Applications of Genetic Sequencing Technologies in Veterinary Diagnostics

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Outline

1. Strengths and weaknesses of NGS relative to traditional tests
   - *Briefly describe the nuts and bolts of the NGS process*
2. Clinical case examples using metagenomics
   - *Mixed or novel agent detection in diagnostic submissions*
3. The role of pathology and direct detection methods in interpretation
   - *Serial testing for novel agents detected by NGS*
4. Using NGS for detailed pathogen characterization
   - *Temporal comparisons, epidemiological investigations, AMR prediction*

Strengths and weaknesses of NGS

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Background

- Pathogen detection in VDLs commonly involves a combination of culture-based and culture-independent techniques.

- Most VDLs have a number of routine assays available for commonly encountered agents and animal species.
  - Significant variability in breadth and depth of assays among labs
  - Can be difficult to stay current on what is available where
    - Forwarding samples between labs increases TAT

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Background

- Multiplex assays / PCR panels:
  - One approach to increase diagnostic sensitivity (bundling)
    - Helps address polymicrobial infections in populations
  - Still limited to what is known

- Unbiased, high-throughput approaches (NGS):
  - Desirable for flexibility and efficiency
    - *Less limited than targeted assays*
  - Currently more expensive than traditional assays
  - Interpretation of results may take significant time

Background

- What exactly is NGS?

	1970s	1990s	2000s	2010s	2020s
Generation	1st generation with gel-based methods	1st generation with capillary methods	Next generation	Third generation	Future generations
Technologies	Sanger sequencing (manual)	Sanger sequencing (automated)	Illumina, Roche 454, Ion Torrent	PacBio SMRT, ONT	Genapsys, MGI
Breakthrough	Gel-based analysis	Capillary analysis	High throughput	Long reads	
Pros	Accuracy	Higher scale, Lower cost, Accuracy	Long reads, Ultra-fast, Portability		Aiming for cost effectiveness, accuracy
Cons	Labour intensive, High cost	Short reads make analysis more difficult		A reduction in accuracy	

https://www.yourgenome.org/theme/timeline-the-past-present-and-future-of-sequencing-technologies/

Massive Parallel Sequencing

NGS = 2nd Gen Sequencing

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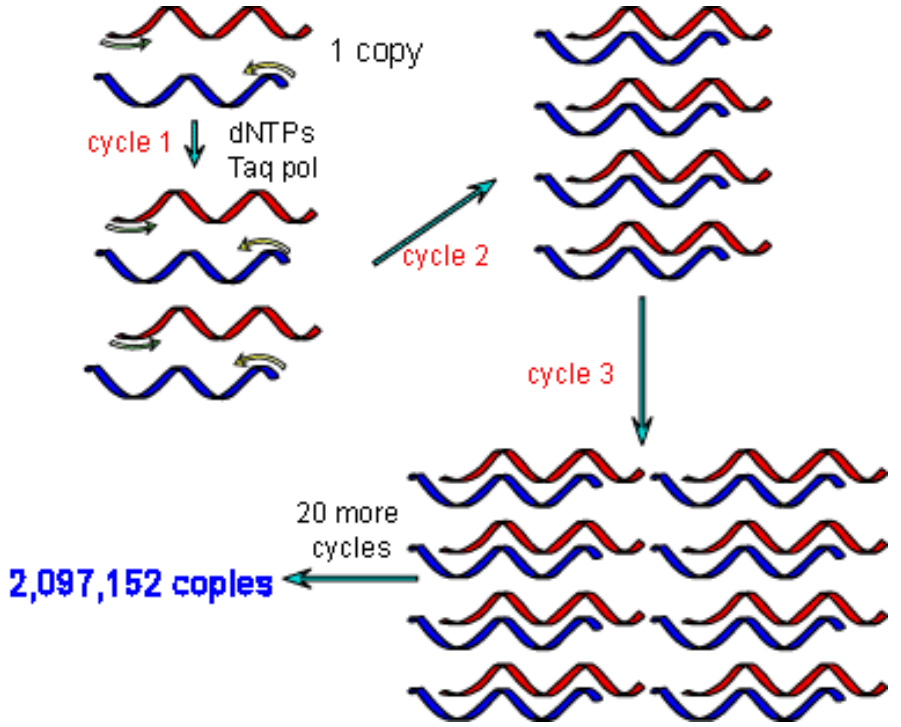
Background

- NGS vs PCR:
  - PCR assays amplify a specific target NA sequence within a sample
    - Contamination is not an issue
    - Well-suited for feces and other dirty samples

Extracted DNA

Amplified DNA



http://www.scq.ubc.ca/polymerase-chain-reaction/

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Background

- NGS vs PCR:
  - NGS detects all nucleic acid sequences indiscriminately
    - Host DNA often consumes a majority of read capacity
    - Contamination may severely interfere with output and interpretation
      - *Follow up with direct detection may be needed with novel agent discovery*

All Reads

Filtered Reads

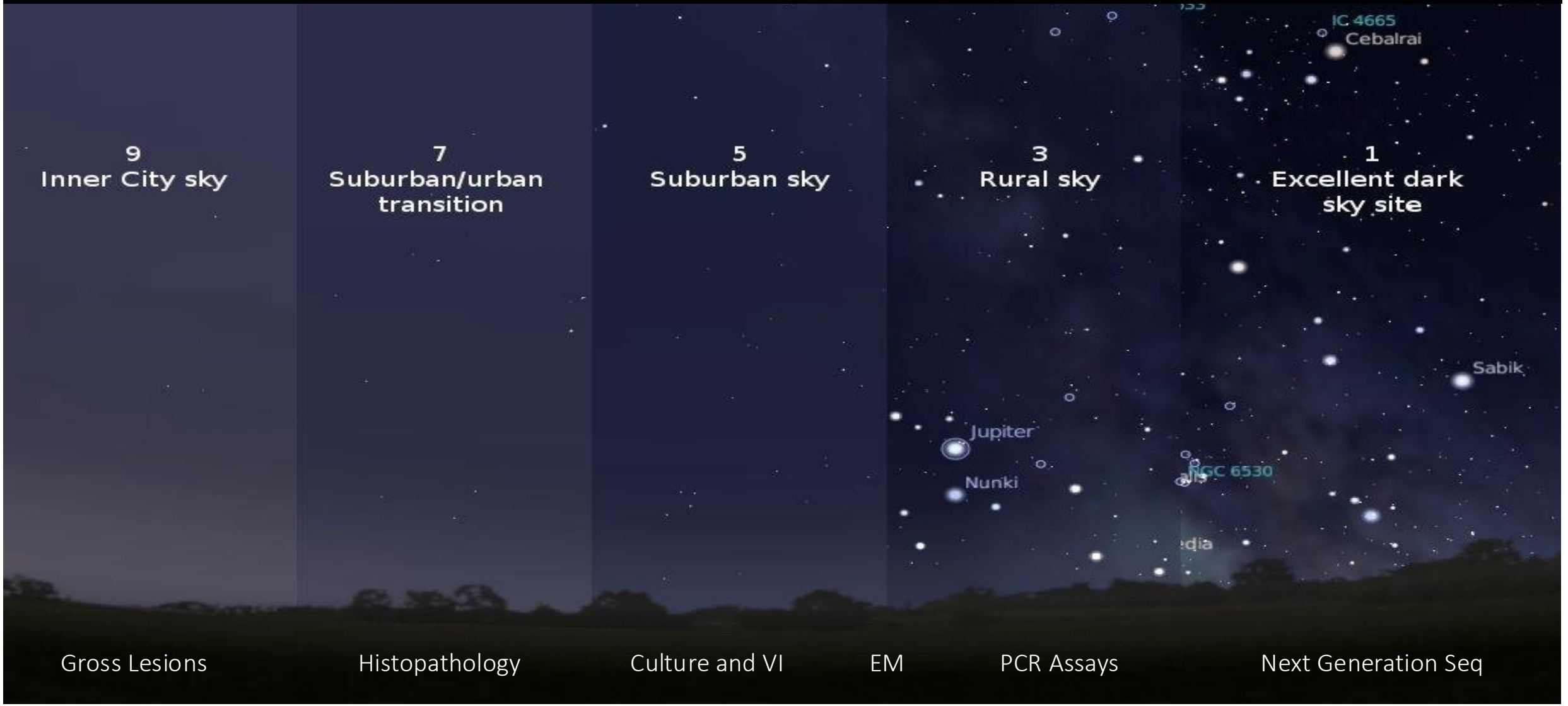
Output to Analyze

Background

Traditional PCR Assays

NGS Approaches

Evolution of Agent Detection



Gross Lesions	Histopathology	Culture and VI	EM	PCR Assays	Next Generation Seq
Avenzoar – ~1020	Virchow – 1850s	Pasteur – 1860s	1940	~1987	~2000

Background

Optimist: "NGS can find ***anything***!"

Realist: "NGS can find <u>everything</u>."

Pessimist: "NGS is too prone to error and misinterpretation."

Background

- As costs continue to come down and new platforms emerge, unbiased high-throughput sequencing approaches are becoming an affordable, efficient reality for routine diagnostic use.

- Advantages of NGS:
  - Detection of multiple classes of known pathogens in one assay
    - *viruses, bacteria, archaea, fungi, protozoa*
  - Allows potential detection of novel agents
  - Fewer individual assays to maintain in a VDL
    - *Readily explore potential etiologies suggested from histopathologic lesions*
  - Pathogen viability not required

Background

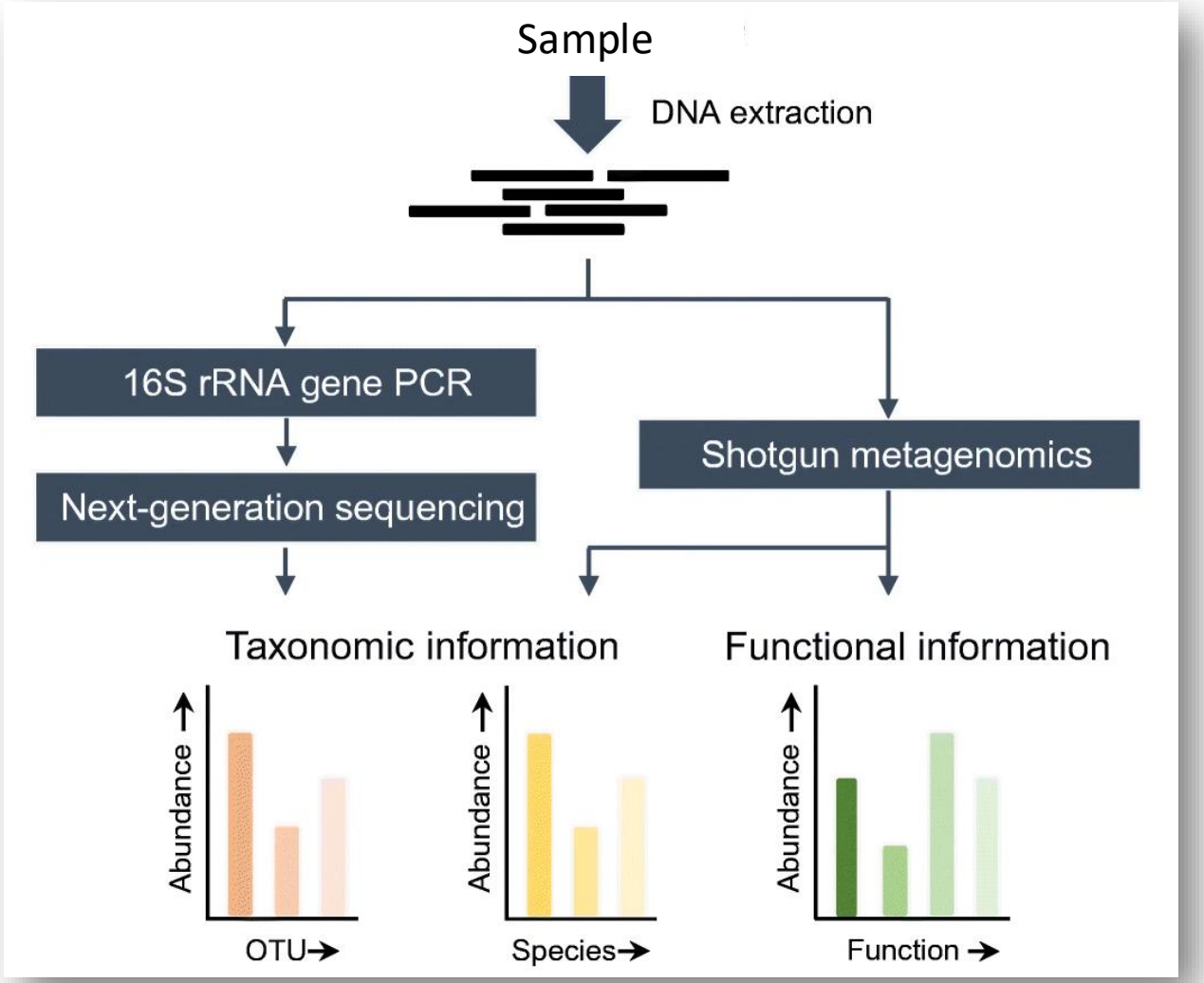
- Disadvantages / Risks of NGS
  - HUGE output to analyze
    - *"Haystack full of needles"*
  - Potential for hypothesis free application
    - *Inductive reasoning*
  - Detection vs disease diagnosis
    - *Lots of things will be detected depending upon sample type*
      - *CSF > Serum > Spleen > Lung > Intestine*
    - *Interpretation may be difficult with serial testing required*
  - Cost
    - *Typically ~$200 per sample but coming down*
    - *Cheaper options often with less sensitivity / specificity*

How It's Done

- NGS Process:
  - High-throughput molecular testing
  - Creates many short NA sequences (~150-300 bp)
    - Products generated are compared against a reference database
  - Many different approaches:
    - Microbial census taking (microbiota; typically16S rRNA)
    - Mixed agent detection (shotgun metagenomics)
    - Novel agent detection (shotgun + de novo assembly)

How It's Done

- NGS Process:



Boers, S.A., Jansen, R. & Hays, J.P. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. *Eur J Clin Microbiol Infect Dis* **38**, 1059–1070 (2019).

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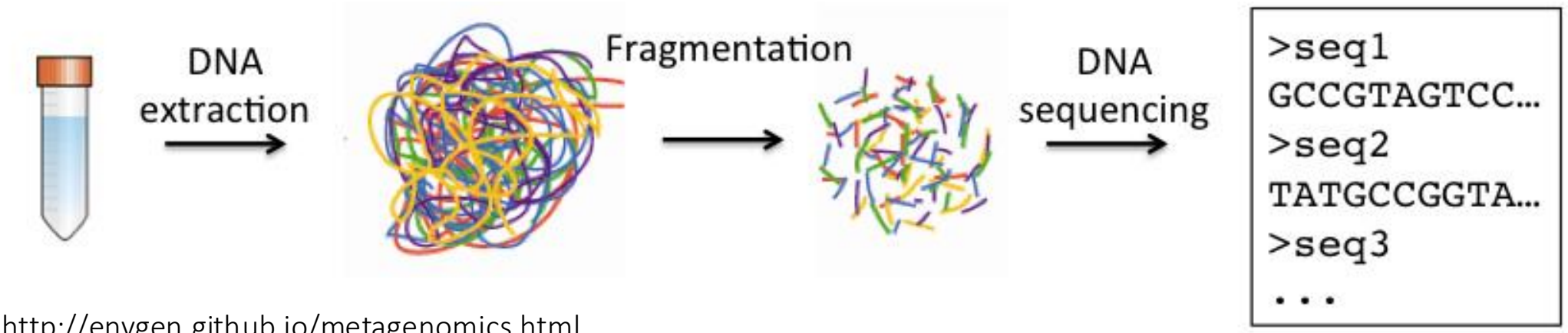
How It's Done

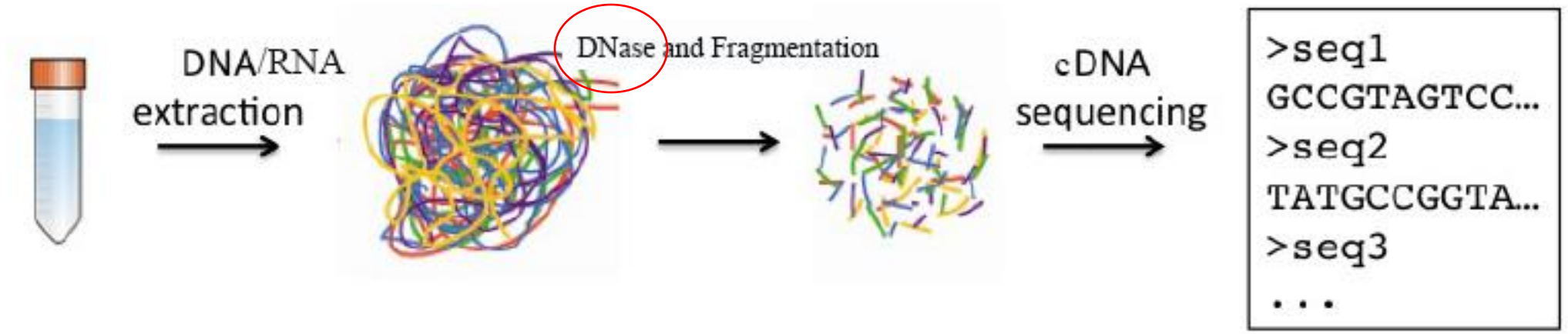
- Before sequencing, a library of all DNA in the sample is prepared
  - Includes fragmenting of DNA and +/- barcoding
  - Library is a collection of DNA fragments that represent the entire genomic content of the sample
    - *For small DNA viruses, obtaining a complete genome sequence may be possible*
    - *RNA libraries can also be prepared*



http://envgen.github.io/metagenomics.html

How It's Done

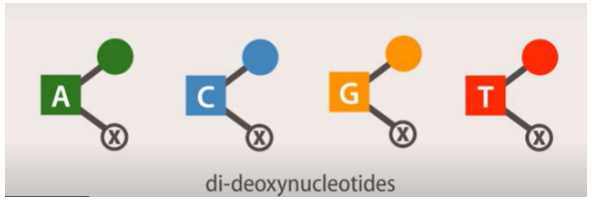
- How RNA sequencing differs from DNA:
  - Extraction is the same, but for RNA sequencing DNase can be applied
    - This removes any host DNA and increases the relevant sequencing yield
      - *Increases the likelihood of obtaining enough reads to generate a full genome sequence for RNA viruses*



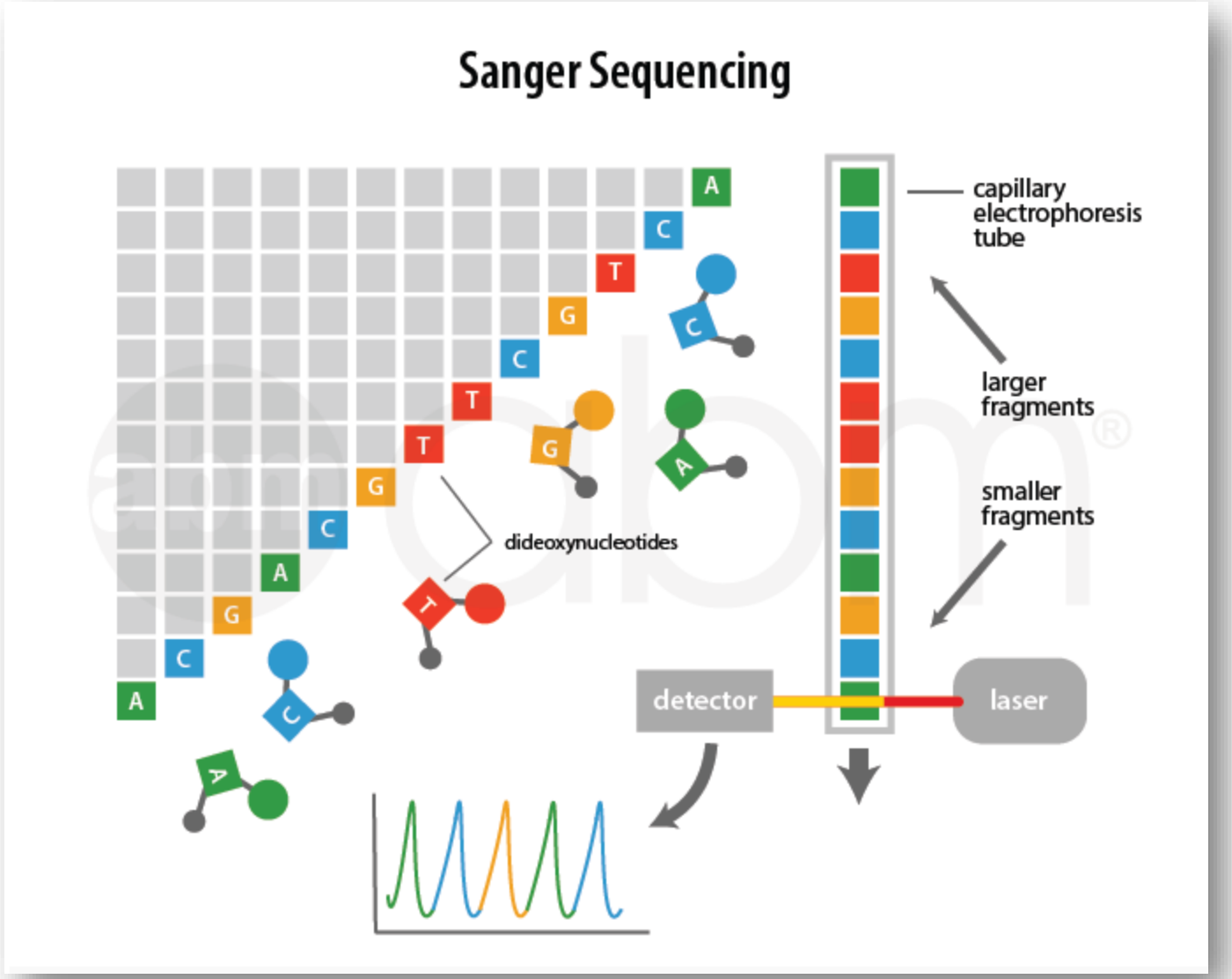
How It's Done

- Sanger Sequencing:

