

Supplementary information

Viral Phylogenomics Using an Alignment-Free Method: A Three-Step Approach to Determine Optimal Length of *k-mer*

Qian Zhang^{2,3}, Se-Ran Jun^{1,3}, Michael Leuze^{4,5}, David Ussery^{1,3}, Intawat Nookaew^{1,3,*}

¹Department of Biomedical Informatics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

²UT-ORNL Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN 37996, USA

³Comparative Genomics Group, Biosciences Division, Oak Ridge National Laboratory Oak Ridge, TN 37831 USA

⁴Joint Institute for Computational Sciences, University of Tennessee, Knoxville, TN 37831, USA

⁵Computational Biomolecular Modeling and Bioinformatics Group, Computer Science and Mathematics Division, Oak Ridge National Laboratories, Oak Ridge, TN 37831, USA

*INookaew@uams.edu

Table S1 Baltimore classification and ICTV Orders Information

| Baltimore Classification | counts | ICTV Order | counts |
|-----------------------------|--------|----------------------------|--------|
| dsDNA viruses, no RNA stage | 1826 | <i>Caudovirales</i> | 1208 |
| (+)ssRNA viruses | 911 | <i>Picornavirales</i> | 157 |
| ssDNA viruses | 649 | <i>Tymovirales</i> | 141 |
| dsRNA viruses | 192 | <i>Mononegavirales</i> | 91 |
| (-)ssRNA viruses | 180 | <i>Herpesvirales</i> | 67 |
| Retro-transcribing viruses | 131 | <i>Nidovirales</i> | 58 |
| Unclassified viruses | 8 | <i>Ligamenvirales</i> | 12 |
| Unclassified virophages | 5 | Unassigned or Unclassified | 2171 |
| Unassigned ssRNA viruses | 3 | | |

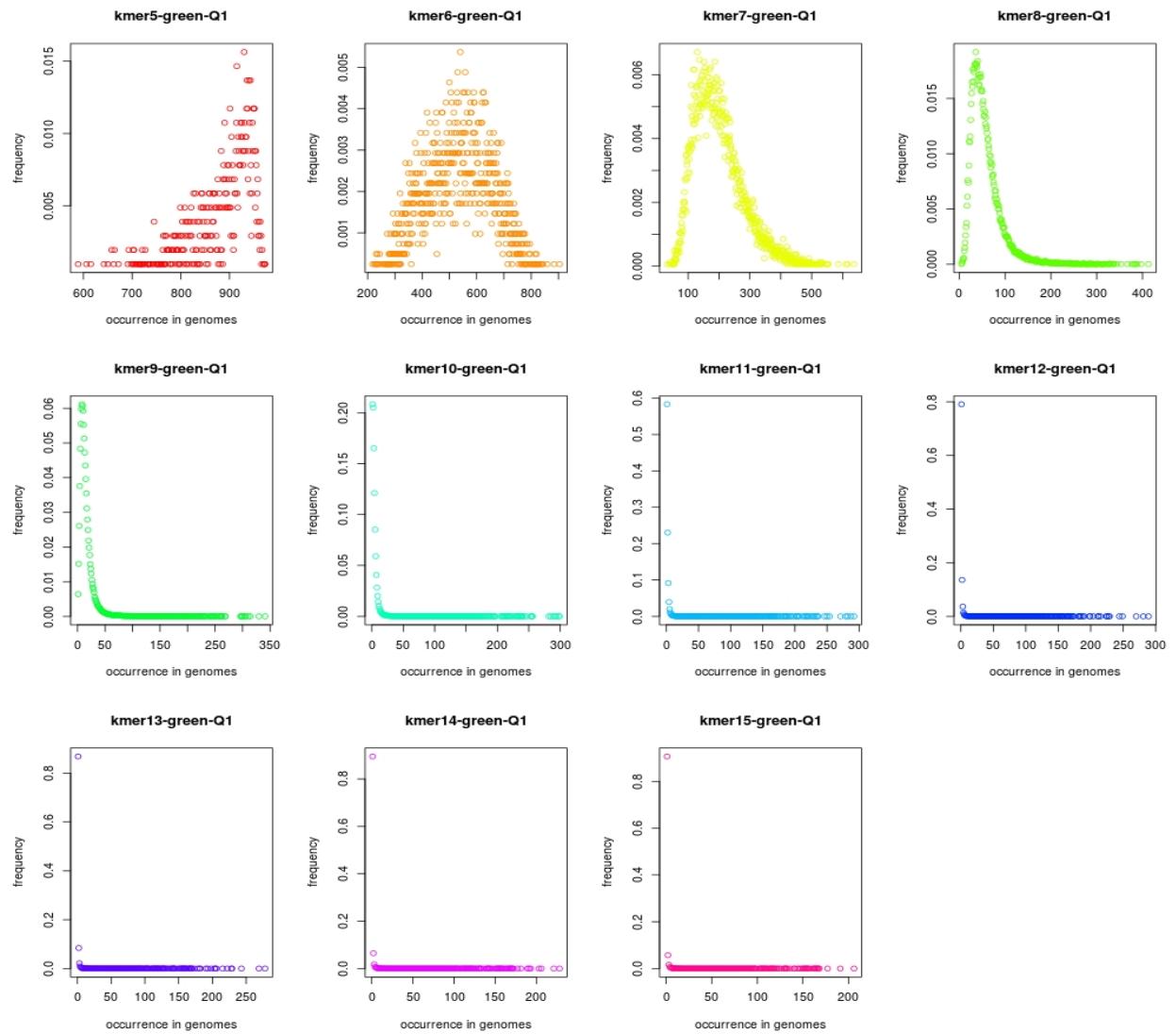


Figure S1 Distribution of feature occurrences in subgroup Q1 (size < 25%)

