



Characterizing the Respiratory Microbiome of Commercial Broilers on the Delmarva Peninsula

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The Avian Respiratory Microbiome

- Microbiomes are complex environments containing bacteria, viruses, fungi/yeast, archaea and protozoa
- Disturbances of this environment by colonization of a new bacteria, virus, or fungi can result in disease
- The avian respiratory disease complex (RDC) is a complex multi-agent syndrome
- Understanding the etiology of RDC requires quantification of the microbial composition of the avian respiratory tract in both healthy and sick flocks

Challenges with Determining the Avian Respiratory Virome

- Sampling the respiratory tract
- Managing dilute samples
- Developing avian-specific bioinformatics tools
 - viral genome databases
 - method for detecting and quantifying viruses
- Aggregating and presenting bacteria, bacteriophage, fungi/yeast and virus data

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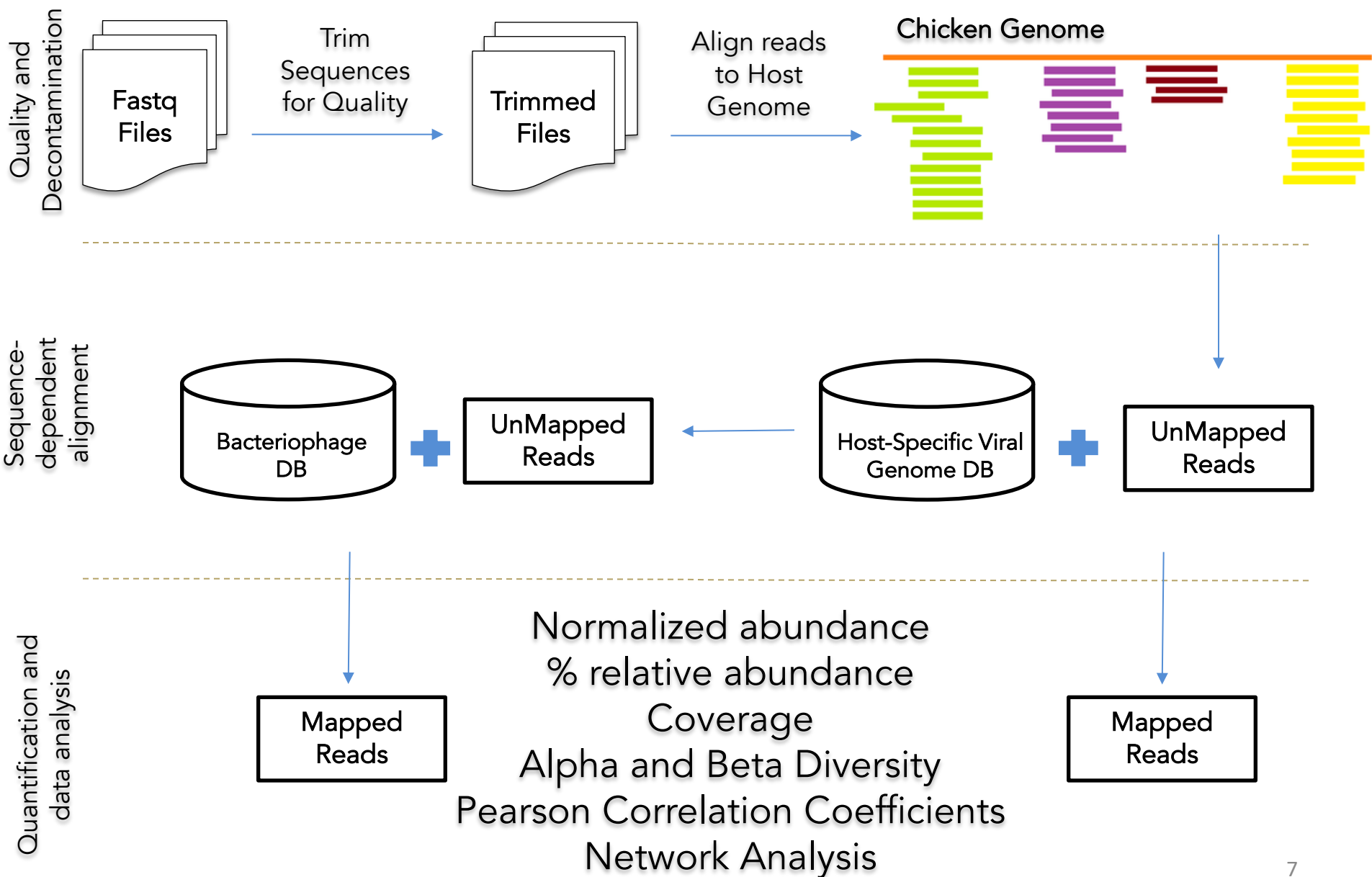
Objectives

1. Develop an automated workflow for identifying viral community abundance within the avian respiratory microbiome with the use of metagenomics NGS data and avian-specific viral genome databases.
2. Use these tools to elucidate microbial changes occurring during the grow out of a healthy broiler flock.

Experimental Design

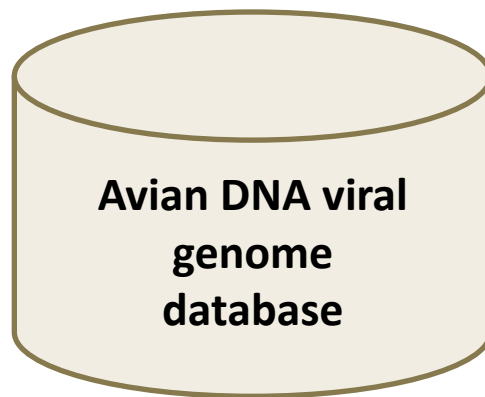
- A healthy, untreated flock of chickens were sampled at placement and again at weeks 1, 2, 3, 4, 5, 6 and 7.
- Tracheal swabs were collected, DNA and RNA was isolated and libraries were created
- DNaseq (DNA viruses, bacteriophage and Yeast/Fungi), RNAseq (RNA viruses), and 16S rRNA (Bacteria) data were collected at each time point.
- DNA and RNAseq was conducted using Illumina HiSeq NGS technology and processed using following viral workflow for virus detection and quantification



