Supplemental Material for Ren et al. "VirFinder: a novel *k*-mer based tool for identifying viral sequences from assembled metagenomic data"

Table S1. VirFinder and VirSorter prediction results for n=45 RefSeq prokaryotic virus genomes sequenced after 1/1/2014 that have no significant blastn similarity (E-value > $1e^{-5}$) to RefSeq prokaryotic virus genomes sequenced before 1/1/2014.

Virus genome	NCBI accession	Length (bp)	VirFinder score	VirFinder *p*-value	VirSorter result[a]	Evaluate VirFinder (VF) and VirSorter (VS) results[b]
Oenococcus phage phi9805	NC_023559_1	46145	0.060	0.422	II	VS but not VF
Oenococcus phage phiS11	NC_023571_1	46243	0.065	0.407	II	VS but not VF
Oenococcus phage phiS13	NC_023560_1	43454	0.094	0.345	II	VS but not VF
Eel River basin pequenovirus isolate c22476	NC_026665_1	6083	0.255	0.187	II	VS but not VF
Mycobacterium phage Adler	NC_023591_1	95705	0.401	0.120	Cat. VI provirus	Neither
Vibrio phage X29	NC_024369_2	41569	0.565	0.073	II	VS but not VF
Uncultured phage WW-nAnB strain 3	NC_026613_1	5210	0.657	0.054	N	Neither
Shewanella sp. phage 1/41	NC_025458_1	43510	0.686	0.049	II	VS but not VF
Rhizobium phage vB_RglS_P106B	NC_023566_1	56024	0.696	0.047	II	VS but not VF
Psychrobacter phage Psymv2	NC_023734_1	35725	0.704	0.045	II	VS but not VF
Erwinia phage Ea35-70	NC_023557_1	271084	0.765	0.034	II	VS but not VF
Arthrobacter phage vB_ArtM-ArV1	NC_026606_1	71200	0.803	0.028	II	VS but not VF
Vibrio phage VpKK5	NC_026610_2	56637	0.894	0.014	II	VS but not VF
Microviridae IME-16	NC_026013_1	5755	0.917	0.012	III	Neither
Croceibacter phage P2559Y	NC_023614_1	43153	0.924	0.010	II	VS but not VF
Lactoccocus phage WP-2	NC_024149_1	18899	0.934	0.009	II	Both
Aeromonas phage pAh6-C	NC_025459_1	53744	0.944	0.008	II	Both
Rhizobium phage vB_RleS_L338C	NC_023502_1	109558	0.946	0.008	II	Both
Rhodococcus phage ReqiPoco6	NC_023694_1	78064	0.948	0.007	II	Both
Erwinia phage PhiEaH1	NC_023610_1	218339	0.951	0.007	II	Both
Uncultured phage WW-nAnB strain 2	NC_026612_1	5077	0.954	0.007	N	**VF but not VS**
Rhodococcus phage ReqiPepy6	NC_023735_1	76797	0.957	0.007	II	Both
Idiomarinaceae phage Phi1M2-2	NC_025471_1	36844	0.957	0.007	II	Both
Shewanella sp. phage 3/49	NC_025466_1	40161	0.963	0.006	II	Both
Idiomarinaceae phage 1N2-2	NC_025439_1	34773	0.966	0.005	II	Both

Clavibacter phage CN1A	NC_023549_1	56789	0.969	0.005	II	Both
Uncultured phage WW-nAnB	NC_026582_1	4817	0.972	0.005	N	**VF but not VS**
Ruegeria phage DSS3-P1	NC_025428_1	59601	0.973	0.005	II	Both
Vibrio phage CHOED	NC_023863_2	66316	0.975	0.004	II	Both
Shewanella sp. phage 1/44	NC_025463_1	49640	0.975	0.004	II	Both
Mesorhizobium phagevB_MloP_Lo5R7ANS	NC_025431_1	45718	0.976	0.004	II	Both
Shewanella phage Spp001	NC_023594_1	54789	0.979	0.004	II	Both
Enterococcus phage vB_Efae230P-4	NC_025467_1	17972	0.980	0.004	II	Both
Uncultured phage crAssphage	NC_024711_1	97065	0.983	0.003	II	Both
Rhizobium phage vB_RleM_P10VF	NC_025429_1	156446	0.986	0.003	II	Both
Vibrio phage SHOU24	NC_023569_1	77837	0.988	0.003	III	**VF but not VS**
Acinetobacter phage IME_AB3	NC_023590_1	43050	0.990	0.002	II	Both
Rhodococcus phage ReqiDocB7	NC_023706_1	75772	0.992	0.002	II	Both
Microbacterium phage vB_MoxS-ISF9	NC_023859_1	59254	0.991	0.002	II	Both
Shewanella sp. phage 1/40	NC_025470_1	139004	0.991	0.002	II	Both
Shewanella sp. phage 1/4	NC_025436_1	133824	0.993	0.002	II	Both
Acinetobacter phage vB_AbaM_Acibel004	NC_025462_1	99730	0.996	0.001	Cat. V provirus	Both
Pseudomonas phage phi_Pto-bp6g	NC_023718_1	26499	0.997	0.001	III	**VF but not VS**
Vibrio phage phi-A318	NC_025822_1	42544	0.999	0.000	II	Both
Anabaena phage A-4L	NC_024358_1	41750	1.000	0.000	II	Both

a – VirSorter prediction result (category I, II, or III, for viruses and IV, V, and VI for category 1, 2, 3 results for detected proviruses). N = no prediction made by VirSorter.
b – Summary of the results comparing the VirFinder (VF, p-value < 0.01) and VirSorter (VS, only cat. I & II predictions) results. "Neither" = neither method predicted the genome as viral, "Both" = both VF and VS predicted it as viral, "VF but not VS" = VF predicted it as viral but VS did not, and "VS but not VF" = VS predicted it as viral but VF did not

Table S2. NCBI accession numbers for prokaryotic host and viral genomes used in the training and evaluation of VirFinder. This table is available as supplemental online material.

Table S3. Table of abundances of 1,562 possible virus and 2,698 complete prokaryotic genomes as determined by read mapping of human gut microbiome metagenome sample SRR061166 from Vázquez-Castellanos et al. 2014. These abundances were used to generate simulated metagenomes. This table is available as supplemental online material.

Table S4. Information about the 2,657 top-scoring predicted viral contigs assembled from 78 human gut microbiome samples from the liver cirrhosis study of Qin et al. 2014.

The table includes general information about the size of contigs and to which bin they belong, VirFinder and VirSorter prediction results, and whether or not those contigs have significantly similarity to other sequences in NCBI's nucleotide nt and protein nr databases. This table is available as supplemental online material.

Supplemental Figure Legends

Figure S1. Area under the receiver operator curve (AUROC) (A) and Area under precision-recall curves (AUPRC) (B) for predictions results made with VirFinder on varying mixtures of viral and host contigs subsampled from viral and host genomes sequenced after 1/1/2014. VirFinder was trained using contigs equal numbers of viral and host contigs subsampled from genomes sequenced before 1/1/2014 as in the results for Fig. 1. Bars depict mean values for 30 replicate bootstrap samples and error bars depict the standard error.

Figure S2. Performance of VirSorter and VirFinder virus prediction for contigs subsampled from virus and prokaryotic genomes as in Fig. 2A, except that evaluation datasets contained 10% (A) or 90% (B) viral contigs. Results are shown for the fraction of true viral contigs (true positive rate, TPR) when using VirSorter category I and II predictions and VirFinder at the same false positive rate (FPR) as VirSorter (listed in or above the VirSorter bars) and at FPRs of 0.001, 0.005, and 0.01. Bars depict mean values for 30 replicate bootstrap samples and error bars depict the standard error. TPRs of VirFinder were all significantly higher than that of VirSorter at the same false positive rate (Wilcoxon signed-rank one sided test, $p < 0.001$).

