



Advanced Molecular Detection Southeast Region Bioinformatics

Decoding Oropouche: Tracking an Emerging Threat

Arnold Rodríguez, MS
Florida's Bureau of Public Health Laboratories

AMD Southeast Region 2025 Bioinformatics Training Series

2-day Kickoff Molecular Epidemiology Workshop

6/24/2025

Funding Statement

This resource was made possible through funding provided under the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC) Cooperative Agreement (CK24-0002), Project D: Advanced Molecular Detection to the Florida Department of Health.

The conclusions, findings, and opinions expressed by authors do not necessarily reflect the official position of the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention.

Outline



Background: Recent OROV Outbreaks



Sequencing Strategy



Bioinformatics Pipeline Development



Phylogenetic Analysis



Genomic Epidemiology Insights



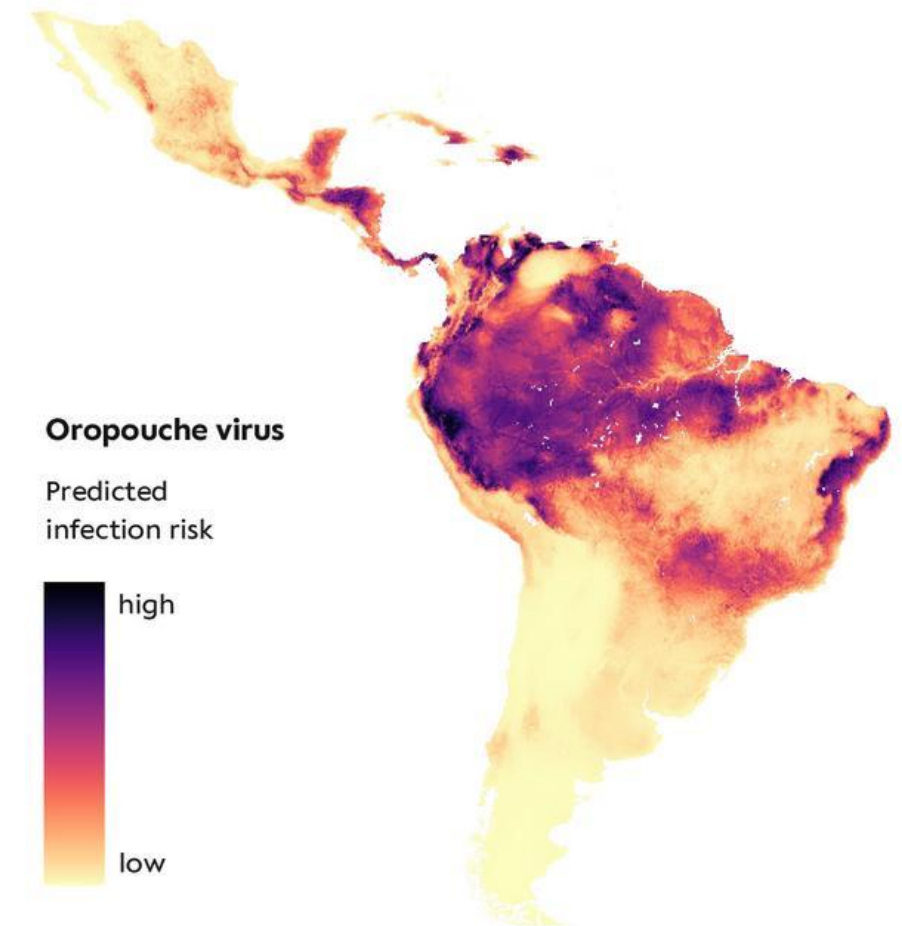
Key Takeaways



Why Oropouche virus (OROV)?