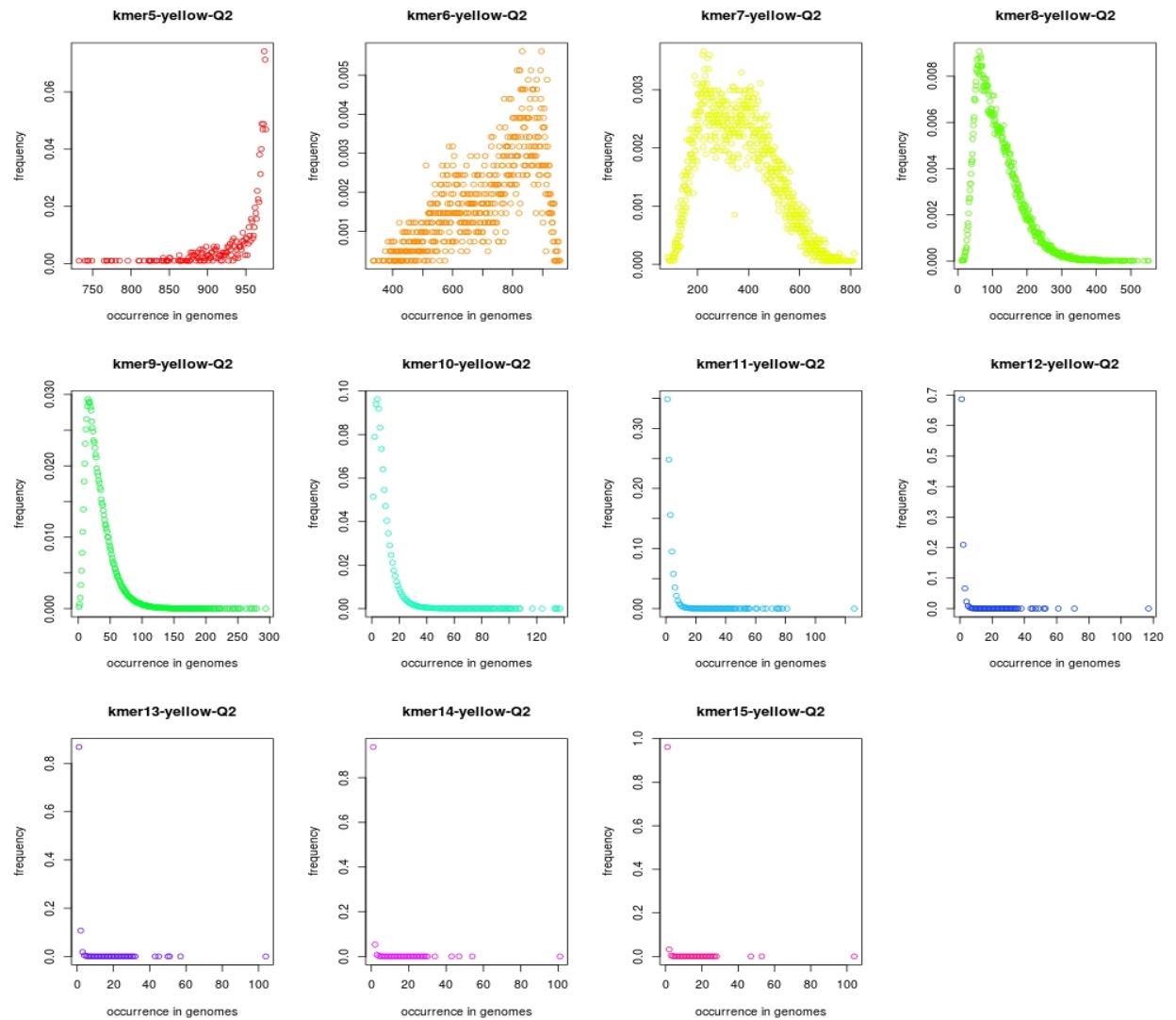


Figure S2 Distribution of feature occurrences in subgroup Q2 ($25\% < \text{size} < 50\%$)