Figure S3. Sensitivity of VirFinder to random mutations applied to evaluation contigs. VirFinder prediction results as evaluated by AUROCs were determined on contigs subsampled from viral and host genomes with no mutations applied vs. when random mutations were applied to the contigs at three different rates (0.0001, 0.001, and 0.01 substitutions per position). Bars represent averages of 30 replicate datasets tested, and error bars indicate standard deviations. Within each contig length group, there was only a significant difference in values between the 0.01 rate and the case of no mutation ($p < 0.01$, *t*-test).

Figure S4. Precision-recall curves and AUPRC for VirFinder results when analyzing contigs assembled from simulated metagenomes. (A) Precision-recall curves for the analysis of equal proportions of viral and host contigs representing genomes sequenced after 1/1/2014. Results are shown for when chimeras were included or excluded from the analysis. (B) AUPRC scores for various VirFinder results when varying the total sequencing depth for the simulated metagenomes (10 M or 20 M reads) and varying the relative abundance of viral and host contigs analyzed. Bars represent averages of 30 replicate datasets tested, and error bars indicate standard deviations.

Figure S5. Evaluation of VirFinder (VF) and VirSorter (VS) predictions on contigs for three length ranges assembled from simulated human gut metagenomes when viral contigs were combined with host contigs at 10% (A) and 90% (B) viral levels. Bars depict true positive rates (TPRs) for VirSorter category I; I and II; and I, II, and III predictions. As in Fig. 5, VirFinder predictions were evaluated at the same false positive rates (FPRs) as corresponding VirSorter results. Thirty replicate bootstrap samples of contigs assembled from simulated metagenomes were tested for each condition. Metagenomes were simulated based on the relative abundance of complete virus and host genomes found in a real human gut metagenome. The horizontal bar displays the median, boxes display the first and third quartiles, and whiskers depict minimum and maximum values. "*" indicates VirFinder's TPRs are significantly larger than VirSorter's (Wilcoxon signed-rank one sided test, $p < 10^{-5}$).

Figure S6. Evaluation of VirFinder (VF) and VirSorter (VS) predictions on contigs assembled from simulated human gut metagenomes when viral contigs were combined with host contigs at 10%, 50% and 90% viral levels. Results are shown for predictions made on all contigs > 500 bp (left column) or all contigs > 1000 bp (right column). Bars depict true positive rates (TPRs) for VirSorter category I ("I"); I and II ("I&II"); and I, II, and III ("I-III") predictions. As in Fig. 5, VirFinder predictions were evaluated at the same false positive rates (FPR) as corresponding VirSorter results. Thirty replicate bootstrap samples of contigs assembled from simulated metagenomes were tested for each condition. Metagenomes were simulated based on the relative abundance of complete virus and host genomes found in a real human gut metagenome. The horizontal bar displays the median, boxes display the first and third quartiles, and whiskers depict minimum and maximum values. "*" indicates VirFinder's TPRs are significantly larger than VirSorter's (Wilcoxon signed-rank one sided test, $p < 0.05$).

Figure S7. Histogram of the lengths of 352,020 contigs that are >1,000 bp generated by cross-assembly of 78 human gut metagenomic samples from 40 healthy and 38 liver cirrhosis patients (Qin et al. 2014).

Figure S8. Histograms depicting the cumulative frequencies for different groups of *k*-mers (length 8) as they occur in viral and host contigs. Panels depict the top 100, 500, 1000 most highly scored *k*-mers or all *k*-mers used by VirFinder (trained with 1,000 bp contigs) to generate prediction scores (n=6269 and 6082 for *k*-mers with positive and negative coefficients respectively). The left column of graphs depicts *k*-mers with positive coefficients in VirFinder's model (i.e. those that are found more frequently among viral sequences) and the right column shows *k*-mers that are negatively scored (those that are found more frequently among host sequences). In each panel, host and viral *k*-mer distributions were significantly different ($p < 10^{-16}$, *t*-test).

Figure S9. Similarity between the prediction proteins on the crAssphage genome (below) and on two contigs belonging to viral bin 64 (above). Grey arrows depict predicted proteins and trapezoids depict the percent amino acid identity between two connected

genes as determined by blastp searches. Numbers in crAssphage genes indicate the annotated locus tag of those genes (UGP_xxx).

Figure S10. VirFinder predictions were made when it was trained on the set of 14,722 prokaryotic host genomes from Roux et al. 2015 and the 1,225 viral genomes sequenced before 1/1/2014 that were used in the rest of our study. The Roux et al. host genomes were used as is or with proviruses identified by VirSorter removed ('proviruses removed'). VirFinder predictions were made on contigs with various lengths of virus genomes sequenced after 1/1/2014 and host genomes subsampled from host genomes after 1/1/2014 at equal proportions, and the resulting AUROC values are shown. The difference in AUROC values among the three datasets are less than 3%. Bars depict the mean of results on 30 replicate evaluation datasets and error bars depict standard deviations.

Figure S11. VirFinder predictions were made when VirFinder was trained with viral and prokaryotic sequences as before or with viral contigs 'spiked' into the host training set to assess the impact of an overabundance of proviruses in host training dataset. VirFinder was trained on host and viral contigs that were subsampled at equal numbers from prokaryotic and viral genomes sequenced before 1/1/2014 ("Control") and when 5% of the host contigs in the training set were replaced with contigs subsampled from viral genomes ("5% viral contigs added to host training database"). Predictions were made on equal numbers of viral and host contigs subsampled from genomes sequenced after 1/1/2014. Bar depict mean AUROC values for 30 replicate sets of subsampled contigs and error bars depict standard deviations.