1. PCR amplification of target
2. Cycle sequencing
   - Generates chain-terminated fragments with ddNTPs



3. Electrophoresis separates the fragments
4. Data analysis
   - Sequence determined based upon location of ddNTPs



https://old.abmgood.com/marketing/knowledge_base/next_generation_sequencing_introduction.php

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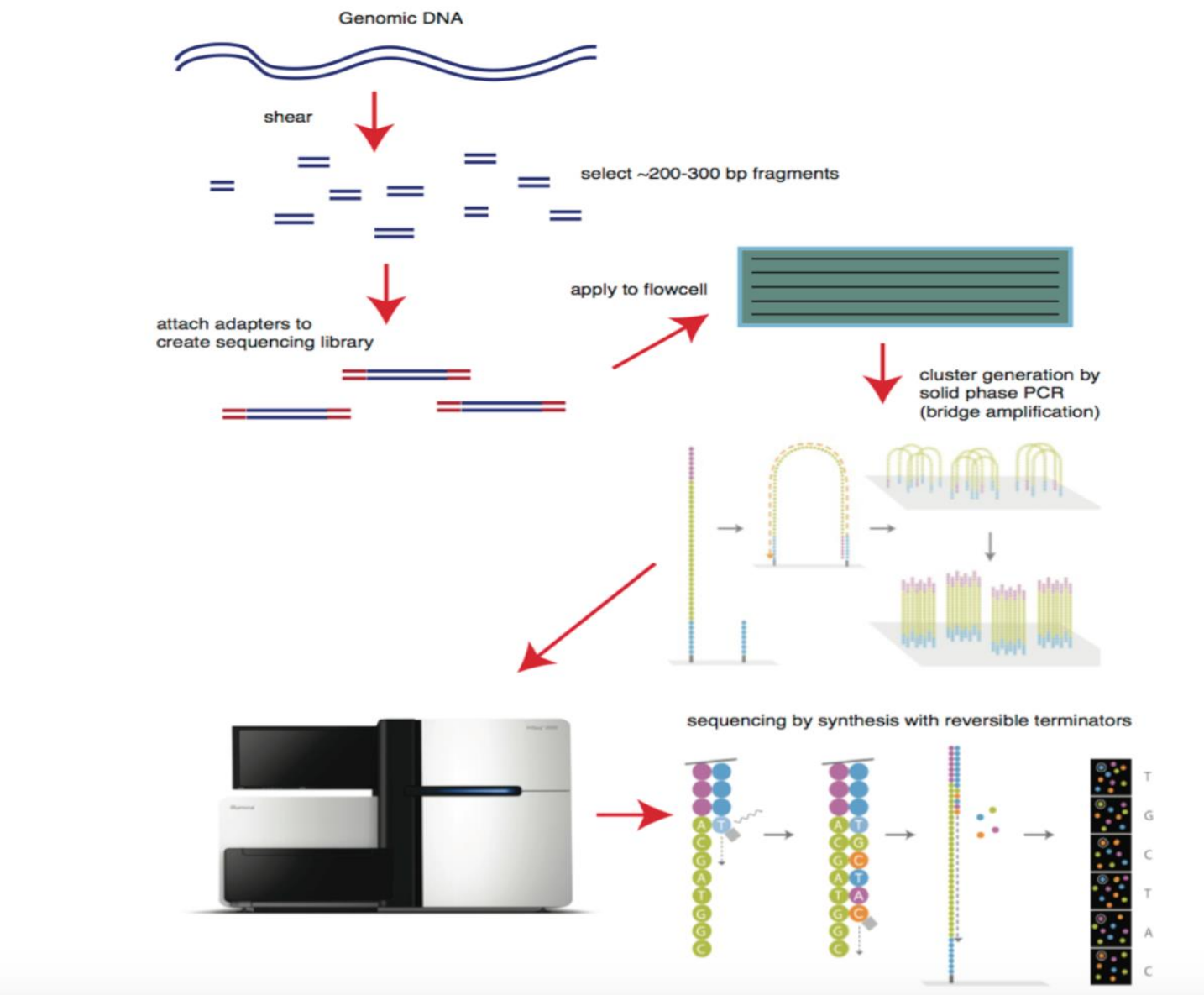
How It's Done

- Sequencing by synthesis:
    1. Library Prep (tagmentation)
    2. Cluster generation
    3. Sequencing
        - Labeled dNTPs with terminators added in repeated cycles
        - Software records the base added at each cluster
    4. Data analysis
        - Quality control
        - Sequences compared to reference database
        - More computational than Sanger

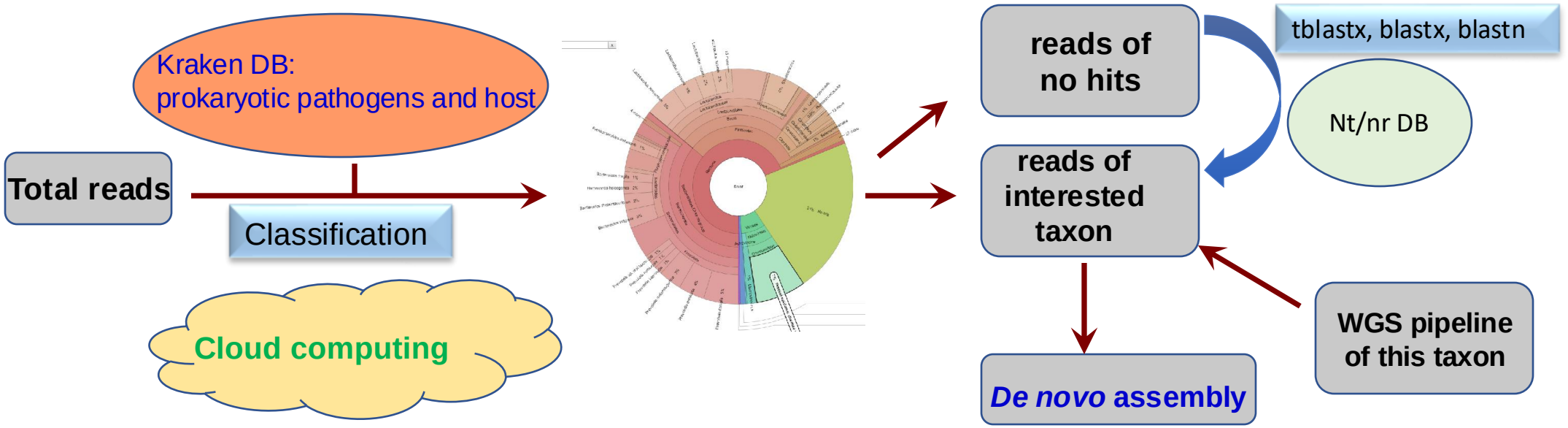
https://youtu.be/womKfikWlxM



https://bitesizebio.com/13546/sequencing-by-synthesis-explaining-the-illumina-sequencing-technology/

How It's Done

- Strategy: NGS total reads classified by Kraken and reads of interested pathogen extracted and fed into the WGS pipeline; "no-hits" reads *de novo* assembled, contigs tblastx against NCBI nt database for further detection.
- Pros: High speed, relatively small number of queries in blast search step;
  Krona plot shows relative abundance of taxonomy.
- Cons: Database only contains **RefSeq complete genome** of virus, bacteria, archaea and host.



Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biology* 2014, 15:R46.

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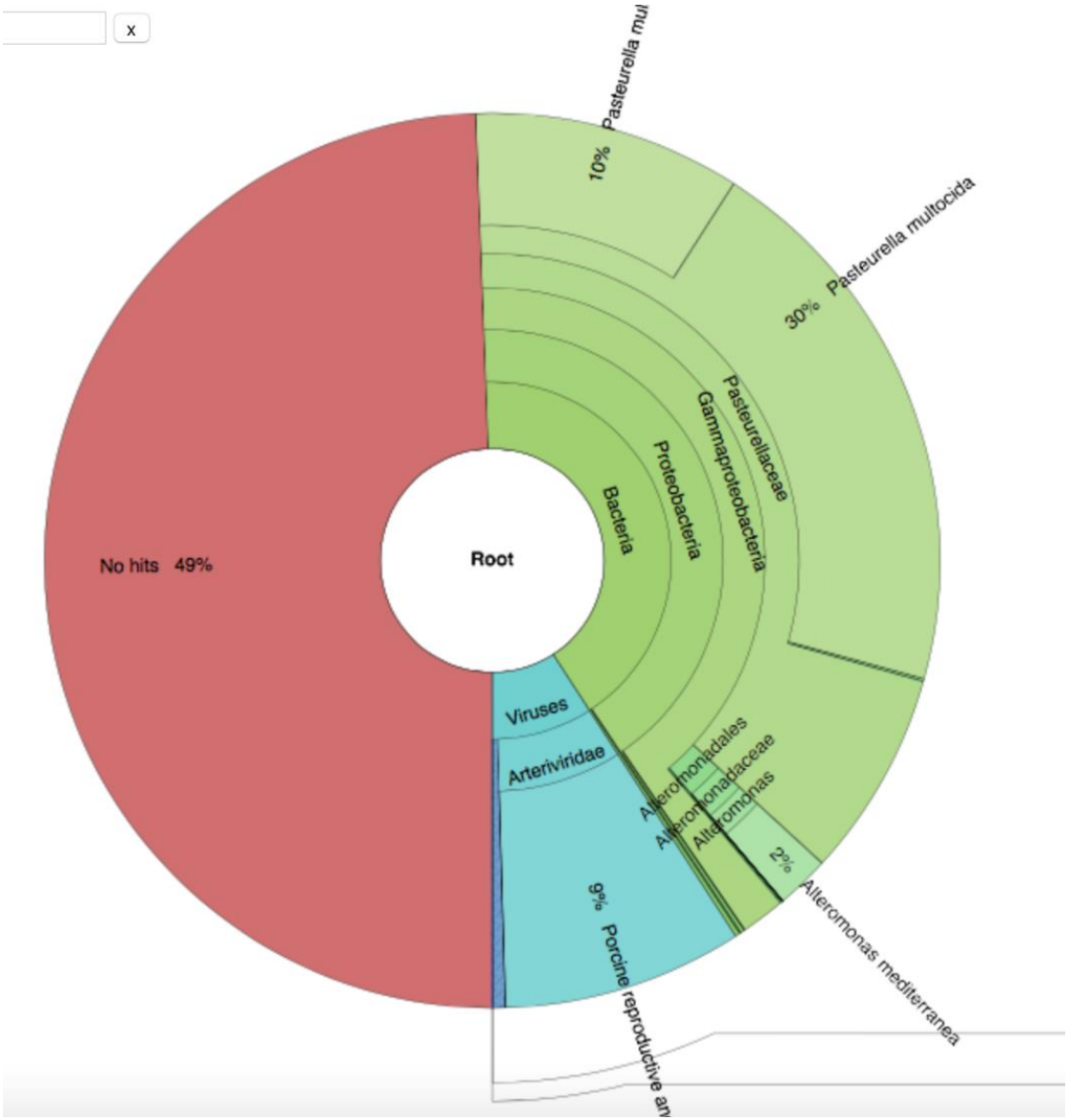
How It's Done

- What exactly is a Krona plot?

1. A tool for visualizing NGS output

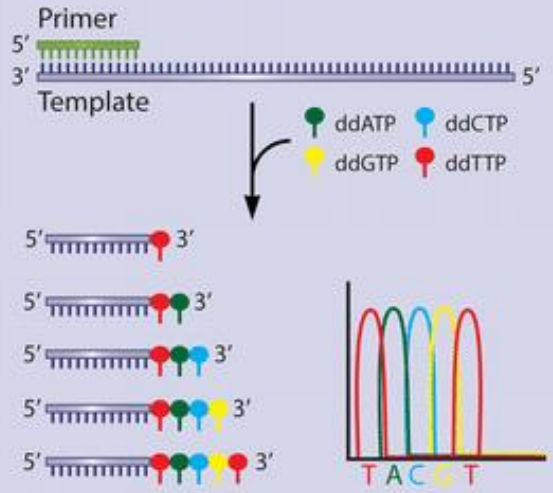
2. Shows relative abundance of taxa
   - *Limited to the reference database*

3. Classification will depend upon stringency criteria selected
   - Sequences from taxa of interest should be further evaluated
   - Contigs created are then subject to BLAST
   - *May or may not be a real finding*



How It's Done (review)

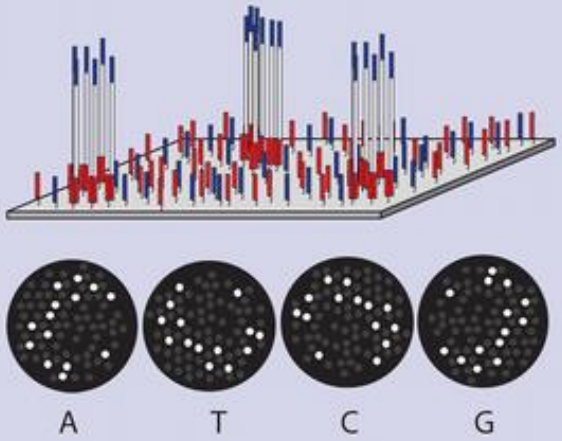
First Generation
Sanger Sequencing



- Sequencing by synthesis
- High accuracy
- Long read lengths
- Relatively small amount of data generated

e.g., ABI capillary sequencer (ABI)

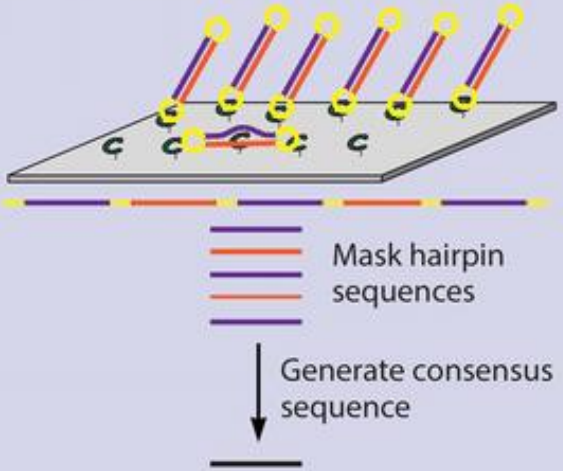
Second Generation
Massively Parallel Sequencing



- Sequencing by synthesis
- Amplified templates are generated during sequencing, reducing the requirements for starting material
- High accuracy
- Short read lengths

e.g., MiSeq (Illumina), Ion Torrent (Thermo Fisher Scientific)

Third Generation
Single-molecule Sequencing



- Single-molecule templates
- Low accuracy
- Long read lengths

e.g., Single-Molecule Real-Time (SMRT) — Sequencing (Pacific Biosciences), MinION (Oxford Nanopore Technologies)

Uses at ISU VDL:

- ORF 5 PRRSV, HA/NA IAV,
- S1 PEDV, 16s rDNA
- Unbiased Pathogen Detection,
- WGS of *S. suis*, GPS, *E. coli*, PRRSV, etc.

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Clinical Case Examples Using NGS

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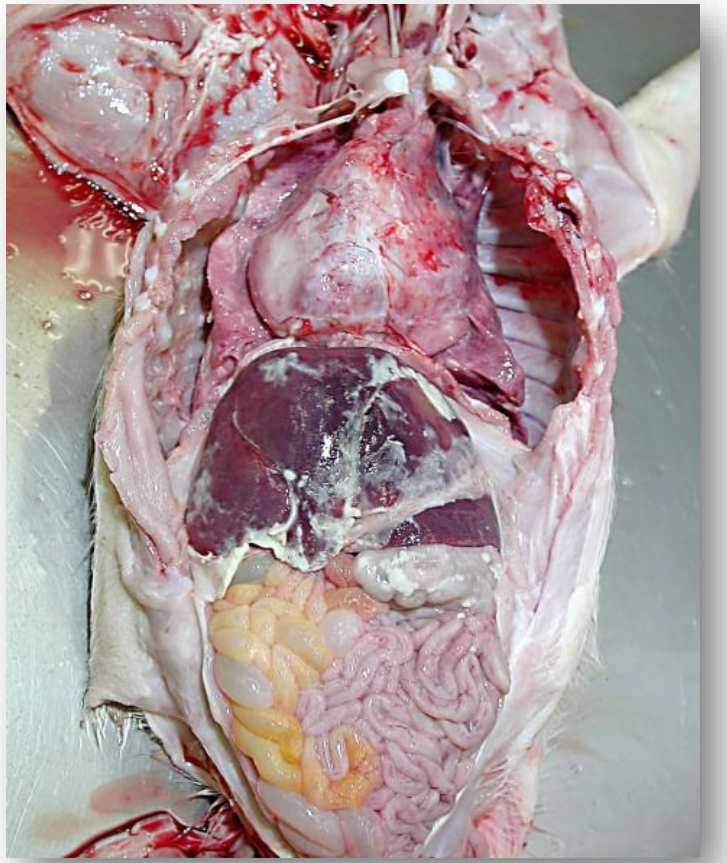
Clinical Case #1

- 6-week-old pigs, mild cough, increased fallout (10%)
  - Recent diagnostics have detected IAV by PCR at a high Ct, various systemic bacteria by culture
  - Diagnostic questions:
    - Is there another underlying viral etiology?
    - What is the contribution of isolated bacteria?
  - Gross lesions:
    - Severe fibrinous polyserositis
    - Multifocal pulmonary consolidation
  - Histopathology:
    - Fibrinopurulent polyserositis, severe
    - Lymphoplasmacytic tracheitis, mild to moderate
    - Bronchial epithelial attenuation, multifocal, mild
    - Neutrophilic and histiocytic bronchopneumonia, mild



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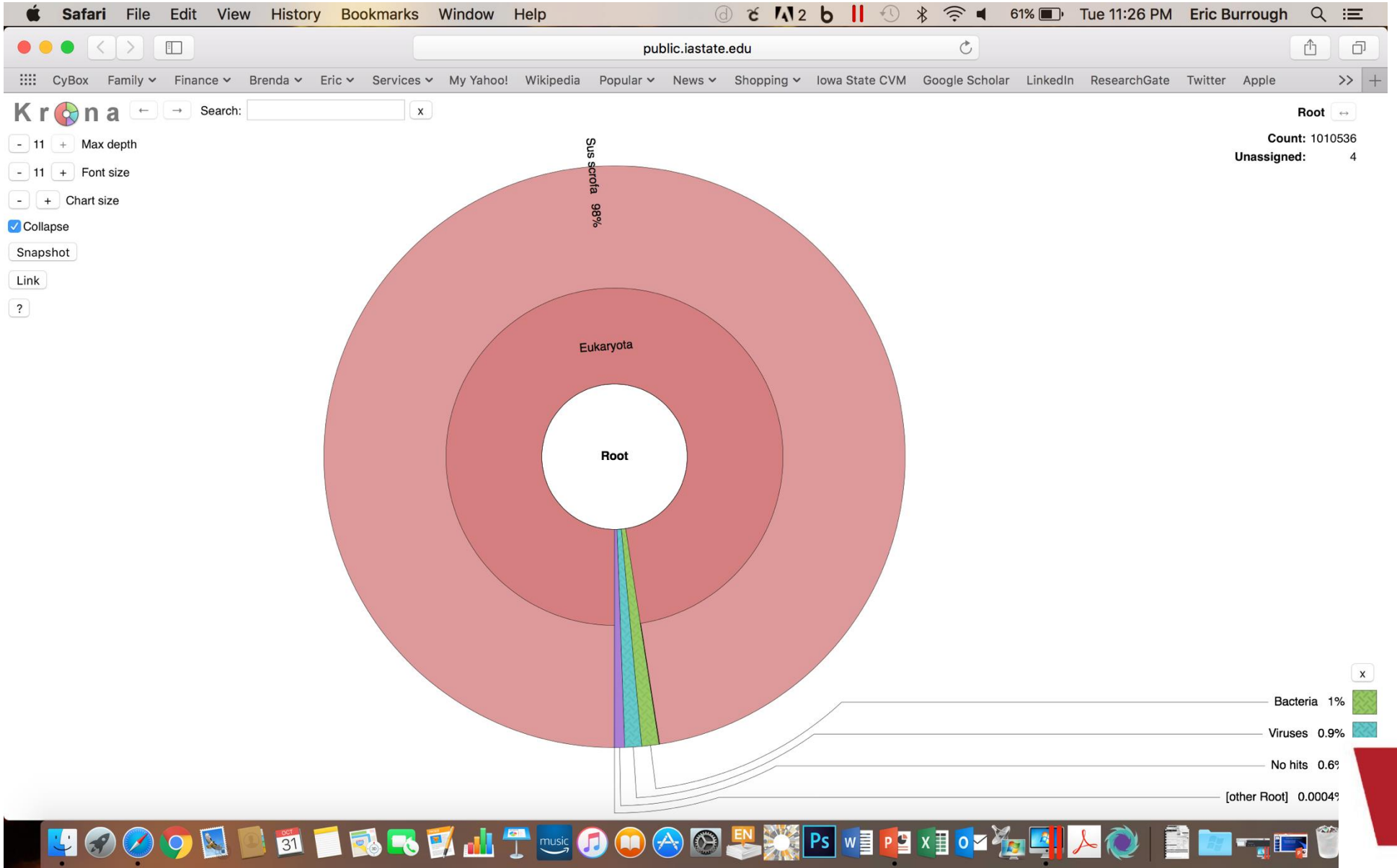