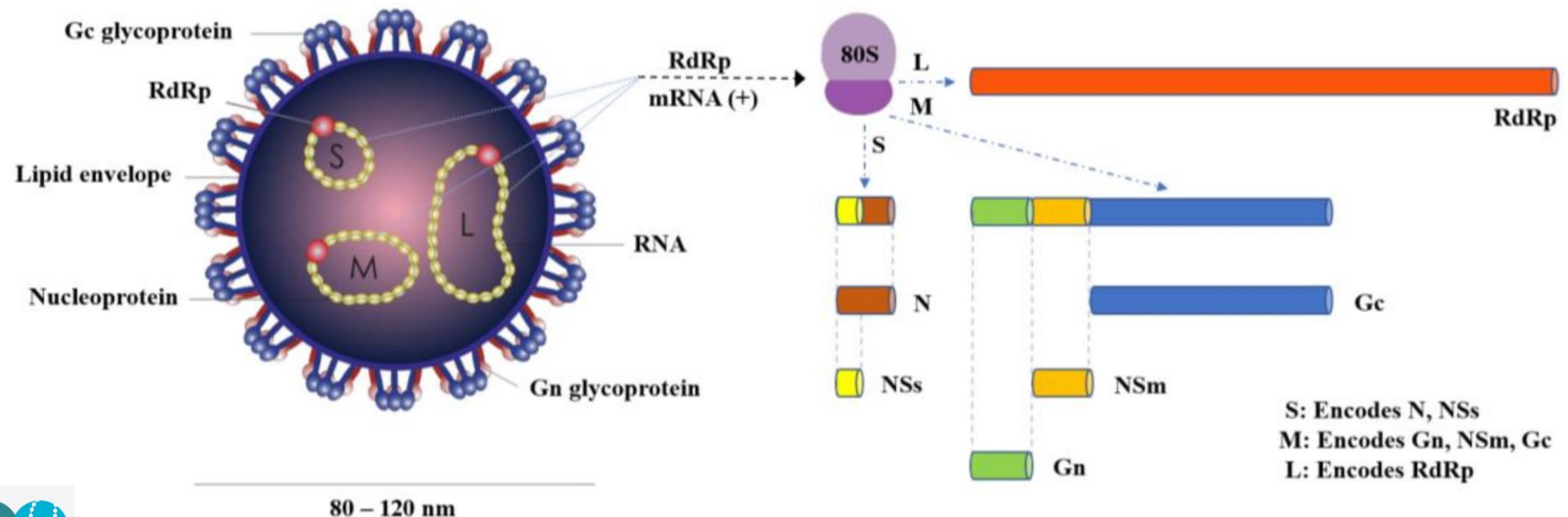
- Endemic arbovirus in the Americas
- Transmitted by biting midges and mosquitoes
 - a febrile illness similar to dengue or chikungunya
- Outbreaks since the 1960s in Brazil
 - 8,000 cases reported in Brazil (2024)
- Recent spread to Caribbean, Europe, U.S.
- No approved vaccine, specific treatment or FDA-cleared diagnostics