Figure S1

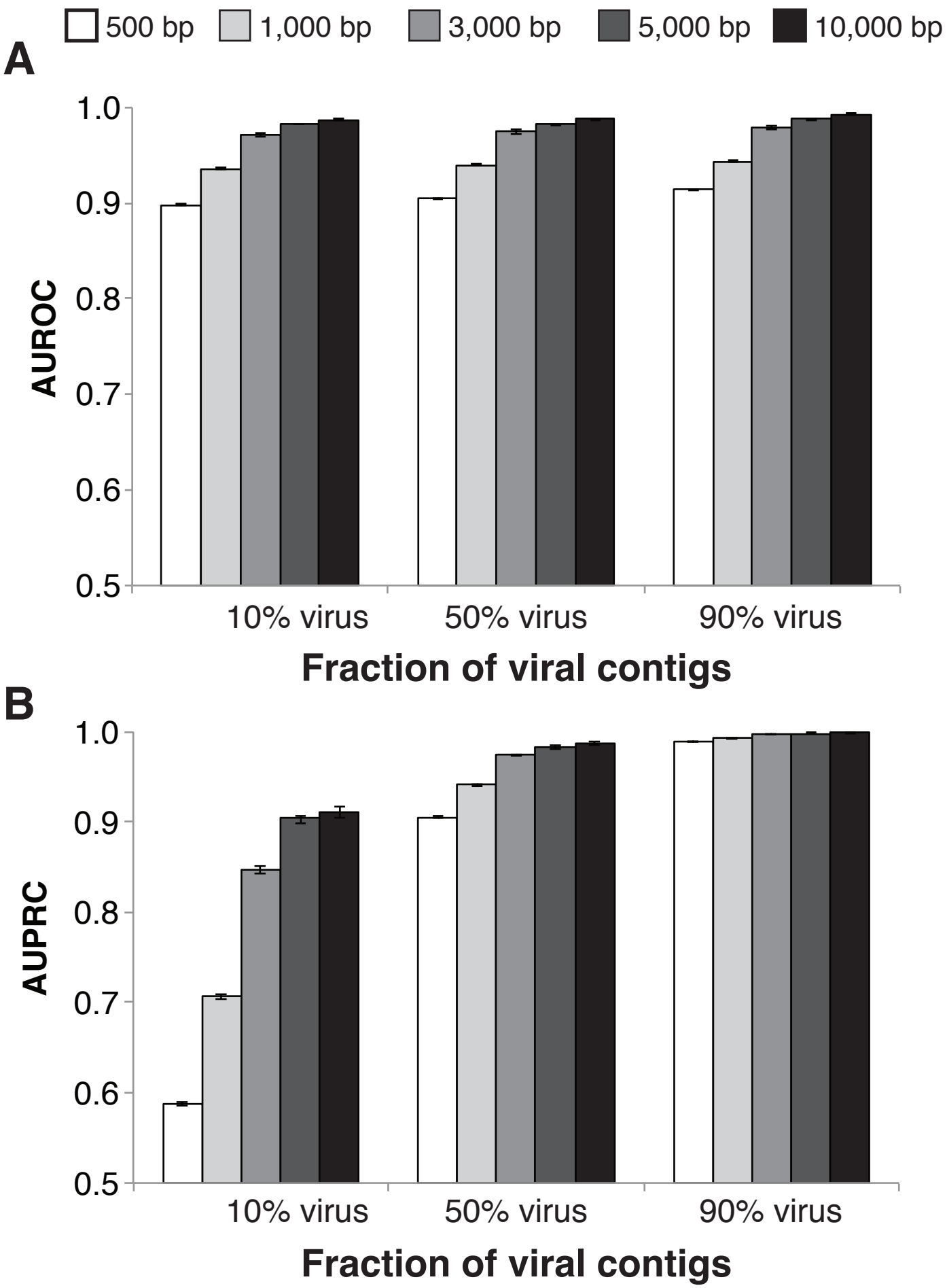
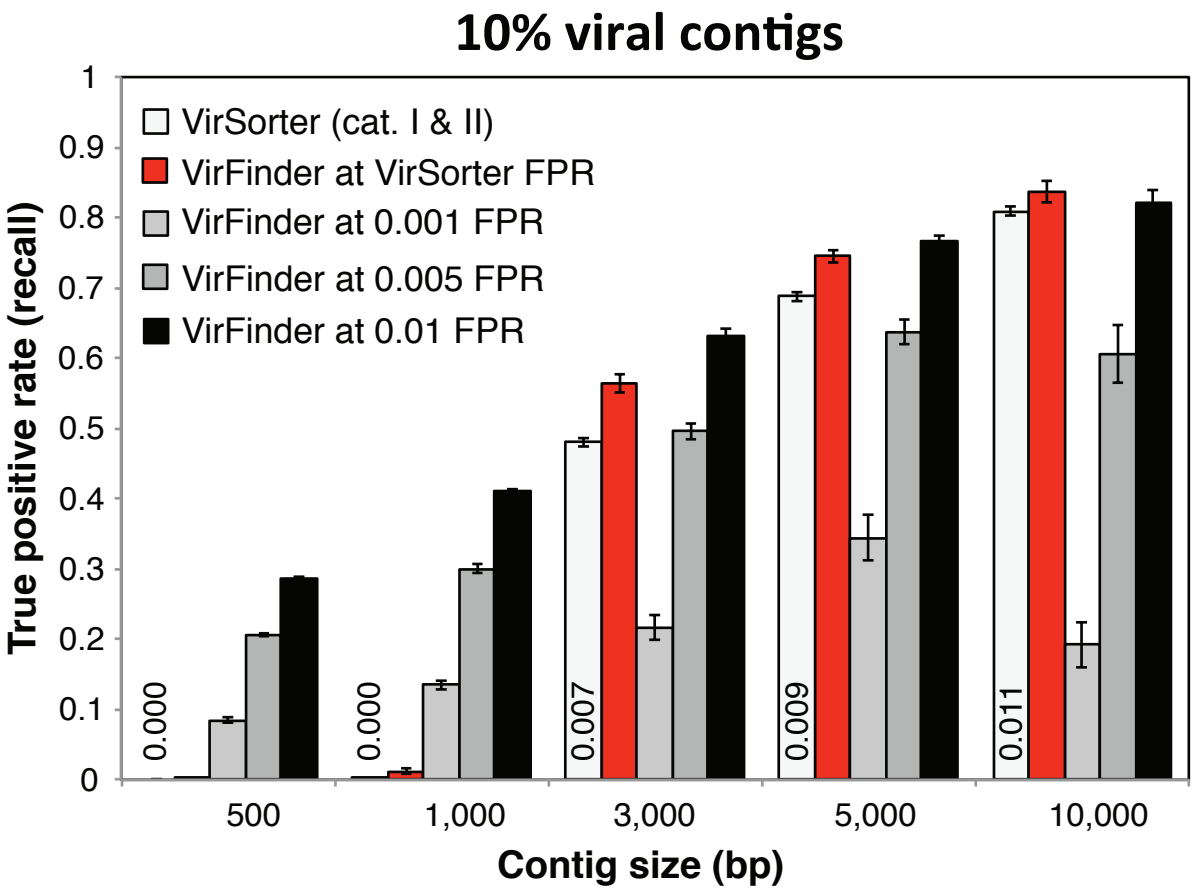

500 bp
1,000 bp
3,000 bp
5,000 bp
10,000 bp
A
AUROC
1.0
0.9
0.8
0.7
0.6
0.5
10% virus
50% virus
90% virus
Fraction of viral contigs
B
AUPRC
1.0
0.9
0.8
0.7
0.6
0.5
10% virus
50% virus
90% virus
Fraction of viral contigs

Figure S2

A


10% viral contigs
VirSorter (cat. I & II)
VirFinder at VirSorter FPR
VirFinder at 0.001 FPR
VirFinder at 0.005 FPR
VirFinder at 0.01 FPR
True positive rate (recall)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.000
0.000
0.007
0.009
0.011
500
1,000
3,000
5,000
10,000
Contig size (bp)