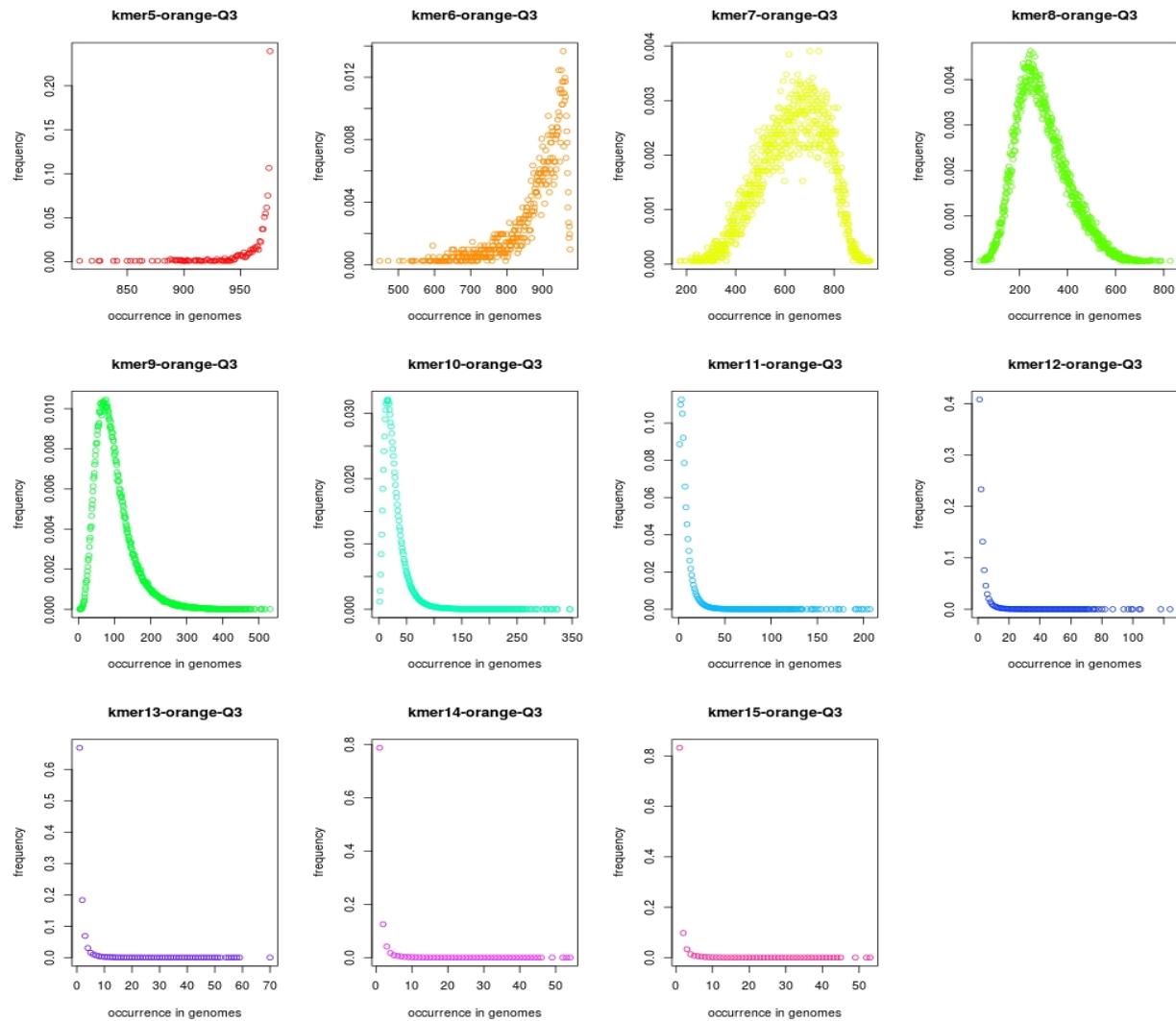


Figure S3 Distribution of feature occurrences in subgroup Q3 (50% < size < 75%)

