

# Understanding the respiratory microbiome of commercial poultry

Ohio State University and University of  
Minnesota  
PRD-CAP



# Overall goals

- Comprehensively define core baseline respiratory microbiota in commercial broilers, layers, and turkeys.
- Define how the respiratory microbiota changes over time.
- Associate respiratory microbiota with susceptibility to disease.



# Year 1

- Identify flocks in Minnesota and Ohio for turkeys and layers.
- Develop methods for collecting biological materials from upper respiratory tract.
- Begin baseline sampling for bacterial and fungal populations.



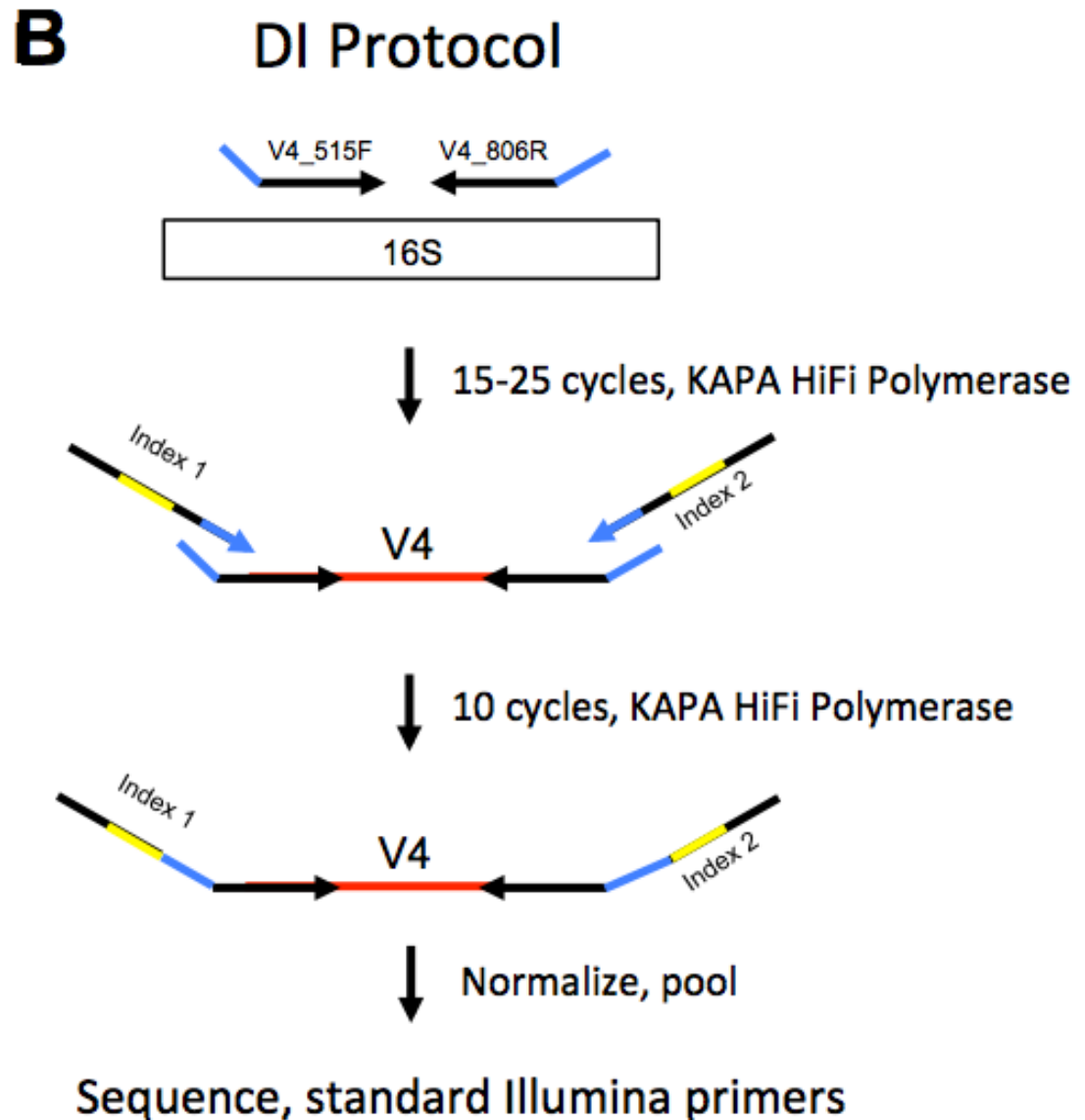
# Approach

- Samples are collected in MN and OH
- DNA is extracted using common protocols
- DNA shipped to UMN
- Optimized PCR and library creation at UMN
- Sequenced using Illumina Miseq
- Data immediately deposited on shared server
- Analyzed collectively in QIIME