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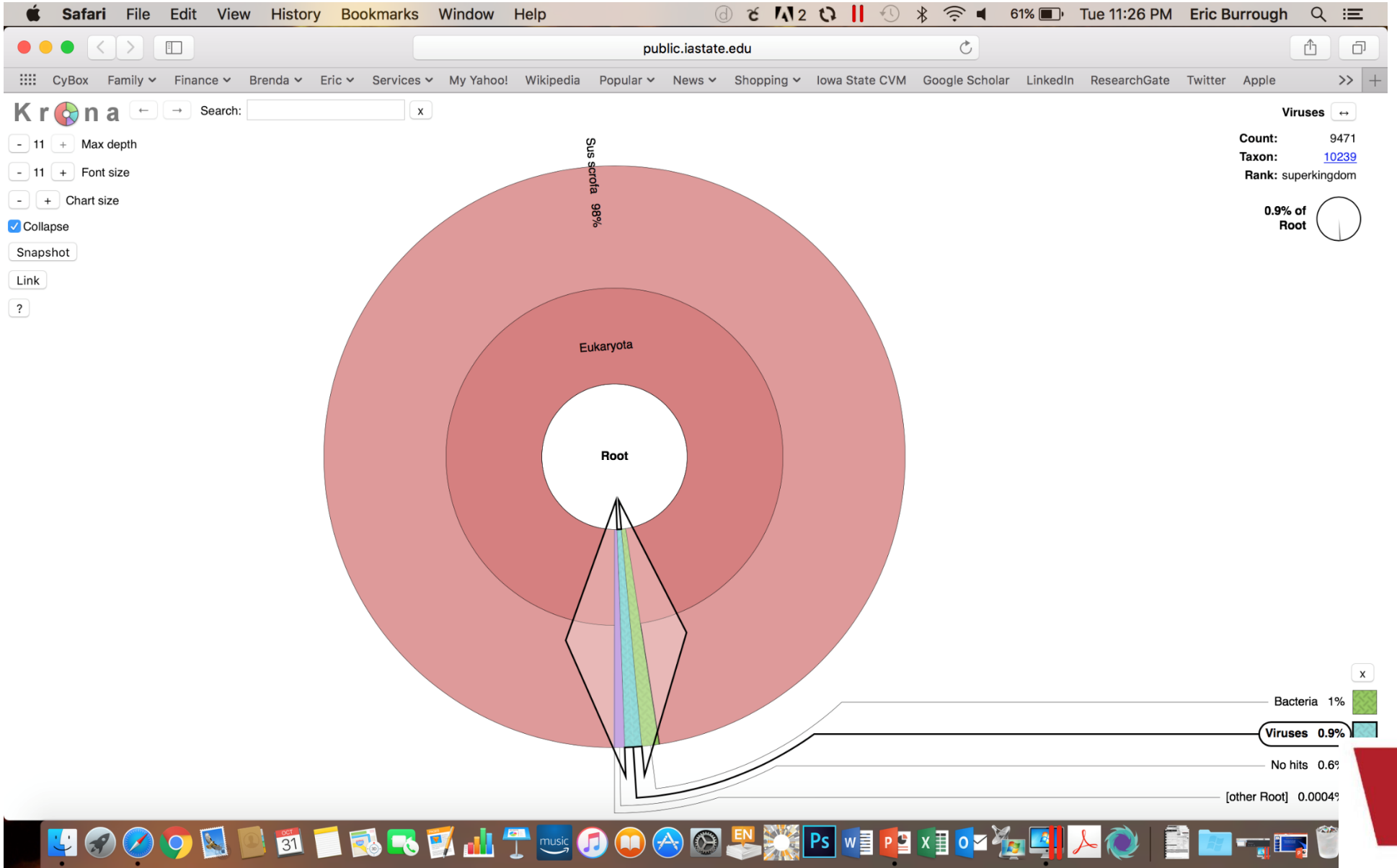

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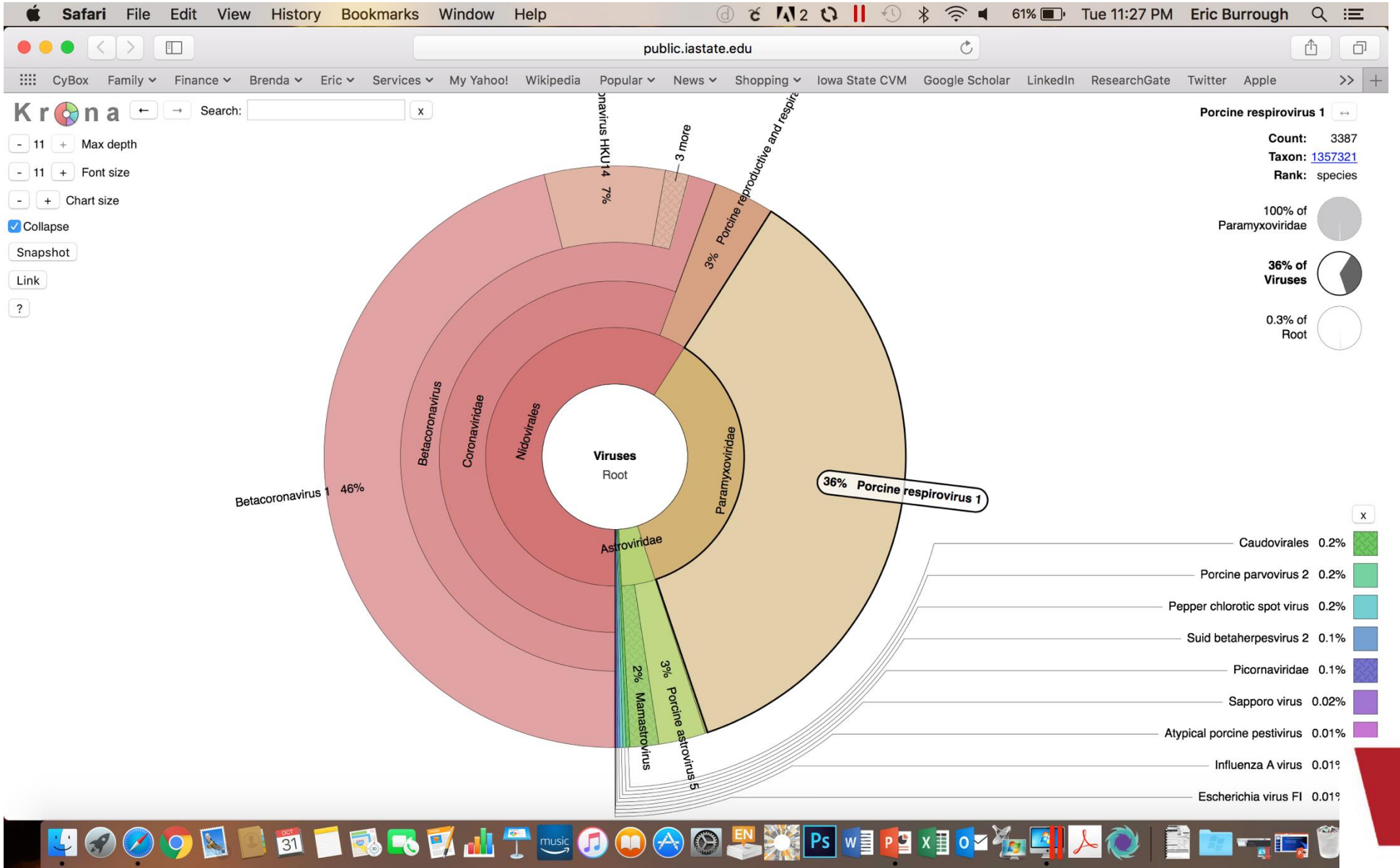

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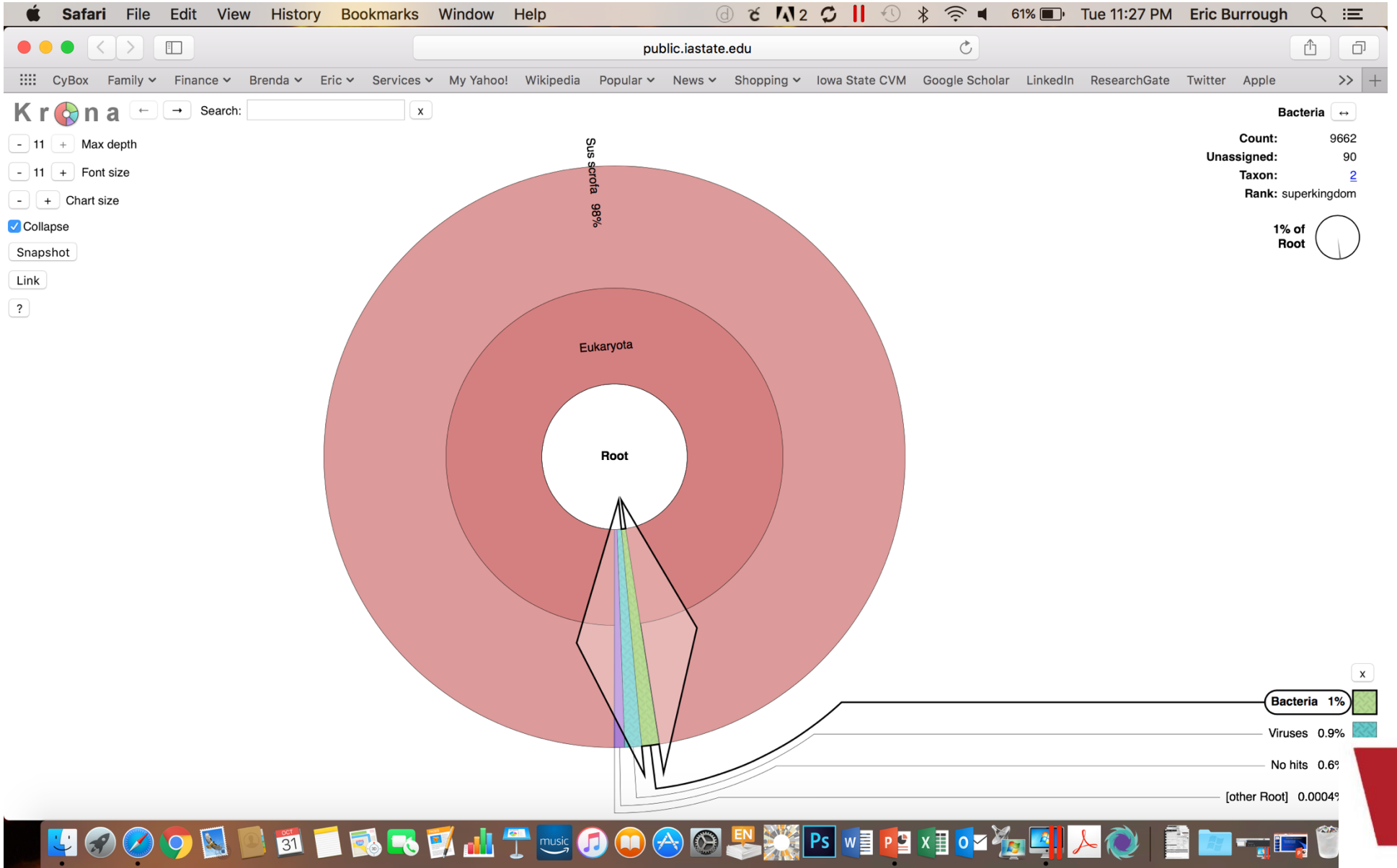

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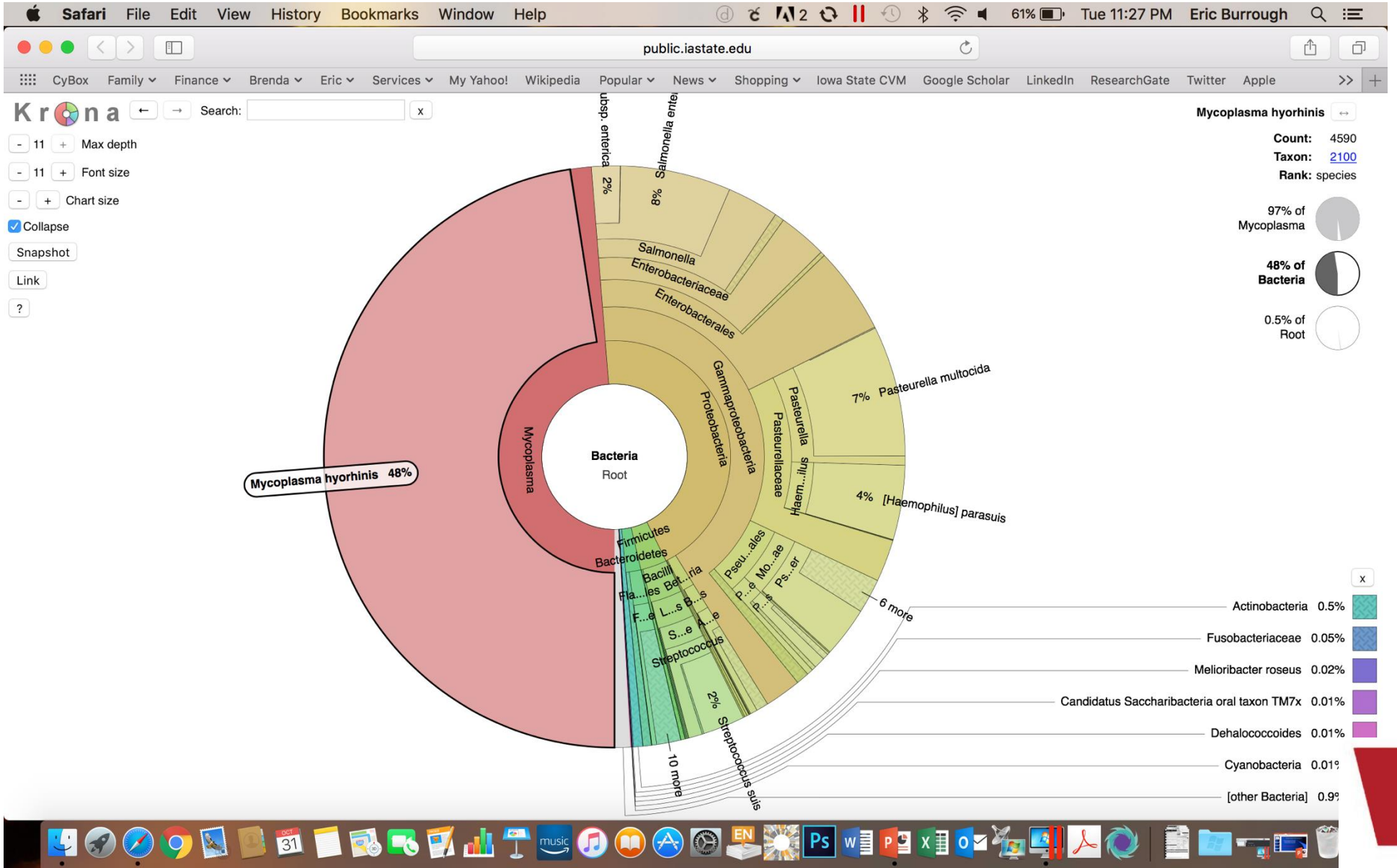

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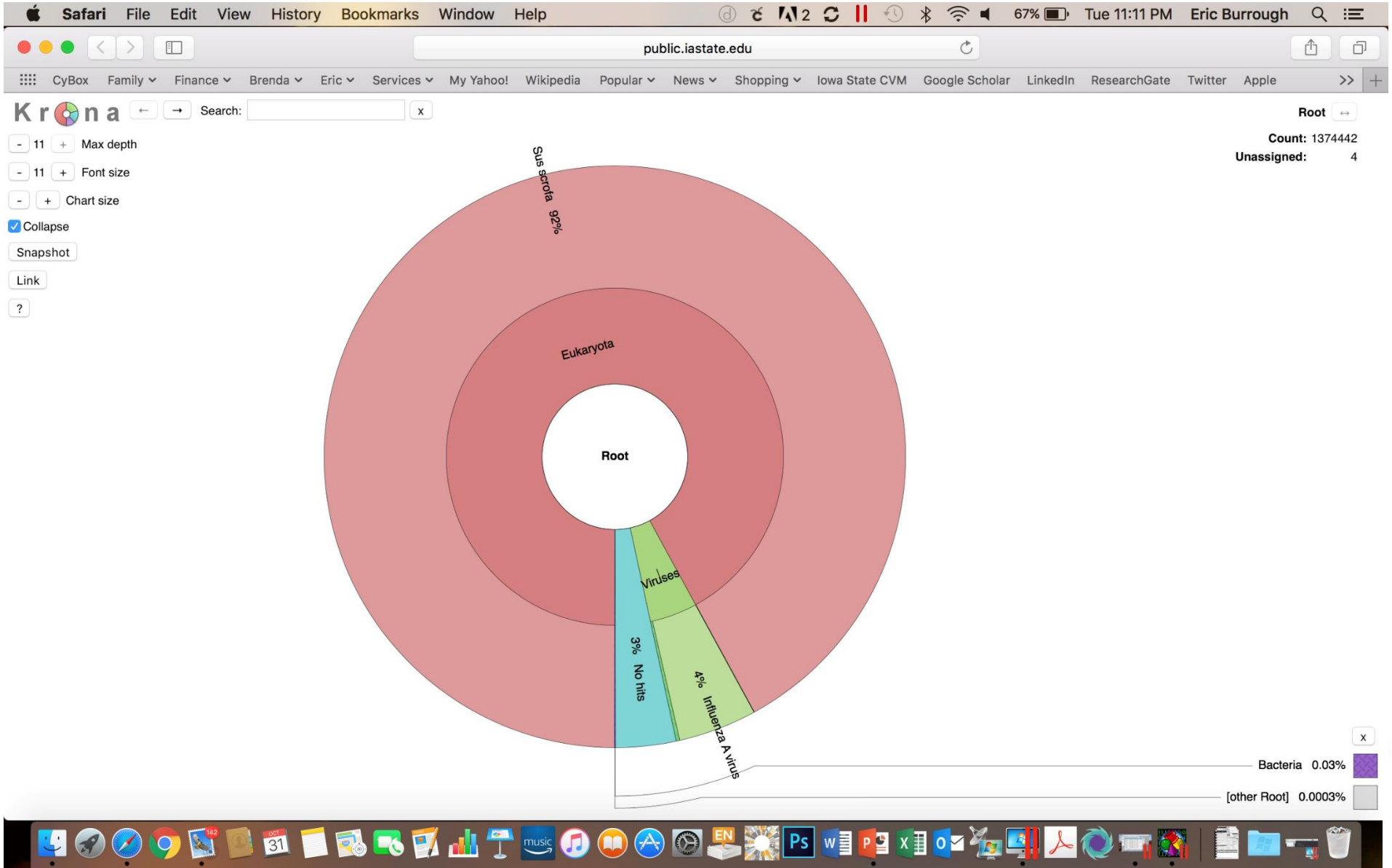
Clinical Case #1

- 6-week-old pigs, mild cough, increased fallout (10%)
  - Recent diagnostics have detected IAV by PCR at a high Ct, various systemic bacteria by culture
  - Diagnostic questions:
    - Is there an underlying viral etiology?
    - What is the contribution of isolated bacteria?
  - Dx:
    - Fibrinosuppurative polyserositis (MHR, GPS, *S. suis*)
    - PPIV1 infection
    - Other viral infections?
      - PHEV, PRRSV?
  - **This case is hypothesis generating (detection ≠ disease)**

Clinical Case #2

- 16-week-old pigs with acute cough
  - 60% of pigs affected
    - IAV suspected, looking for secondaries
  - PM: lungs mildly non-collpasing with multifocal checkerboard hemorrhage