B

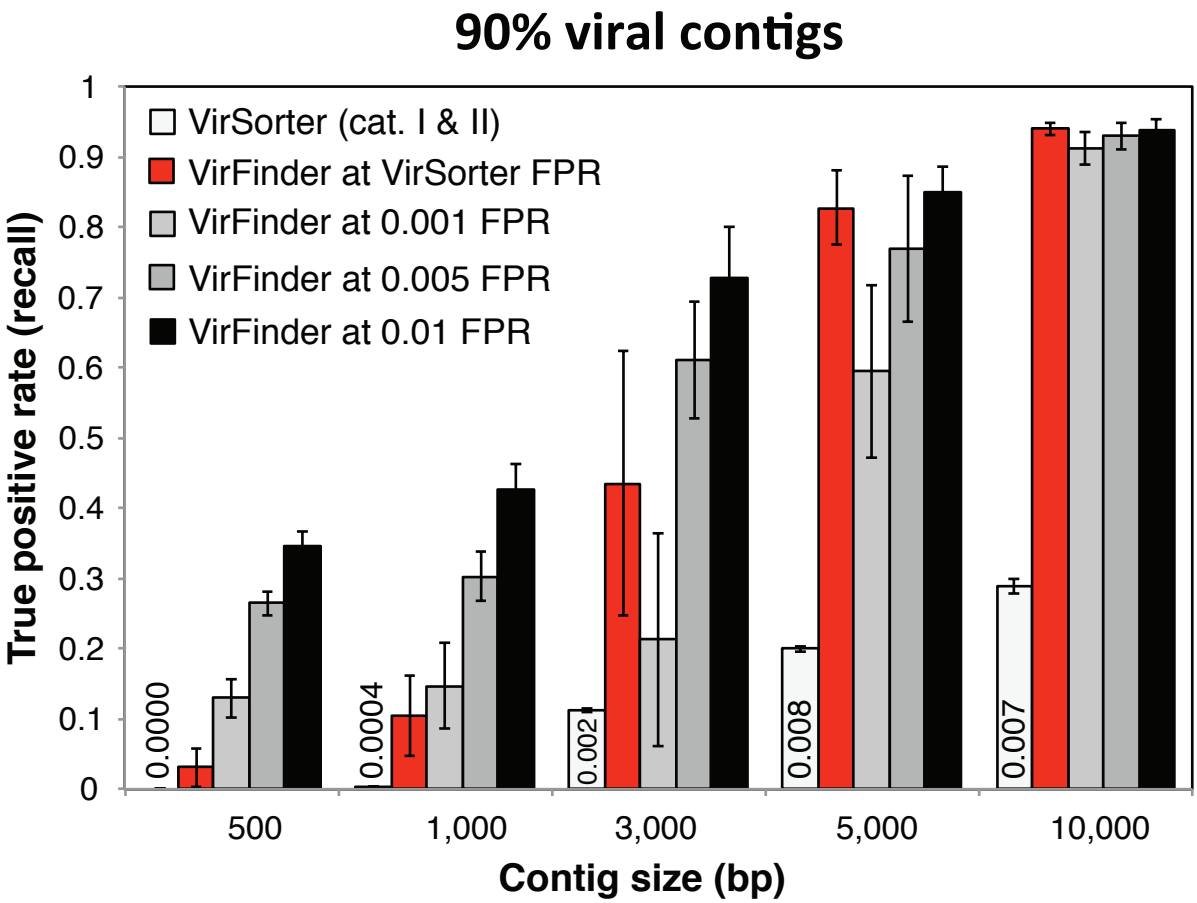

90% viral contigs
VirSorter (cat. I & II)
VirFinder at VirSorter FPR
VirFinder at 0.001 FPR
VirFinder at 0.005 FPR
VirFinder at 0.01 FPR
True positive rate (recall)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.0000
0.0004
0.002
0.008
0.007
500
1,000
3,000
5,000
10,000
Contig size (bp)

Figure S3

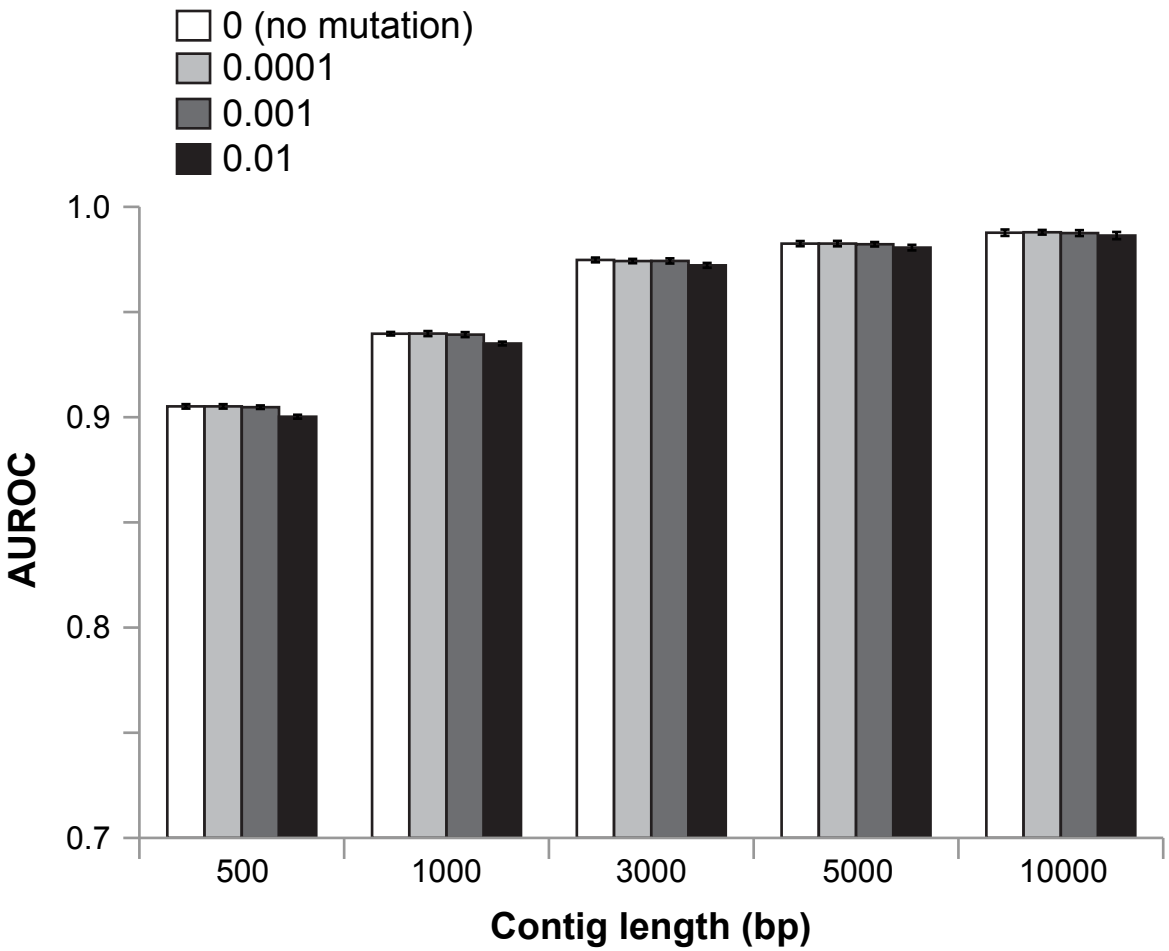

0 (no mutation)
0.0001
0.001
0.01
1.0
0.9
0.8
0.7
AUROC
500
1000
3000
5000
10000
Contig length (bp)