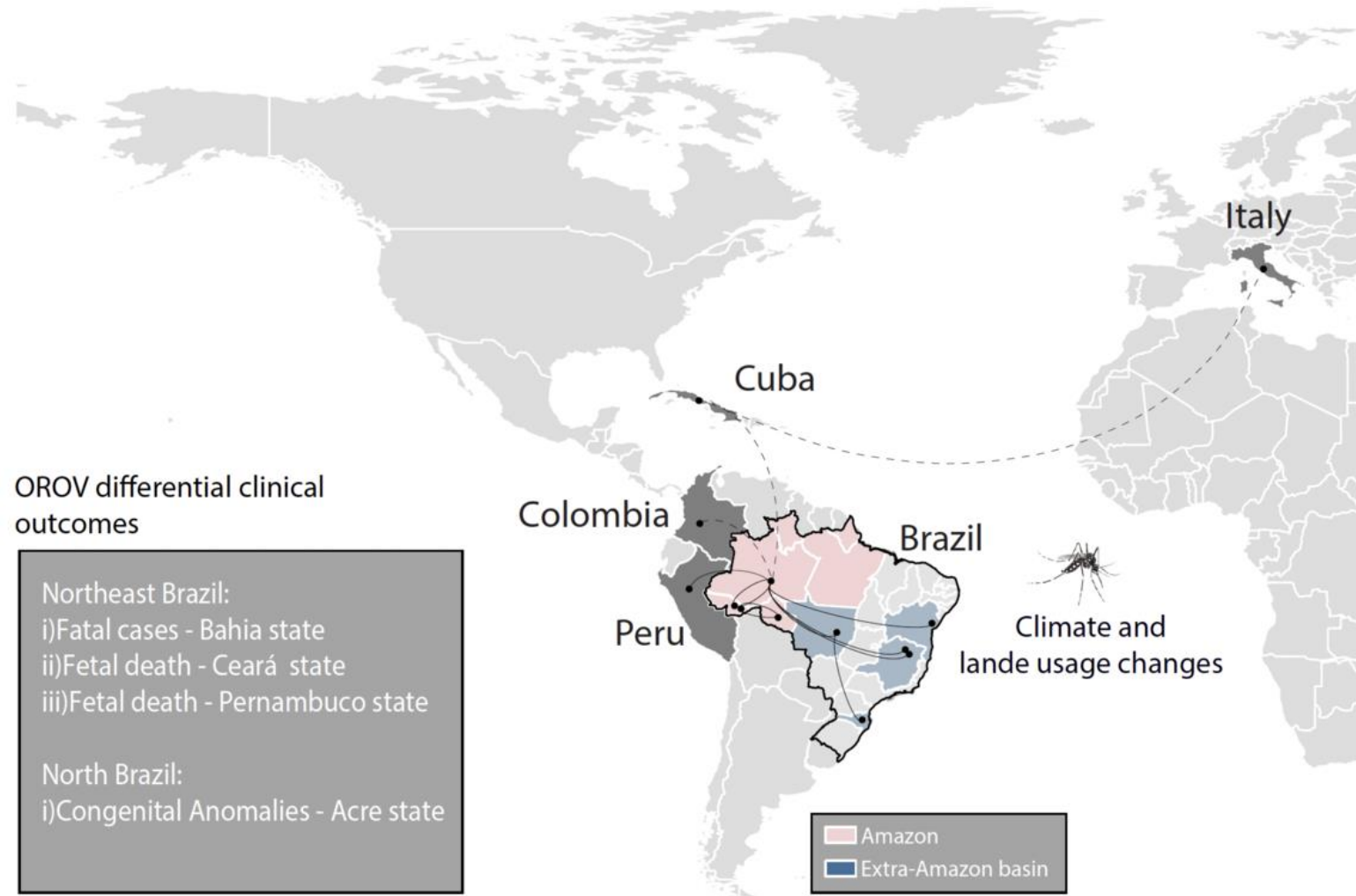
Inside the Virus: The OROV Genome

3 RNA segments: **L, M, S**

- **L**: Makes viral polymerase (RdRp)
- **M**: Makes envelope proteins (Gn, Gc) + NSm
- **S**: Makes nucleoprotein (N) + NSs



Resurgence and Spread (2023–2024)



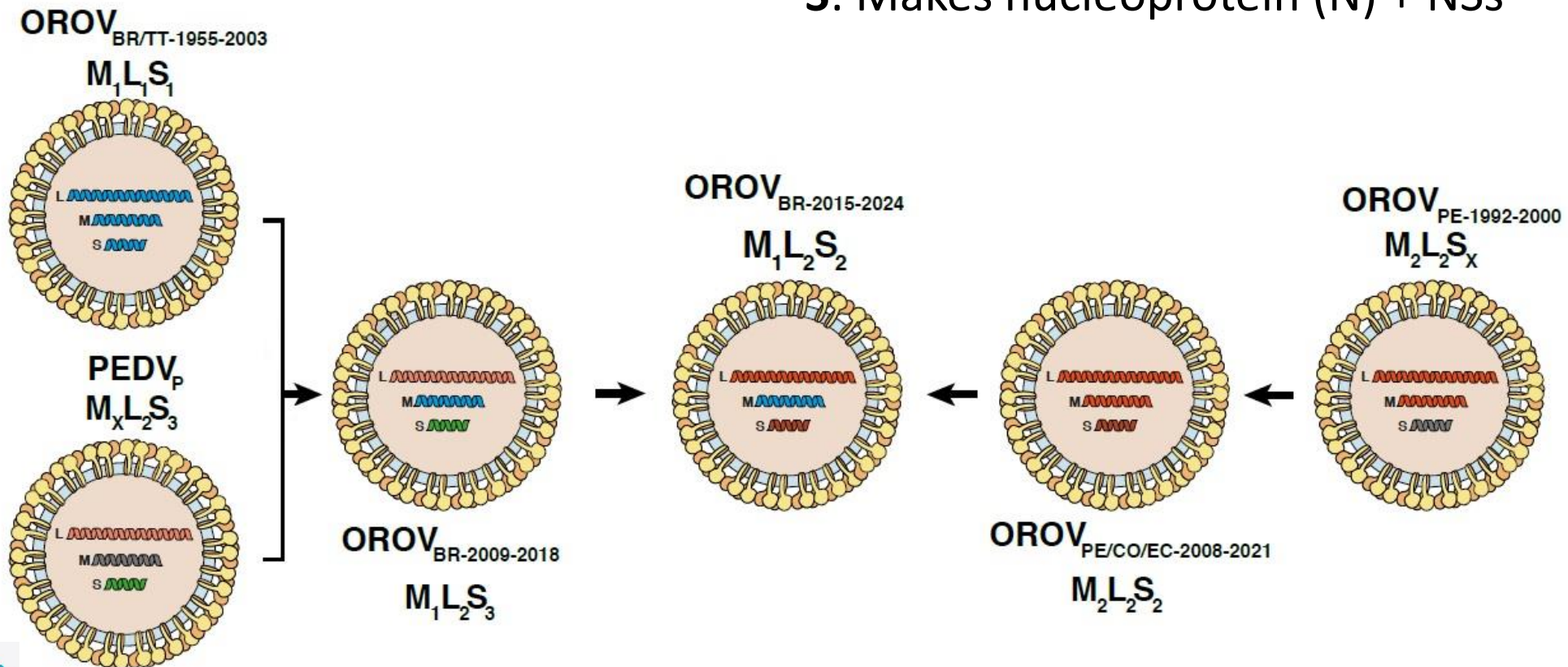
Giovanetti M. Oropouche virus and the urgent need for global surveillance. *Nat Microbiol.* (2025)