Clinical Case #2



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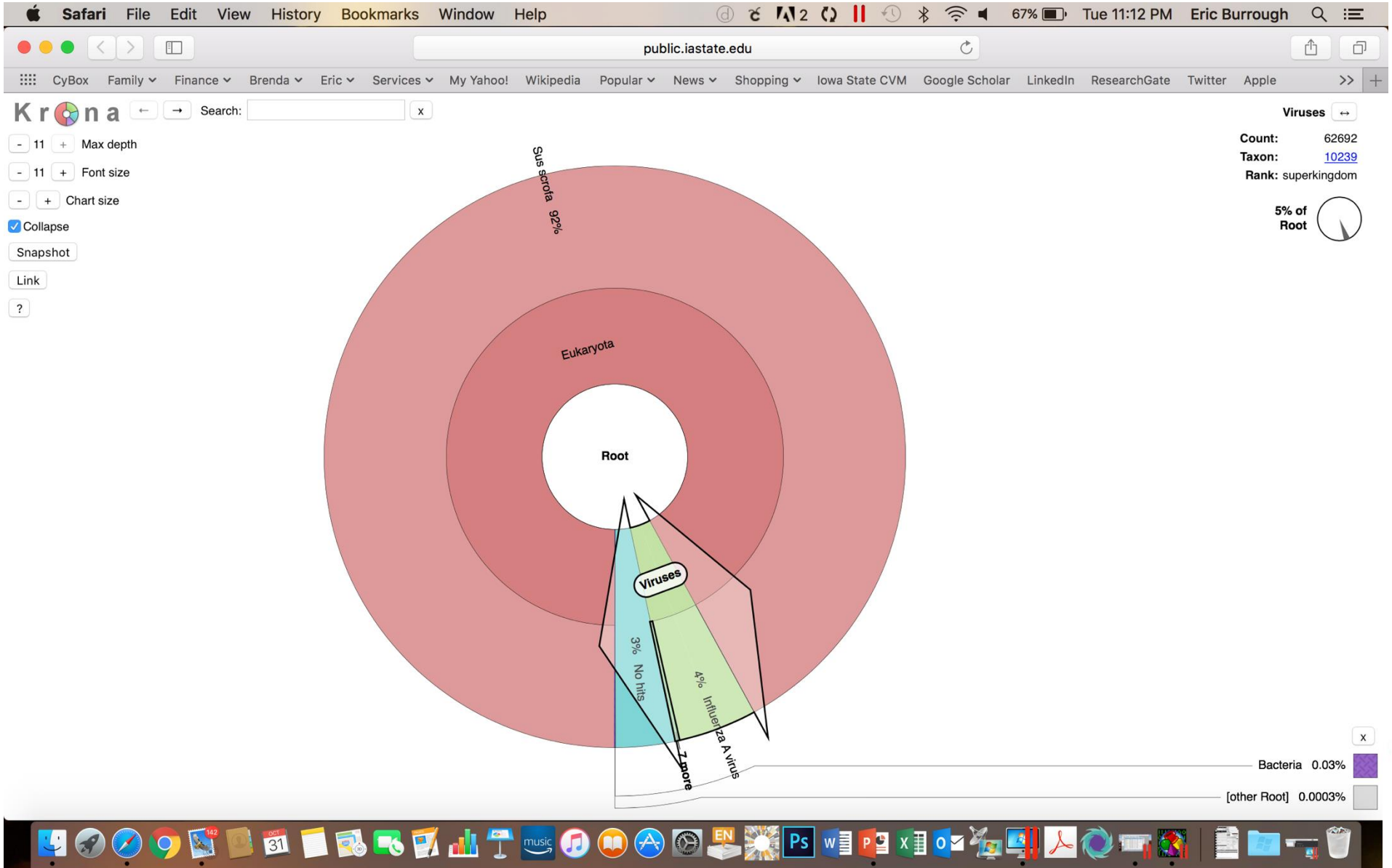

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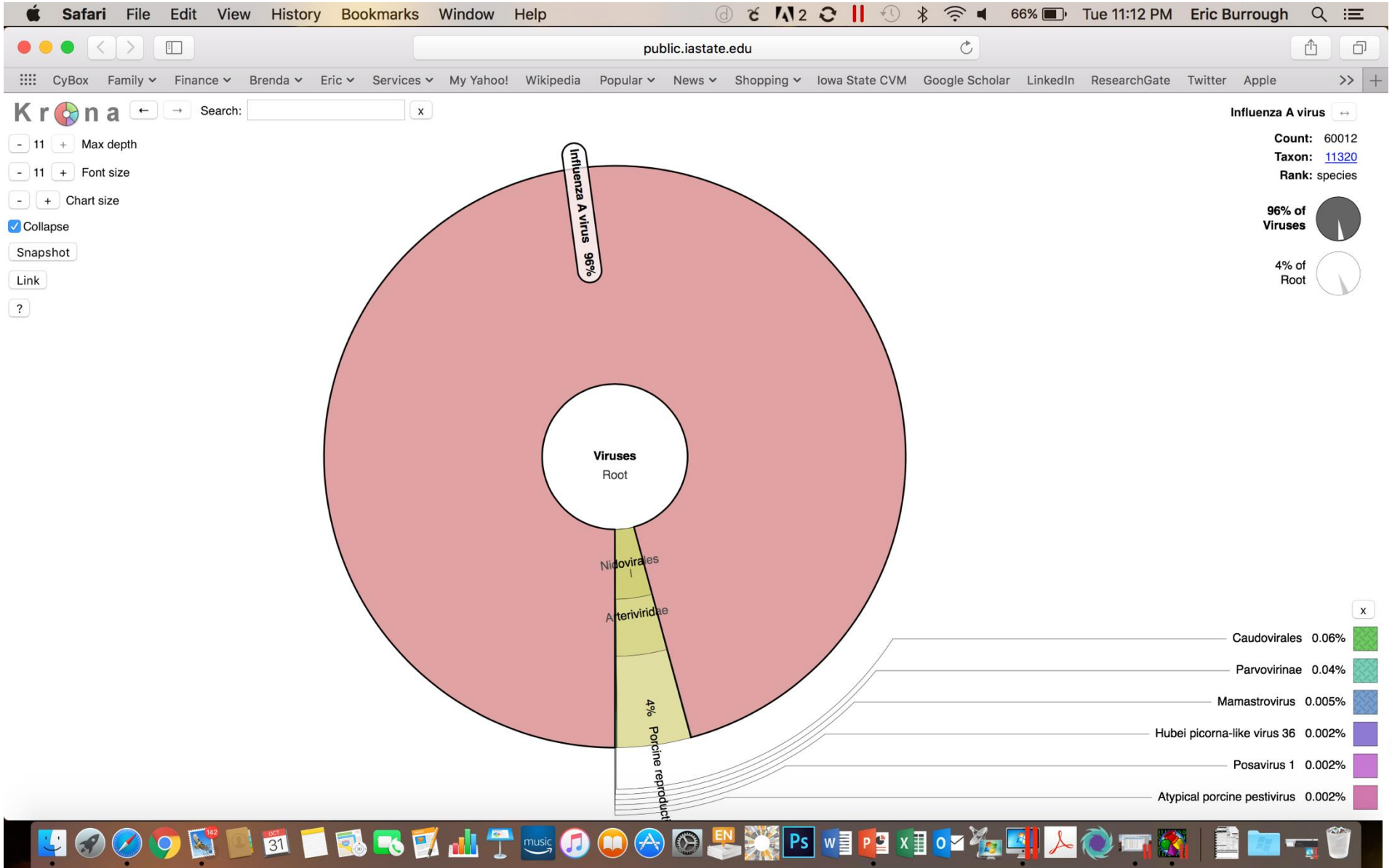
Clinical Case #2



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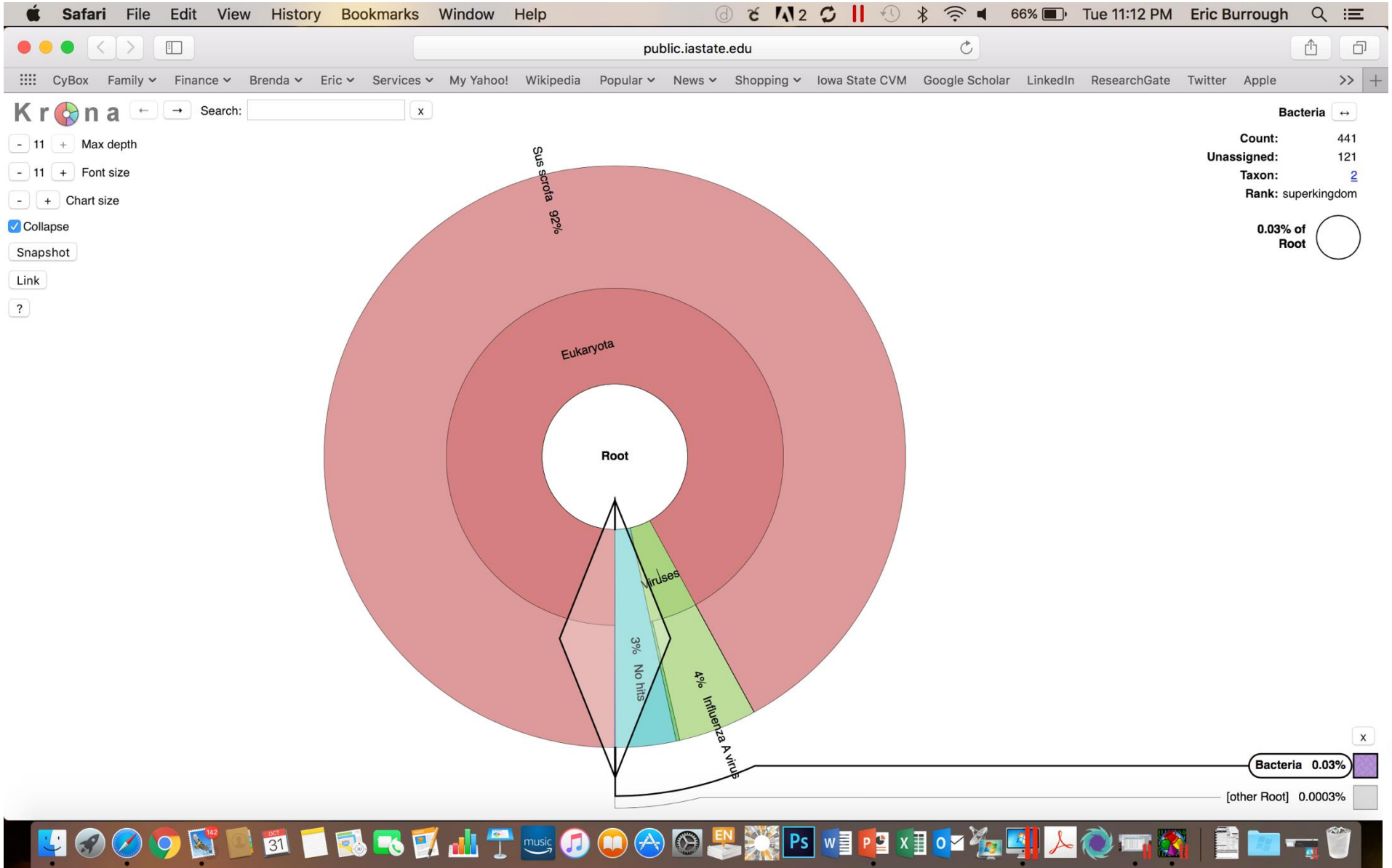

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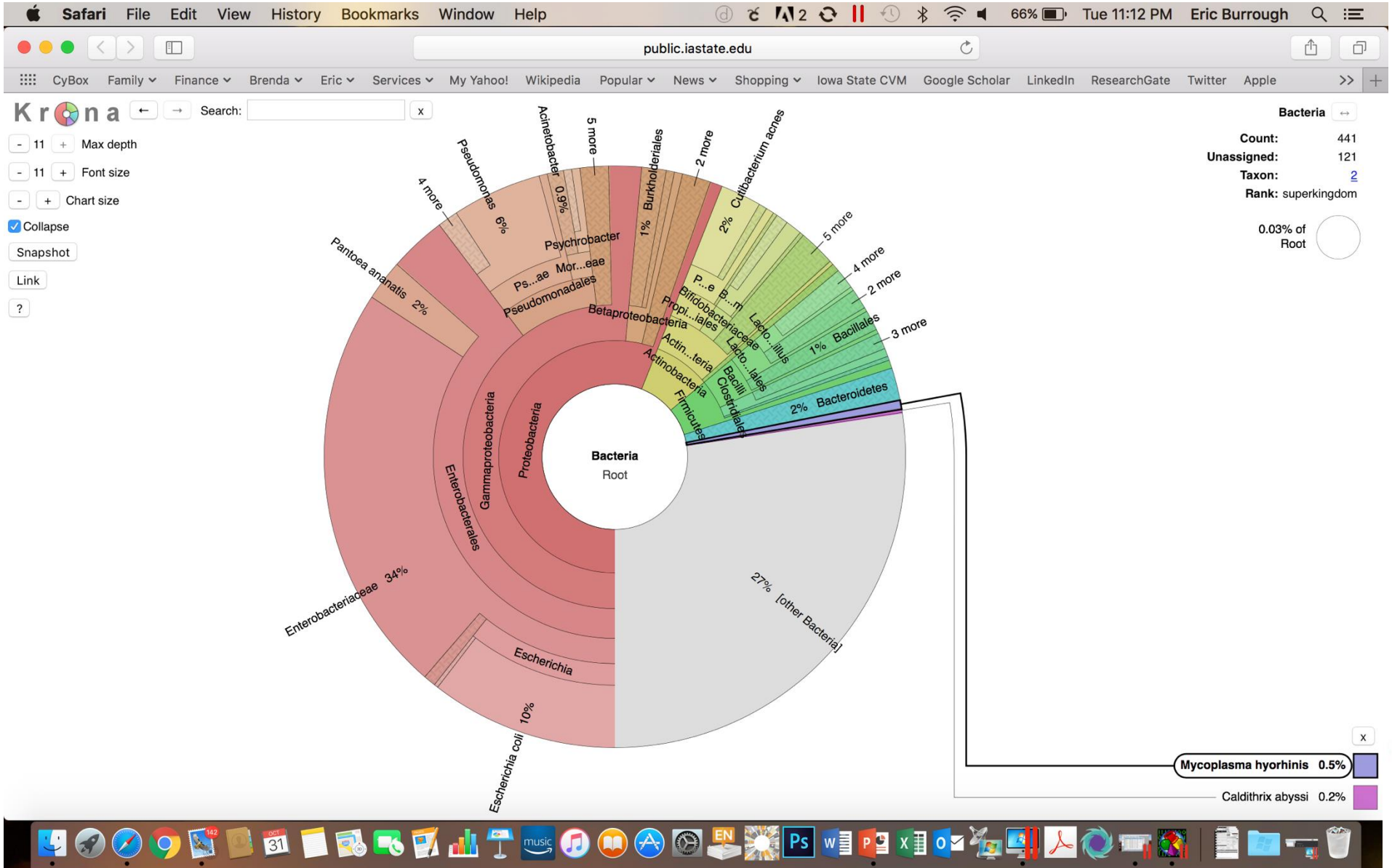
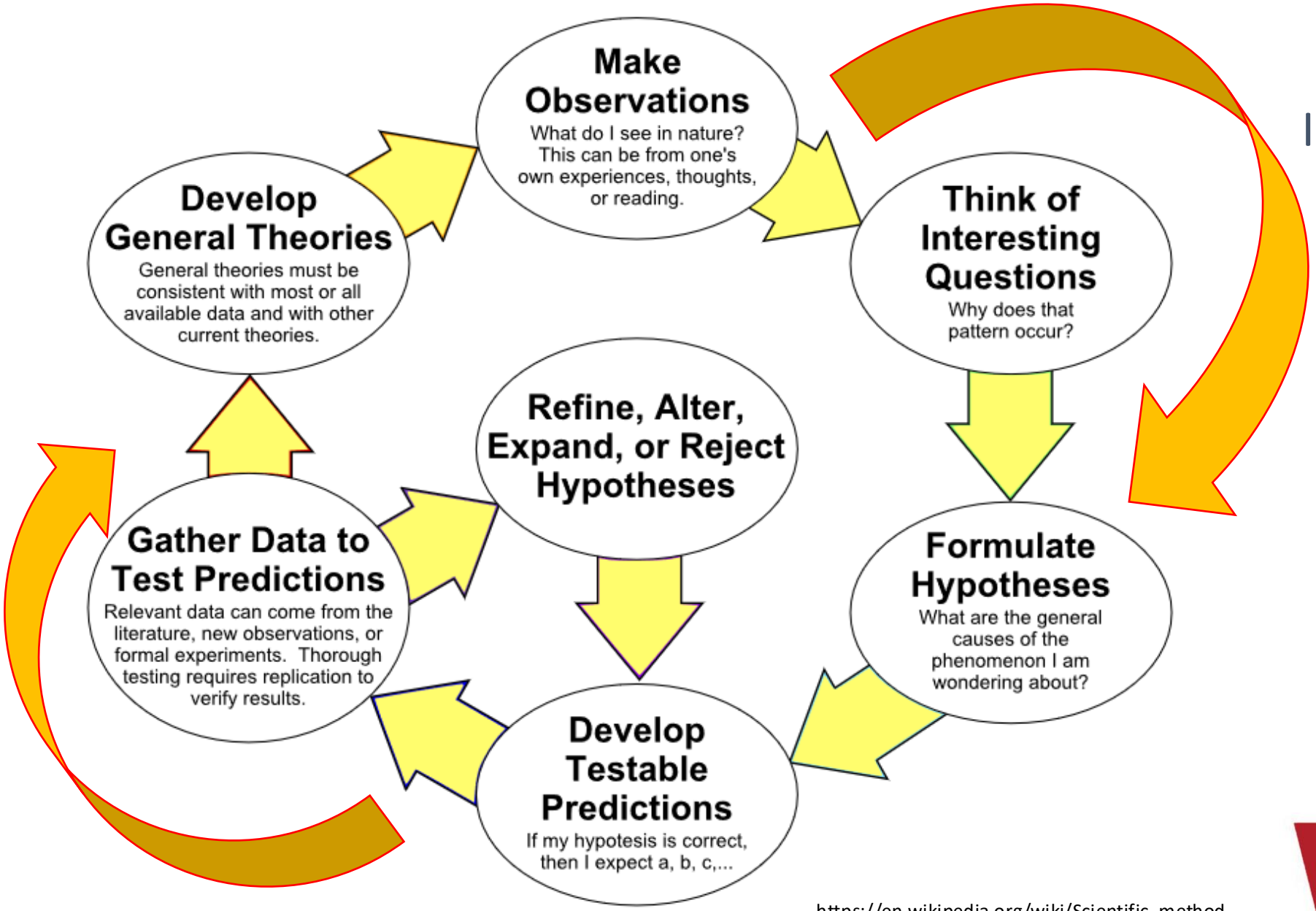


Clinical Case #2

- 16-week-old pigs with acute cough
  - 60% of pigs affected
    - IAV suspected, looking for secondaries
  - PM: lungs mildly non-collpasing with multifocal checkerboard hemorrhage
  - Histopathology:
    - Necrotizing bronchiolitis, acute

- DX:
  - Swine influenza and MLV PRRSV

The Scientific Method as an Ongoing Process



Induction

Deduction

https://en.wikipedia.org/wiki/Scientific_method

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Importance of Pathology & Direct Detection with NGS

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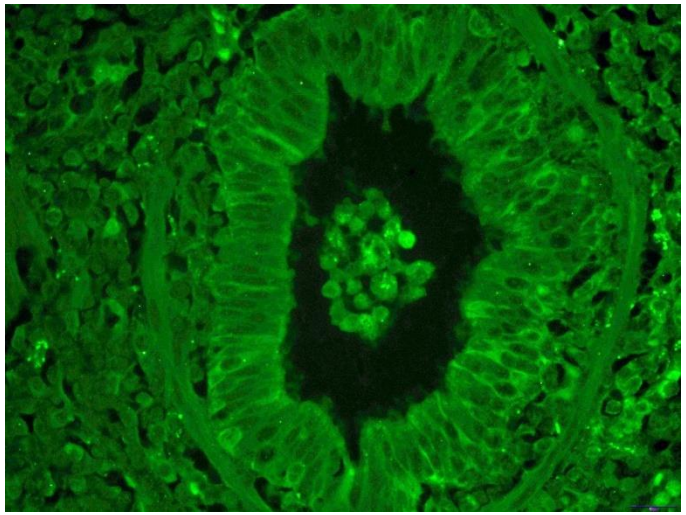
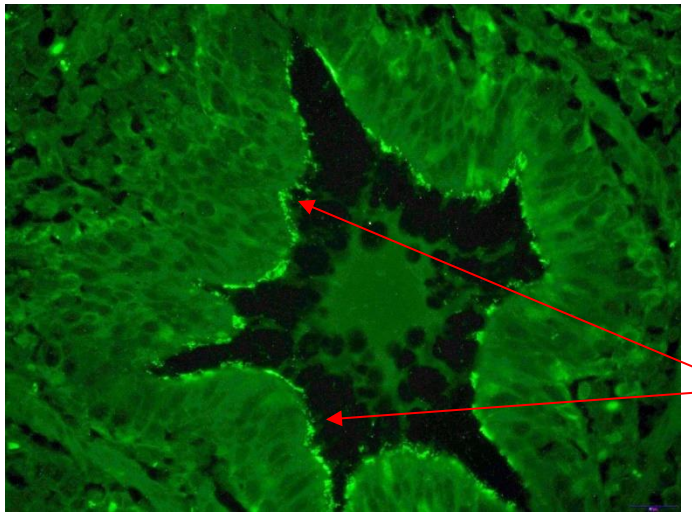
Direct Detection and NGS

