669	Viruses	gi|139424470|ref|NC_009334.1|	1254.28	0
C120960358_1_673.0:1-671	671	Viruses	gi|82503188|ref|NC_007605.1|	1290.81	0
C121286040_1_791.0:1-789	789	Viruses	gi|82503188|ref|NC_007605.1|	1517.69	0
C121288274_1_792.0:1-790	790	Viruses	gi|82503188|ref|NC_007605.1|	1506.15	0
C121293122_1_794.0:1-792	792	Viruses	gi|82503188|ref|NC_007605.1|	1517.69	0
C121319800_1_805.0:1-803	803	Viruses	gi|82503188|ref|NC_007605.1|	1544.61	0
C121320126_1_805.0:1-803	803	Viruses	gi|82503188|ref|NC_007605.1|	1544.61	0
C121347810_1_817.0:1-815	815	Viruses	gi|139424470|ref|NC_009334.1|	1554.22	0
C121428246_1_852.0:1-850	850	Viruses	gi|82503188|ref|NC_007605.1|	1634.97	0
C121453377_1_864.0:1-862	862	Viruses	gi|82503188|ref|NC_007605.1|	1171.6	0
C121497526_1_885.0:1-883	883	Viruses	gi|82503188|ref|NC_007605.1|	1698.42	0
C121554558_1_914.0:1-912	912	Viruses	gi|82503188|ref|NC_007605.1|	1738.8	0
C121571538_1_923.0:1-921	921	Viruses	gi|82503188|ref|NC_007605.1|	1769.56	0
C121651310_1_966.0:1-964	964	Viruses	gi|82503188|ref|NC_007605.1|	1854.16	0
C121664332_1_973.0:1-971	971	Viruses	gi|82503188|ref|NC_007605.1|	1867.62	0
C121768448_1_1036.0:1-1034	1034	Viruses	gi|82503188|ref|NC_007605.1|	1988.75	0

C121866248_1_1102.0:1-1100	1100	Viruses	gi|82503188|ref|NC_007605.1|	2115.64	0
C121868598_1_1104.0:1-1102	1102	Viruses	gi|82503188|ref|NC_007605.1|	2119.49	0
C121889809_1_1119.0:1-1117	1117	Viruses	gi|82503188|ref|NC_007605.1|	2148.33	0
C121895511_1_1123.0:1-1121	1121	Viruses	gi|82503188|ref|NC_007605.1|	2156.02	0
C121913385_1_1137.0:1-1135	1135	Viruses	gi|82503188|ref|NC_007605.1|	2182.94	0
C121922687_1_1144.0:1-1142	1142	Viruses	gi|82503188|ref|NC_007605.1|	2192.55	0
C121941707_1_1158.0:1-1156	1156	Viruses	gi|82503188|ref|NC_007605.1|	2207.93	0
C122032117_1_1233.0:1-1231	1231	Viruses	gi|139424470|ref|NC_009334.1|	2352.13	0
C122181441_1_1381.0:1-1379	1379	Viruses	gi|139424470|ref|NC_009334.1|	2652.07	0
C122299164_1_1526.0:1-1524	1524	Viruses	gi|82503188|ref|NC_007605.1|	2930.86	0
C122328330_1_1567.0:1-1565	1565	Viruses	gi|82503188|ref|NC_007605.1|	3009.69	0
C122330180_1_1570.0:1-1568	1568	Viruses	gi|82503188|ref|NC_007605.1|	3015.46	0
C122362574_1_1620.0:1-1618	1618	Viruses	gi|82503188|ref|NC_007605.1|	3111.6	0
C122435276_1_1745.0:1-1743	1743	Viruses	gi|82503188|ref|NC_007605.1|	3351.93	0
C122441380_1_1757.0:1-1755	1755	Viruses	gi|82503188|ref|NC_007605.1|	3375	0
C122442982_1_1760.0:1-1758	1758	Viruses	gi|82503188|ref|NC_007605.1|	3380.77	0
C122455201_1_1784.0:1-1782	1782	Viruses	gi|82503188|ref|NC_007605.1|	3178.89	0
C122458643_1_1791.0:1-1789	1789	Viruses	gi|82503188|ref|NC_007605.1|	3440.38	0
C122460615_1_1795.0:1-1793	1793	Viruses	gi|82503188|ref|NC_007605.1|	3448.07	0
C122480117_1_1836.0:1-1834	1834	Viruses	gi|82503188|ref|NC_007605.1|	3526.9	0
C122499557_1_1880.0:1-1878	1878	Viruses	gi|82503188|ref|NC_007605.1|	3611.5	0
C122500603_1_1882.0:1-1880	1880	Viruses	gi|82503188|ref|NC_007605.1|	3615.34	0
C122532157_1_1960.0:1-1958	1958	Viruses	gi|82503188|ref|NC_007605.1|	3765.31	0
C122536387_1_1970.0:1-1968	1968	Viruses	gi|82503188|ref|NC_007605.1|	3782.61	0