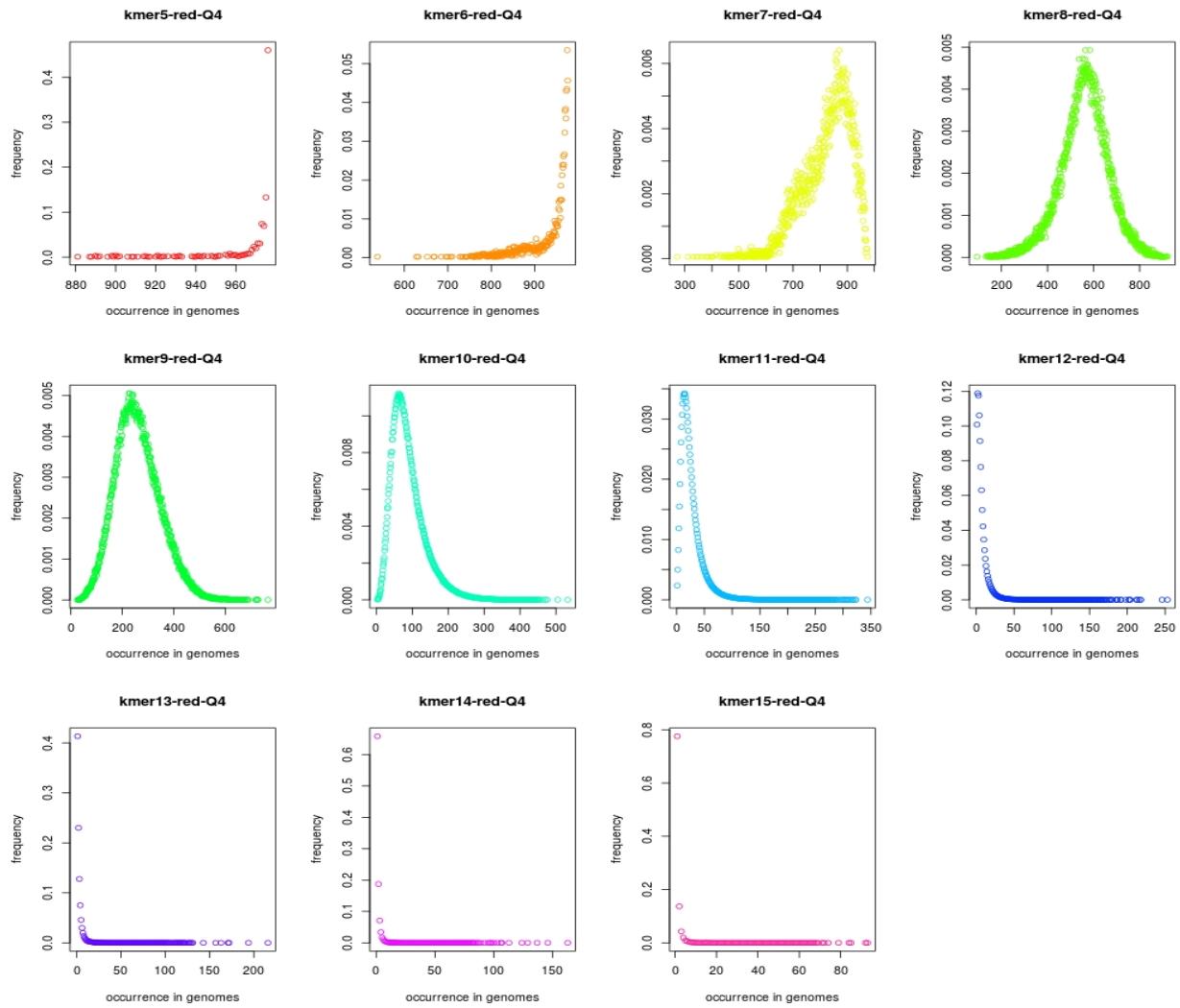


Figure S4 Distribution of feature occurrences in subgroup Q4 (size > 75%)