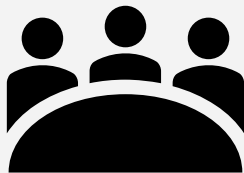
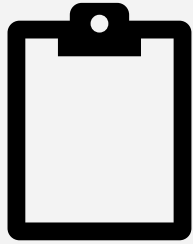


2023–2024 Reassortant strain

3 RNA segments: **L**, **M**, **S**

- **L**: Makes viral polymerase (RdRp)
- **M**: Makes envelope proteins (Gn, Gc) + NSm
- **S**: Makes nucleoprotein (N) + NSs





Then Vs Now: Surveillance Approaches

Conventional:

- Misdiagnosed
 - Underreporting
- No geographic clustering
- Limited outbreak tracking

Genomic:

- Transmission chains
- Evolutionary relationships
- Geographic origins
- Real-time outbreak tracking



Genomic Surveillance Strategy

- Collect serum samples from suspected orov patients
- RNA extracted (QIAGEN DSP Kit)
- Determine positivity via RT-PCR
- cDNA synthesis of PCR positive samples from earlier extracted RNA (NEB kits)
- Purified with AMPure XP beads
- Libraries prepped using Illumina DNA Prep
- Sequencing using Illumina instruments (NextSeq, MiSeq, etc.)

Florida BPHL's Juno Pipeline [\(<https://github.com/BPHL-Molecular/Juno>\)](https://github.com/BPHL-Molecular/Juno)

- Generic pipelines miss Oropouche specifics
- Need for rapid, automated assembly
 - Manual processes too slow for public health response
- Dual mode (reference/ de novo assembly)

Juno 🦟 🌿 🧬 📊 - A Nextflow Pipeline for the Assembly of Oropouche Virus (OROV) Genomes

Juno is a flexible pipeline for Illumina data that supports both reference-based and de novo assembly of Oropouche virus genomes.

⚡ Usage

```
$ nextflow run juno.nf -profile singularity -params-file params.yaml
```