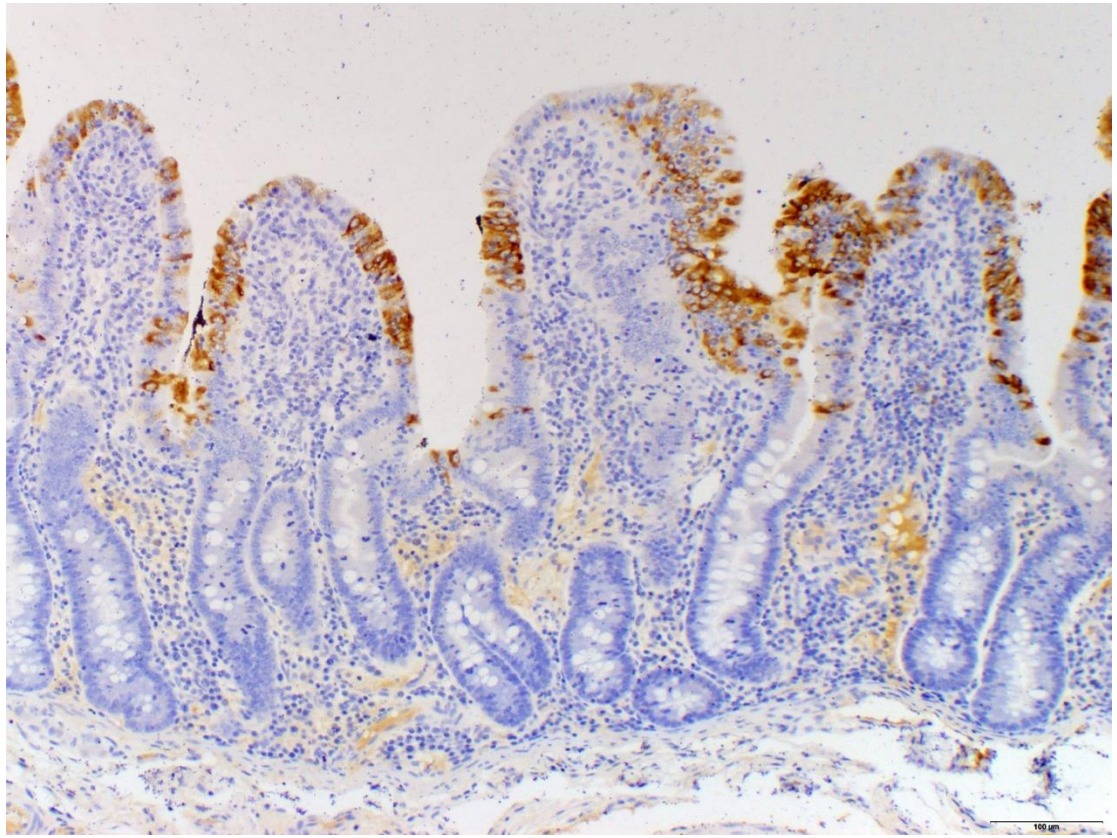
- Direct detection after NGS increases diagnostic specificity (serial approach)
  - *In situ* hybridization
    - Direct detection of pathogens with nucleic acid specificity
      - Development time can be faster then IHC as antibodies are not required (sequence-based probes)
      - Probes can be readily designed from sequences obtained via NGS
    - Fluorescent techniques allow for detection at very low levels of template



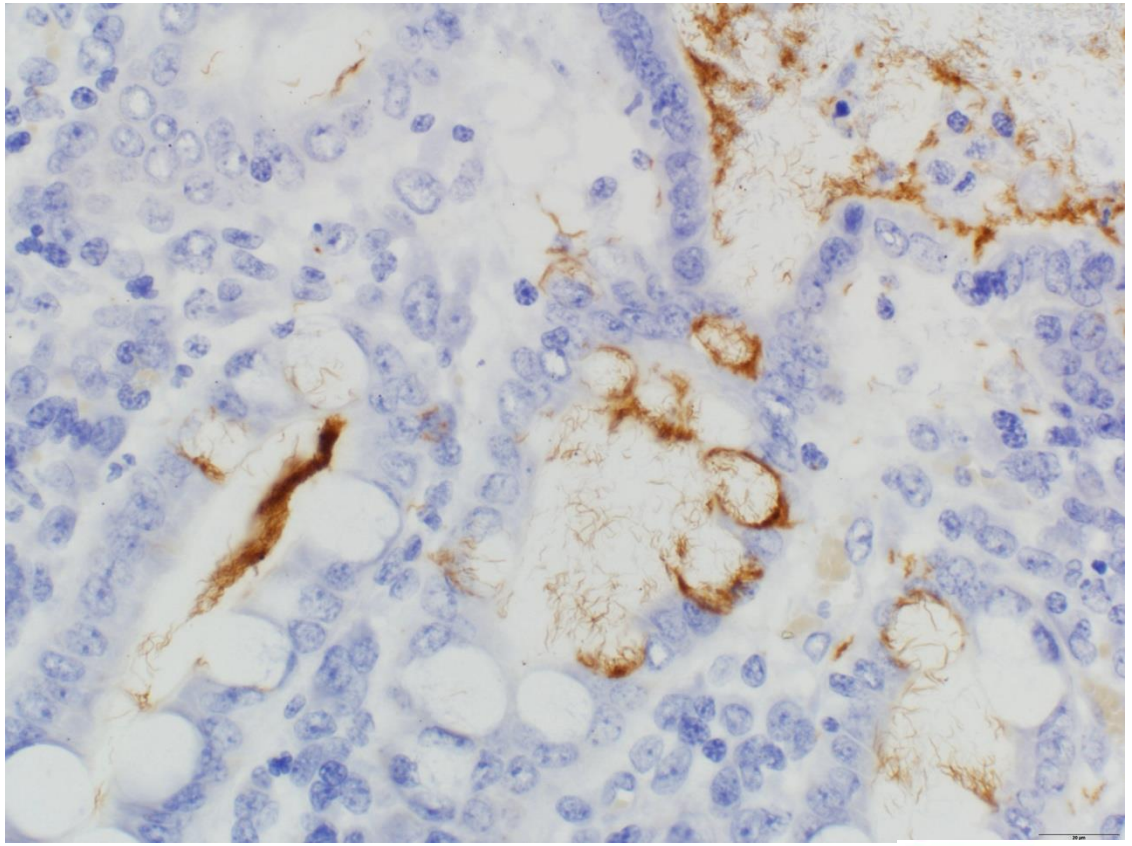


Direct Detection and NGS

- FISH converted to ISH with anti-fluorophore antibodies



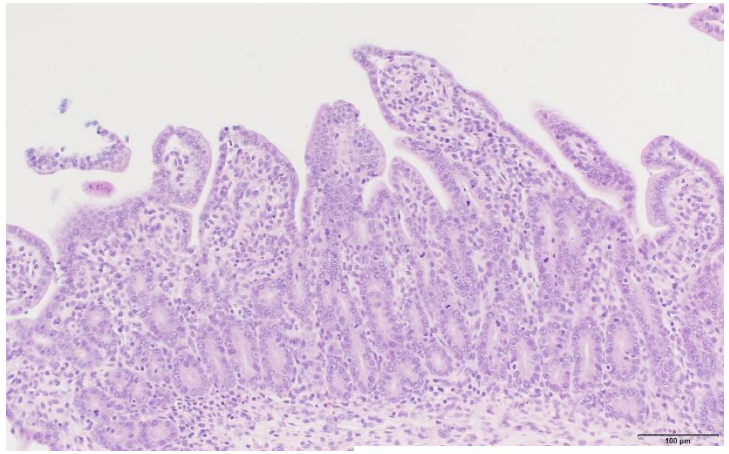
PEDV



Brachyspira hampsonii

Putting It All Together

- Diarrhea in 14-day-old pigs
  - This farm was experiencing an increased number of litters with creamy to pasty yellow diarrhea
    - Onset typically between 12 and 14 days of age
    - Diarrhea that is generally self-limiting, but affected pigs have reduced weaning weights
  - Histopathology:
    - Mild to moderate villous atrophy and fusion
      - Retraction of stroma at villus tips
      - Epithelial attenuation



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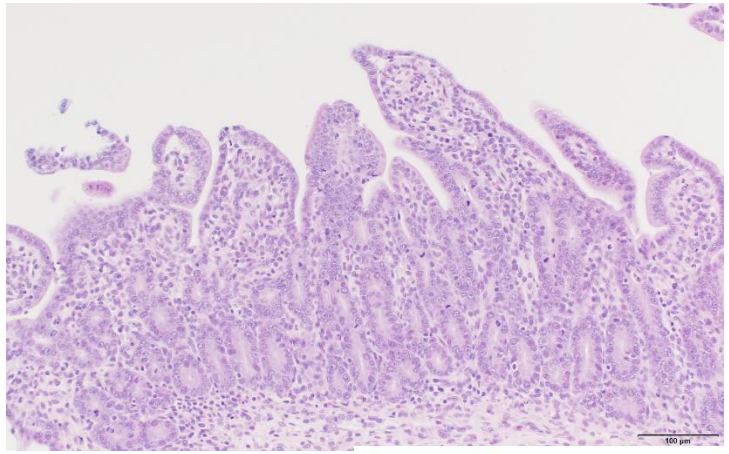

Molecular Diagnostic

PCR - Porcine Rotavirus

Animal ID	Specimen	Target Agents	Ct / Result
A Pig 1, SID #1	Feces	Rotavirus group A	>=36 / Negative
		Rotavirus group B	>=36 / Negative
		Rotavirus group C	>=36 / Negative
Comment:			
B Pig 2, SID #2	Feces	Rotavirus group A	>=36 / Negative
		Rotavirus group B	>=36 / Negative
		Rotavirus group C	>=36 / Negative

PCR - PEDV/PDCoV/TGEV Multiplex Applied Biosystems

Animal ID	Specimen	PEDV / Result	PDCoV / Result	TGEV / Result
A Pig 1, SID #1	Feces	>=36 / Negative	>=36 / Negative	>=36 / Negative
B Pig 2, SID #2	Feces	>=36 / Negative	>=36 / Negative	>=36 / Negative

- With negative PCRs and lesions suggesting a viral etiology, NGS was performed:

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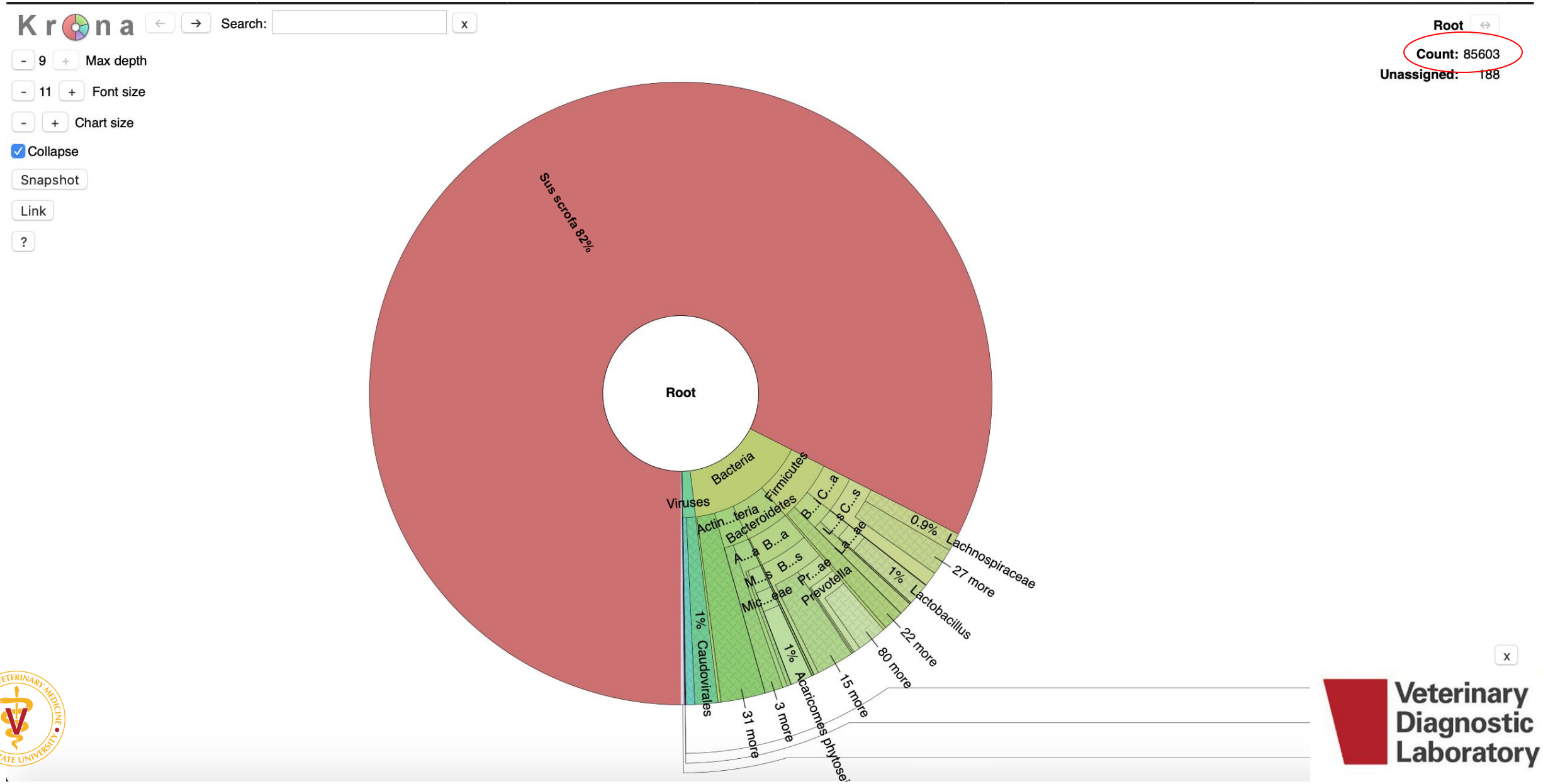
DNA Library: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Snapshot
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Root
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Unassigned: 188
Sus scrofa 82%
Root
Bacteria
Viruses
Firmicutes
Actin...teria
Bacteroidetes
B...i
C...a
L...s
C...s
L...ae
L...ae
B...a
A...a
M...s
B...s
Pr...ae
Mic...eae
Prevotella
0.9%
Lachnospiraceae
27 more
1%
Lactobacillus
22 more
80 more
15 more
1%
Caudovirales
1%
Acaricomes phytoseiuli
31 more
3 more
x

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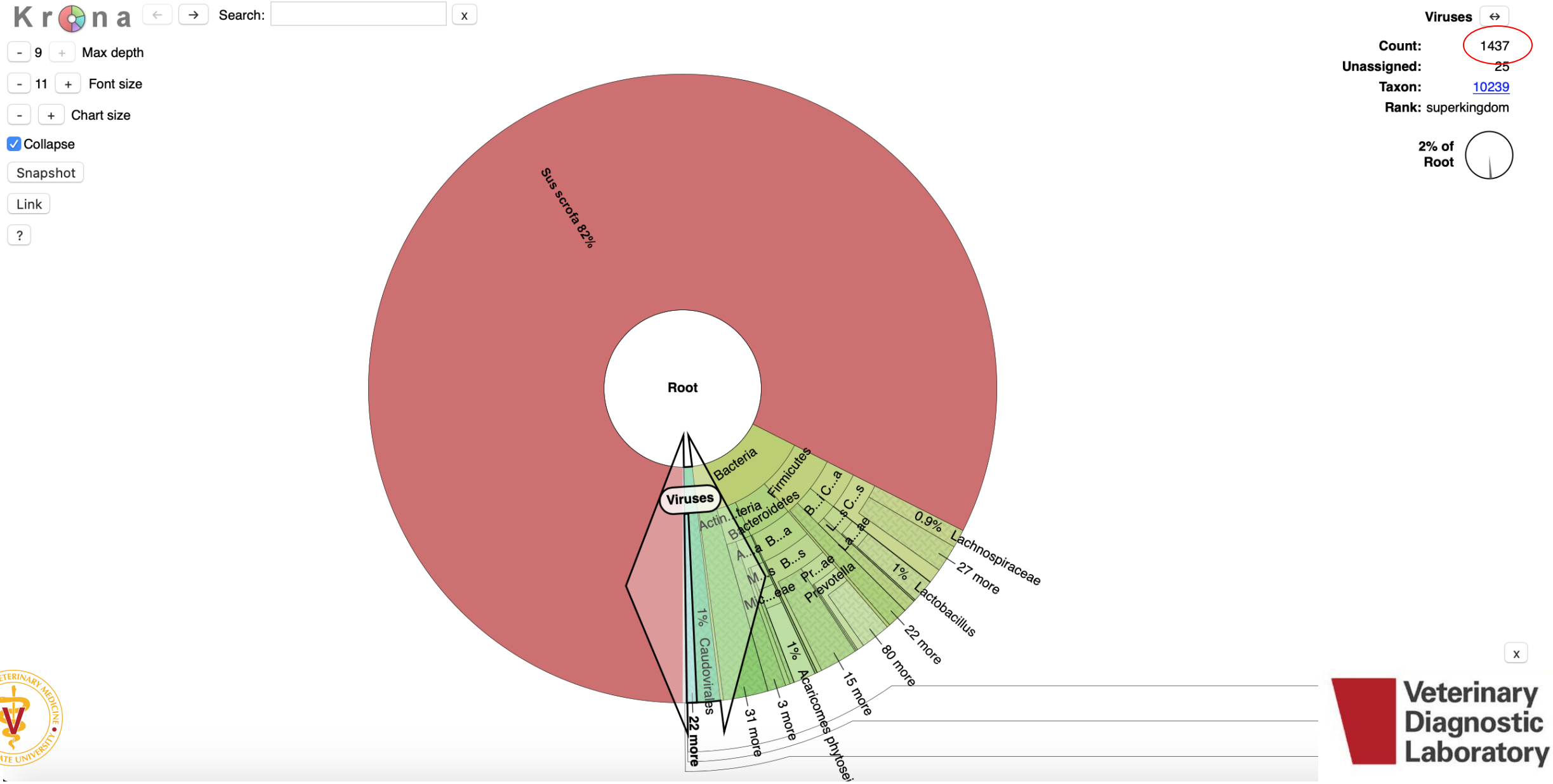

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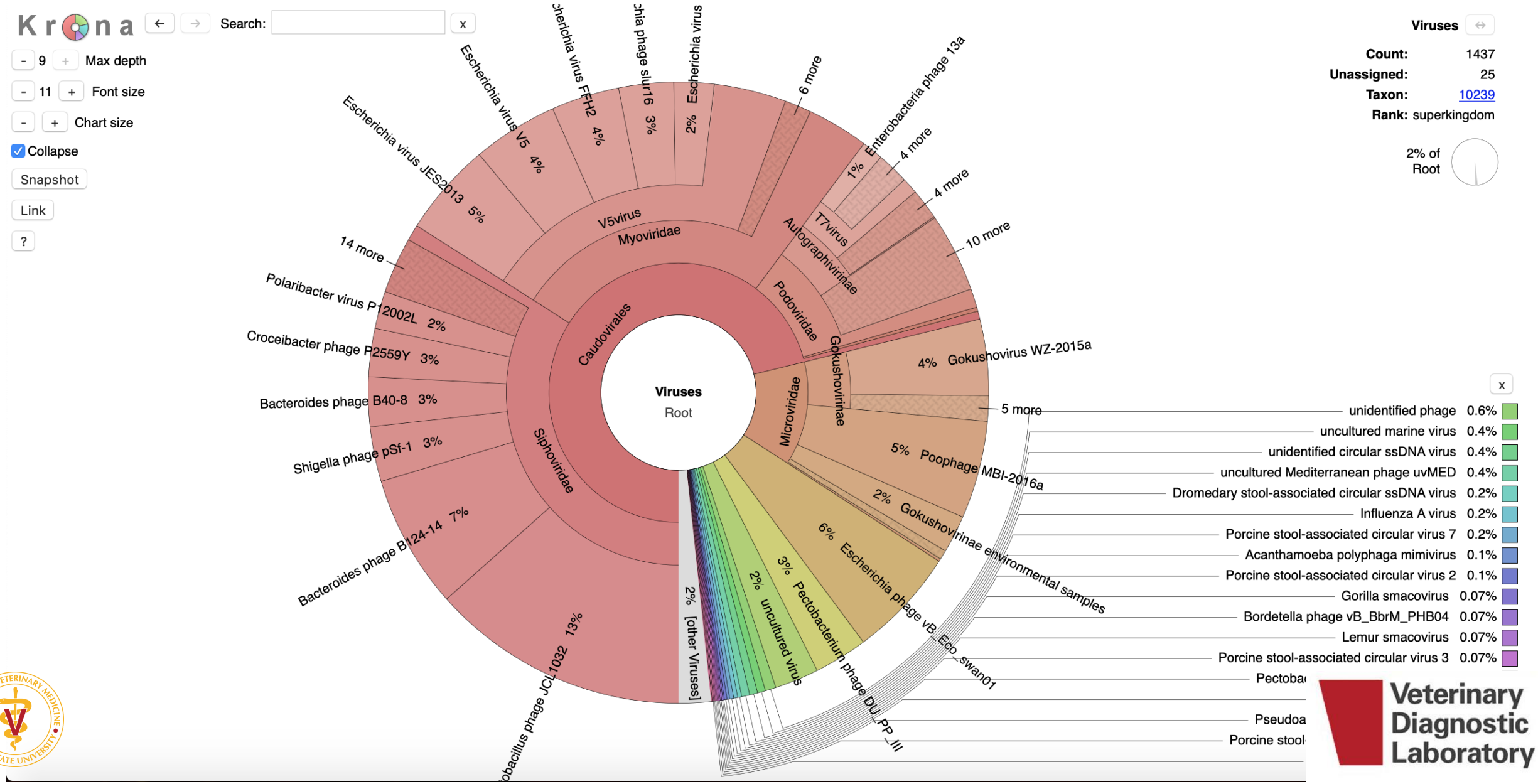