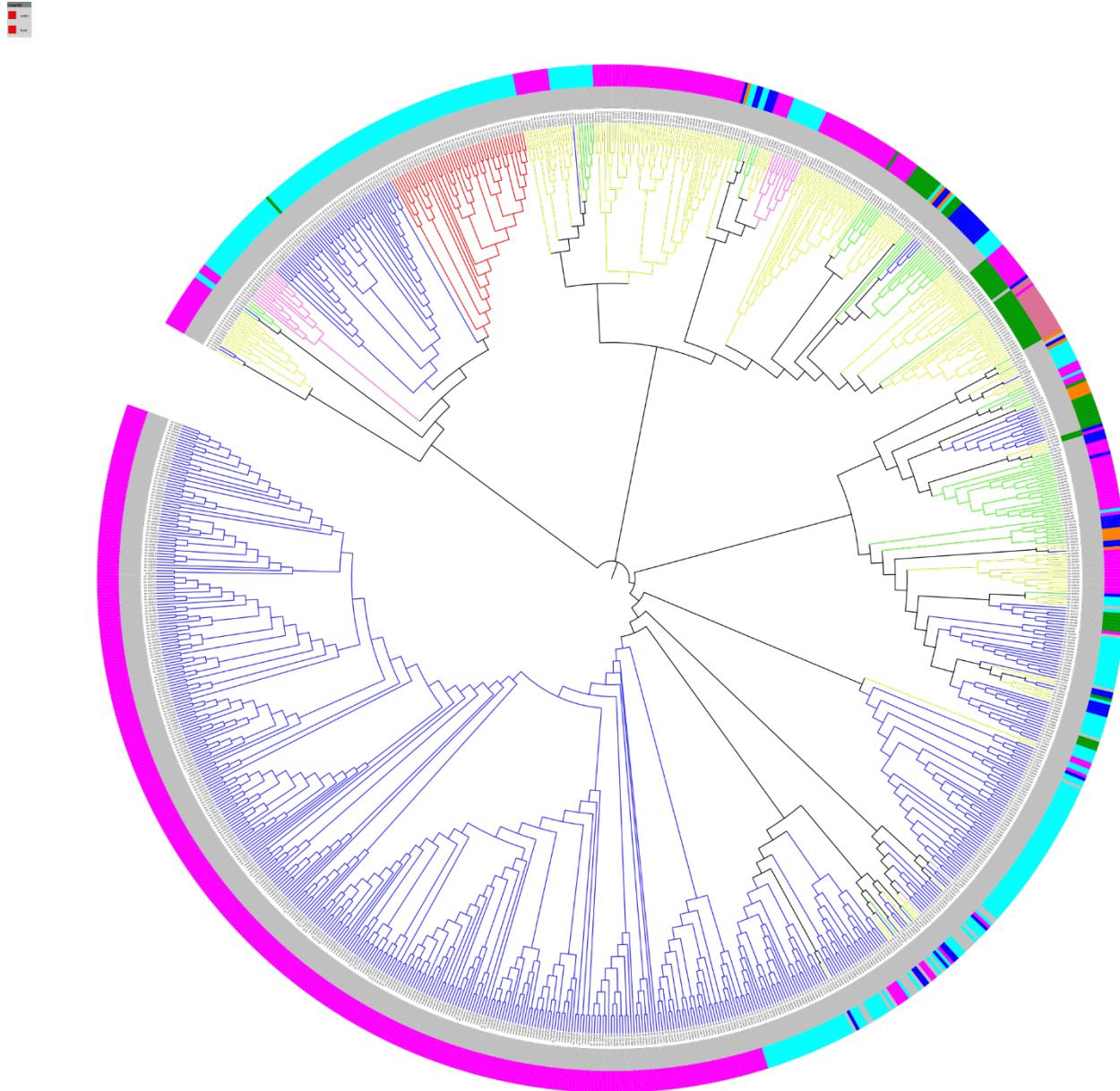


Figure S5 Dendrogram of 976 RefSeq viral genomes in subgroup Q1 (genome size < 25%), when k=9. The branches are colored by Baltimore Classifications. The circles, from inside to outside, are colored by different orders and hosts. [Color information: (A) Branch: Baltimore Classification; dsDNA, no RNA stage: red; dsRNA: green; Retro-transcribing viruses: pink; ssDNA: blue; ssRNA negative-strand: bright blue; ssRNA positive-strand: yellow. (B) From inside to outside, first circle: Order; Caudovirales: red; Herpesvirales: green; Ligamenvirales: blue; Mononegavirales: orange; Nidovirales: cyan; Picornavirales: pink; Tymovirales: dark green; unclassified: silver; (C) From inside to outside, second circle: Host; protest: orange; archaea: red; bacteria: dark green; fungi: blue; animal: cyan; animal and plants: pale violet red; plant: pink; environment or NA: silver.]

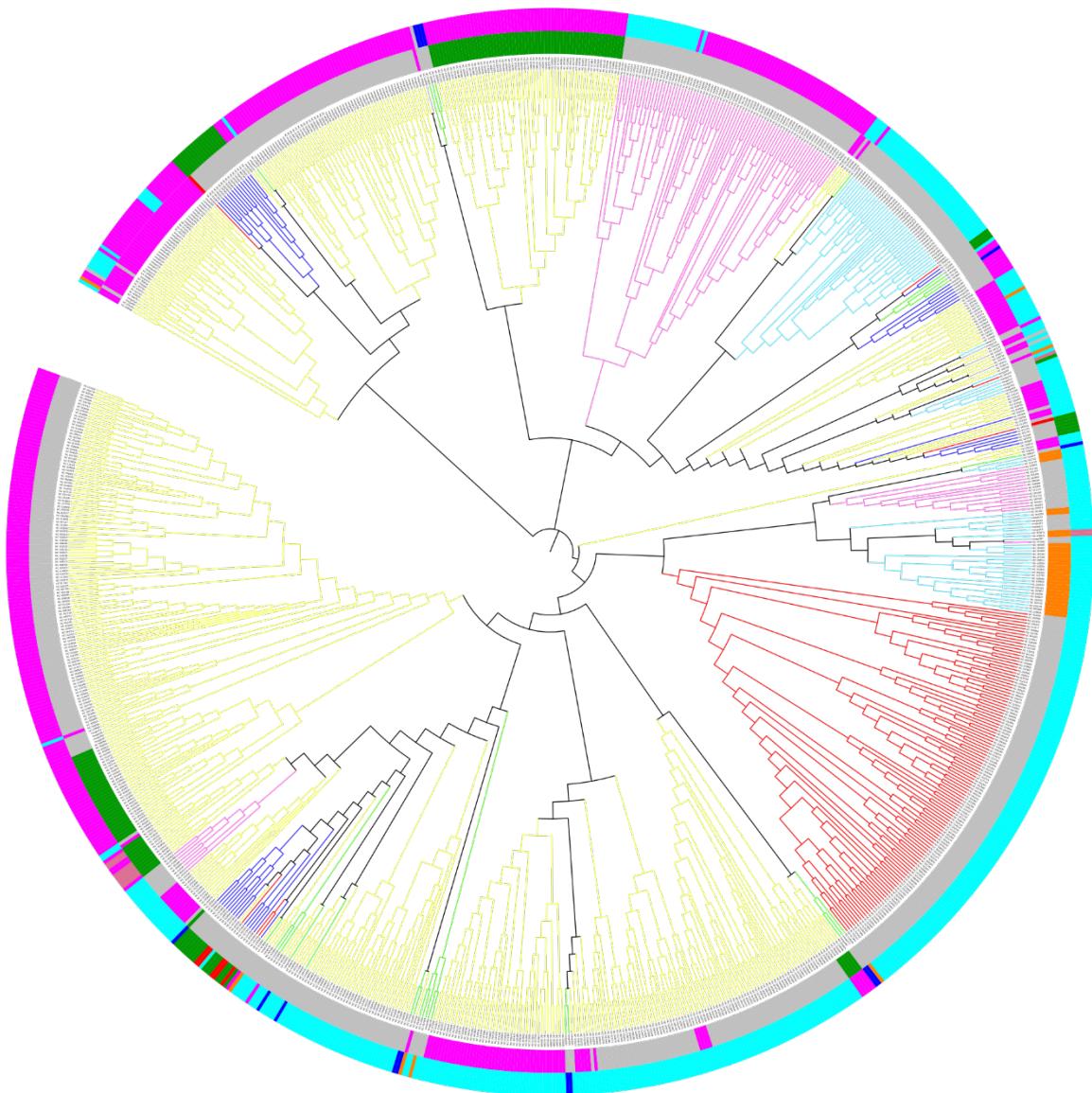


Figure S6 Dendrogram of 977 RefSeq viral genomes in subgroup Q2 (genome size: 25%-50%), when k=10. The branches are colored by Baltimore Classifications. The circles, from inside to outside, are colored by different orders and hosts. [Color information: (A) Branch: Baltimore Classification; dsDNA, no RNA stage: red; dsRNA: green; Retro-transcribing viruses: pink; ssDNA: blue; ssRNA negative-strand: bright blue; ssRNA positive-strand: yellow. (B) From inside to outside, first circle: Order; Caudovirales: red; Herpesvirales: green; Ligamenvirales: blue; Mononegavirales: orange; Nidovirales: cyan; Picornavirales: pink; Tymovirales: dark green; unclassified: silver; (C) From inside to outside, second circle: Host; prokaryote: orange; archaea: red; bacteria: dark green; fungi: blue; animal: cyan; animal and plants: pale violet red; plant: pink; environment or NA: silver.]