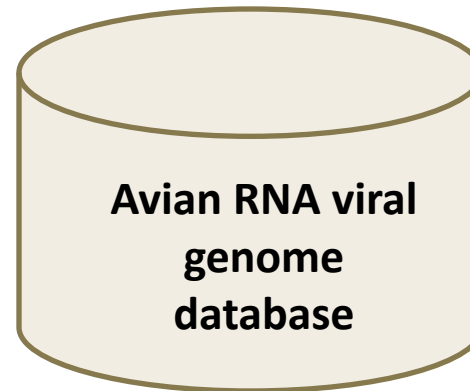


Database Construction

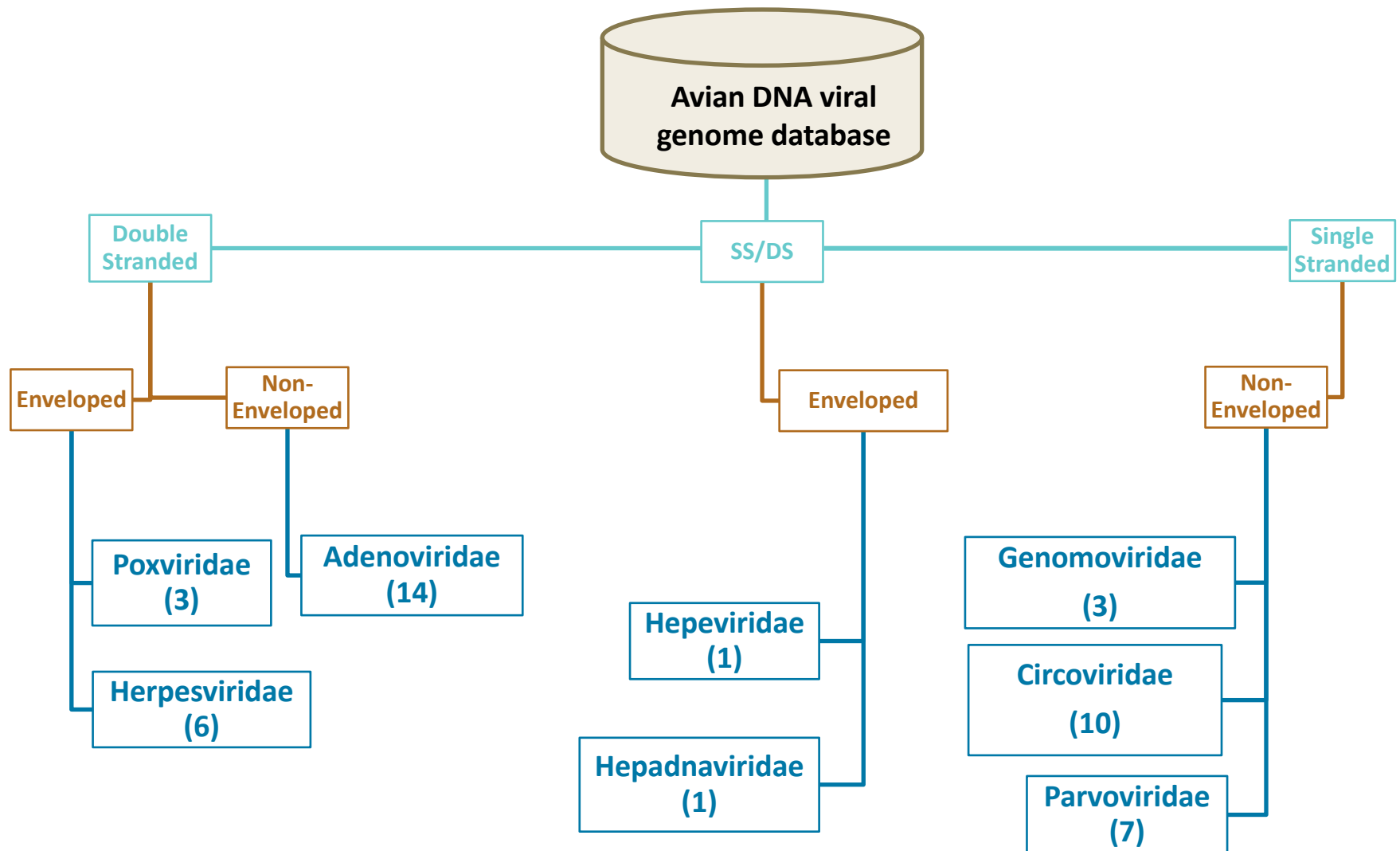
- **A robust, curated avian viral genome databases is critical to virome studies**
- To resolve this issue, databases were constructed using viral reference genomes from NCBI and contain 1-4 families for every virus class