C122640872_1_2310.0:1-2308	2308	Viruses	gi|82503188|ref|NC_007605.1|	4438.25	0
C122682056_1_2501.0:1-2499	2499	Viruses	gi|82503188|ref|NC_007605.1|	4805.48	0
C122683436_1_2508.0:1-2506	2506	Viruses	gi|82503188|ref|NC_007605.1|	4818.94	0
C122691220_1_2551.0:1-2549	2549	Viruses	gi|82503188|ref|NC_007605.1|	4892.01	0
C122698812_1_2596.0:1-2594	2594	Viruses	gi|82503188|ref|NC_007605.1|	4988.14	0
C122715178_1_2699.0:1-2697	2697	Viruses	gi|82503188|ref|NC_007605.1|	5186.18	0
C122720738_1_2737.0:1-2735	2735	Viruses	gi|82503188|ref|NC_007605.1|	5259.24	0
C122742268_1_2909.0:1-2907	2907	Viruses	gi|82503188|ref|NC_007605.1|	5589.94	0
C122747872_1_2961.0:1-2959	2959	Viruses	gi|82503188|ref|NC_007605.1|	5689.92	0
C122766940_1_3166.0:1-3164	3164	Viruses	gi|82503188|ref|NC_007605.1|	6078.3	0
C122770662_1_3216.0:1-3214	3214	Viruses	gi|82503188|ref|NC_007605.1|	6180.21	0
C122779453_1_3342.0:1-3340	3340	Viruses	gi|82503188|ref|NC_007605.1|	6422.47	0
C122807074_1_3934.0:1-3932	3932	Viruses	gi|82503188|ref|NC_007605.1|	7560.7	0
C122816684_1_4307.0:1-4305	4305	Viruses	gi|82503188|ref|NC_007605.1|	8264.4	0
C122823252_1_4697.0:1-4695	4695	Viruses	gi|82503188|ref|NC_007605.1|	9027.71	0
C122832902_1_6215.0:1-6213	6213	Viruses	gi|82503188|ref|NC_007605.1|	11946.3	0
C122833702_1_6620.0:1-6618	6618	Viruses	gi|82503188|ref|NC_007605.1|	12725	0
C122833934_1_6767.0:1-6765	6765	Viruses	gi|82503188|ref|NC_007605.1|	13007.7	0
scaffold168760_1_552.0:1-468	468	Viruses	gi|82503188|ref|NC_007605.1|	888.969	0
scaffold25509_1_5582.10:1-717	717	Viruses	gi|9626372|ref|NC_001422.1|	1379.25	0
scaffold25509_1_5582.12:1-874	874	Viruses	gi|9626372|ref|NC_001422.1|	1617.67	0
scaffold25509_1_5582.15:1-457	457	Viruses	gi|9626372|ref|NC_001422.1|	879.356	0
scaffold25509_1_5582.2:1-837	837	Viruses	gi|9626372|ref|NC_001422.1|	1609.98	0
C154349094_1_379.0:1-377	377	Viruses	gi|9626158|ref|NC_001405.1|	714.005	0

<table>
<tr><th></th><th></th><th></th><th></th><th></th><th></th></tr>
<tr><td>scaffold25509_1_5582.11:1-297</td><td>297</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>571.726</td><td>1.58E-162</td></tr>
<tr><td>C119074009_1_306.0:1-304</td><td>304</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>569.803</td><td>6.16E-162</td></tr>
<tr><td>C118836010_1_281.0:1-279</td><td>279</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>537.117</td><td>3.88E-152</td></tr>
<tr><td>C118994787_1_297.0:1-295</td><td>295</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>537.117</td><td>4.12E-152</td></tr>
<tr><td>C118747645_1_272.0:1-270</td><td>270</td><td>Viruses</td><td>gi|139424470|ref|NC_009334.1|</td><td>519.813</td><td>6.06E-147</td></tr>
<tr><td>C118700636_1_268.0:1-266</td><td>266</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>512.122</td><td>1.23E-144</td></tr>
<tr><td>scaffold25509_1_5582.6:1-264</td><td>264</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>490.973</td><td>2.84E-138</td></tr>
<tr><td>scaffold25509_1_5582.4:1-208</td><td>208</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>400.606</td><td>3.49E-111</td></tr>