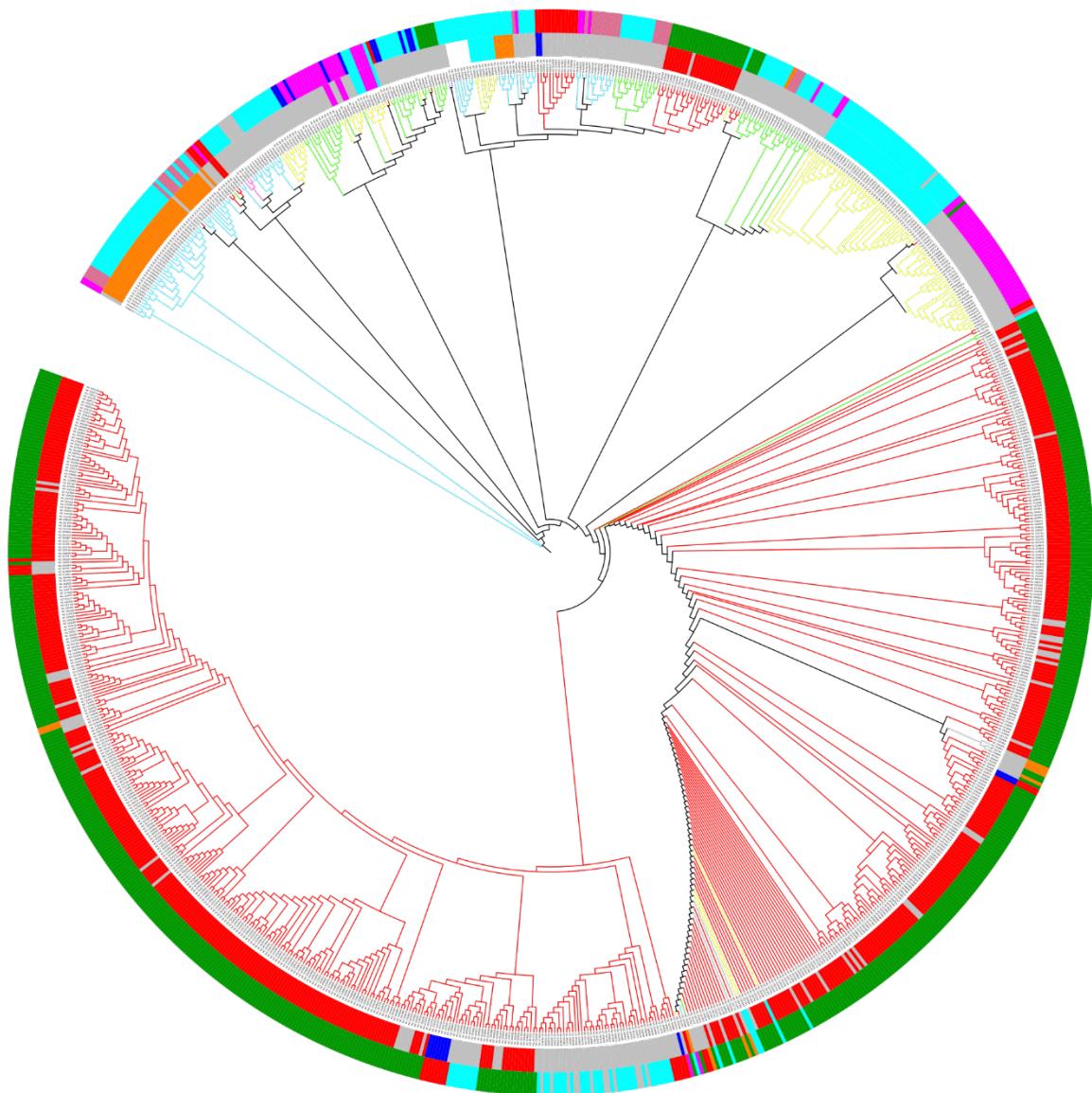


Figure S7 Dendrogram of 977 RefSeq viral genomes in subgroup Q3 (genome size: 50%-75%), when $k=11$. The branches are colored by Baltimore Classifications. The circles, from inside to outside, are colored by different orders and hosts. [Color information: (A) Branch: Baltimore Classification; dsDNA, no RNA stage: red; dsRNA: green; Retro-transcribing viruses: pink; ssDNA: blue; ssRNA negative-strand: bright blue; ssRNA positive-strand: yellow. (B) From inside to outside, first circle: Order; Caudovirales: red; Herpesvirales: green; Ligamenvirales: blue; Mononegavirales: orange; Nidovirales: cyan; Picornavirales: pink; Tymovirales: dark green; unclassified: silver; (C) From inside to outside, second circle: Host; protest: orange; archaea: red; bacteria: dark green; fungi: blue; animal: cyan; animal and plants: pale violet red; plant: pink; environment or NA: silver.]