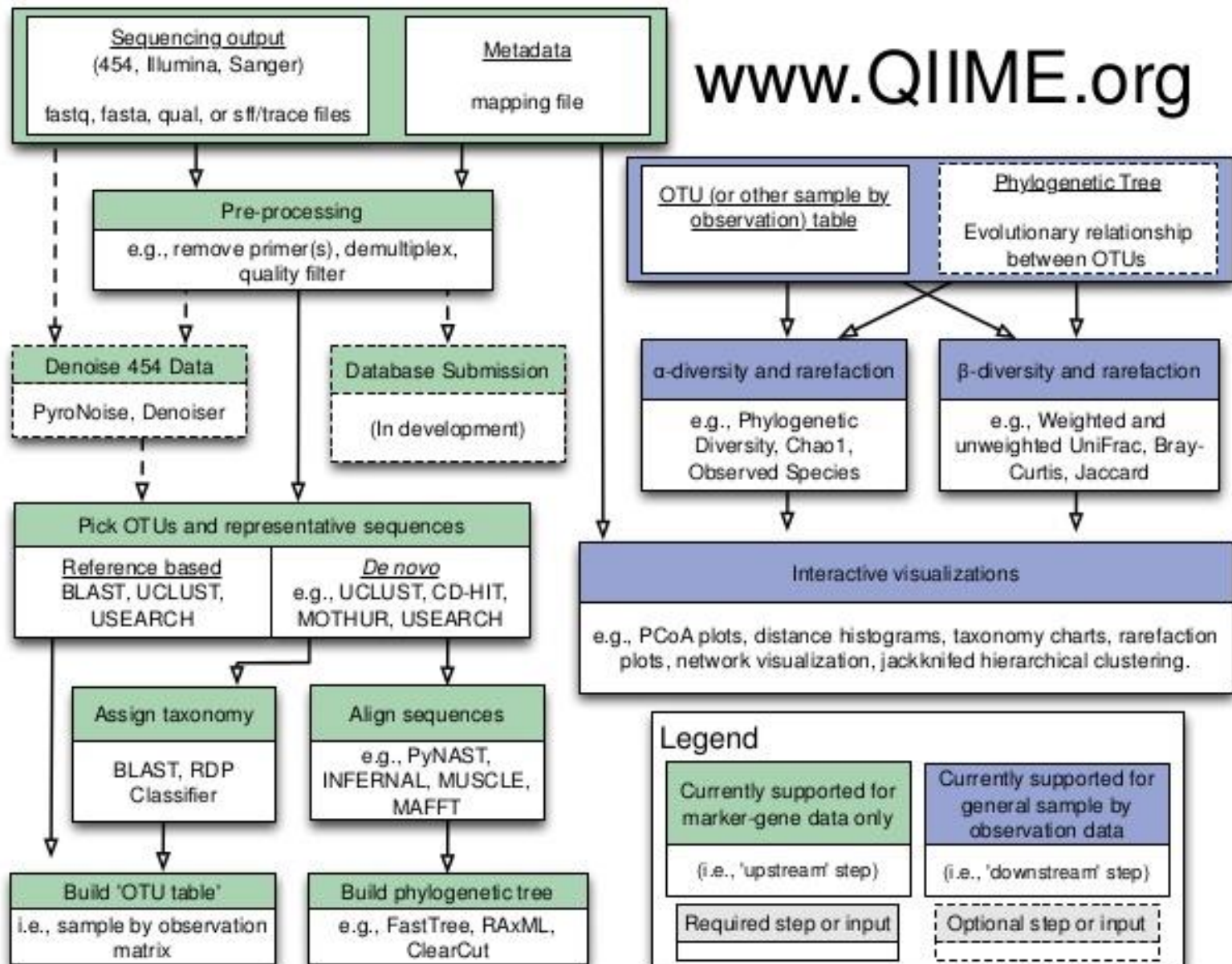
# Optimized PCR to minimize bias

16S rRNA = V4  
Fungal = ITS1



Grohl et al., UMN, unpublished data

# QIIME workflow



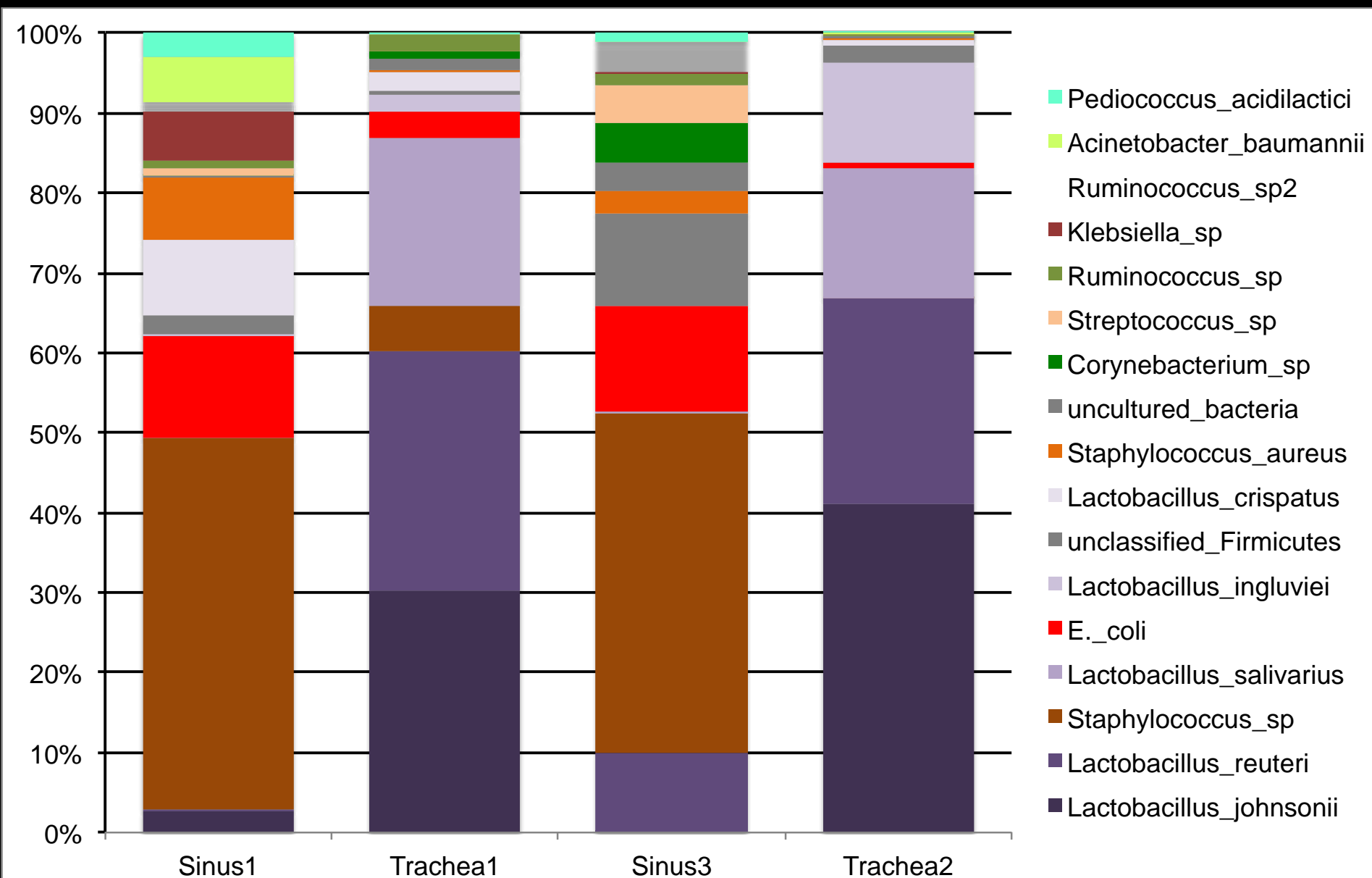


# Pilot sampling

- Commercial turkeys
- Sinus cavity and trachea aseptically collected
- Washed in 10mL PBS
- Centrifuged to pellet cells
- DNA extracted from pellet