<tr><td>C117842211_1_203.0:1-201</td><td>201</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>387.148</td><td>3.79E-107</td></tr>
<tr><td>C117849537_1_203.0:1-201</td><td>201</td><td>Viruses</td><td>gi|139424470|ref|NC_009334.1|</td><td>387.148</td><td>3.79E-107</td></tr>
<tr><td>scaffold25509_1_5582.7:1-197</td><td>197</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>379.457</td><td>7.65E-105</td></tr>
<tr><td>C117426534_1_182.0:1-180</td><td>180</td><td>Viruses</td><td>gi|139424470|ref|NC_009334.1|</td><td>346.771</td><td>4.78E-95</td></tr>
<tr><td>scaffold25509_1_5582.16:1-173</td><td>173</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>333.312</td><td>5.14E-91</td></tr>
<tr><td>scaffold25509_1_5582.5:1-150</td><td>150</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>289.091</td><td>8.97E-78</td></tr>
<tr><td>scaffold25509_1_5582.9:1-142</td><td>142</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>273.709</td><td>3.62E-73</td></tr>
<tr><td>C116026170_1_133.0:1-131</td><td>131</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>252.559</td><td>7.67E-67</td></tr>
<tr><td>C115756264_1_127.0:1-125</td><td>125</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>241.023</td><td>2.16E-63</td></tr>
<tr><td>C115761692_1_127.0:1-125</td><td>125</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>241.023</td><td>2.16E-63</td></tr>
<tr><td>scaffold62567_1_922.3:1-125</td><td>125</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>241.023</td><td>2.16E-63</td></tr>
<tr><td>scaffold25509_1_5582.14:1-124</td><td>124</td><td>Viruses</td><td>gi|9626372|ref|NC_001422.1|</td><td>239.101</td><td>8.10E-63</td></tr>
<tr><td>C151024180_1_126.0:1-124</td><td>124</td><td>Viruses</td><td>gi|9626243|ref|NC_001416.1|</td><td>235.255</td><td>1.16E-61</td></tr>
<tr><td>scaffold62567_1_922.4:1-119</td><td>119</td><td>Viruses</td><td>gi|82503188|ref|NC_007605.1|</td><td>229.487</td><td>6.04E-60</td></tr>
<tr><td>C154594504_1_424.0:1-422</td><td>422</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>812.062</td><td>0</td></tr>
<tr><td>C154645116_1_434.0:1-432</td><td>432</td><td>Bacteria</td><td>gi|209916806|ref|NC_011407.1|</td><td>642.865</td><td>0</td></tr>
<tr><td>C155261927_1_583.0:1-581</td><td>581</td><td>Bacteria</td><td>gi|209921952|ref|NC_011419.1|</td><td>1112</td><td>0</td></tr>
<tr><td>C155828458_1_777.0:1-775</td><td>775</td><td>Bacteria</td><td>gi|152973607|ref|NC_009650.1|</td><td>1479.23</td><td>0</td></tr>
<tr><td>C153974801_1_321.0:1-319</td><td>319</td><td>Bacteria</td><td>gi|253750923|ref|NC_012924.1|</td><td>515.968</td><td>5.42E-144</td></tr>
<tr><td>C153399044_1_253.0:1-251</td><td>251</td><td>Bacteria</td><td>gi|253750923|ref|NC_012924.1|</td><td>471.746</td><td>8.56E-131</td></tr>
<tr><td>C152979378_1_217.0:1-215</td><td>215</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>396.761</td><td>2.71E-108</td></tr>
<tr><td>C152733830_1_200.0:1-198</td><td>198</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>381.38</td><td>1.05E-103</td></tr>