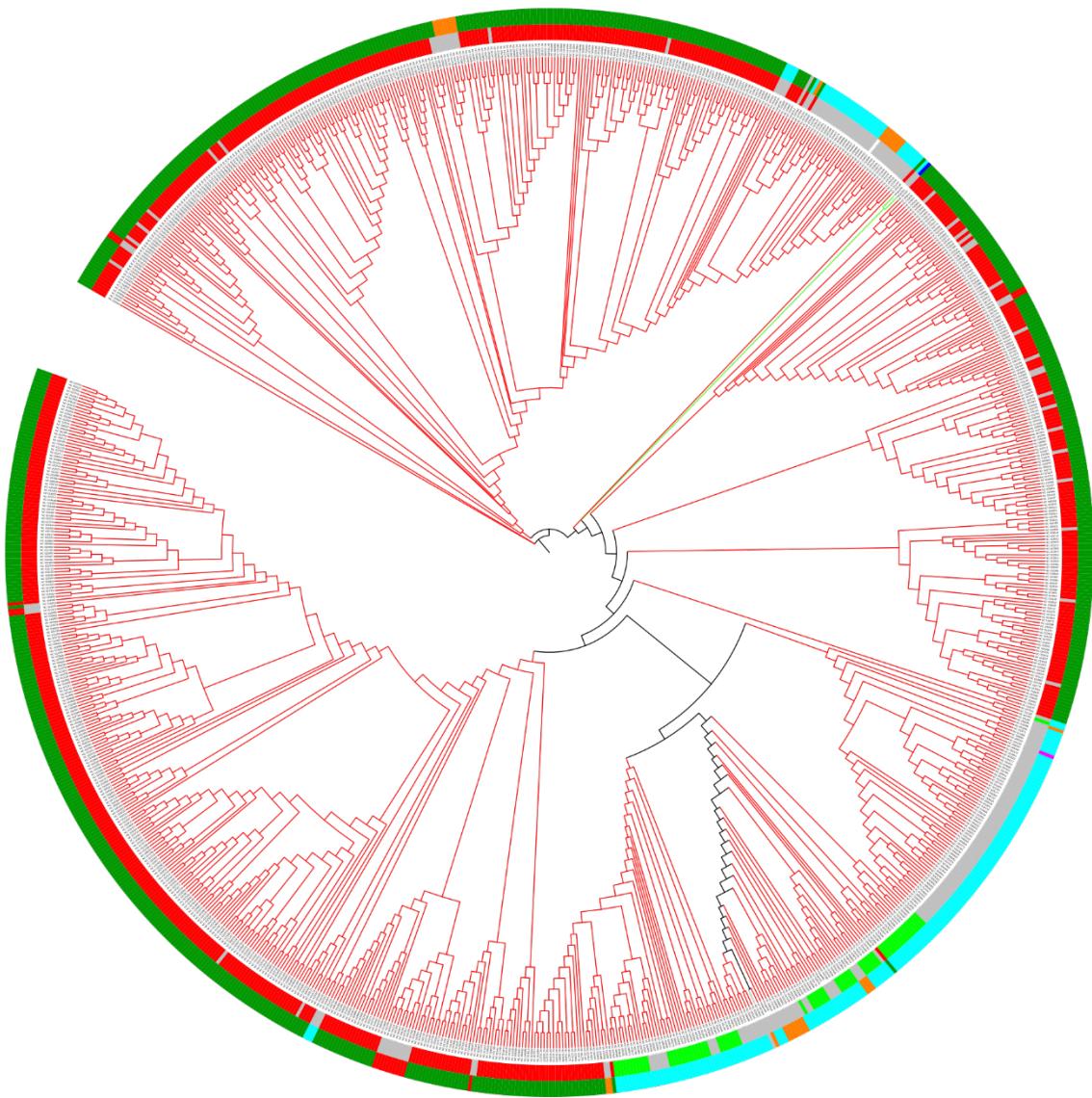


Figure S8 Dendrogram of 977 RefSeq viral genomes in subgroup Q4 (genome size: >75%), when k=12. The branches are colored by Baltimore Classifications. The circles, from inside to outside, are colored by different orders and hosts. [Color information: (A) Branch: Baltimore Classification; dsDNA, no RNA stage: red; dsRNA: green; Retro-transcribing viruses: pink; ssDNA: blue; ssRNA negative-strand: bright blue; ssRNA positive-strand: yellow. (B) From inside to outside, first circle: Order; Caudovirales: red; Herpesvirales: green; Ligamenvirales: blue; Mononegavirales: orange; Nidovirales: cyan; Picornavirales: pink; Tymovirales: dark green; unclassified: silver; (C) From inside to outside, second circle: Host; protest: orange; archaea: red; bacteria: dark green; fungi: blue; animal: cyan; animal and plants: pale violet red; plant: pink; environment or NA: silver.]

