

Understanding the Respiratory Microbiome of Commercial Poultry (Broilers)

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The Avian Respiratory Microbiome

- The avian respiratory disease complex (RDC) is a complex multi-agent syndrome involving a combination of bacterial and viral agents
- The classic experimental approach to RDC has been to study respiratory disease agents individually
- The development of new experimental paradigms requires an understanding of the actual microbial composition of the avian respiratory tract in both healthy and sick flocks

Issues in Determining the Virome

- Methods exist for examining the avian intestinal virome but the avian respiratory virome presents unique challenges
- Smaller sample volumes (swab material)
- With more dilute concentrations of viruses
- Resulting in the need to develop methods for virus enrichment, nucleic acid extraction, and sequencing library construction

First Generation Protocol

- Tracheal swabs obtained from clinical samples submitted to the University of Delaware Poultry Diagnostic Laboratory (Dr. Bautista)
 - Samples confirmed as positive for ILTV and/or IBV or of unknown disease etiology
- Centrifuge at 7,000 x g for 20 min and extract nucleic acids from pellet with Qiagen Blood & Tissue Kit
- 16S sequencing analysis performed at the University of Minnesota
- DNaseq library construction and Illumina sequencing performed at the University of Delaware

Clinical Samples

Accession Number	Date Submitted	Company	Flock Age	ILTV Vaccination Status	Diagnosis
15-12667	11/23/2015	B	7W-4D	No	ILT
15-12033	12/01/2015	C	5W-6D	Yes	Respiratory Disease
16-0982	1/25/2016	A	7W-0D	No	ILT

16S β -diversity

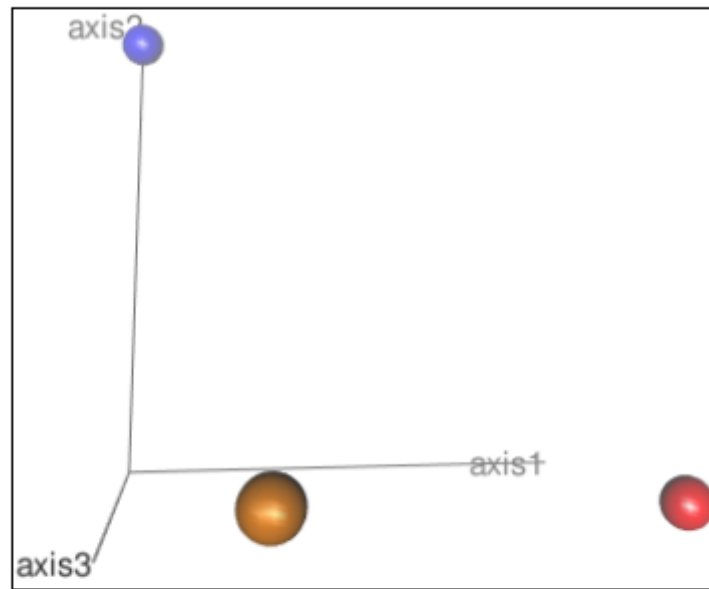


Figure 3.7: Differences in community structure between samples. Blue: 15-12033;
Red: 15-12667L; Orange: 16-0982L

- The two samples from birds known to be infected with ILTV were more similar in community structure than the third sample

16S Analysis

OTU	Classification	15-12033	15-12667	16-0982
1	<i>Gallibacterium</i>	8%	57%	44%
2	<i>Ornithobacterium</i>	33%	22%	7%
3	<i>Avibacterium</i>	35%	0.1%	0.1%
4	<i>Escherichia-Shigella</i>	5%	20%	0.4%
5	<i>Avibacterium</i>	19%	0.03%	0%
6	Lactobacillales	0.1%	0.3%	14%

- Six most abundant OTUs and their abundance in each 16S rDNA sample
- The predominant bacterial species in the two ILTV-positive samples was *Gallibacterium*
- The dominant bacterial species in the undiagnosed sample was *Avibacterium* (infectious coryza?)

DNaseq Analysis

Sample	Total Sequences	Total Nucleotides	Chicken Sequences		Non-Chicken Sequences	
15-12033	7.98×10^7	4.07×10^9	7.17×10^7	90%	8.18×10^6	10%
15-1266	7.63×10^7	3.87×10^9	6.93×10^7	91%	7.04×10^6	9%
16-0982	9.23×10^7	4.71×10^9	8.41×10^7	91%	8.24×10^6	9%

- Approximately 10% of the generated sequences ($\sim 4 \times 10^7$ nucleotides per sample) were not from the chicken genome