<tr><td>C152632972_1_193.0:1-191</td><td>191</td><td>Bacteria</td><td>gi|157149504|ref|NC_009790.1|</td><td>367.921</td><td>1.14E-99</td></tr>
<tr><td>C152435528_1_182.0:1-180</td><td>180</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>346.771</td><td>2.47E-93</td></tr>
<tr><td>C152451524_1_183.0:1-181</td><td>181</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>337.158</td><td>1.95E-90</td></tr>
<tr><td>C152085975_1_164.0:1-162</td><td>162</td><td>Bacteria</td><td>gi|18466424|ref|NC_003384.1|</td><td>312.163</td><td>5.74E-83</td></tr>
<tr><td>C151524210_1_142.0:1-140</td><td>140</td><td>Bacteria</td><td>gi|18466424|ref|NC_003384.1|</td><td>269.864</td><td>2.61E-70</td></tr>
<tr><td>C151497500_1_141.0:1-139</td><td>139</td><td>Bacteria</td><td>gi|152973607|ref|NC_009650.1|</td><td>267.941</td><td>9.82E-70</td></tr>
<tr><td>C151911791_1_156.0:1-154</td><td>154</td><td>Bacteria</td><td>gi|146317663|ref|NC_009442.1|</td><td>262.173</td><td>6.05E-68</td></tr>
<tr><td>C151376510_1_137.0:1-135</td><td>135</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>260.25</td><td>1.96E-67</td></tr>
<tr><td>C151136665_1_130.0:1-128</td><td>128</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>246.791</td><td>2.07E-63</td></tr>
<tr><td>C151162069_1_130.0:1-128</td><td>128</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>241.023</td><td>1.13E-61</td></tr>
<tr><td>C151164119_1_130.0:1-128</td><td>128</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>235.255</td><td>6.14E-60</td></tr>
<tr><td>C150834658_1_122.0:1-120</td><td>120</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>231.41</td><td>8.16E-59</td></tr>
<tr><td>C150539079_1_115.0:1-113</td><td>113</td><td>Bacteria</td><td>gi|253750923|ref|NC_012924.1|</td><td>217.951</td><td>8.52E-55</td></tr>
<tr><td>C150516123_1_114.0:1-112</td><td>112</td><td>Bacteria</td><td>gi|219682499|ref|NC_011835.1|</td><td>216.028</td><td>3.19E-54</td></tr>
<tr><td>C150634133_1_117.0:1-115</td><td>115</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>216.028</td><td>3.30E-54</td></tr>
<tr><td>C150437527_1_113.0:1-111</td><td>111</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>214.106</td><td>1.20E-53</td></tr>
<tr><td>C150438245_1_113.0:1-111</td><td>111</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>214.106</td><td>1.20E-53</td></tr>
<tr><td>C150340425_1_111.0:1-109</td><td>109</td><td>Bacteria</td><td>gi|254667448|ref|NC_013010.1|</td><td>210.26</td><td>1.68E-52</td></tr>
</table>

C149877309_1_103.0:1-101	101	Bacteria	gi|253750923|ref|NC_012924.1|	189.111	3.55E-46
C152535080_1_187.0:1-185	185	Bacteria	gi|146317663|ref|NC_009442.1|	285.245	8.48E-75
C151256372_1_133.0:1-131	131	Bacteria	gi|254667448|ref|NC_013010.1|	244.869	8.07E-63
C150829637_1_121.0:1-119	119	Bacteria	gi|157149504|ref|NC_009790.1|	227.565	1.16E-57
C153075744_1_225.0:1-223	223	Bacteria	gi|209917191|ref|NC_011415.1|	425.601	5.87E-117
C153834674_1_302.0:1-300	300	Bacteria	gi|209921952|ref|NC_011419.1|	558.267	9.37E-157
C151702247_1_148.0:1-146	146	Bacteria	gi|18466424|ref|NC_003384.1|	277.554	1.33E-72