Table S2 Wilcoxon rank sum test result of the top 10 highest members of viral family

| | Siphoviridae | Geminiviridae | Myoviridae | Podoviridae | Papillomaviridae | Potyviridae | Parvoviridae | Picornaviridae | Flaviviridae | Betaflexiviridae |
|---------------------------------------|--------------|---------------|------------|-------------|------------------|-------------|--------------|----------------|--------------|------------------|
| Siphoviridae vs. Geminiviridae | < 2.2 E-16 | < 2.2 E-16 | | | | | | | | |
| Siphoviridae vs. Myoviridae | < 2.2 E-16 | | < 2.2 E-16 | | | | | | | |
| Siphoviridae vs. Podoviridae | < 2.2 E-16 | | | < 2.2 E-16 | | | | | | |
| Siphoviridae vs. Papillomaviridae | < 2.2 E-16 | | | | < 2.2 E-16 | | | | | |
| Siphoviridae vs. Potyviridae | < 2.2 E-16 | | | | | < 2.2 E-16 | | | | |
| Siphoviridae vs. Parvoviridae | < 2.2 E-16 | | | | | | < 2.2 E-16 | | | |
| Siphoviridae vs. Picornaviridae | < 2.2 E-16 | | | | | | | < 2.2 E-16 | | |
| Siphoviridae vs. Flaviviridae | < 2.2 E-16 | | | | | | | | < 2.2 E-16 | |
| Siphoviridae vs. Betaflexiviridae | < 2.2 E-16 | | | | | | | | | < 2.2 E-16 |
| Geminiviridae vs. Myoviridae | | < 2.2 E-16 | < 2.2 E-16 | | | | | | | |
| Geminiviridae vs. Podoviridae | | < 2.2 E-16 | | < 2.2 E-16 | | | | | | |
| Geminiviridae vs. Papillomaviridae | | < 2.2 E-16 | | | < 2.2 E-16 | | | | | |
| Geminiviridae vs. Potyviridae | | < 2.2 E-16 | | | | < 2.2 E-16 | | | | |
| Geminiviridae vs. Parvoviridae | | < 2.2 E-16 | | | | | < 2.2 E-16 | | | |
| Geminiviridae vs. Picornaviridae | | < 2.2 E-16 | | | | | | < 2.2 E-16 | | |
| Geminiviridae vs. Flaviviridae | | < 2.2 E-16 | | | | | | | < 2.2 E-16 | |
| Geminiviridae vs. Betaflexiviridae | | < 2.2 E-16 | | | | | | | | < 2.2 E-16 |
| Myoviridae vs. Podoviridae | | | < 2.2 E-16 | < 2.2 E-16 | | | | | | |
| Myoviridae vs. Papillomaviridae | | | < 2.2 E-16 | | < 2.2 E-16 | | | | | |
| Myoviridae vs. Potyviridae | | | < 2.2 E-16 | | | < 2.2 E-16 | | | | |
| Myoviridae vs. Parvoviridae | | | < 2.2 E-16 | | | | < 2.2 E-16 | | | |
| Myoviridae vs. Picornaviridae | | | < 2.2 E-16 | | | | | < 2.2 E-16 | | |
| Myoviridae vs. Flaviviridae | | | < 2.2 E-16 | | | | | | < 2.2 E-16 | |
| Myoviridae vs. Betaflexiviridae | | | < 2.2 E-16 | | | | | | | < 2.2 E-16 |
| Podoviridae vs. Papillomaviridae | | | | < 2.2 E-16 | < 2.2 E-16 | | | | | |
| Podoviridae vs. Potyviridae | | | | < 2.2 E-16 | | < 2.2 E-16 | | | | |
| Podoviridae vs. Parvoviridae | | | | < 2.2 E-16 | | | < 2.2 E-16 | | | |
| Podoviridae vs. Picornaviridae | | | | < 2.2 E-16 | | | | < 2.2 E-16 | | |
| Podoviridae vs. Flaviviridae | | | | < 2.2 E-16 | | | | | < 2.2 E-16 | |
| Podoviridae vs. Betaflexiviridae | | | | < 2.2 E-16 | | | | | | < 2.2 E-16 |
| Papillomaviridae vs. Potyviridae | | | | | < 2.2 E-16 | < 2.2 E-16 | | | | |
| Papillomaviridae vs. Parvoviridae | | | | | < 2.2 E-16 | | < 2.2 E-16 | | | |
| Papillomaviridae vs. Picornaviridae | | | | | < 2.2 E-16 | | | < 2.2 E-16 | | |
| Papillomaviridae vs. Flaviviridae | | | | | < 2.2 E-16 | | | | < 2.2 E-16 | |
| Papillomaviridae vs. Betaflexiviridae | | | | | < 2.2 E-16 | | | | | < 2.2 E-16 |
| Potyviridae vs. Parvoviridae | | | | | | < 2.2 E-16 | < 2.2 E-16 | | | |
| Potyviridae vs. Picornaviridae | | | | | | < 2.2 E-16 | | 0.249381472 | | |
| Potyviridae vs. Flaviviridae | | | | | | < 2.2 E-16 | | | < 2.2 E-16 | |
| Potyviridae vs. Betaflexiviridae | | | | | | < 2.2 E-16 | | | | < 2.2 E-16 |
| Parvoviridae vs. Picornaviridae | | | | | | | 0.40400024 | < 2.2 E-16 | | |
| Parvoviridae vs. Flaviviridae | | | | | | | < 2.2 E-16 | | < 2.2 E-16 | |
| Parvoviridae vs. Betaflexiviridae | | | | | | | 9.69E-14 | | | < 2.2 E-16 |
| Picornaviridae vs. Flaviviridae | | | | | | | | 0.017555005 | < 2.2 E-16 | |
| Picornaviridae vs. Betaflexiviridae | | | | | | | | < 2.2 E-16 | | < 2.2 E-16 |
| Flaviviridae vs. Betaflexiviridae | | | | | | | | | < 2.2 E-16 | < 2.2 E-16 |