Veterinary Diagnostic Laboratory

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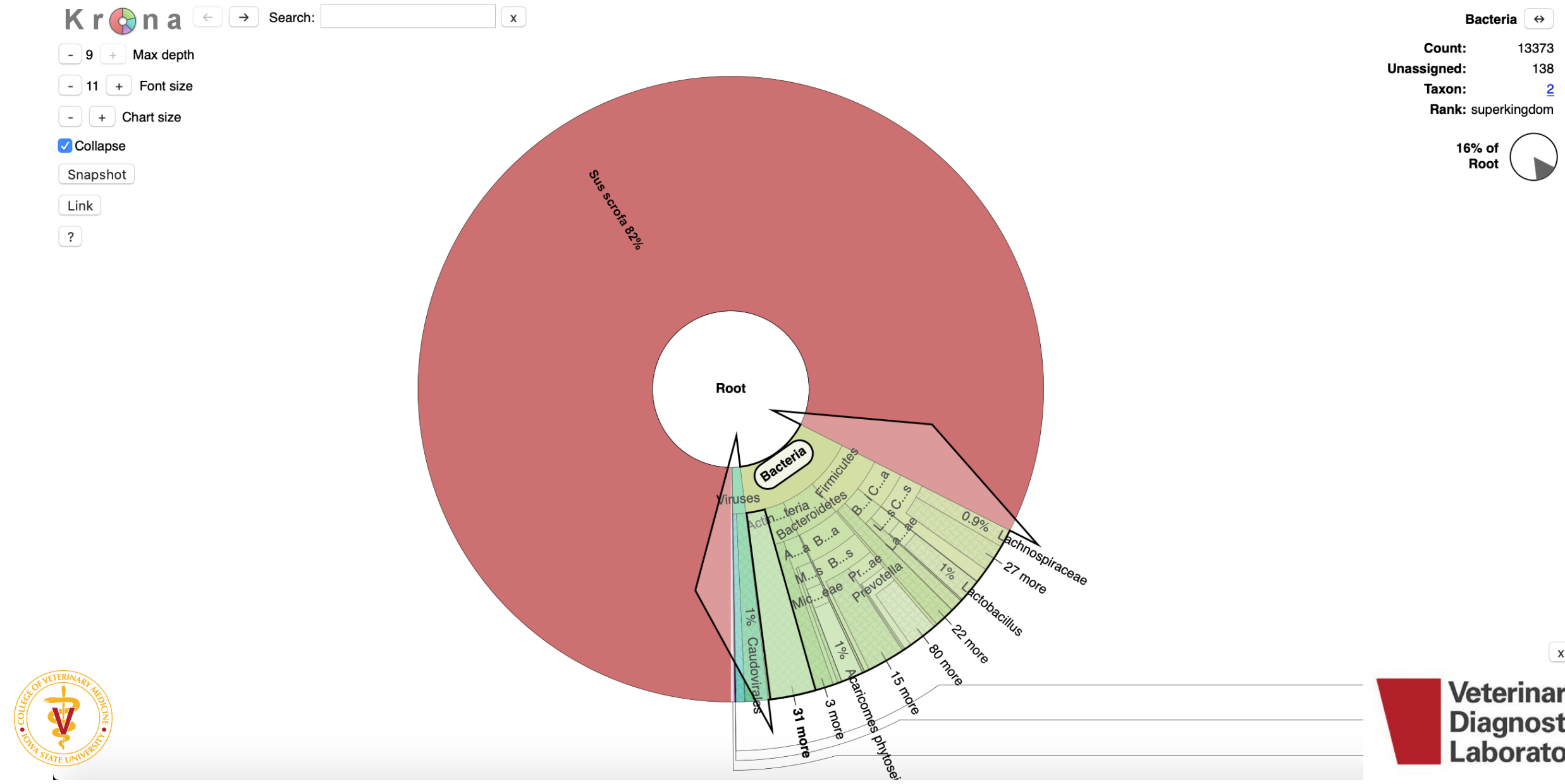
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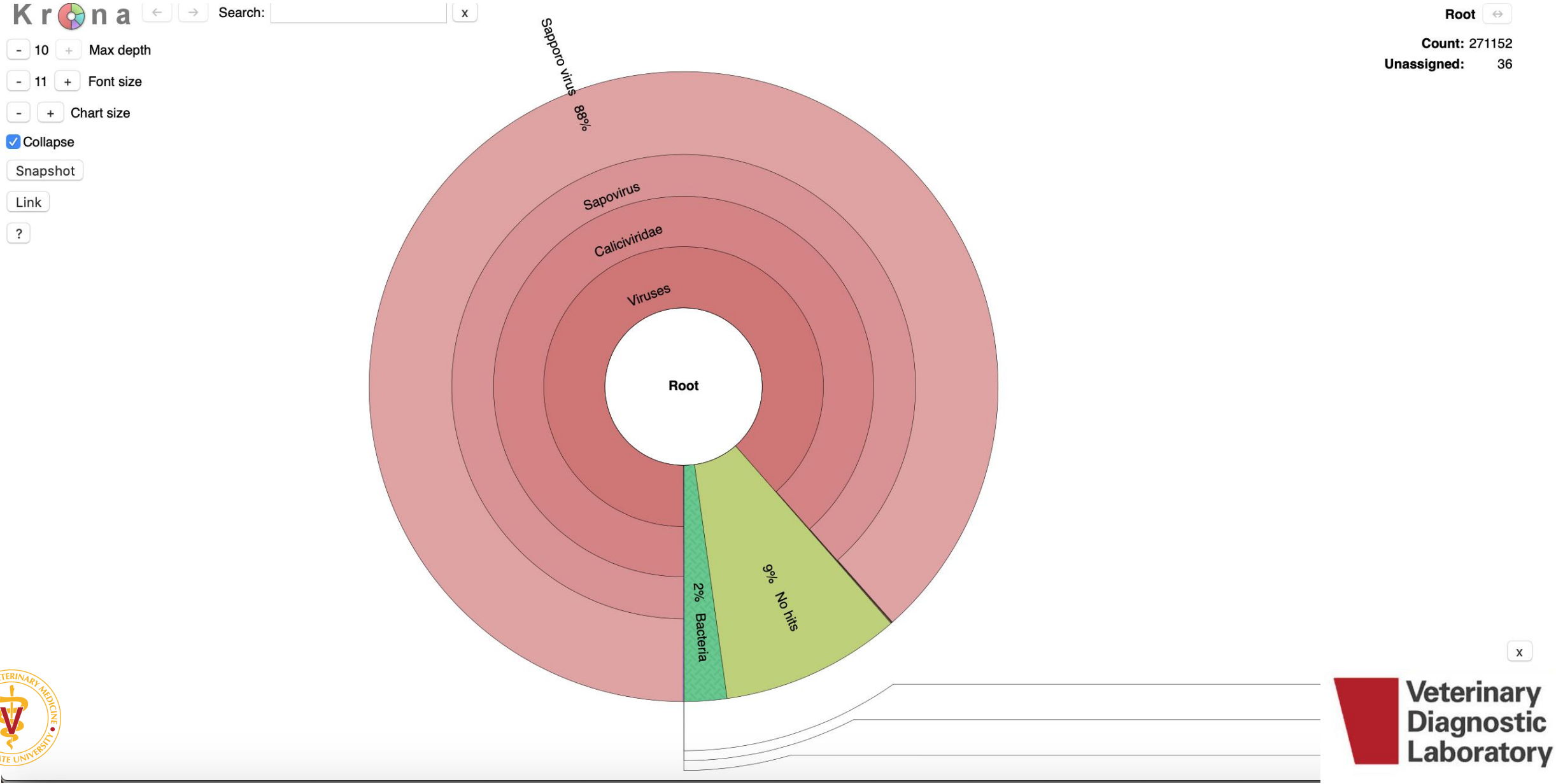
Snapshot
Link
?
Bacteria
Count: 13373
Unassigned: 138
Taxon: 2
Rank: superkingdom
16% of Root
Sus scrofa 82%
Root
Bacteria
Viruses
Firmicutes
Bacteroidetes
Acti...teria
A...a
B...a
M...s
B...s
Mic...eae
Pr...ae
Prevotella
B...i
C...a
L...s
C...s
L...ae
L...ae
0.9%
Lachnospiraceae
27 more
1%
Lactobacillus
22 more
80 more
15 more
3 more
31 more
1%
Acaricomes phytoseiuli
1%
Caudovirales
x

DNA Library:

Veterinary Diagnostic Laboratory

RNA Library: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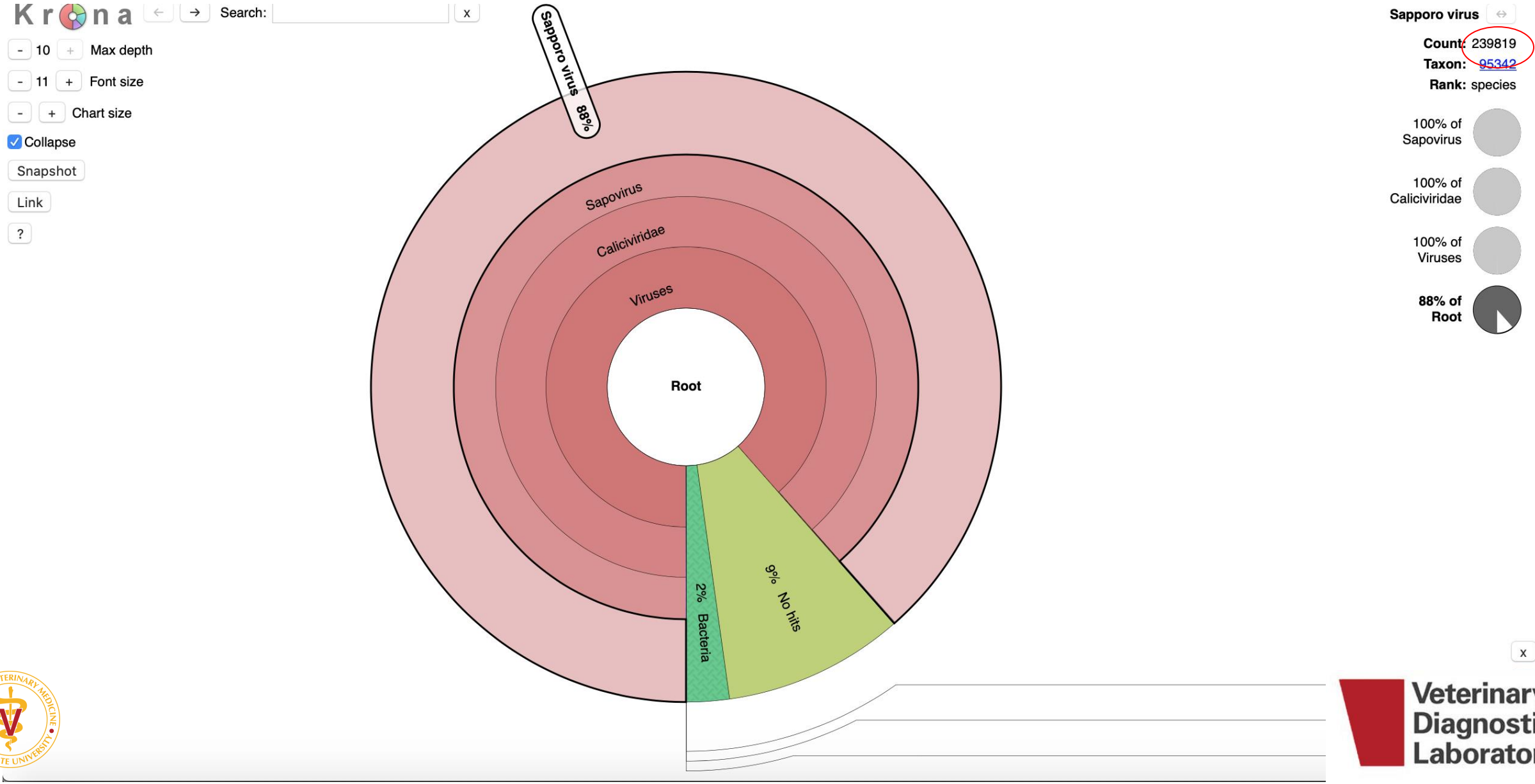
Snapshot
Link
?
Root
Count: 271152
Unassigned: 36
Sapporo virus 88%
Sapovirus
Caliciviridae
Viruses
Root
2% Bacteria
9% No hits
x

RNA Library:


Krona
Search:
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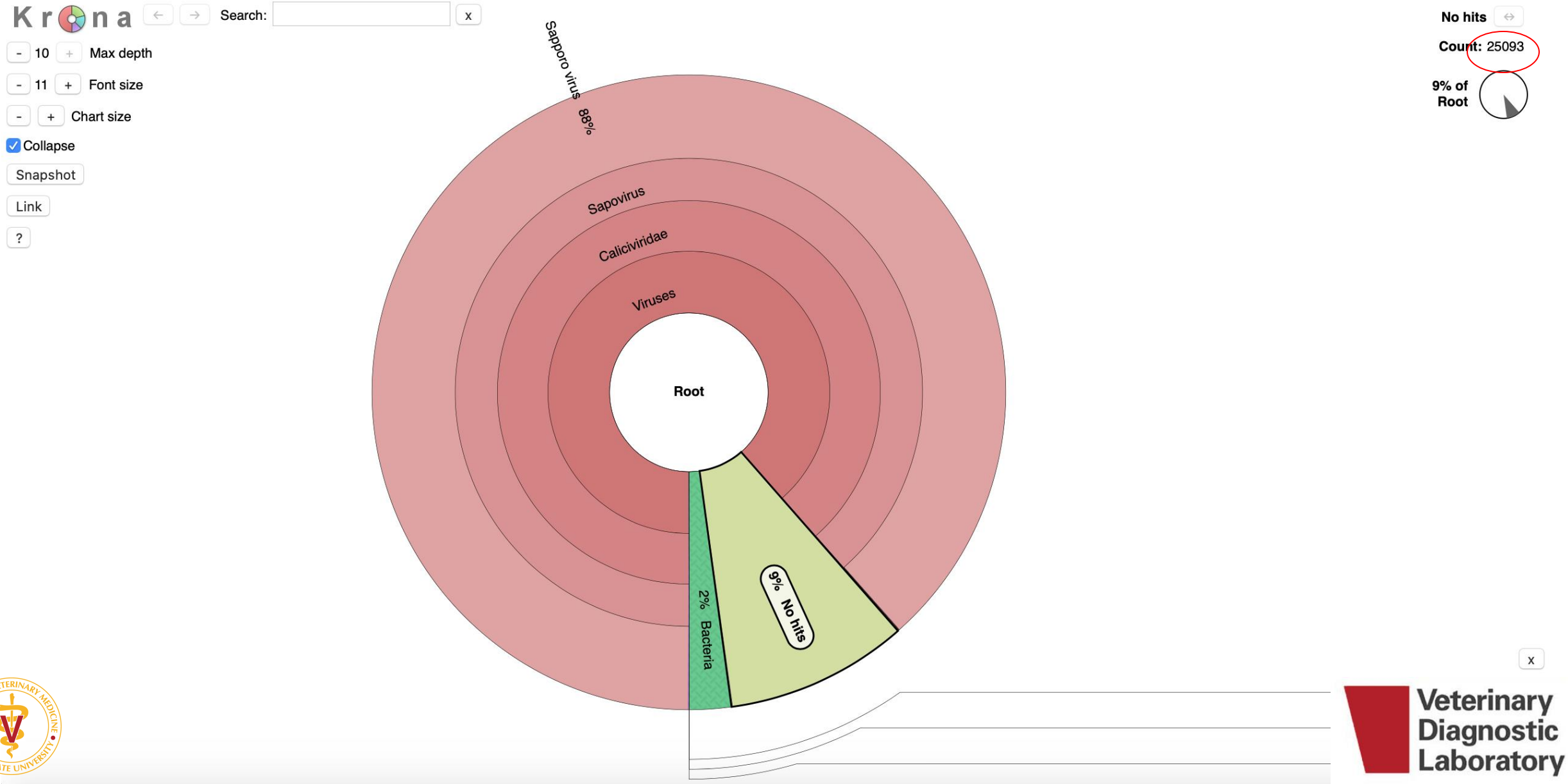
- 10 + Max depth
- 11 + Font size
- + Chart size
Collapse
Snapshot
Link
?
Sapporo virus 88%
Sapovirus
Caliciviridae
Viruses
Root
2% Bacteria
9% No hits
Sapporo virus
Count: 239819
Taxon: 95342
Rank: species
100% of Sapovirus
100% of Caliciviridae
100% of Viruses
88% of Root
x

RNA Library: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Snapshot
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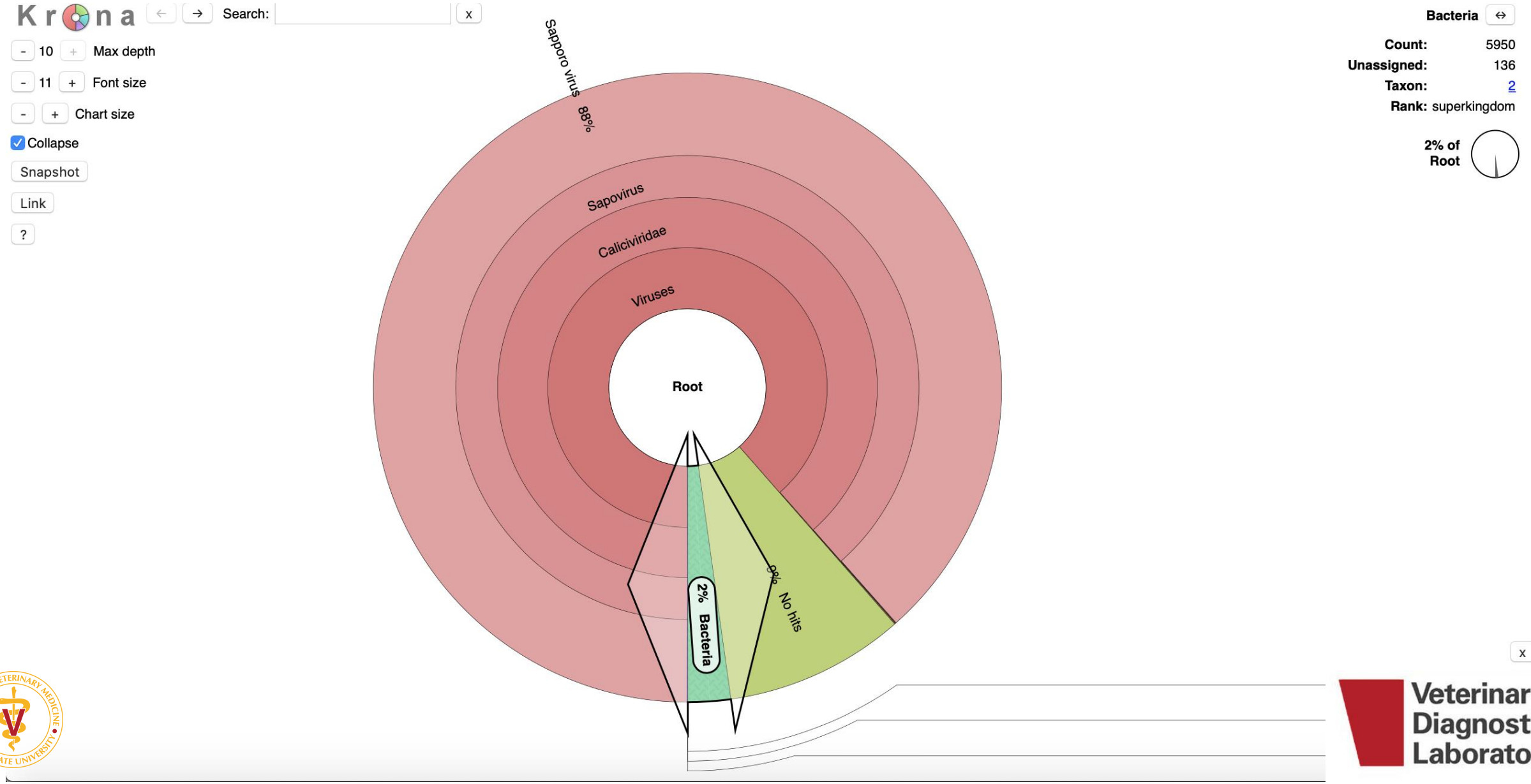
?
No hits
Count: 25093
9% of Root
Sapporo virus 88%
Sapovirus
Caliciviridae
Viruses
Root
2% Bacteria
9% No hits
x