C151024180_1_126.0:1-124	124	Bacteria	gi|238899406|ref|NC_012759.1|	235.255	5.91E-60
C152460830_1_183.0:1-181	181	Bacteria	gi|182433793|ref|NC_010572.1|	173.729	3.07E-41
C153118340_1_228.0:1-226	226	Bacteria	gi|209921952|ref|NC_011419.1|	425.601	5.95E-117
C153176499_1_233.0:1-231	231	Bacteria	gi|253750923|ref|NC_012924.1|	415.988	4.78E-114
C150012630_1_105.0:1-103	103	Bacteria	gi|170679574|ref|NC_010498.1|	192.956	2.53E-47
C152090971_1_164.0:1-162	162	Bacteria	gi|209921952|ref|NC_011419.1|	302.549	4.49E-80
C152369833_1_178.0:1-176	176	Bacteria	gi|89106884|ref|AC_000091.1|	321.776	8.06E-86
C153494416_1_263.0:1-261	261	Bacteria	gi|206575367|ref|NC_011281.1|	292.936	6.00E-77
C151132801_1_129.0:1-127	127	Bacteria	gi|253750923|ref|NC_012924.1|	223.719	1.81E-56
C150990962_1_126.0:1-124	124	Bacteria	gi|238899406|ref|NC_012759.1|	214.106	1.37E-53
C152036216_1_162.0:1-160	160	Bacteria	gi|157149574|ref|NC_009791.1|	237.178	2.11E-60
C156518102_1_1159.0:1-1102	1102	Fungi	gi|160338784|ref|NZ_AACM02000411.1|	1788.79	0
C154601314_1_425.0:1-423	423	Fungi	gi|160338799|ref|NZ_AACM02000426.1|	744.768	0
C154321982_1_374.0:1-318	318	Fungi	gi|160338805|ref|NZ_AACM02000432.1|	606.334	1.63E-171
scaffold32896_1_1686.5:1-273	273	Fungi	gi|160338808|ref|NZ_AACM02000435.1|	469.823	1.72E-130
scaffold32896_1_1686.2:1-241	241	Fungi	gi|160338808|ref|NZ_AACM02000435.1|	464.055	8.16E-129
C153540141_1_268.0:1-230	230	Fungi	gi|160338799|ref|NZ_AACM02000426.1|	402.529	2.57E-110
C153492428_1_263.0:34-237	204	Fungi	gi|160338805|ref|NZ_AACM02000432.1|	381.38	5.23E-104
C152632064_1_193.0:1-191	191	Fungi	gi|160338794|ref|NZ_AACM02000421.1|	367.921	5.47E-100
C152529756_1_187.0:1-185	185	Fungi	gi|160338803|ref|NZ_AACM02000430.1|	344.849	4.65E-93
C152392335_1_179.0:1-177	177	Fungi	gi|160338797|ref|NZ_AACM02000424.1|	341.003	6.35E-92
C152336937_1_176.0:1-174	174	Fungi	gi|160338803|ref|NZ_AACM02000430.1|	335.235	3.39E-90
C152085975_1_164.0:1-162	162	Fungi	gi|221228878|ref|NZ_AABX02000085.1|	312.163	2.78E-83
C152749880_1_201.0:53-199	147	Fungi	gi|160338805|ref|NZ_AACM02000432.1|	283.322	1.19E-74
C151718669_1_149.0:1-147	147	Fungi	gi|160338797|ref|NZ_AACM02000424.1|	277.554	6.49E-73
C151503302_1_141.0:1-139	139	Fungi	gi|160338805|ref|NZ_AACM02000432.1|	267.941	4.76E-70
C151364952_1_136.0:1-134	134	Fungi	gi|160338807|ref|NZ_AACM02000434.1|	258.328	3.57E-67
C151164119_1_130.0:1-128	128	Fungi	gi|121697430|ref|NW_001510354.1|	246.791	1.00E-63
C151321645_1_135.0:1-133	133	Fungi	gi|160338790|ref|NZ_AACM02000417.1|	244.869	3.98E-63
scaffold32896_1_1686.0:1-118	118	Fungi	gi|160338808|ref|NZ_AACM02000435.1|	227.565	5.57E-58
C151479640_1_140.0:1-138	138	Fungi	gi|160338808|ref|NZ_AACM02000435.1|	214.106	7.58E-54
C150339437_1_111.0:1-109	109	Fungi	gi|160338805|ref|NZ_AACM02000432.1|	210.26	8.18E-53
C150340425_1_111.0:1-109	109	Fungi	gi|145606593|ref|NW_001798731.1|	210.26	8.18E-53
C150285373_1_110.0:1-108	108	Fungi	gi|160338797|ref|NZ_AACM02000424.1|	208.338	3.06E-52
C150516123_1_114.0:1-112	112	Fungi	gi|145606593|ref|NW_001798731.1|	200.647	6.62E-50