# Pilot data – trachea and sinus

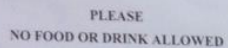




# Mid-Central Research and Outreach Center (MCROC) Lab

- Willmar, MN
- Newly renovated BSL-2 laboratory
- Offices and space for UMN faculty and staff
- Two full-time personnel, two more being hired
- Research-service model
- Mission: enable industry-academia relationships through direct connection, favorable budget model, IP benefits





PLEASE  
NO FOOD OR DRINK ALLOWED

[illegible]

University of Minnesota  
Veterinary Research Lab

Avian disease researcher Dr. Carol Cardona, veterinary and biomedical sciences professor and Ben Pomeroy Chair in Avian Medicine

Mid-Central Research  
and Outreach Center  
UNIVERSITY OF MINNESOTA  
Driven to Discover™

150







UMN poultry  
faculty

Allied industry

BLT industry

MCROC

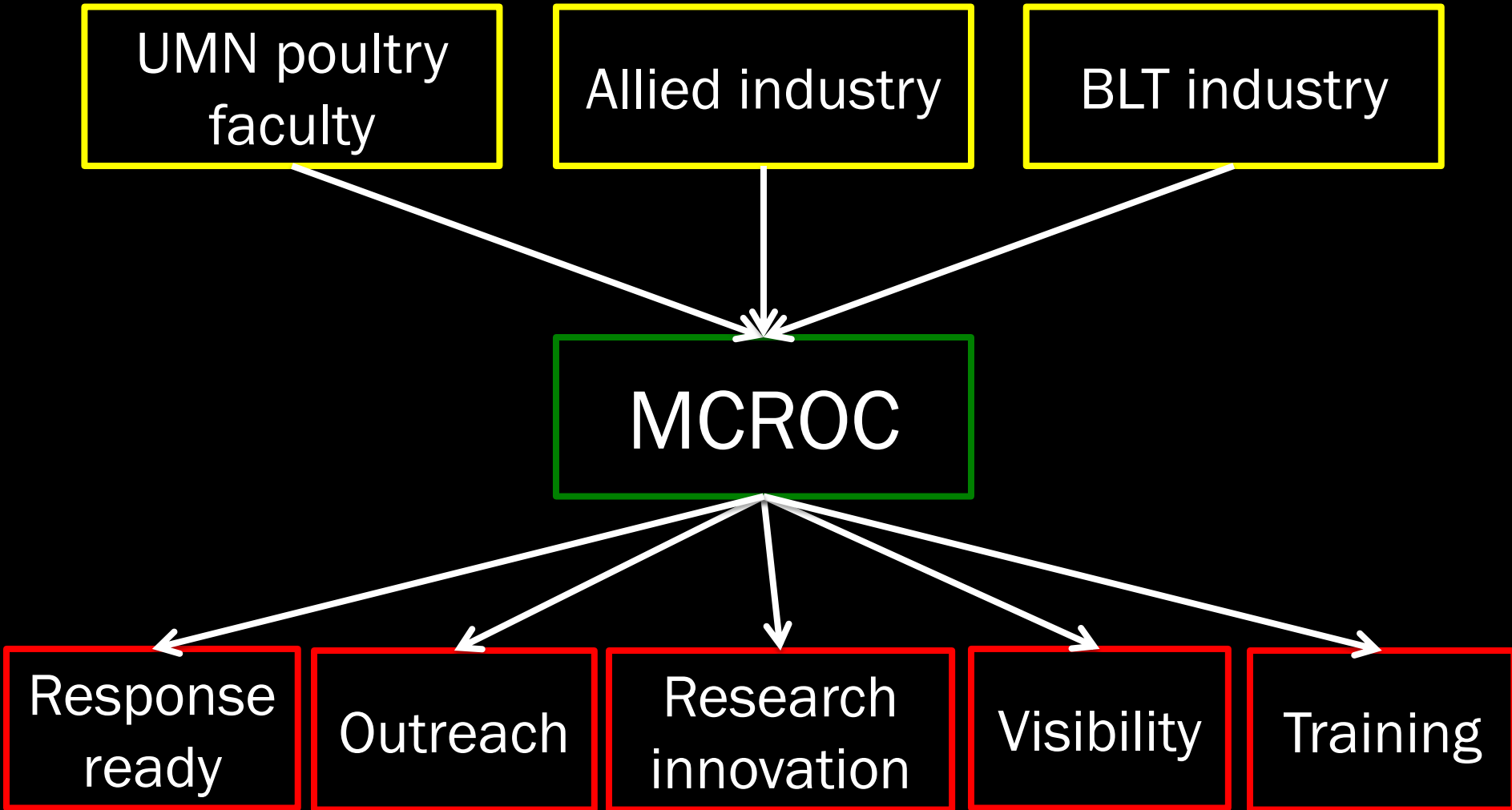
Response  
ready

Outreach

Research  
innovation

Visibility

Training





# MCROC sustainability





# Sampling in OH/MN (Years 1-3)

- MN Turkey partner = Willmar Poultry Company
- MN Layer partner = Sparboe
- OH has identified 2 farms each for turkey and layer
- Flocks will be followed temporally
  - Turkeys: weeks 0, 1, 2, 3, 4, 5, 8, 10, 15
  - Layers: weeks 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45
- Samples collected: sinus, trachea, ileum, ceca, spleen
- Tissue archive

# Microbiome in response to challenge (Years 4-5)

- PIs from PRD-CAP performing challenge studies