Figure S4

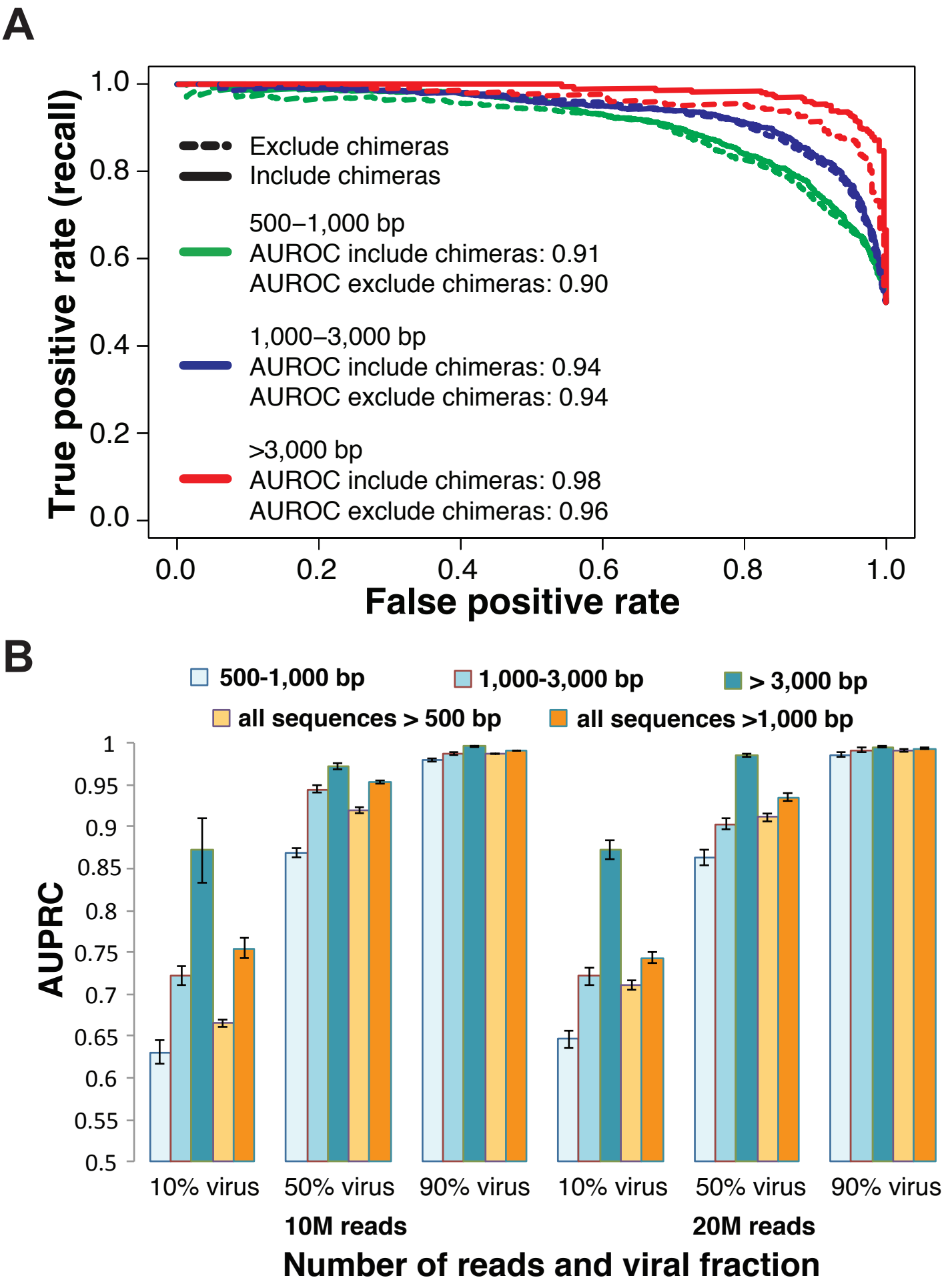
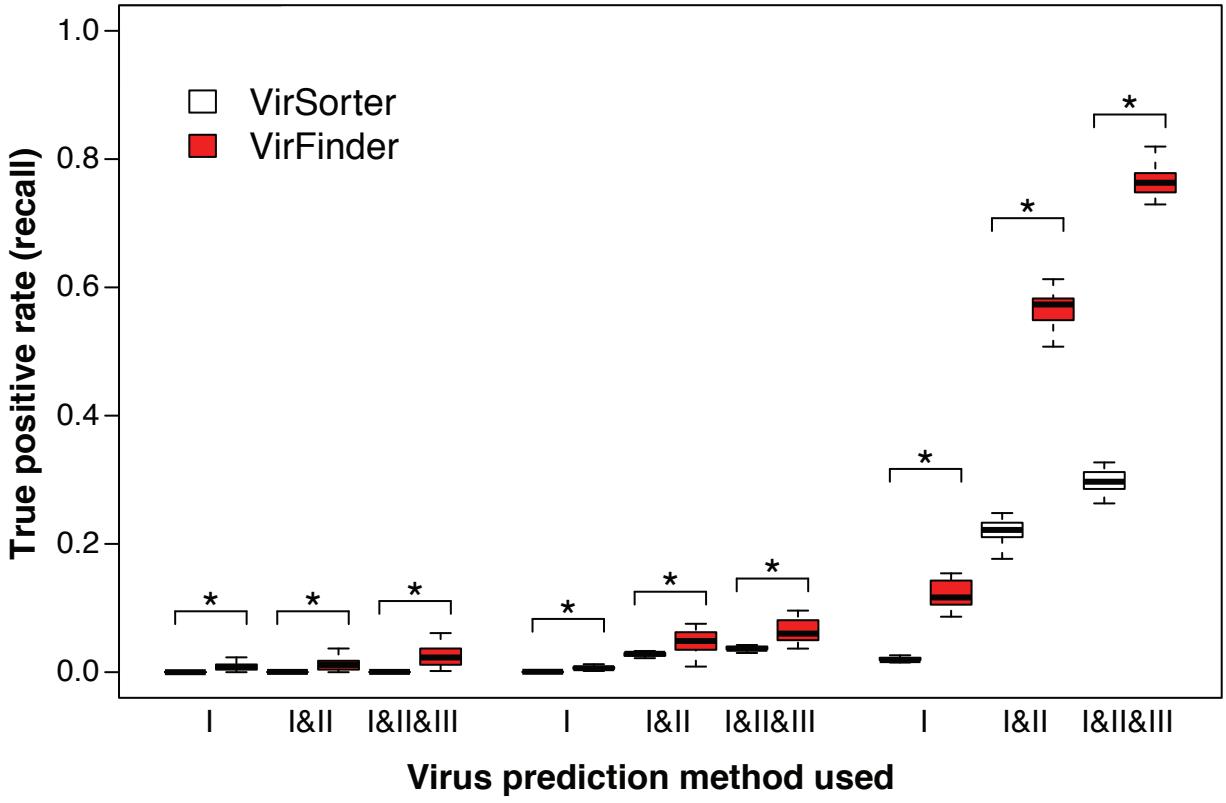

A
True positive rate (recall)
False positive rate
Exclude chimeras
Include chimeras
500–1,000 bp
AUROC include chimeras: 0.91
AUROC exclude chimeras: 0.90
1,000–3,000 bp
AUROC include chimeras: 0.94
AUROC exclude chimeras: 0.94
>3,000 bp
AUROC include chimeras: 0.98
AUROC exclude chimeras: 0.96
B
500-1,000 bp
1,000-3,000 bp
> 3,000 bp
all sequences > 500 bp
all sequences >1,000 bp
AUPRC
10% virus
50% virus
90% virus
10M reads
20M reads
Number of reads and viral fraction