C152460830_1_183.0:1-181	181	Fungi	gi|115433601|ref|XM_001216938.1|	198.724	4.41E-49
C149941624_1_104.0:1-102	102	Fungi	gi|145606593|ref|NW_001798731.1|	196.802	8.46E-49
scaffold39416_1_3596.2:1-506	506	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	183.343	5.74E-44
C151256372_1_133.0:1-131	131	Fungi	gi|121697430|ref|NW_001510354.1|	250.637	7.18E-65
C151702247_1_148.0:1-146	146	Fungi	gi|121697430|ref|NW_001510354.1|	277.554	6.44E-73
scaffold41546_1_908.0:1-145	145	Fungi	gi|160338807|ref|NZ_AACM02000434.1|	237.178	9.12E-61

scaffold6349_1_7434.2:211-717	507	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	216.028	8.33E-54
C151948881_1_158.0:1-156	156	Fungi	gi|155017865|ref|NC_005788.3|	194.879	5.38E-48
scaffold60353_1_623.0:100-455	356	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	196.802	3.52E-48
scaffold31885_1_3636.3:1-350	350	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	216.028	5.64E-54
scaffold3678_491179_492217.1:1-207	207	Fungi	gi|145602701|ref|NW_001798719.1|	212.183	4.56E-53
scaffold61091_1_2316.1:1-485	485	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	177.575	2.99E-42
scaffold76524_1_4387.4:1-513	513	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	198.724	1.36E-48
C152369833_1_178.0:1-176	176	Fungi	gi|221228837|ref|NZ_AABX02000044.1|	308.317	4.36E-82
scaffold31764_1_478.0:1-116	116	Fungi	gi|160338807|ref|NZ_AACM02000434.1|	202.57	1.83E-50
scaffold11464_1_800.1:1-627	627	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	204.492	3.09E-50
scaffold34194_1_9437.2:1-566	566	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	221.796	1.71E-55
scaffold42760_1_2579.2:1-331	331	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	187.188	2.55E-45
scaffold19674_1_351.0:1-349	349	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	194.879	1.31E-47
C151524210_1_142.0:1-140	140	Fungi	gi|121697430|ref|NW_001510354.1|	239.101	2.31E-61
scaffold21232_1_21043.3:1-533	533	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	175.652	1.25E-41
scaffold19121_1_9786.2:1-445	445	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	210.26	3.96E-52
scaffold27701_1_2435.2:1-385	385	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	183.343	4.31E-44
scaffold42760_1_2579.1:1-276	276	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	175.652	6.23E-42
scaffold40930_1_1010.1:1-261	261	Fungi	gi|160338807|ref|NZ_AACM02000434.1|	385.225	4.79E-105
scaffold8637_1_20241.10:1-597	597	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	173.729	5.34E-41
scaffold6349_1_7434.4:1-816	816	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	202.57	1.54E-49
scaffold31075_1_3643.1:54-990	937	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	242.946	1.24E-61
scaffold21232_1_21043.19:1-649	649	Fungi	gi|221247333|ref|NZ_AAYY01000048.1|	260.25	5.25E-67

Supplementary Data Set 3. Microbial sequences identified among the ‘no-hit’ contigs from *Wheeler et al*. We aligned the complete set of ‘no hit’ contigs against viral, bacterial, and fungal databases by MegaBlast. The most significant alignments are reported.