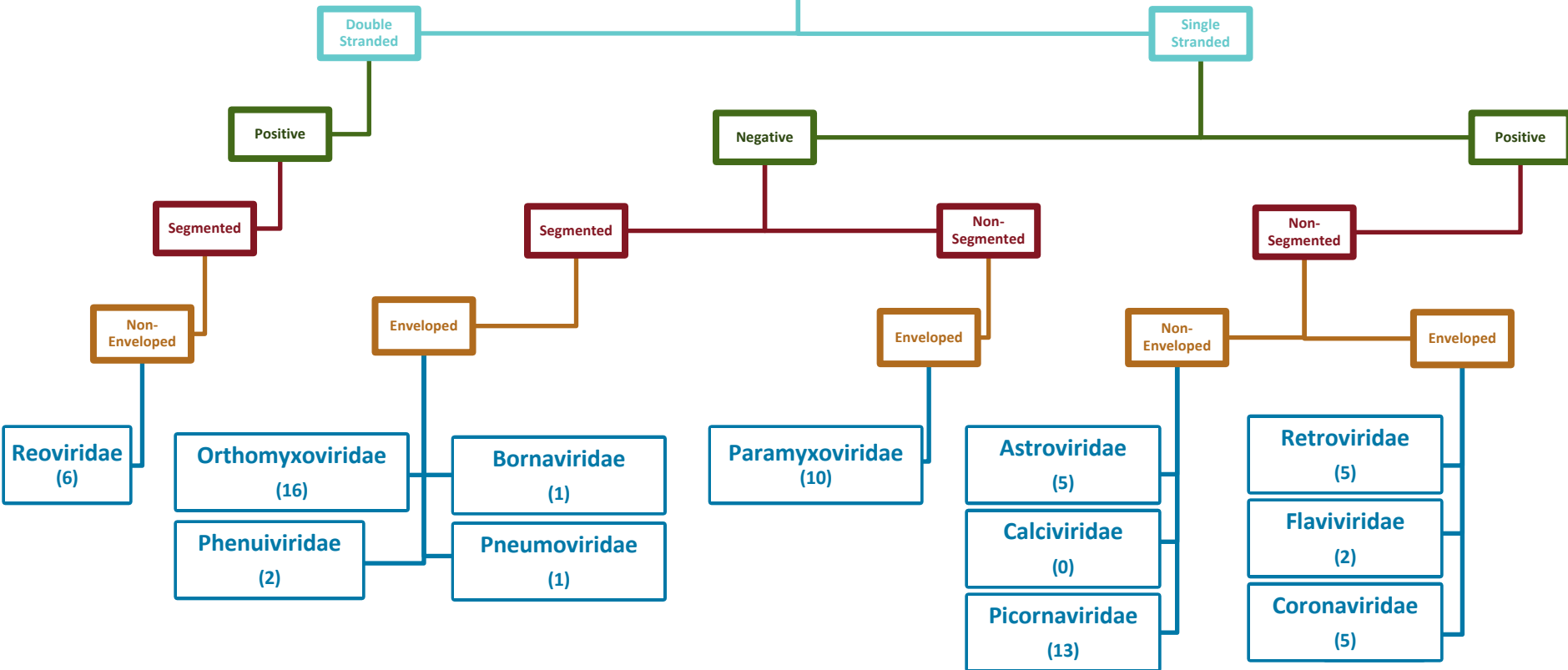
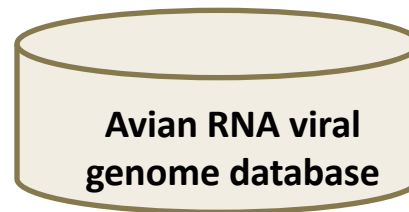


~35 viruses
8 families



~65 viruses
12 families





Sample Statistics

Sample	Total Reads	After Trimming	% Mapped Chicken	% UnMapped Chicken	Mapped to Viral Reads
Week 0 DNA	46,586,608	46,568,465	86.43	13.61	1
Week 0 RNA	62,375,955	62,351,619	49.21	50.79	416
Week1 DNA	43,940,202	43,911,476	81.11	18.95	0
Week1 RNA	54,496,930	54,477,530	44.30	55.70	16,569
Week 2 DNA	33,471,831	33,442,860	89.80	10.28	1
Week 2 RNA	51,426,090	51,415,545	65.54	34.46	10,016
Week 3 DNA	45,131,953	45,108,639	84.81	15.24	2
Week 3 RNA	57,566,851	57,551,710	28.76	71.24	977
Week 4 DNA	44,621,969	44,600,956	90.00	10.05	387
Week 4 RNA	56,334,014	56,321,058	61.62	38.38	3,660
Week 5 DNA	38,630,592	38,590,484	88.99	11.11	7
Week 5 RNA	50,937,821	50,926,463	57.56	42.44	4,579
Week 6 DNA	45,915,721	45,900,494	89.73	10.30	4,634
Week 6 RNA	53,248,431	53,236,536	59.08	40.92	29,476
Week 7 DNA	41,215,207	41,196,338	90.18	9.87	131
Week 7 RNA	54,173,052	54,162,138	61.44	38.56	6,243