Figure S5

A 10% viral contigs



B 90% viral contigs

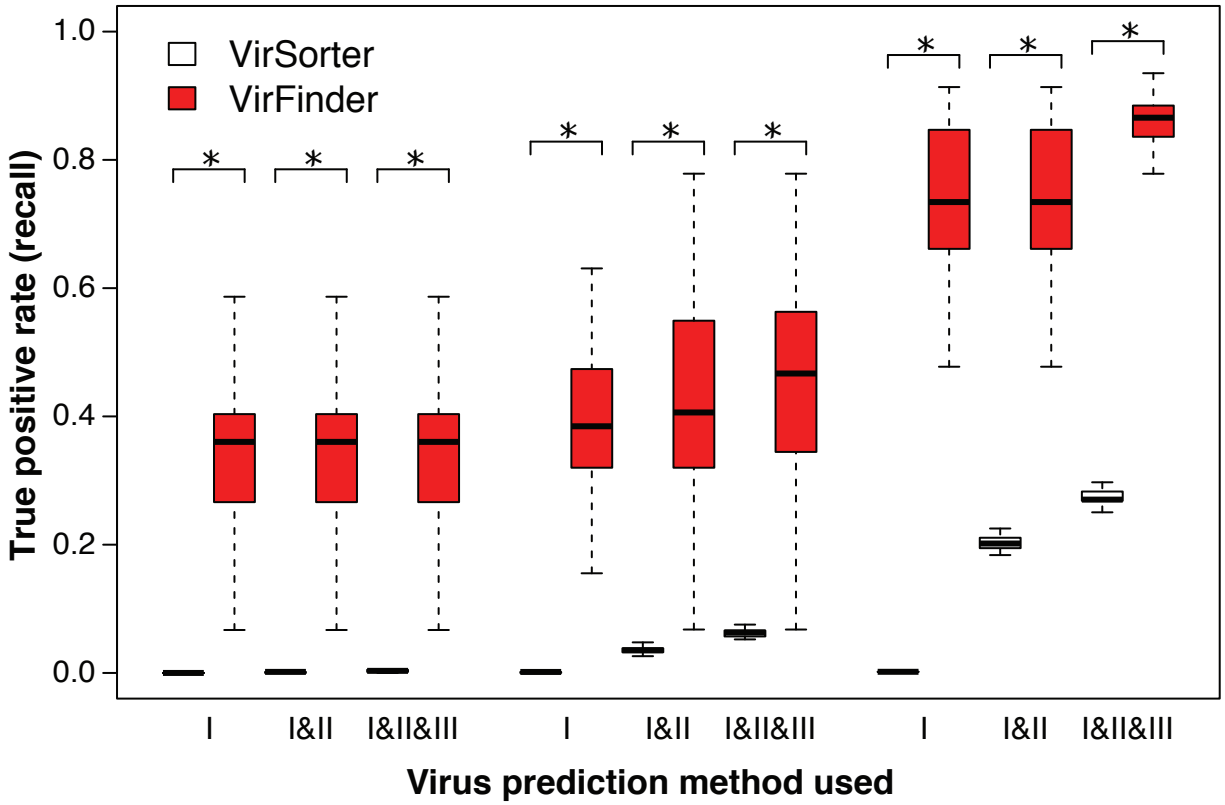


Figure S6

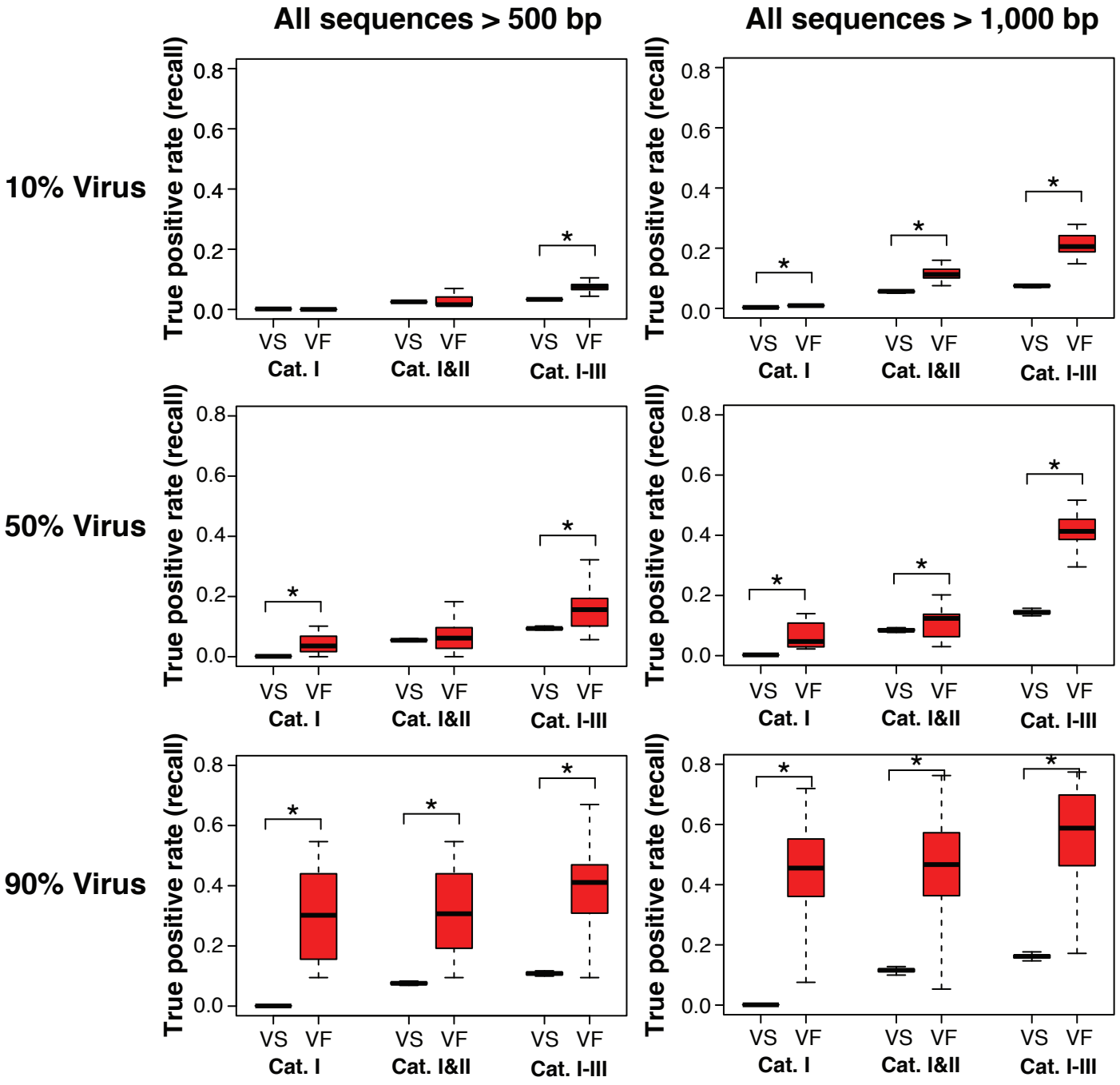

All sequences > 500 bp
All sequences > 1,000 bp
10% Virus
50% Virus
90% Virus
True positive rate (recall)
0.0
0.2
0.4
0.6
0.8
VS
VF
Cat. I
Cat. I&II
Cat. I-III
*

Figure S7

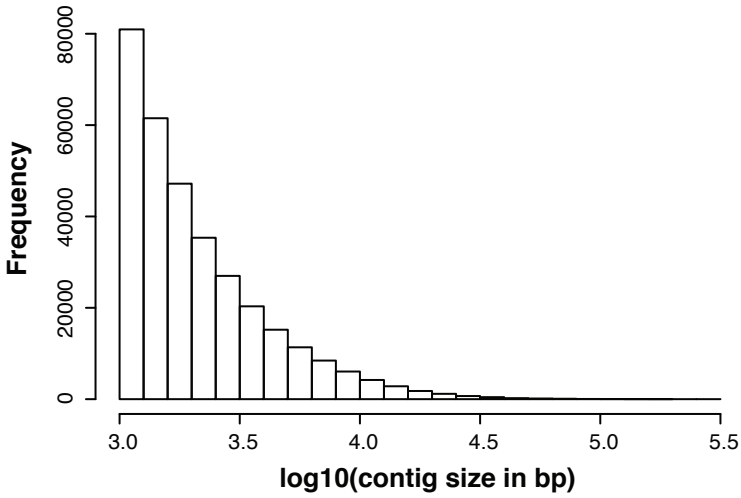

Frequency
0
20000
40000
60000
80000
3.0
3.5
4.0
4.5
5.0
5.5
log10(contig size in bp)