RNA Library: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Search:
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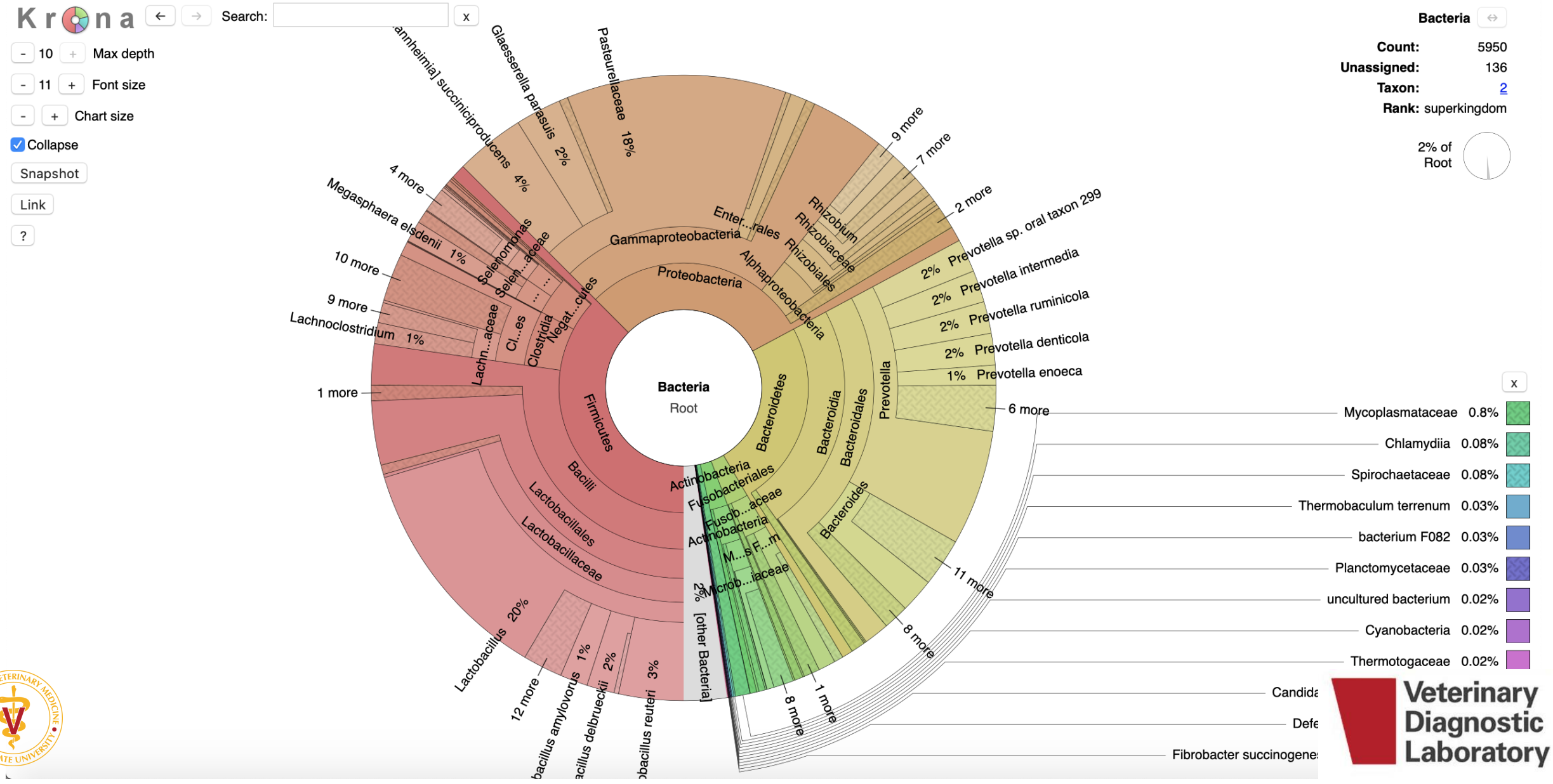
Collapse
Snapshot
Link
?
Sapporo virus 88%
Sapovirus
Caliciviridae
Viruses
Root
2% Bacteria
9% No hits
Bacteria
Count: 5950
Unassigned: 136
Taxon: 2
Rank: superkingdom
2% of Root
x

RNA Library:



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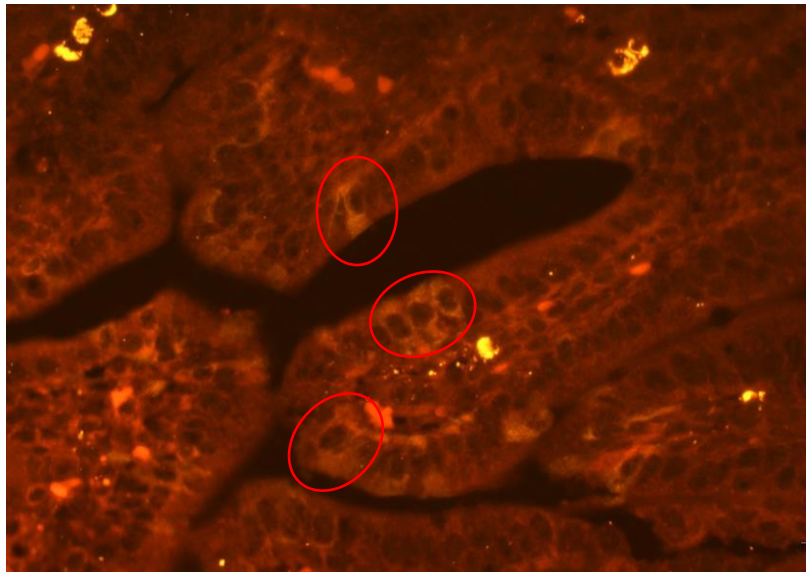
Putting It All Together

- With porcine sapovirus detected as the only known viral agent in these samples:
  - a oligonucleotide probe for *in situ* hybridization was prepared:
  - Targeting ORF1 (contains VP1 gene)
- With sapovirus now a clinically relevant etiology in suckling piglets, a direct sample PCR was also developed for clinical use.
  - When applied to the original samples in this case that had tested negative for rotaviruses and coronaviruses:



Research & Development

QA/QC testing

Animal ID	Specimen	Test	Target Agent	Result	Comment
A Pig 1, SID #1	Feces	PCR	Other	Positive	Sapovirus PCR, Ct = 14.0
B Pig 2, SID #2	Feces	PCR	Other	Positive	Sapovirus PCR, Ct = 17.7

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Putting It All Together

Received: 31 May 2019 | Revised: 19 August 2019 | Accepted: 20 August 2019
DOI: 10.1111/tbed.13337

RAPID COMMUNICATION

Transboundary and Emerging Diseases WILEY

Genetically divergent porcine sapovirus identified in pigs, United States

Leyi Wang[1] | Douglas Marthaler[2] | Richard Fredrickson[1] | Phillip C. Gauger[3] | Jianqiang Zhang[3] | Eric R. Burrough[3] | Thomas Petznick[4] | Ganwu Li[3,5]

Received: 10 February 2021 | Revised: 25 March 2021 | Accepted: 26 March 2021
DOI: 10.1111/tbed.14087

ORIGINAL ARTICLE

Transboundary and Emerging Diseases WILEY

Genetic characterization of porcine sapoviruses identified from pigs during a diarrhoea outbreak in Iowa, 2019

Huigang Shen[1] | Jianfeng Zhang[1,2] | Phillip C. Gauger[1] | Eric R Burrough[1] | Jianqiang Zhang[1] | Karen Harmon | Leyi Wang[3] | Ying Zheng[1] | Thomas Petznick[4] | Ganwu Li[1]

College of Veterinary Medicine, Iowa State University

Veterinary Diagnostic Laboratory

NGS for Detailed Pathogen Characterization

Veterinary Diagnostic Laboratory

NGS for Viral Characterization

RESEARCH

Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus Infection in Domestic Dairy Cattle and Cats, United States, 2024

Eric R. Burrough, Drew R. Magstadt, Barbara Petersen, Simon J. Timmermans, Phillip C. Gauger, Jianqiang Zhang, Chris Siepker, Marta Mainenti, Ganwu Li, Alexis C. Thompson, Patrick J. Gorden, Paul J. Plummer, Rodger Main

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 30, No. 7, July 2024

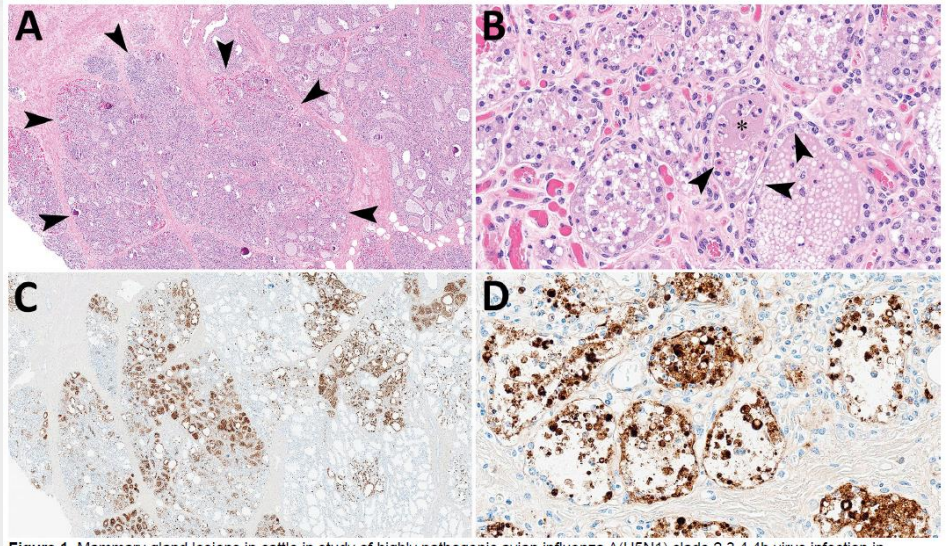

Figure 1. Mammary gland lesions in cattle in study of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus infection in domestic dairy cattle and cats, United States, 2024. A, B) Mammary gland tissue sections stained with hematoxylin and eosin. A) Arrowheads indicate segmental loss within open secretory mammary alveoli. Original magnification ×40. B) Arrowheads indicate epithelial degeneration and necrosis lining alveoli with intraluminal sloughing. Asterisk indicates intraluminal neutrophilic inflammation. Original magnification ×400. C, D) Mammary gland tissue sections stained by using avian influenza A immunohistochemistry. C) Brown staining indicates lobular distribution of avian influenza A virus. Original magnification ×40. D) Brown staining indicates strong nuclear and intracytoplasmic immunoreactivity of intact and sloughed epithelial cells within mammary alveoli. Original magnification ×400.

Emerging Microbes & Infections
2024, VOL. 13, 2380421 (8 pages)
https://doi.org/10.1080/22221751.2024.2380421

EMi Taylor & Francis Taylor & Francis Group

OPEN ACCESS Check for updates

Genomic characterization of highly pathogenic avian influenza A H5N1 virus newly emerged in dairy cattle

Xiao Hu, Anugrah Saxena, Drew R. Magstadt, Phillip C. Gauger, Eric R. Burrough, Jianqiang Zhang, Chris Siepker, Marta Mainenti, Patrick J. Gorden, Paul J. Plummer and Ganwu Li

Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ABSTRACT
In March 2024, the emergence of highly pathogenic avian influenza (HPAI) A (H5N1) infections in dairy cattle was detected in the United Sates for the first time. We genetically characterize HPAI viruses from dairy cattle showing an abrupt drop in milk production, as well as from two cats, six wild birds, and one skunk. They share nearly identical genome sequences, forming a new genotype B3.13 within the 2.3.4.4b clade. B3.13 viruses underwent two reassortment events since 2023 and exhibit critical mutations in HA, M1, and NS genes but lack critical mutations in PB2 and PB1 genes, which enhance virulence or adaptation to mammals. The PB2 E627 K mutation in a human case associated with cattle underscores the potential for rapid evolution post infection, highlighting the need for continued surveillance to monitor public health threats.

ARTICLE HISTORY Received 6 June 2024; Revised 8 July 2024; Accepted 10 July 2024

KEYWORDS Highly pathogenic avian influenza (HPAI); H5N1; clade 2.3.4.4b; dairy cattle; reassortment events; genome sequence

College of Veterinary Medicine • Iowa State University

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NGS for Viral Characterization

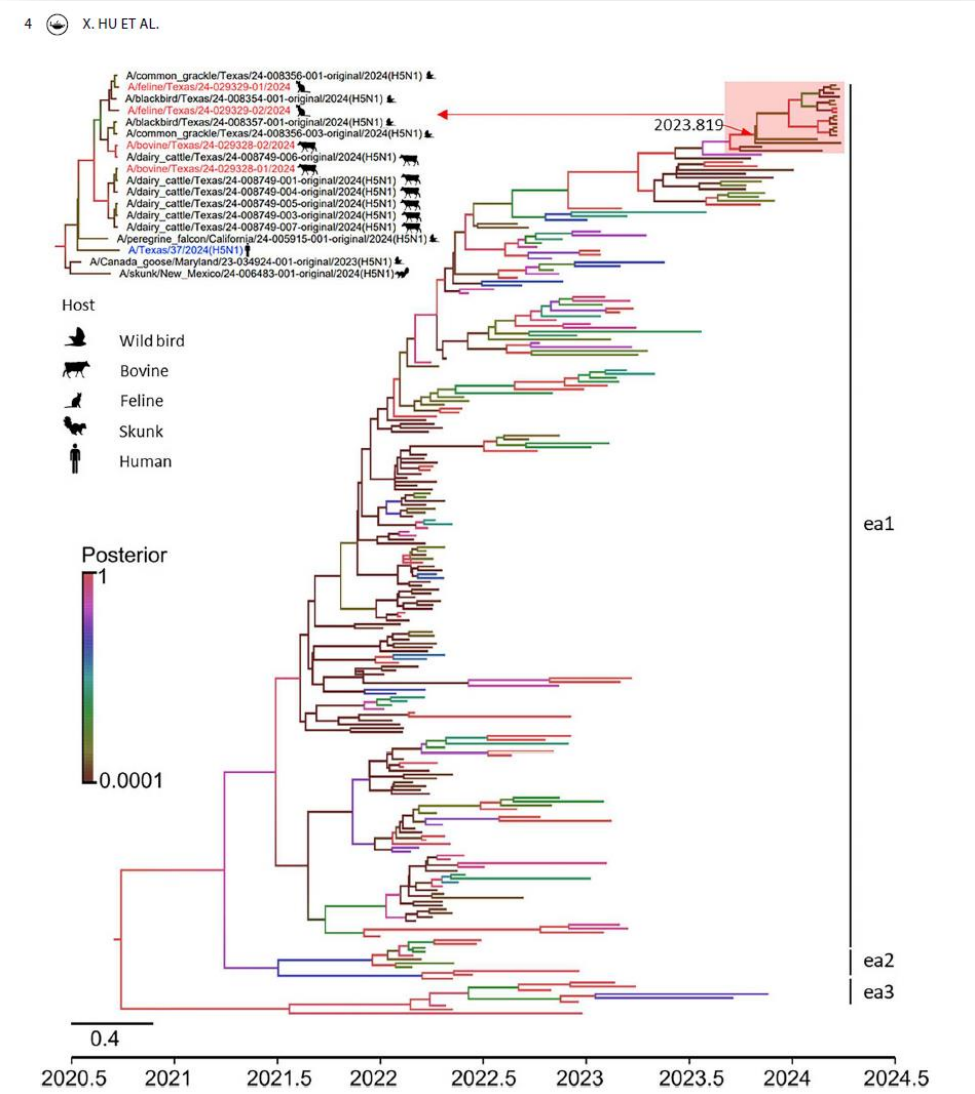


Figure 1. MCC tree of the HA genes of clade 2.3.4.4b H5N1 viruses in the United States since 2021. The MCC tree is constructed by using BEAST v1.10.4 software package. Each branch is coloured using posterior probability. The red frame represents H5N1 of Texas in 2024. The H5N1 viruses isolated in this study are shown in red, human isolate is shown in blue.

NGS for Bacterial Characterization

Bacterial pathogens regularly sequenced at ISU VDL:

- ***Streptococcus suis***
- *Streptococcus zooepidemicus*
- ***Glaesserella parasuis* (GPS)**
- ***Brachyspira hyodysenteriae***
- *Actinobacillus pleuropneumoniae (APP)*
- *Actinobacillus suis*
- *Mycoplasma hyopneumoniae*
- *Mycoplasma hyorhinis*
- *Mycoplasma hyosynoviae*
- *Salmonella*
- ***E. coli* (Enterotoxigenic *E. coli*)**
- *Clostridium perfringens*

NGS analyses:

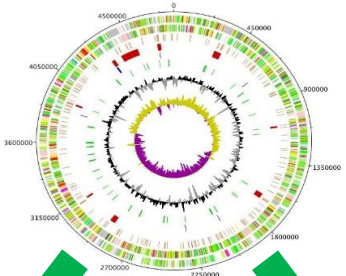
- Bacterial species identification
- Phylogenetic analysis
- Virulence gene identification
- Resistance gene detection
- Serotyping
- MLST typing

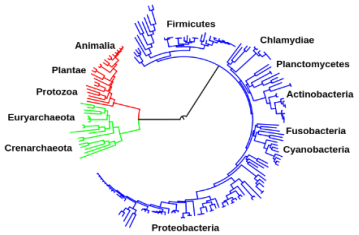
Compare two isolates:

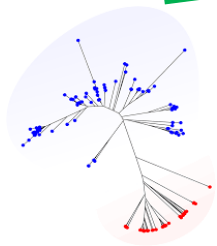
- Average nucleotide identity
- Deletions and insertions
- Pathogenicity islands

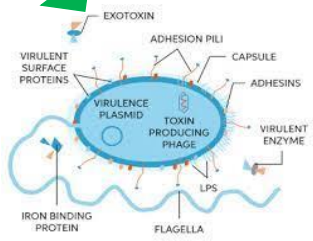
NGS for Bacterial Characterization

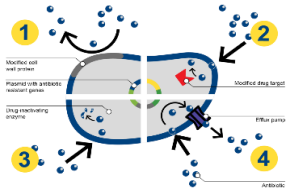
Sequencing of Isolates: ‘All in One Technology’











Bacterial species ID | Genotype and evolution | Virulence genes | Resistance genes

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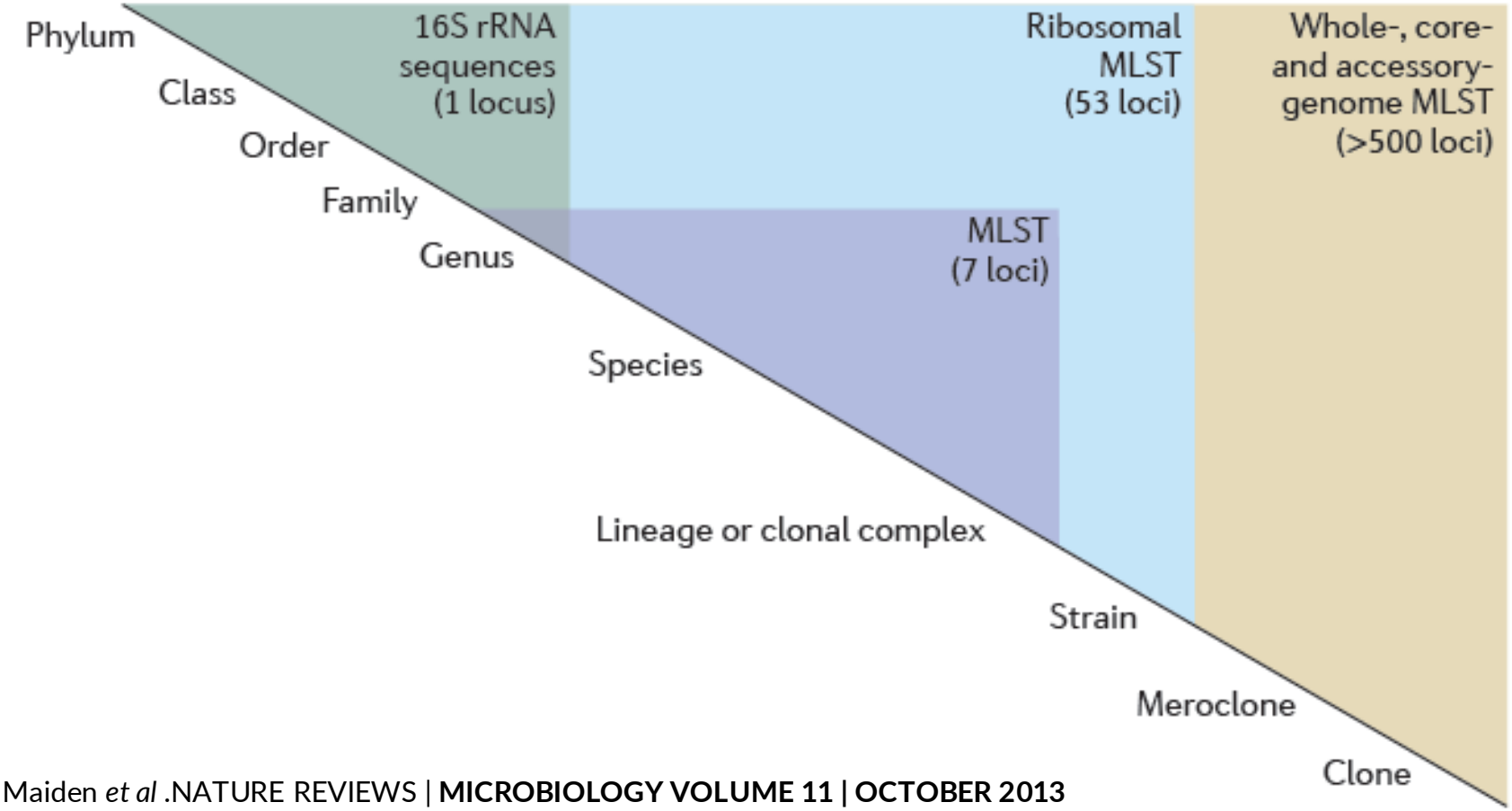
NGS for Bacterial Characterization