Total Raw Reads: ~780 Million

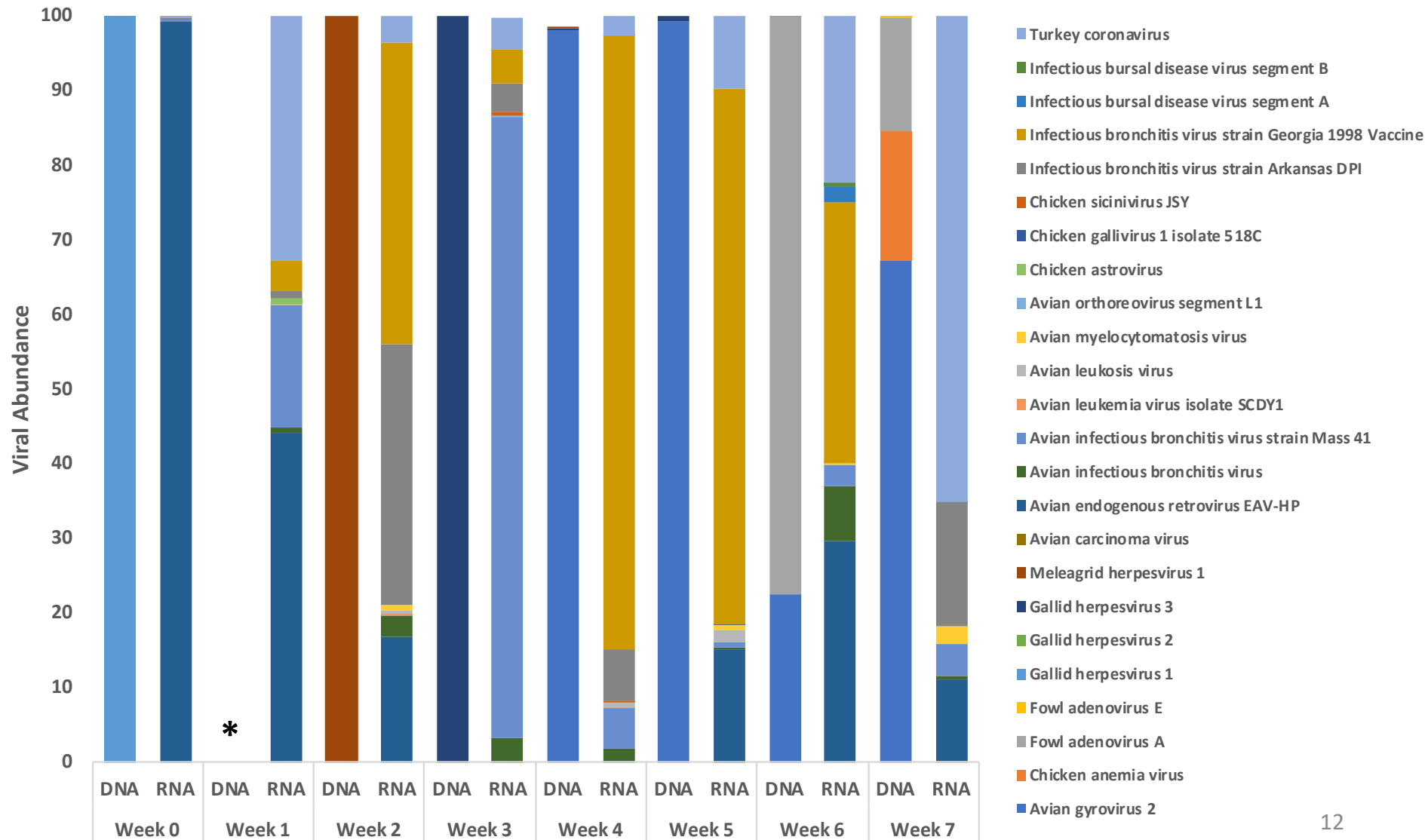
Average % DNA UnMapped:

12.42%