Figure S8

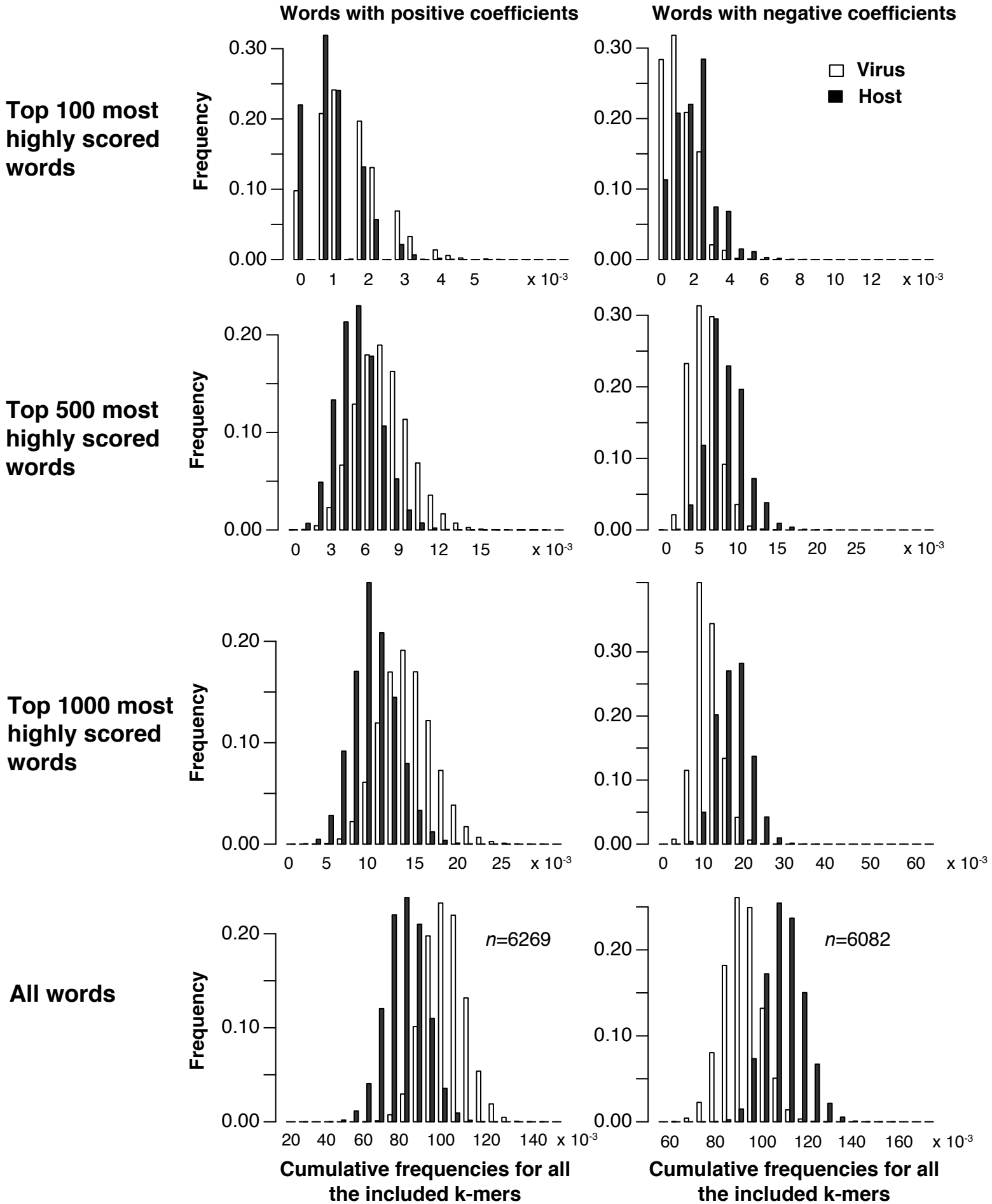
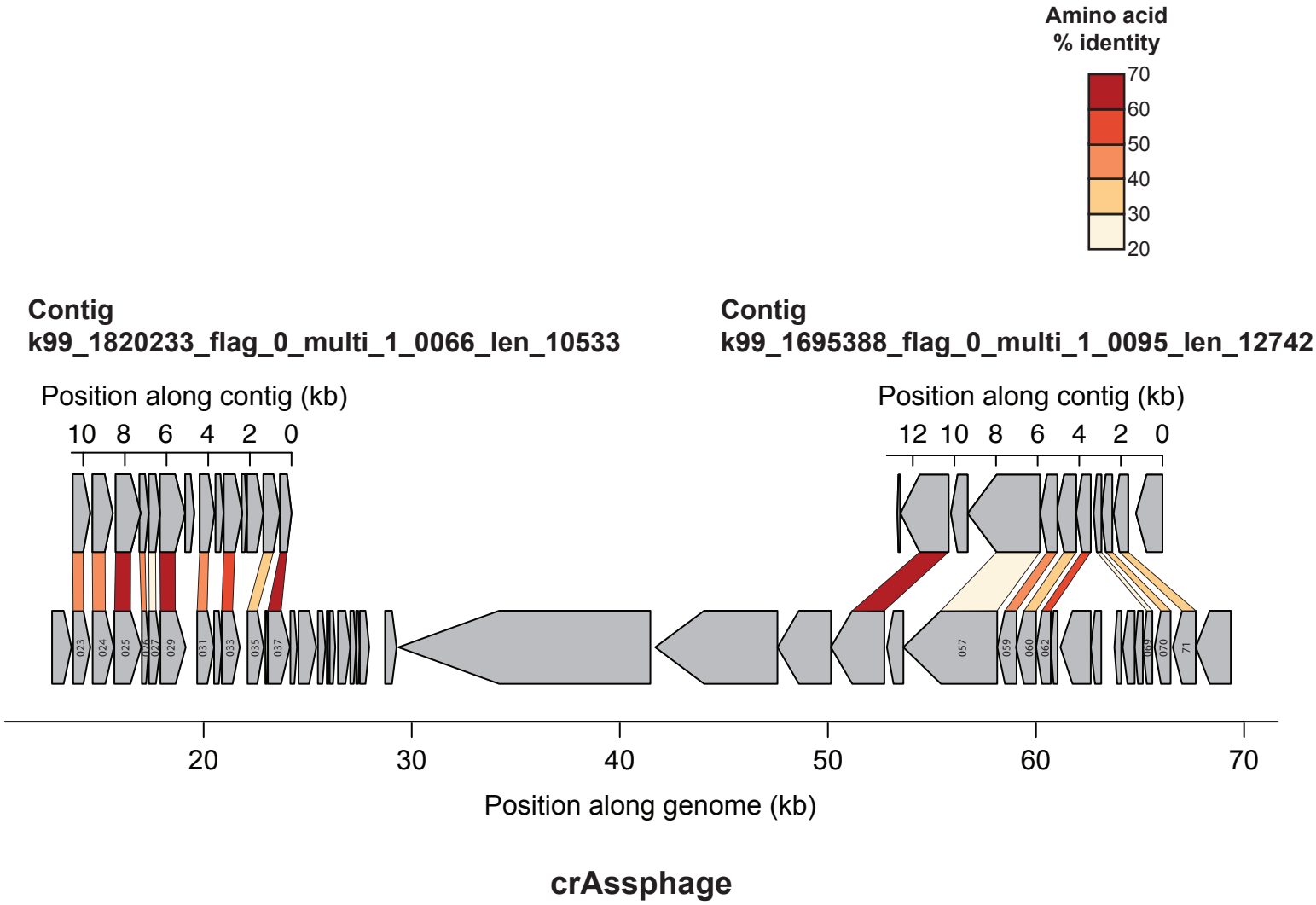

Words with positive coefficients
Words with negative coefficients
Virus
Host
Top 100 most highly scored words
Top 500 most highly scored words
Top 1000 most highly scored words
All words
Frequency
0.30
0.20
0.10
0.00
0 1 2 3 4 5 x 10-3
0 2 4 6 8 10 12 x 10-3
0 3 6 9 12 15 x 10-3
0 5 10 15 20 25 x 10-3
0 5 10 15 20 25 x 10-3
0 10 20 30 40 50 60 x 10-3
n=6269
n=6082
20 40 60 80 100 120 140 x 10-3
60 80 100 120 140 160 x 10-3
Cumulative frequencies for all the included k-mers
Cumulative frequencies for all the included k-mers