Analysis of Avian Viral DNA Sequences

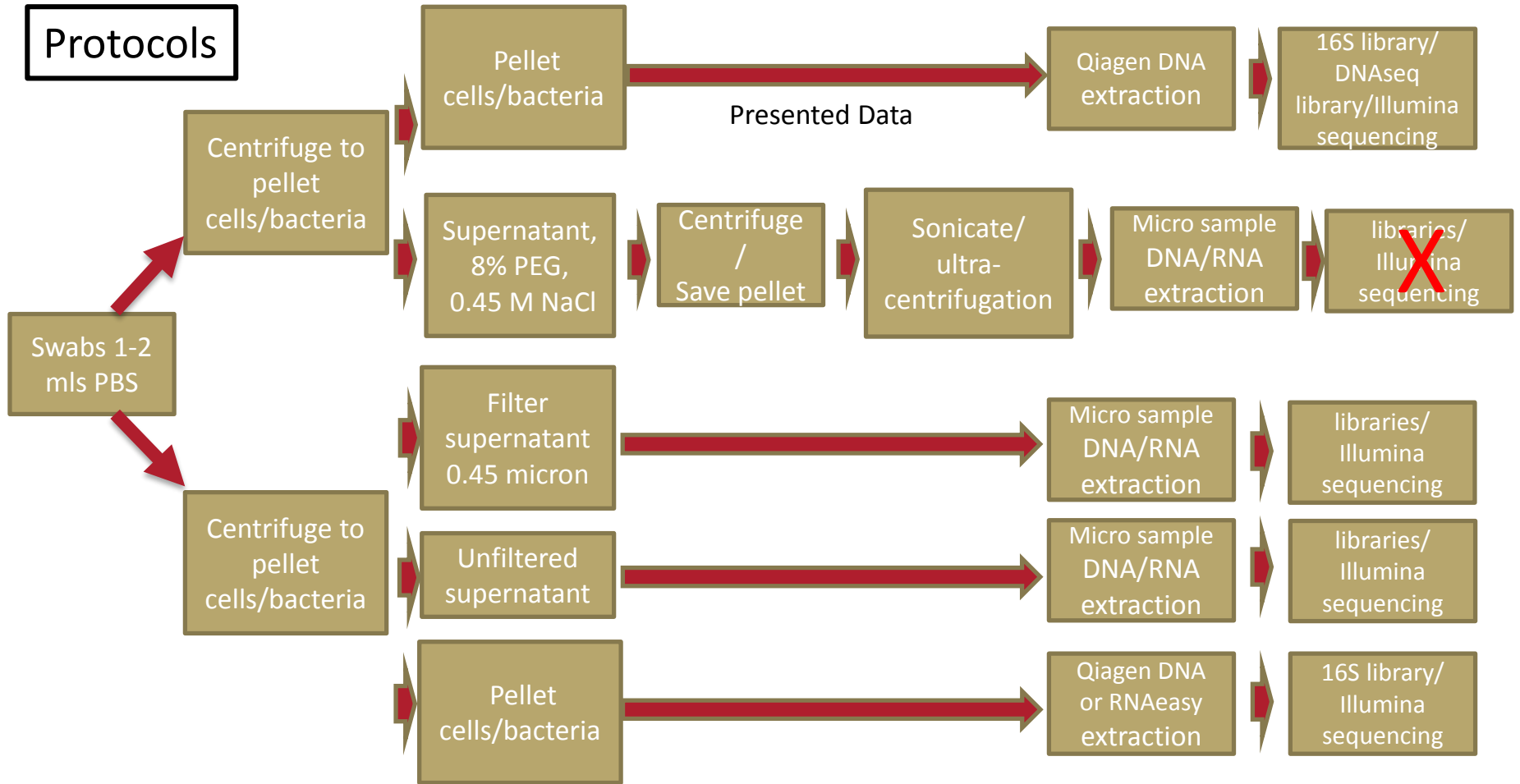
Sample	Non-Chicken Sequences		DNA Virus Sequences		Adenovirus		ILTV		MDV	
	Count	%	Count	%	Count	%	Count	%	Count	%
15-12033	8.07x10 ⁶	99%	110,104	1%	1	<0.01%	332	0.30%	109,771	99.70%
15-1266	6.60x10 ⁶	94%	432,186	6%	2	<0.01%	308,924	71.48%	123,260	28.52%
16-0982	7.80x10 ⁶	95%	437,557	5%	65	0.01%	315,288	72.06%	122,204	27.93%

- 1. Two ILTV+ samples were confirmed as containing ILTV**
- 2. ILTV sequences were identified in a bird vaccinated with ILTV 4 weeks previously but ILTV-**
- 3. All three samples contained MDV**
- 4. One sample contained adenovirus**

What this Experiment Demonstrates

- Using a simple approach the composition of the bacterial and DNA virus avian respiratory microbiome can be determined
- ILTV can be identified from known positive clinical samples
- A respiratory submission which was unidentified through standard methods was potentially identified as being infectious coryza by the abundance of *Avibacterium* 16S sequences found in the tracheal swab sample.
- As expected, the avian upper respiratory tract may contain other virus species – MDV and adenovirus

Protocols



Thank You



Table 2.3 Avian viral DNA sequences. This table lists the seven DNA virus genomes used for Burris Wheeler Alignment and Accession numbers for each strain used is indicated.

Type	Genome Length	Virus family	Strain	Accession Number
Non-enveloped dsDNA	~44,000 bp	Adenovirus	Adenovirus CELO	U46933.1
Enveloped dsDNA	~300,000 bp	Poxviridae	Fowlpox virus	NC_002188
Enveloped dsDNA	~150,000bp	Herpesviridae	Marek's disease virus	CVI988
			Infectious laryngotracheitis virus	NC_006623.1
Non-enveloped ssDNA	~2,000 nt	Circoviridae	NG Chicken38	HQ738642.1
Non-enveloped ssDNA	~5,000 nt	Parvoviridae	Chicken parvovirus ADL120686	KJ486491.1
Enveloped ss/dsDNA	~3,000 nt	Hepadnaviridae	Duck hepatitis B virus	AJ000350.1