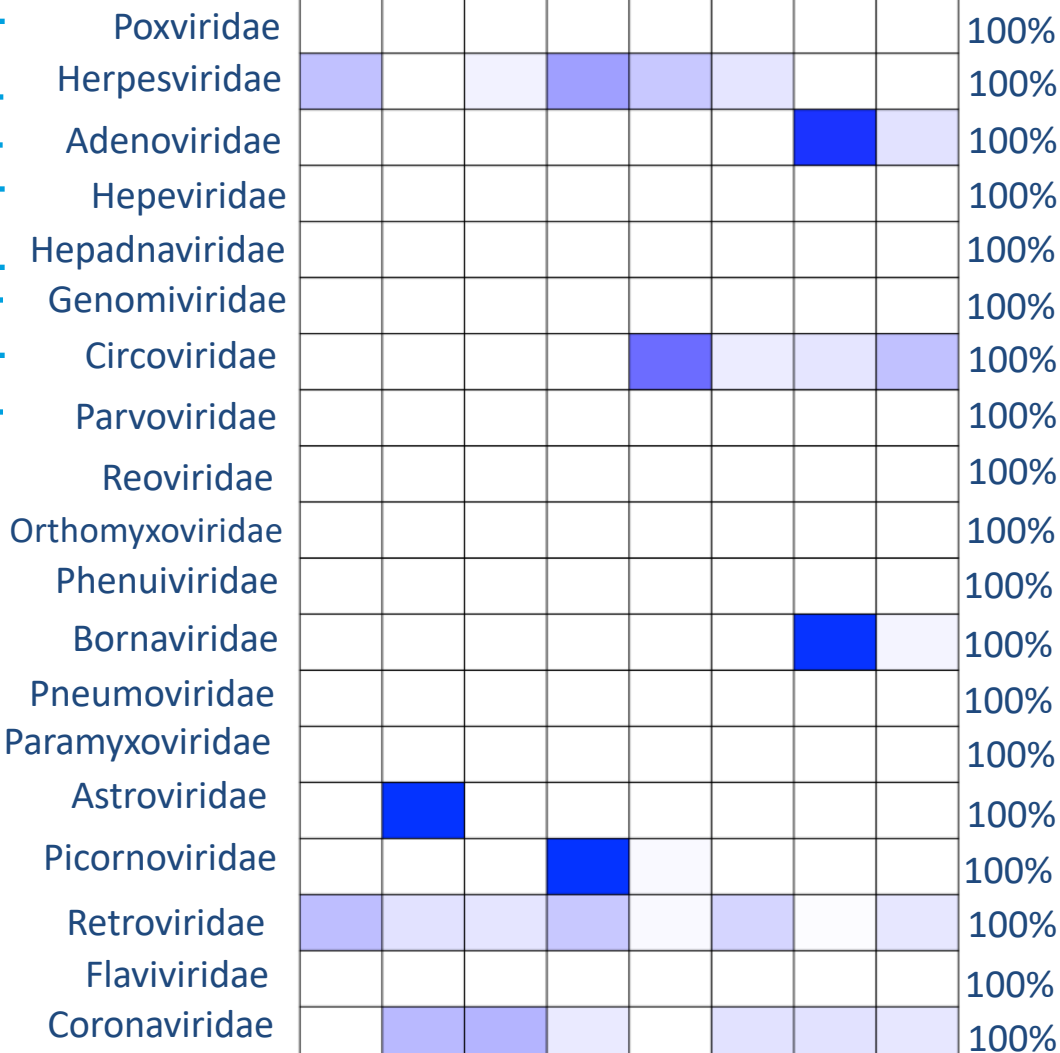
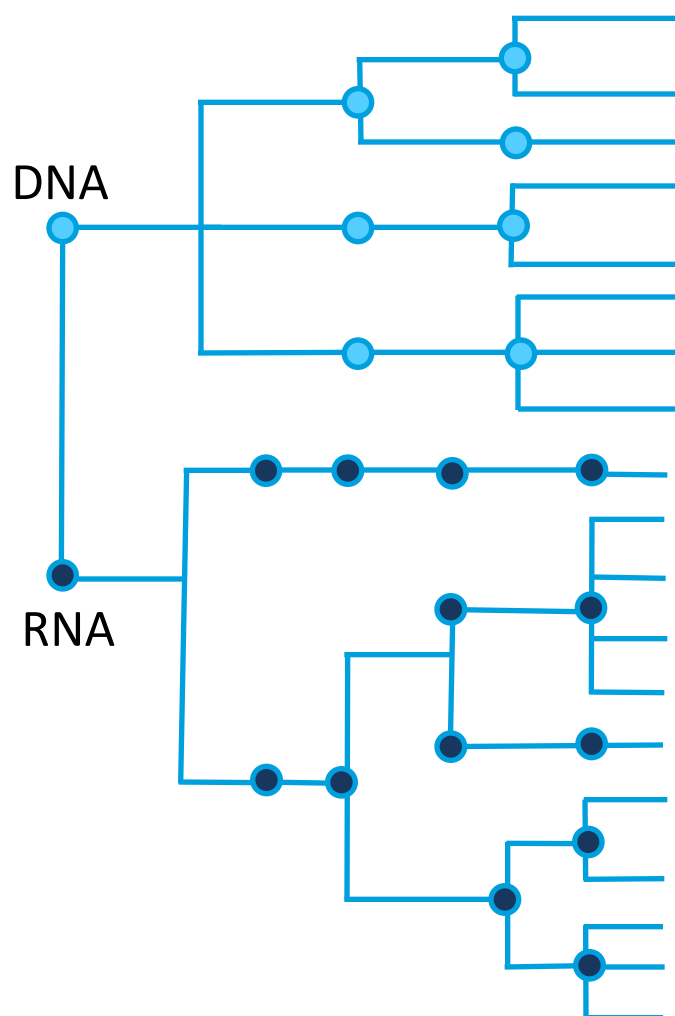
Average % RNA UnMapped:

46.56%

DNA/RNA Viruses Detected During Grow Out

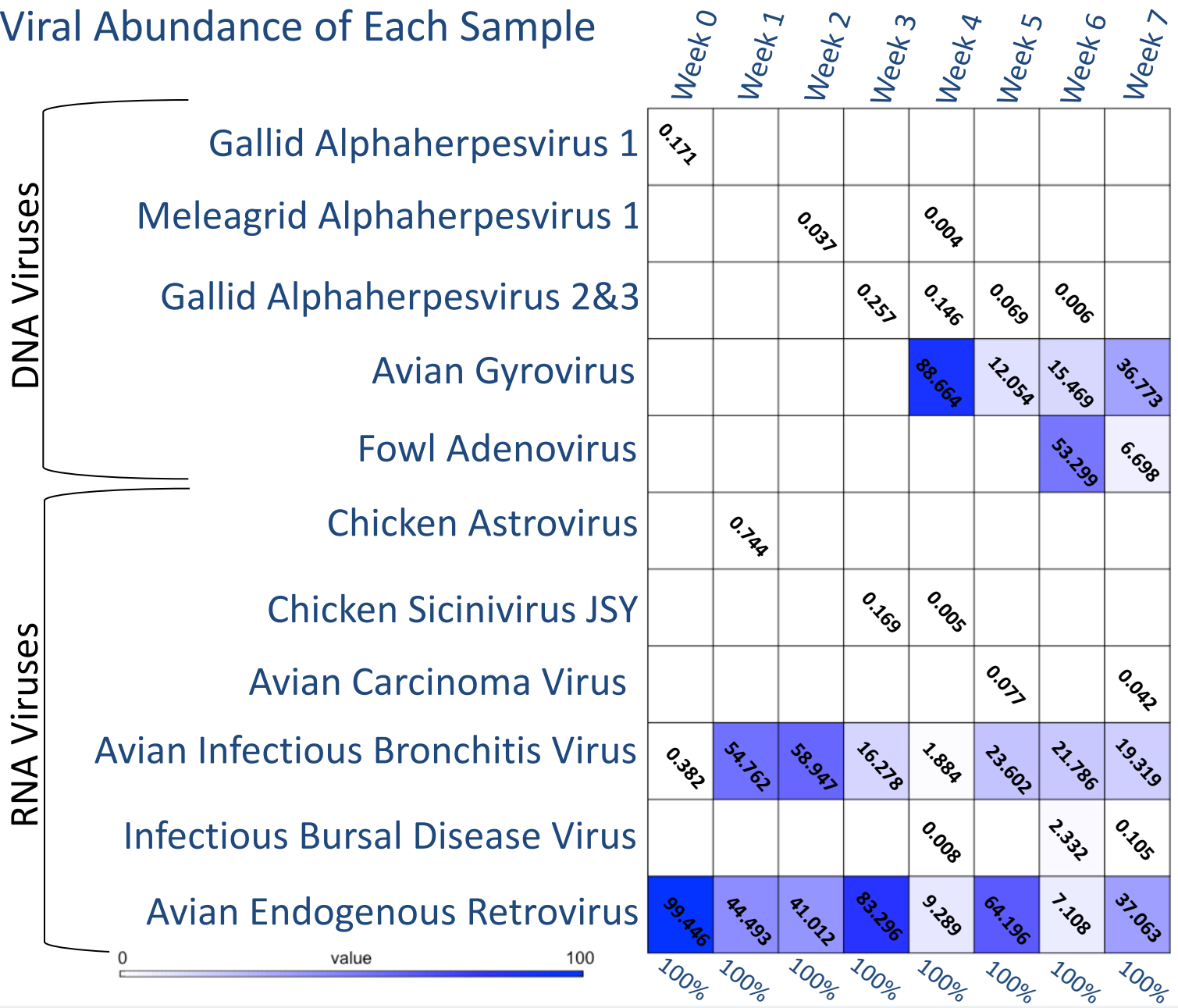


Viral Families

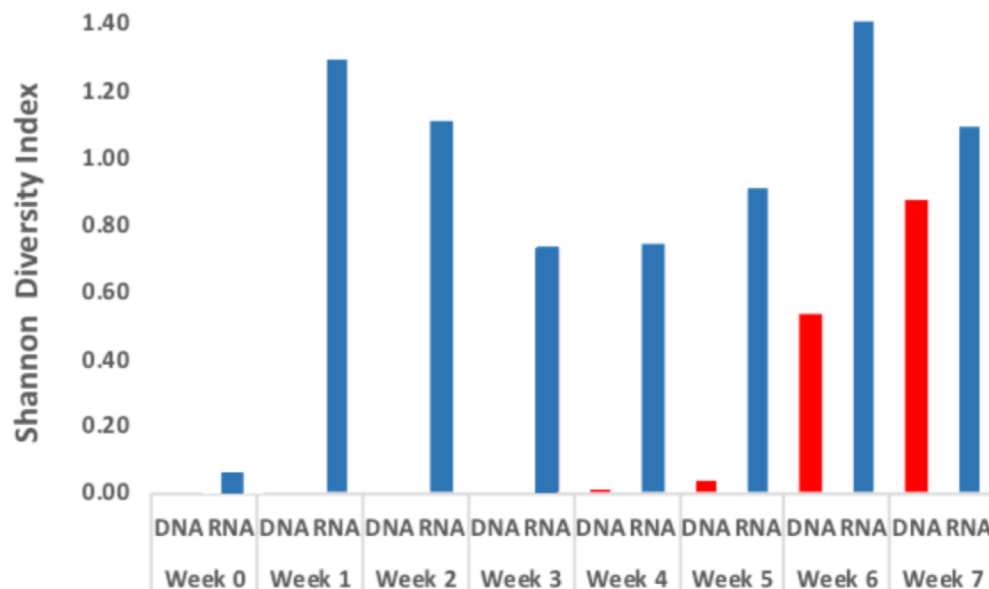


0 value 100

Quantifying Viral Abundance of Each Sample



Viral Alpha Diversity

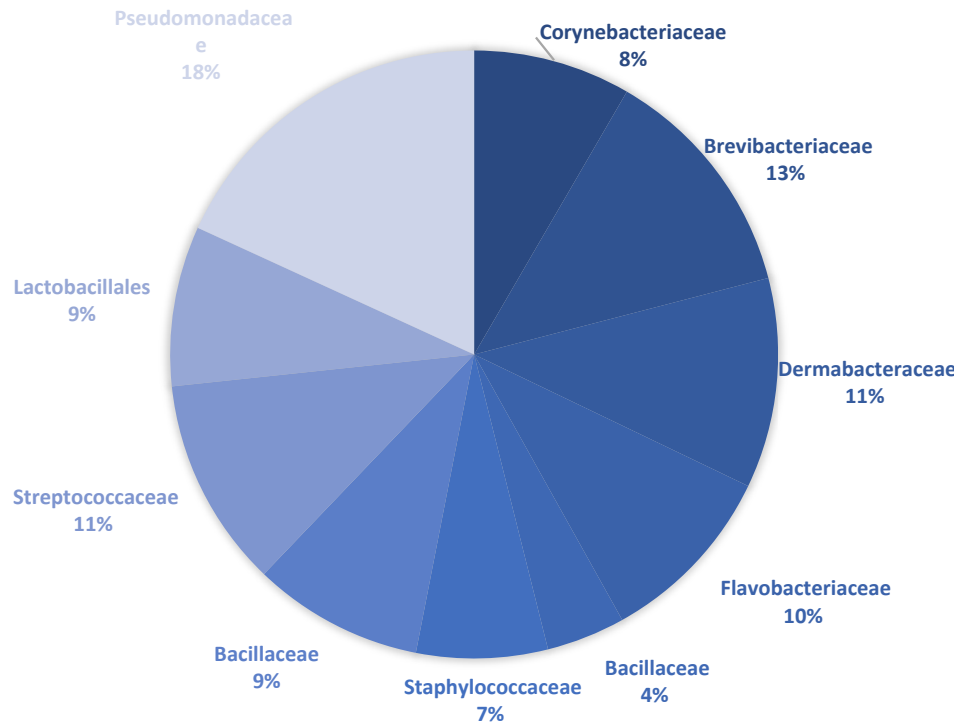


Alpha diversity was measured for viruses utilizing the Shannon Diversity Index:

