

~~Complete RNA Virus Genomes Assembled from Murine Cecal Meta~~

Running title: RNA Viral Genomes from Murine Metatranscriptomes

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Abstract

Efforts to catalog viral diversity in the gut microbiome have largely ~~ignored RNA viruses~~ focused on DNA viruses, while RNA viruses remain understudied. To address this, we screened assemblies of previously published mouse gut metatranscriptomes for the presence of RNA viruses. We identified the ~~complete coding-complete~~ genomes of an Astrovirus and 5 Mitovirus-like viruses.

The viral fraction of the mammalian gut microbiome forms a crucial component in the relationship between microbe and host. Bacterial viruses serve as an important source of genetic diversity and population control for the microbiota, driving its ecology and evolution (1). Mammalian viruses disrupt the gut environment through infection and the response of the host immune system (2). Bacterial and mammalian viruses make significant contributions to host health and disease. Current efforts to describe the diversity of viruses present in the gut have focused on using shotgun metagenomics to identify double-stranded DNA viruses, predominantly bacteriophage and host pathogens (3). However, this method ignores viruses with RNA genomes, which make up a considerable portion of the environmental viromes (4).

We re-analyzed deeply-sequenced metatranscriptome data produced by our lab for the study of microbiome dynamics in a mouse model of *Clostridioides difficile* infection (5, 6). Briefly, C57Bl/6 mice from a breeding colony we maintain at the University of Michigan were treated with one of three different antibiotics (clindamycin, streptomycin, or cefoperazone). After a 24 hour recovery period, the mice were infected with *C. difficile* strain 630. Germ free C57Bl/6 mice were also mono-associated with *C. difficile* strain 630. Cecal contents were removed from each animal 18 hours post infection and frozen for RNA extraction and sequencing. RNA sequences from each sample were trimmed of adapter sequences and low-quality bases using Trimmomatic v0.39 and assembled individually using rnaSPAdes v3.13.1 (7) and concatenated for dereplication, resulting in 70,779 contigs longer than 1 kb. Contigs were ~~then screened against a custom RefSeq database of viral RNA-dependent RNA polymerase (RdRP) protein sequences screened for the presence of RdRP coding sequences using Blast v2.9.0 against a database containing all Viral RefSeq protein sequences annotated as RdRP (screening database available online as described below)~~ with a maximum e-value of 10^{-20} , resulting in 29 contigs. RdRP is conserved amongst almost all RNA viruses without a DNA stage in genome replication. These contigs were then annotated with Interproscan v5.39-77.0 (8, 9). We constructed phylogenetic trees from RdRP protein sequences using IQ-TREE v1.6.12 (10).

Two classes of RNA viruses were assembled with high coverage with sequences originating from most of the mouse treatment groups, including germ-free mice. First, a 6,811 base-long astrovirus genome (GC 56.6%) was obtained with 1,683.5-fold coverage (Figure 1A). The genome contained 3

35 predicted open reading frames encoding a capsid, RdRP, and a trypsin-like peptidase, and appears
36 to be closely related to Murine Astrovirus in *Astroviridae*. Second, 5 distinct, but closely related
37 RNA virus genomes, designated Putative Mitovirus JS1 through JS5, ranging in length from 2,309
38 to 2,447 bases with 4.6 to 16,078.8-fold coverage and average GC content of 46.2% belonged to
39 a previously undescribed clade of ~~Narnaviridae~~ *Narnaviridae* adjacent to the Mitoviruses (Figure
40 1B). These RNA virus genomes will facilitate future studies of RNA virus biology in the murine
41 microbiome.

42 **Data Availability.** The RNA-seq data are available the NCBI Sequence Read Archive
43 (SRA) database under the accession numbers PRJNA354635 (*C. difficile* infected mice)
44 and PRJNA415307 (mock-infected mice). The assembled genomes are available at the
45 National Center for Biotechnology Information (NCBI) GenBank under the accession numbers
46 MN780842-MN780847. All of the scripts and software used to perform this analysis are available at
47 https://github.com/SchlossLab/Stough_Mouse_RNA_Virome_MRA_2019.

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81 **Figure 1. Phylogenetic trees showing the relatives of the metatranscriptome assembled**
82 **genomes.** Maximum Likelihood phylogenetic trees constructed from RdRP amino acid sequences
83 for (A) Astroviruses and (B) Narnaviruses. Node annotations represent IQTree Ultra-fast Bootstrap
84 statistics, values less than 50% were excluded from the tree. Scale bars are marked in red to the left
85 of each tree. Highlight colors in (B) represent major Narnavirus taxa: Orange - Ourmiaviruses, Pink
86 - Ourmia-like Mycoviruses, Gray - Narnaviruses, Blue - Mitoviruses, Purple - Murine Mitovirus-like
87 viruses, Green - Leviviruses.