- Partner to collect respiratory and gut tissue
- Identify shifts in response to challenge
- Identify predictors of disease susceptibility
- OH has sent samples from SPF flock with/without dexamethasone treatment

# Viral sampling

Thoroughly homogenize intestinal tissue in cold 0.9 % saline  
↓  
Spike the homogenate with **Influenza A virus**  
↓  
Centrifuge to discard tissue debris  
↓  
Filter sequentially through 0.8, 0.45 and 0.2 µm pore filters

**This filtrate was treated in three different ways to concentrate viral particles**

## 1<sup>st</sup> Processing method

Add PEG –6000 to the filtrate and stir overnight at 4°C to **concentrate** the VPs  
↓  
**Centrifuge** to get the PEG and VP pellet

## 2<sup>nd</sup> Processing method

Add PEG –6000 to the filtrate and stir overnight at 4 °C to **concentrate** the VPs  
↓  
**Pellet** PEG and VP pellet by centrifugation  
↓  
Re-suspend the precipitate in cold saline, **sonicate to separate** VPs from PEG  
↓  
**Pellet** the PEG by centrifugation  
↓  
**Ultracentrifuge** the supernatant to **concentrate/pellet** the VPs

## 3<sup>rd</sup> Processing method

**Ultracentrifuge** the supernatant to **concentrate/pellet** the VPs

**The viral particles from each of the three methods were treated similarly to extract viral RNA**

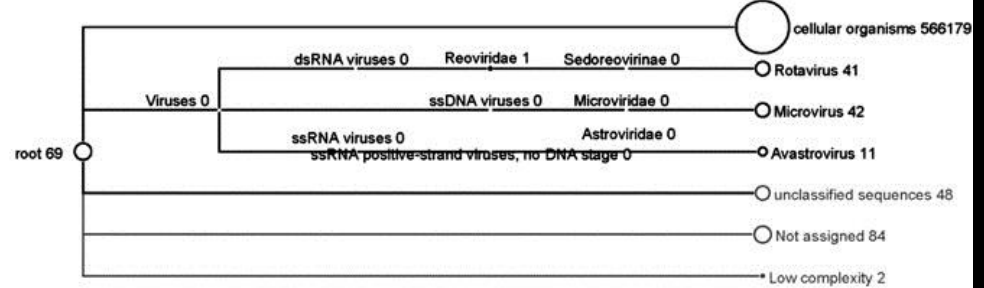
Treat the above pellets of VPs with RNase to degrade the contaminating non-viral RNA  
↓