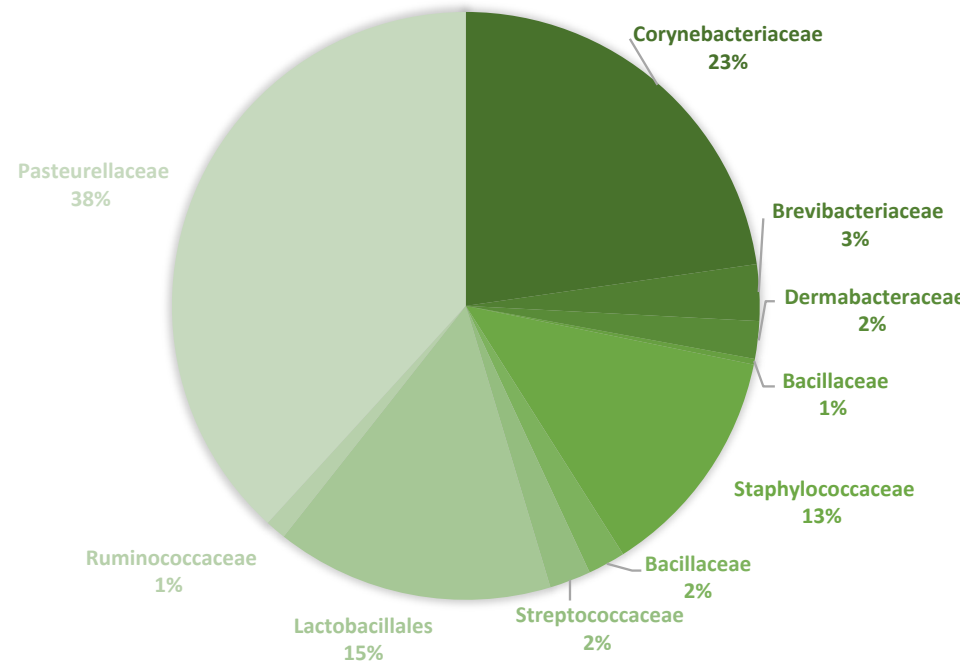
$$H' = - \sum_{i=1}^S p_i \ln p_i$$

Bacteria - 16s rRNA

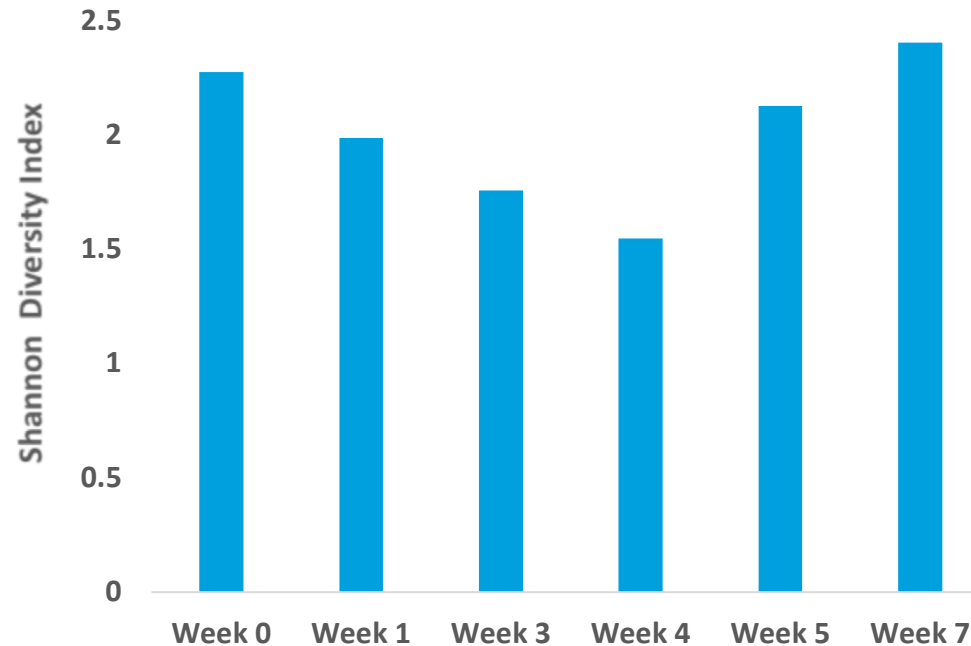
Week 1



Week 7



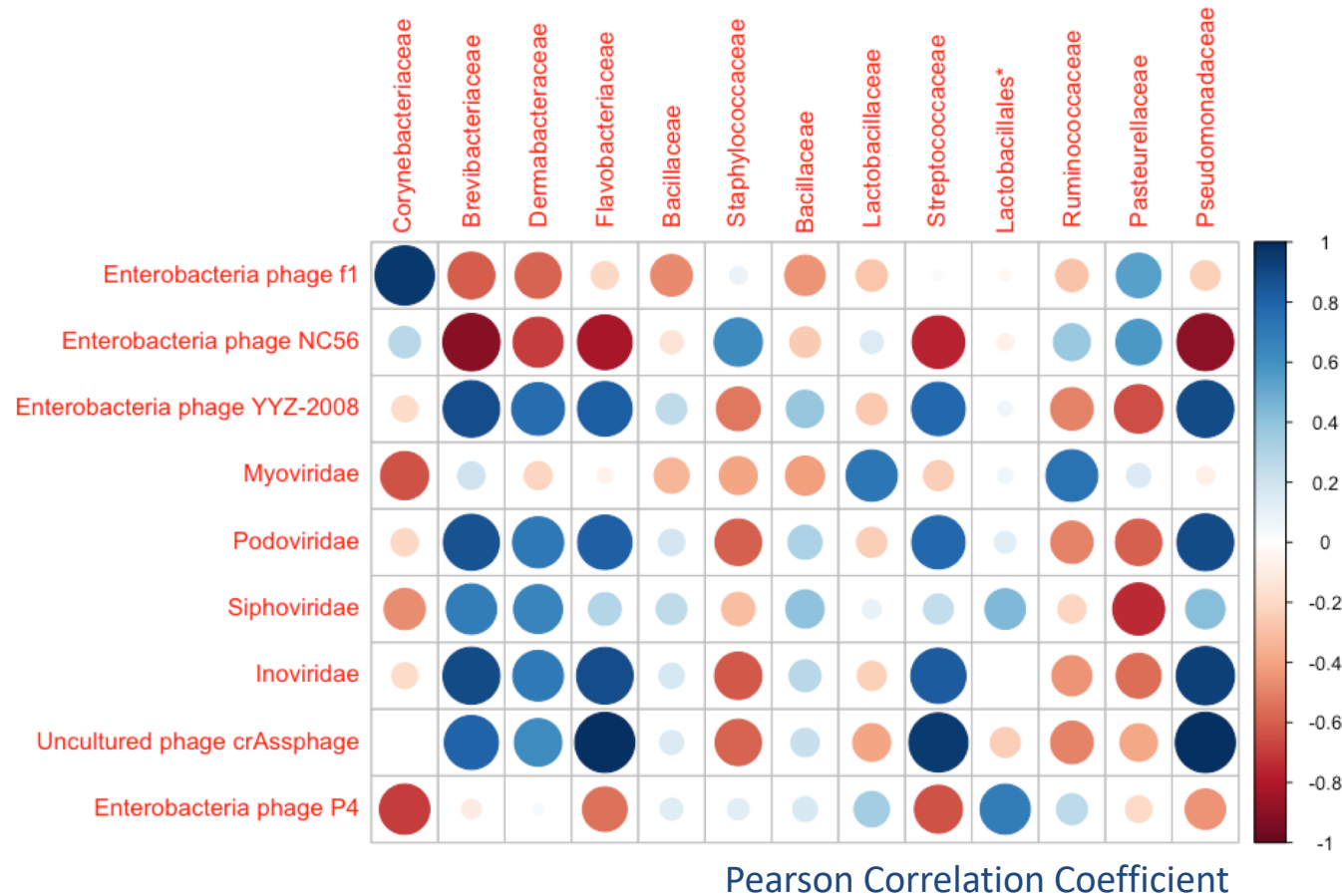
Bacterial Alpha Diversity

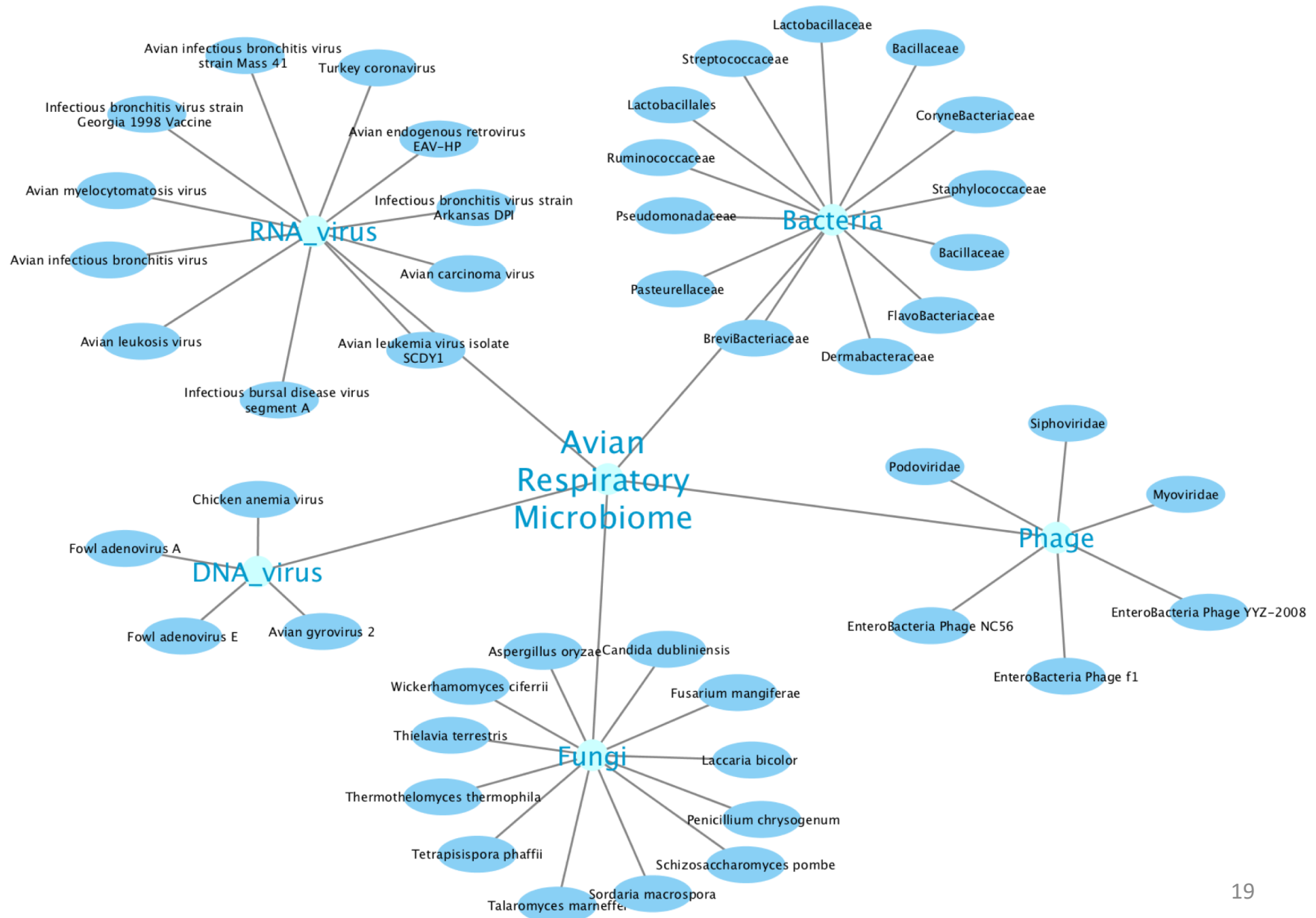


Alpha diversity was measured for bacteria utilizing the
Shannon Diversity Index:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

Bacteria – Bacteriophage Correlations





Conclusions/Future Directions

- Developed avian-specific bioinformatics tools and applied them to detect the microbial shift in the respiratory tract of a healthy broiler flock during grow out
- Identify microbial shift between a healthy flock and a flock exhibiting respiratory disease complex. Conduct a comparison of identified viruses, bacteria, fungi and phage.
- Compare respiratory microbiota detected in a healthy flock to gastrointestinal microbiota
- Implement the present workflow and databases into a user-friendly interface to facilitate future investigators interested in rapidly characterizing and quantifying the viral component of a microbiome

Thank You



Quantifying Abundance of Each Virus