Query name	Query length	BLAST Database	Hit ID	Bit score	E-value
Contig142158	206	Viruses	gi|118496614|ref|NC_008603.1|	362.153	1.30E-99
Contig5230	196	Bacteria	gi|163854304|ref|NC_010170.1|	279.477	4.93E-73
Contig9647	203	Bacteria	gi|50841496|ref|NC_006085.1|	362.153	6.65E-98
Contig94873	187	Bacteria	gi|222093774|ref|NC_011969.1|	262.173	7.57E-68
Contig144673	182	Bacteria	gi|255534169|ref|NC_013062.1|	183.343	3.94E-44
Contig147766	105	Bacteria	gi|172062142|ref|NC_010552.1|	100.667	1.57E-19
Contig149702	190	Bacteria	gi|194363778|ref|NC_011071.1|	244.869	1.25E-62
Contig63162	245	Fungi	gi|145616316|ref|NW_001798830.1|	187.188	1.84E-45
Contig125249	201	Fungi	gi|145616316|ref|NW_001798830.1|	175.652	4.38E-42
Contig75100	192	Fungi	gi|145616316|ref|NW_001798830.1|	179.497	2.89E-43
Contig162560	274	Fungi	gi|145616316|ref|NW_001798830.1|	225.642	5.52E-57
Contig136490	270	Fungi	gi|145616316|ref|NW_001798830.1|	204.492	1.26E-50
Contig857	248	Fungi	gi|145601699|ref|NW_001798706.1|	214.106	1.47E-53
Contig81457	236	Fungi	gi|145616316|ref|NW_001798830.1|	229.487	3.26E-58
Contig98682	211	Fungi	gi|145616316|ref|NW_001798830.1|	210.26	1.77E-52
Contig168296	175	Fungi	gi|145616316|ref|NW_001798830.1|	187.188	1.26E-45
Contig15975	194	Fungi	gi|145615709|ref|NW_001798760.1|	225.642	3.76E-57
Contig108148	177	Fungi	gi|145616316|ref|NW_001798830.1|	214.106	1.01E-53
Contig71361	243	Fungi	gi|145616316|ref|NW_001798830.1|	200.647	1.62E-49
Contig98900	202	Fungi	gi|145616316|ref|NW_001798830.1|	177.575	1.16E-42
Contig49443	207	Fungi	gi|145616319|ref|NW_001798834.1|	187.188	1.52E-45
Contig78945	210	Fungi	gi|145616316|ref|NW_001798830.1|	183.343	2.23E-44
Contig3736	243	Fungi	gi|145616316|ref|NW_001798830.1|	262.173	4.88E-68
Contig8215	247	Fungi	gi|145616316|ref|NW_001798830.1|	235.255	6.29E-60
Contig93429	203	Fungi	gi|145616316|ref|NW_001798830.1|	191.033	1.04E-46
Contig106445	149	Fungi	gi|145616316|ref|NW_001798830.1|	173.729	1.19E-41
Contig45474	256	Fungi	gi|145616316|ref|NW_001798830.1|	194.879	9.34E-48
Contig96785	277	Fungi	gi|145615707|ref|XM_001414736.1|	325.622	4.47E-87
Contig125439	191	Fungi	gi|145616316|ref|NW_001798830.1|	179.497	2.88E-43
Contig122859	193	Fungi	gi|145616316|ref|NW_001798830.1|	196.802	1.80E-48
Contig11322	194	Fungi	gi|145616316|ref|NW_001798830.1|	208.338	6.09E-52
Contig40437	195	Fungi	gi|145616316|ref|NW_001798830.1|	214.106	1.12E-53
Contig91838	197	Fungi	gi|145616316|ref|NW_001798830.1|	177.575	1.13E-42
Contig120141	198	Fungi	gi|145616316|ref|NW_001798830.1|	214.106	1.14E-53
Contig90170	206	Fungi	gi|145616316|ref|NW_001798830.1|	229.487	2.80E-58
Contig78748	206	Fungi	gi|145601699|ref|NW_001798706.1|	196.802	1.94E-48
Contig16738	208	Fungi	gi|145616316|ref|NW_001798830.1|	246.791	1.75E-63
Contig25077	208	Fungi	gi|145616316|ref|NW_001798830.1|	223.719	1.54E-56
Contig85093	208	Fungi	gi|145601699|ref|NW_001798706.1|	175.652	4.55E-42
Contig90475	209	Fungi	gi|145616316|ref|NW_001798830.1|	200.647	1.37E-49
Contig90120	210	Fungi	gi|145616316|ref|NW_001798830.1|	206.415	2.53E-51