Figure S9



crAssphage

Figure S10

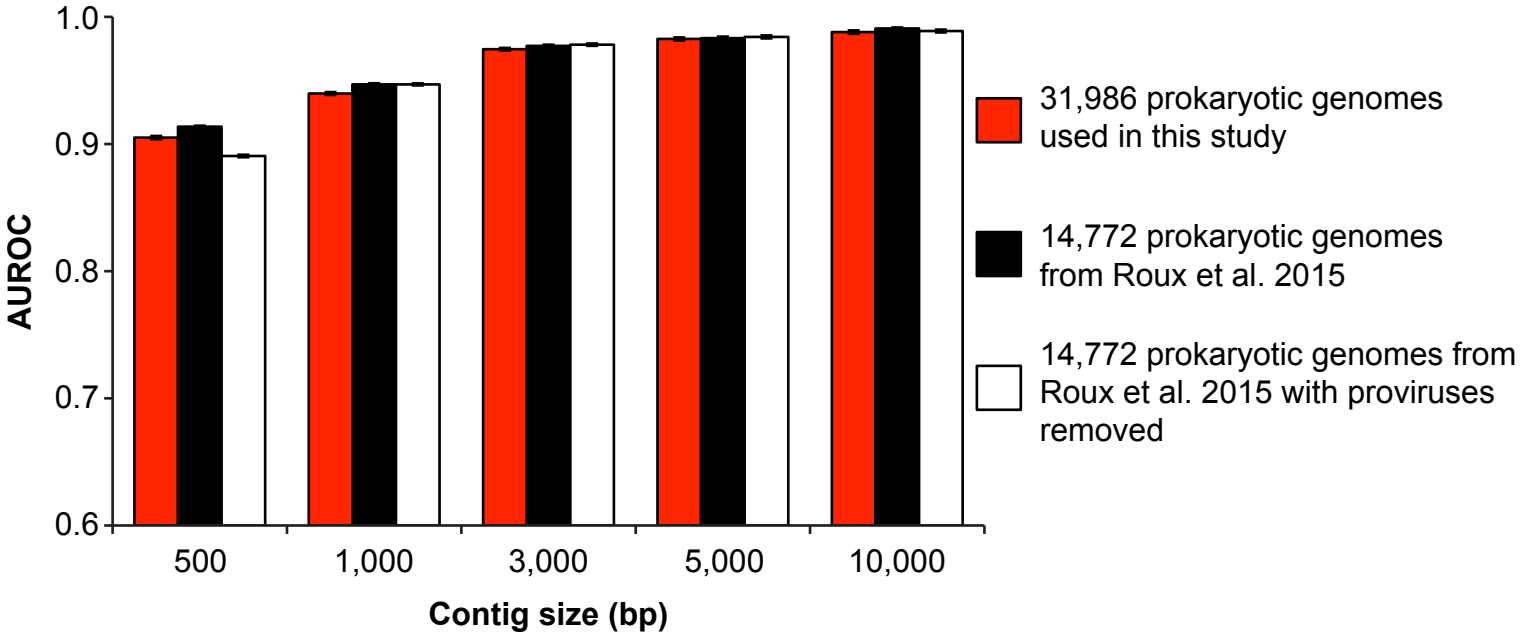
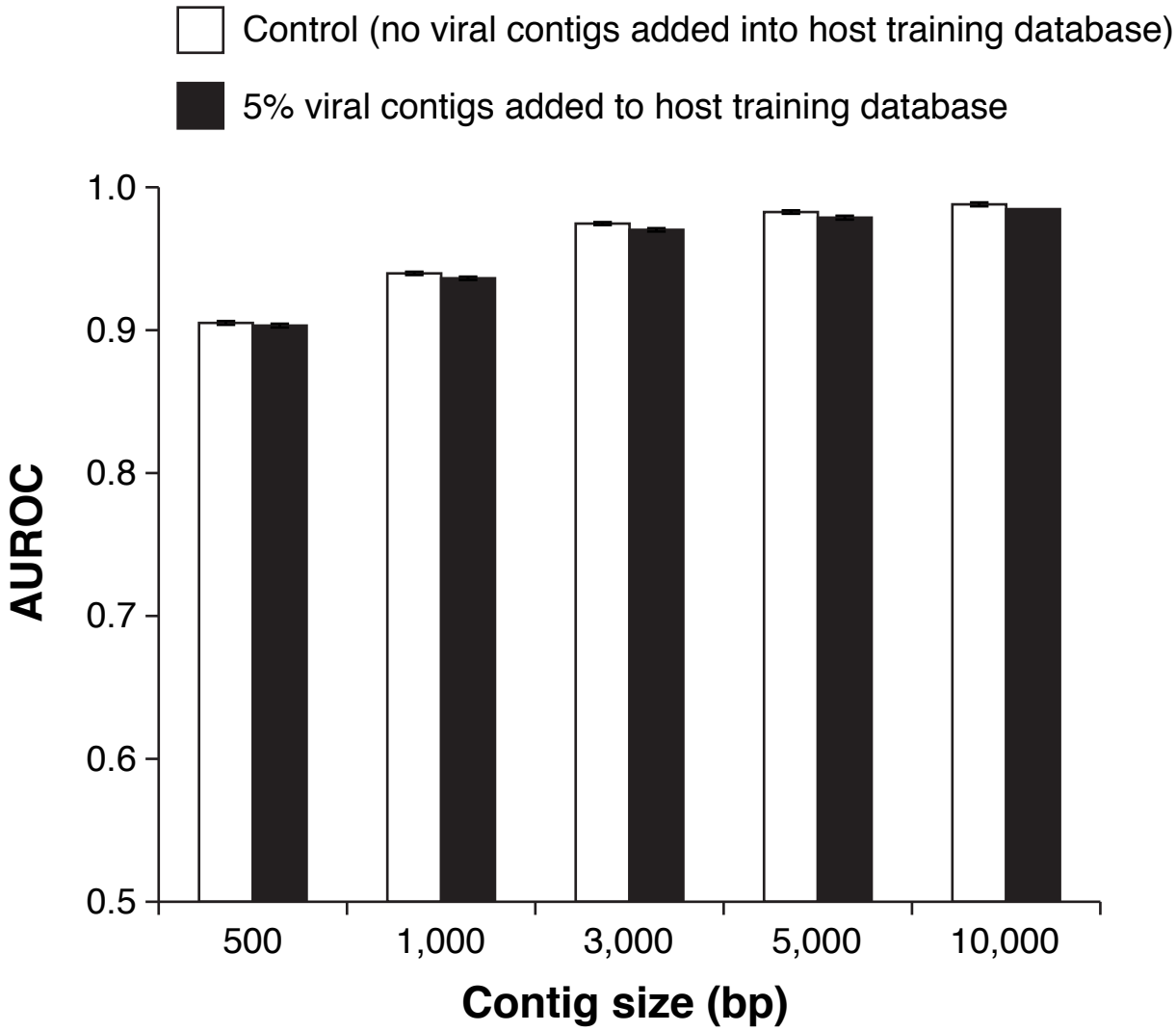

1.0
0.9
0.8
0.7
0.6
AUROC
500
1,000
3,000
5,000
10,000
Contig size (bp)
31,986 prokaryotic genomes used in this study
14,772 prokaryotic genomes from Roux et al. 2015
14,772 prokaryotic genomes from Roux et al. 2015 with proviruses removed

Figure S11


Control (no viral contigs added into host training database)
5% viral contigs added to host training database
AUROC
1.0
0.9
0.8
0.7
0.6
0.5
500
1,000
3,000
5,000
10,000
Contig size (bp)