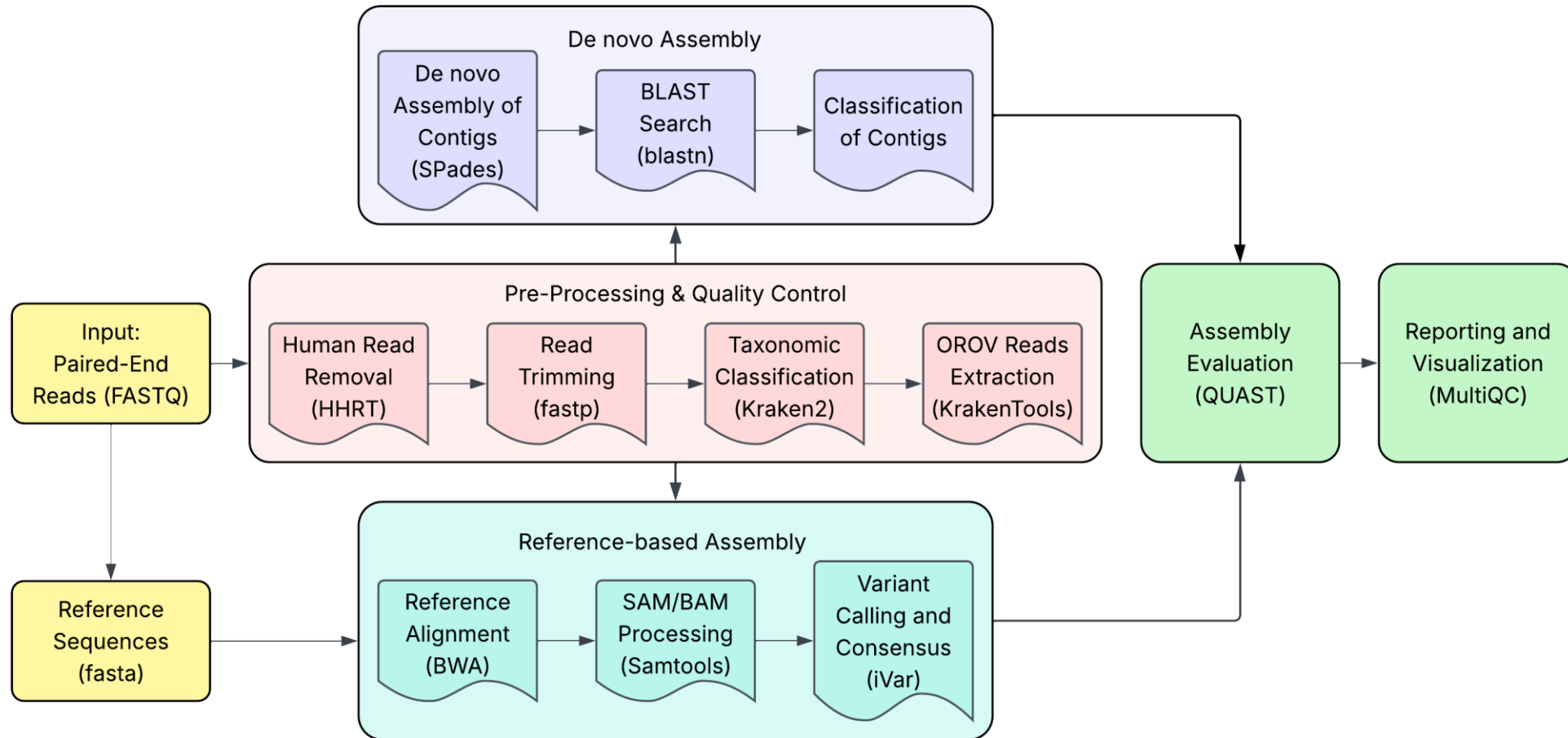
🦘 HiPerGator Usage

```
$ sbatch ./juno.sh
```

📦 Dependencies

- [Nextflow 23.04.0+](#)
- [Singularity](#) or [Docker](#)
- [Python 3.6+](#)
- [Slurm](#) (This applies only if HiPerGator is used)

Juno Pipeline – Key Steps and Tools



Juno Pipeline – Output and Results (reference)

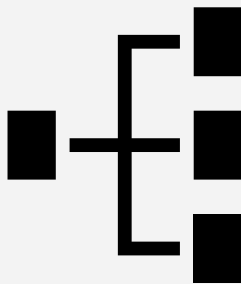
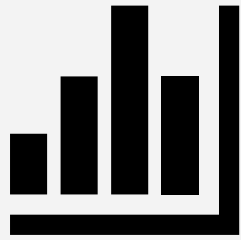
- juno_output/
 - |— hrirt/ -> FASTQ files without human reads
 - |— fastp/ -> Read trimming & QC reports
 - |— kraken2/ -> Taxonomic classification
 - |— krakentools/ -> OROV read extraction
 - |— bwa/ -> Alignment files (SAM/BAM)
 - |— samtools/ -> Sorted BAMs + alignment stats
 - |— ivar/ -> Variants & consensus sequences
 - |— quast/ -> Assembly quality metrics
 - |— summary_report/ -> Summary of key assembly and QC metrics
 - |— multiqc/ -> Aggregate QC report (interactive html)