Re-suspend the pellet with VPs in Trizol for lysis and extract total RNA  
↓

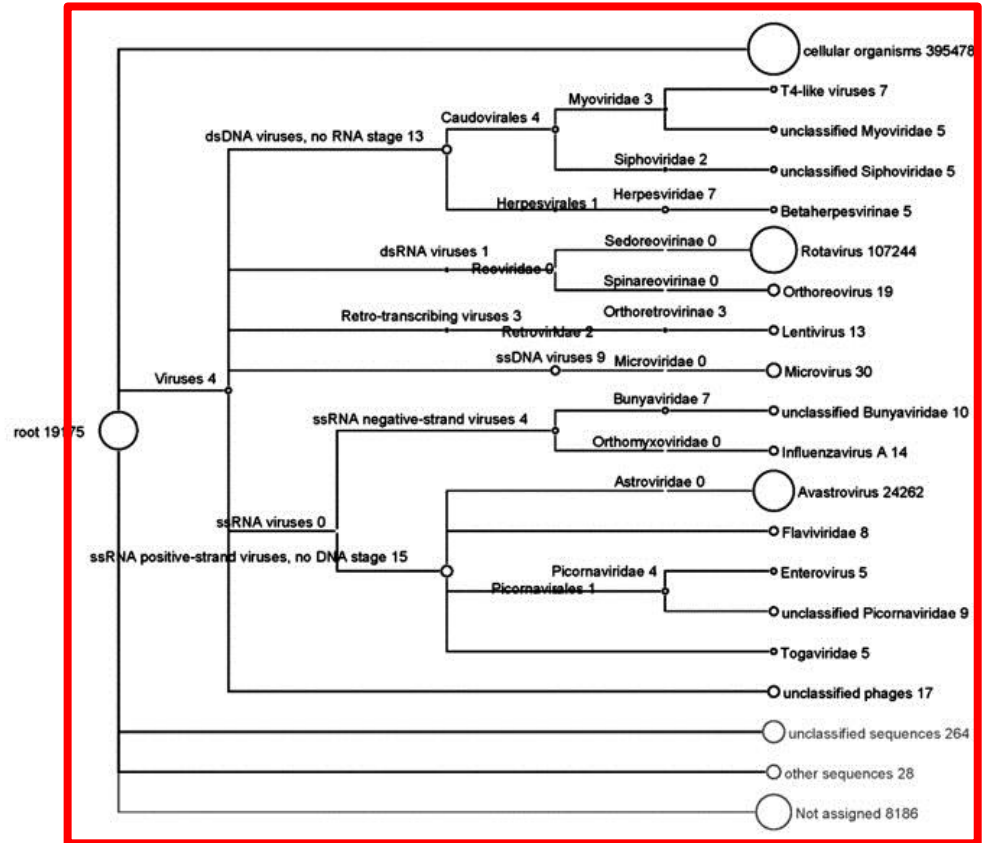
Sequence total RNA with Illumina Miseq PE 150 cycles (2 biological reps for each modification i.e. 6 samples in total)

# Viral shotgun analysis

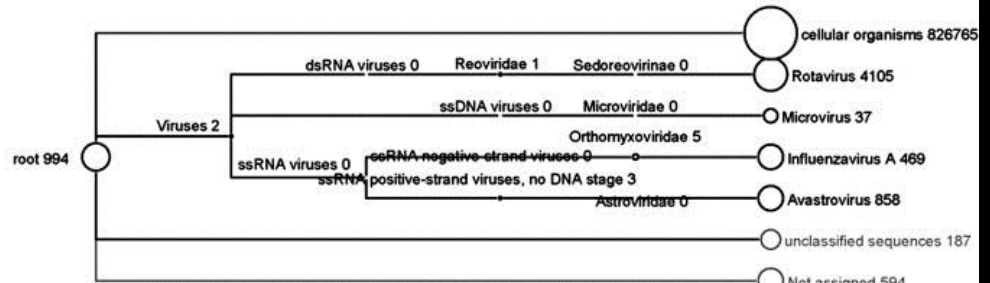
(a)



(b)



(c)



# Percentage of reads by taxon node