Relating Sequence Data to Nomenclature Schemes



Maiden *et al* .NATURE REVIEWS | **MICROBIOLOGY VOLUME 11 | OCTOBER 2013**

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NGS for Bacterial Characterization

Examples of NGS Clinical Applications

- As part of a routine surveillance platform
  - Virulence gene profiling of enterotoxigenic *E. coli* (ETEC)
  - Autogenous vaccine strain selection:
    - *Streptococcus suis*
    - *Glaesserella parasuis*

- Outbreak or epidemiological investigation
  - WGS analysis of *Streptococcus equi zooepidemicus* associated with high mortalities
  - Temporal comparison of *Brachyspira hyodysenteriae* before and after mitigation

NGS for Bacterial Characterization

Brief Communication

Cases of high mortality in cull sows and feeder pigs associated with *Streptococcus equi* subsp. *zooepidemicus* septicemia

Panchan Sitthicharoenchai, Rachel Derscheid, Kent Schwartz, Nubia Macedo, Orhan Sahin, Xuhua Chen, Ganwu Li, Rodger Main, and Eric Burrough[1]

Journal of Veterinary Diagnostic Investigation
2020, Vol. 32(4) 565–571

DOI: 10.1177/1040638720927669
jvdi.sagepub.com

Received: 24 March 2020 | Revised: 21 April 2020 | Accepted: 15 May 2020
DOI: 10.1111/tbed.13645

ORIGINAL ARTICLE

Transboundary and Emerging Diseases
WILEY

Genetic characterization of *Streptococcus equi* subspecies *zooepidemicus* associated with high swine mortality in the United States

Xuhua Chen[1] | Nubia Resende-De-Macedo[1] | Panchan Sitthicharoenchai[1] | Orhan Sahin[1] | Eric Burrough[1] | Maria Clavijo[1] | Rachel Derscheid[1] | Kent Schwartz[1] | Kristina Lantz[2] | Suelee Robbe-Austerman[2] | Rodger Main[1] | Ganwu Li[1]

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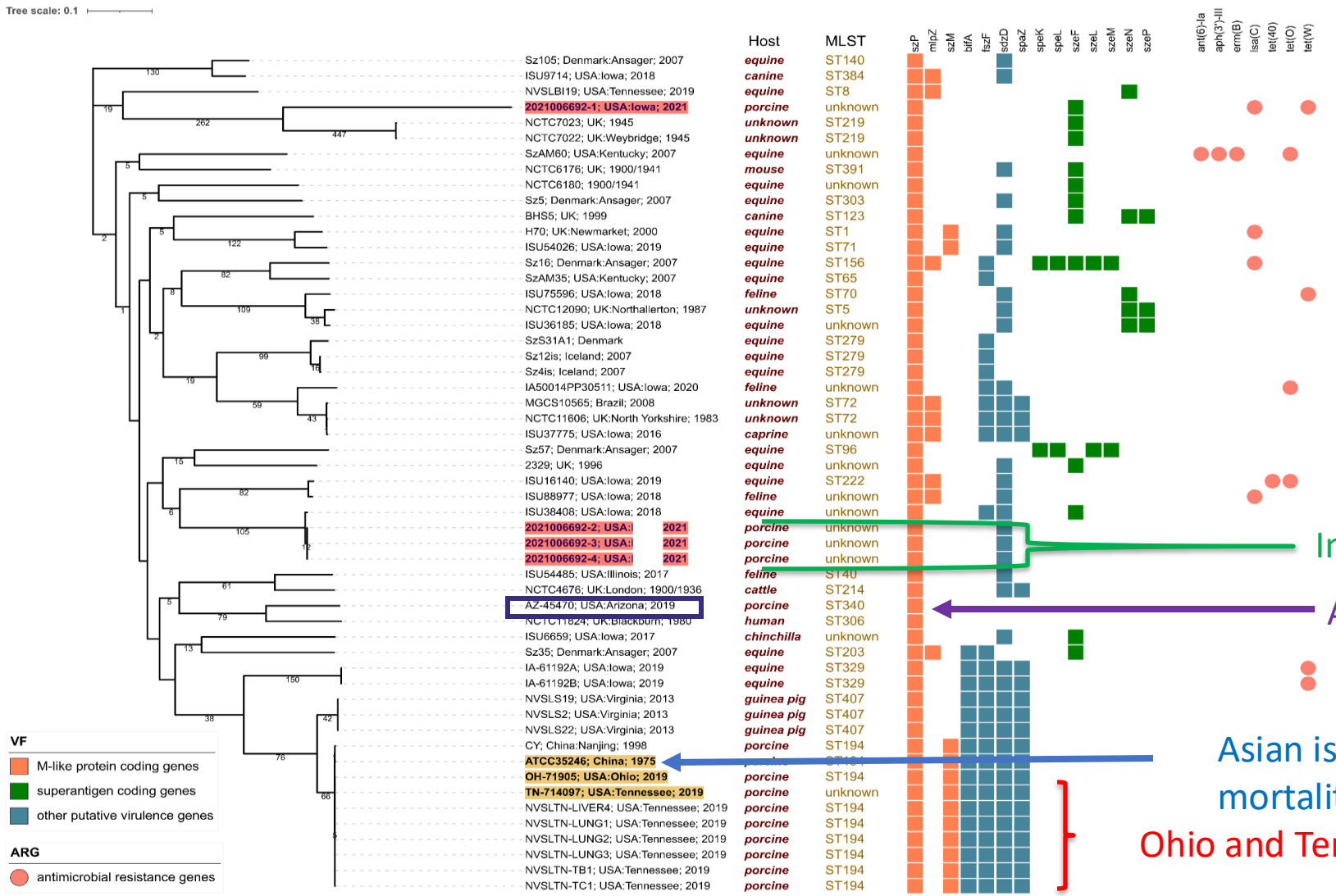
NGS for Bacterial Characterization



NGS for Bacterial Characterization

Unexpected increase in *B. hyodysenteriae* isolations in a pig production system

- Dysentery clean up applied 5 years prior at multiple sites within the system
  - *B. hyodysenteriae* no longer detected for a period but detection occurred again in 2021.
- Diagnostic question:
  - *Did the clean ups fail, or is this the results of one or more new introductions?*
- Research question:
  - Can WGS be used to answer this question as routine MALDI identification and *nox* and 16S sequencing are insufficient?

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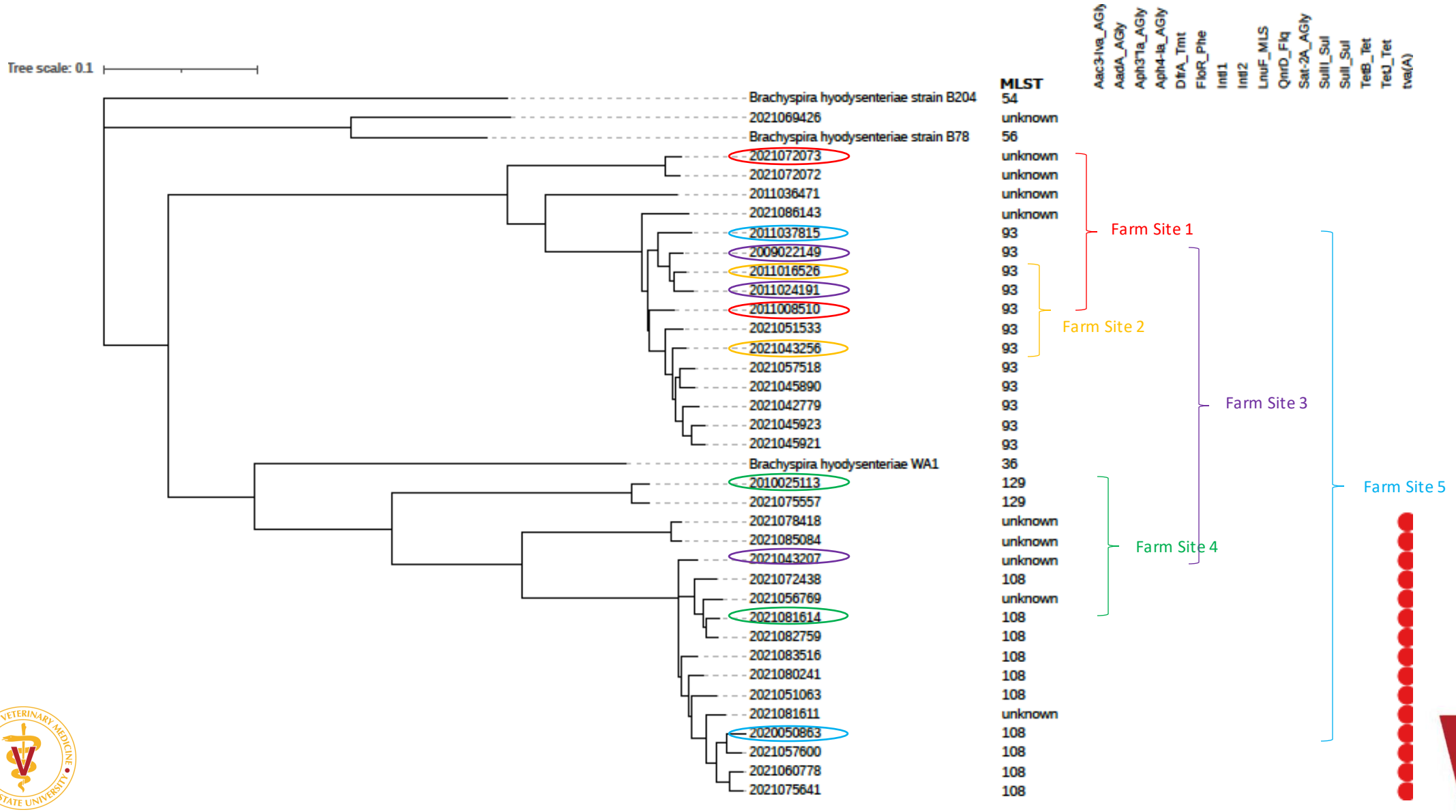
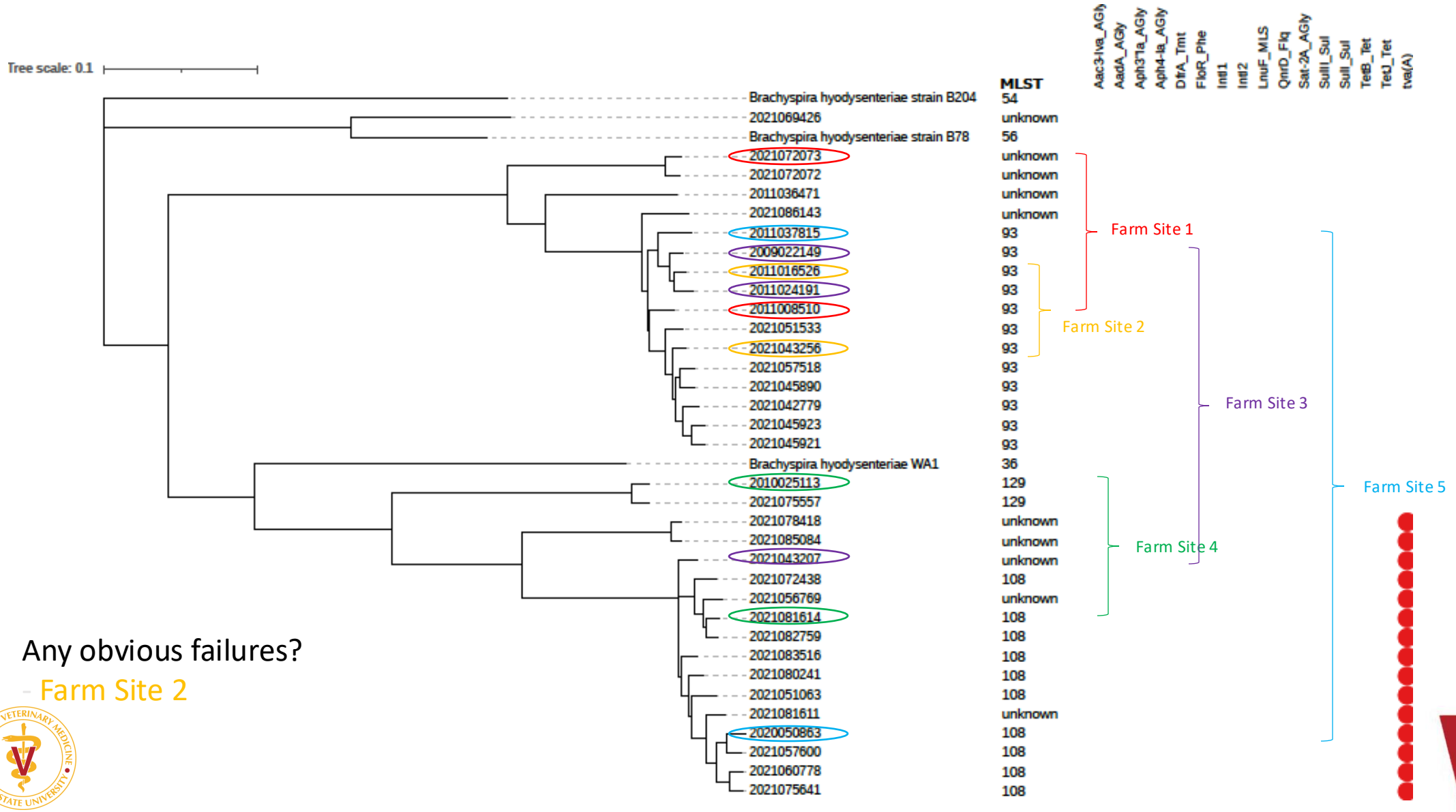
WGS of temporal *B. hyo* isolates from the same system



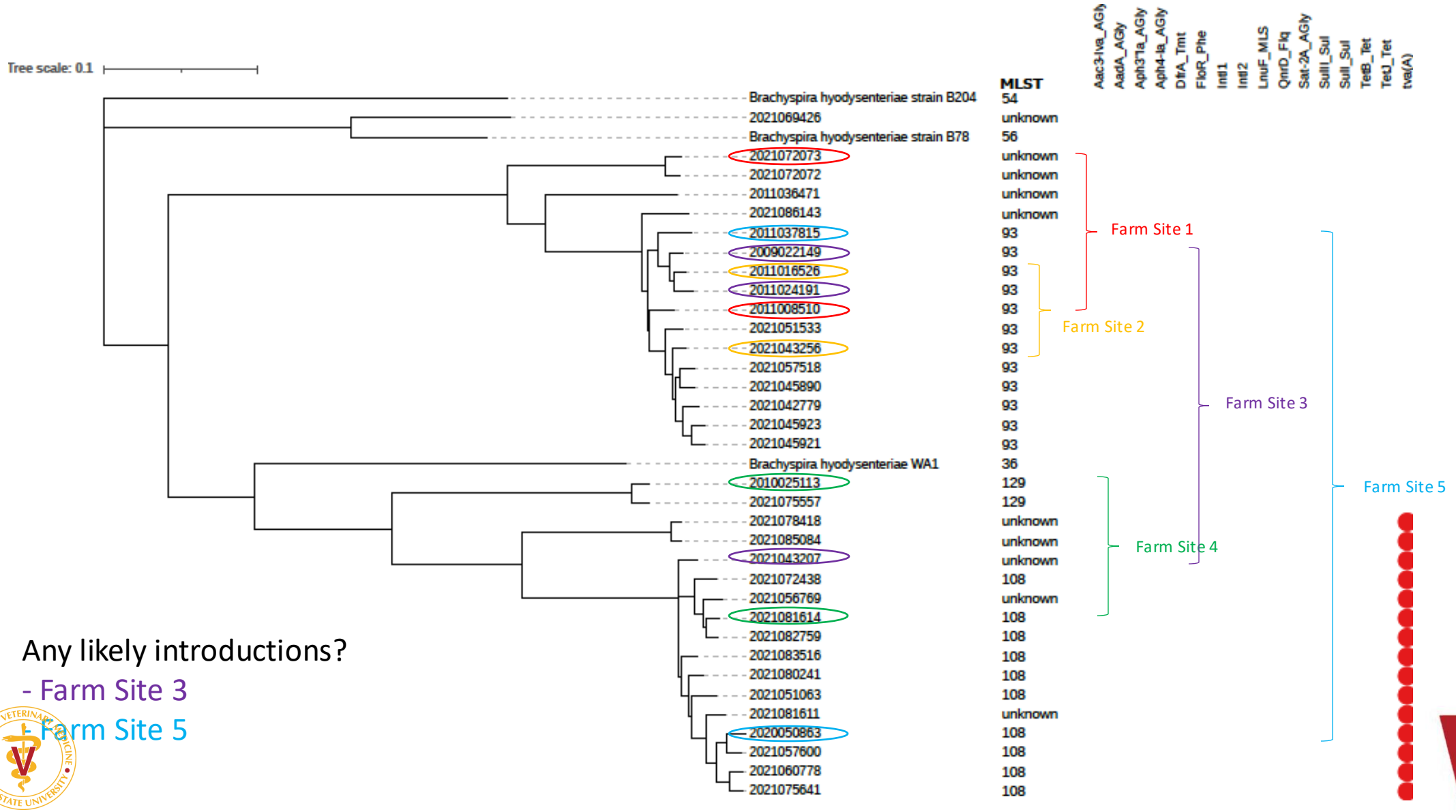
WGS of temporal *B. hyo* isolates from the same system



Any obvious failures?

- Farm Site 2

WGS of temporal *B. hyo* isolates from the same system



Any likely introductions?

- Farm Site 3
- Farm Site 5

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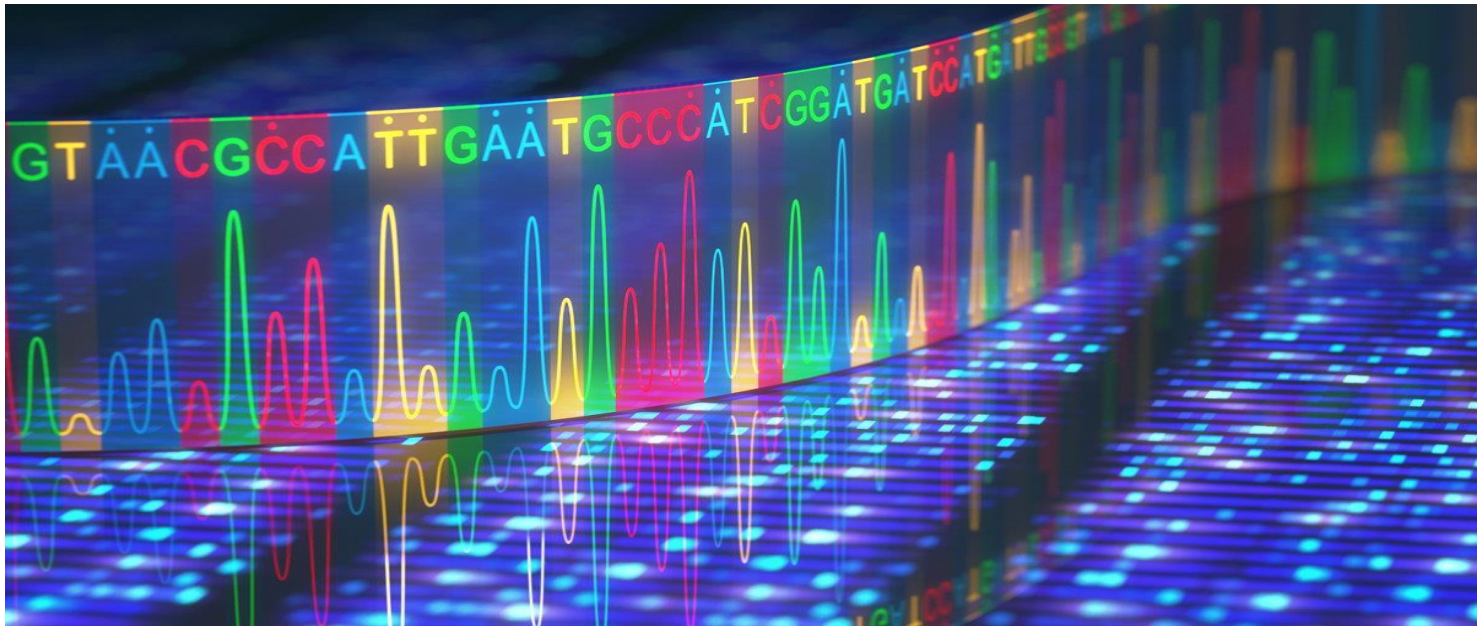
Summary

- NGS technology provides the potential for simultaneous detection of an unlimited number of agents.
- Agents detected by NGS must be interpreted with context.
  - Detection does not always equal disease.
- NGS techniques often produce a massive amount of data.
  - Etiologic agent may go undetected or not be readily identifiable within the output.
- As NGS technology becomes more affordable and widely available:
  - It may improve diagnostic sensitivity for known pathogens.
    - Targeted approaches (pathogens panels) may become commonplace.
  - Detailed pathogen characterization can become routine in veterinary diagnostics.

Acknowledgements

- ISU VDL NGS Section
  - Ganwu Li
  - Ying Zheng
- ISU VDL Director Dr. Rodger Main
- Other ISU-VDL faculty and staff



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Questions?

Eric R. Burrough, DVM, PhD, DACVP | Professor
Diagnostic Pathologist | Pathology Section Leader
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