Juno Pipeline – Output and Results (de novo)

- junos_output/
 - |— hrst/ -> FASTQ files without human reads
 - |— fastp/ -> Read trimming & QC reports
 - |— kraken2/ -> Taxonomic classification
 - |— krakentools/ -> OROV read extraction
 - |— assembly/ -> SPAdes contigs
 - |— blast_db/ -> BLAST reference database
 - |— blast/ -> BLAST search results
 - |— classification/ -> Classified segments
 - |— quast/ -> Assembly quality metrics
 - |— summary_report/ -> Summary of key assembly and QC metrics
 - |— multiqc/ -> Aggregate QC report (interactive html)





From Genomes to Phylogenies: Why It Matters

- Assembled genomes reveal genetic variation
- Phylogenetics traces viral lineage over time
- Segment-specific trees reflect evolutionary dynamics
- Clustering reveals spread, importation, reassortment
- Enables genomic surveillance across outbreaks
- Enabled by tools like Nextstrain, Microbetrace, Microreact etc.



Nextstrain Oropouche: Custom Build

- Adapted Nextstrain for segmented OROV genome
- Custom JSONs for L, M, and S segments
- Segment-specific trees generated separately
- Metadata harmonized across all sequences
- Treetime used for temporal signal in S
- Color-coded by region, segment, and clade



About us

Open-source project to harness the scientific and public health value of pathogen genome data

Core pathogens

Continually updated views of a range of pathogens maintained by the Nextstrain team

SARS-CoV-2

Up-to-date analyses and a range of resources for the virus responsible for COVID-19 disease

Open source tooling

Open-source workflows, analysis tools and visualization apps for the community

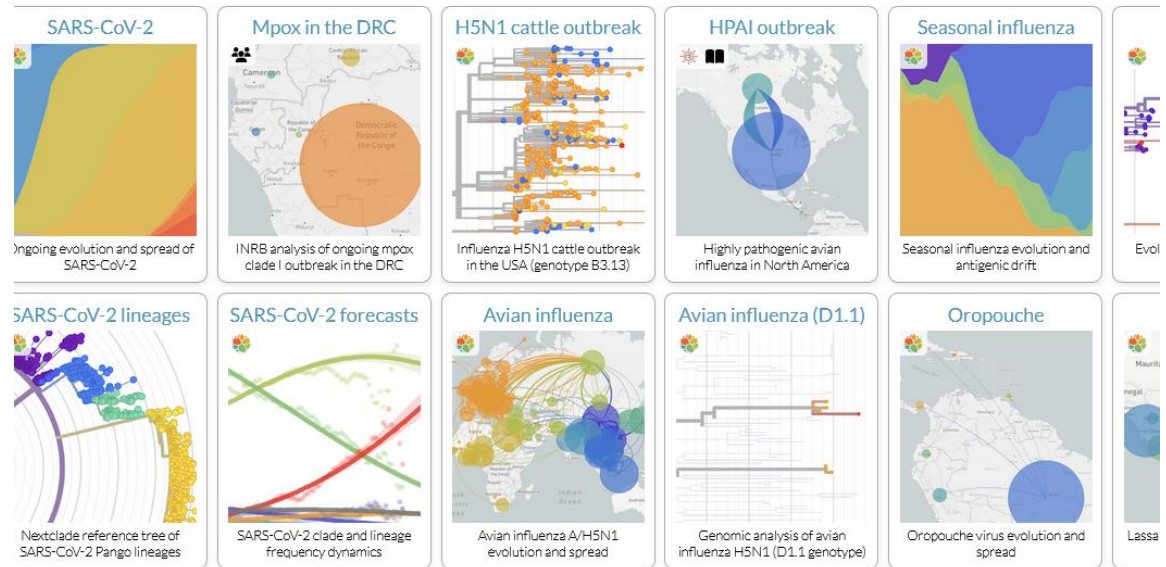
Nextclade

In-browser phylogenetic placement, clade assignment, mutation calling and sequence quality checks

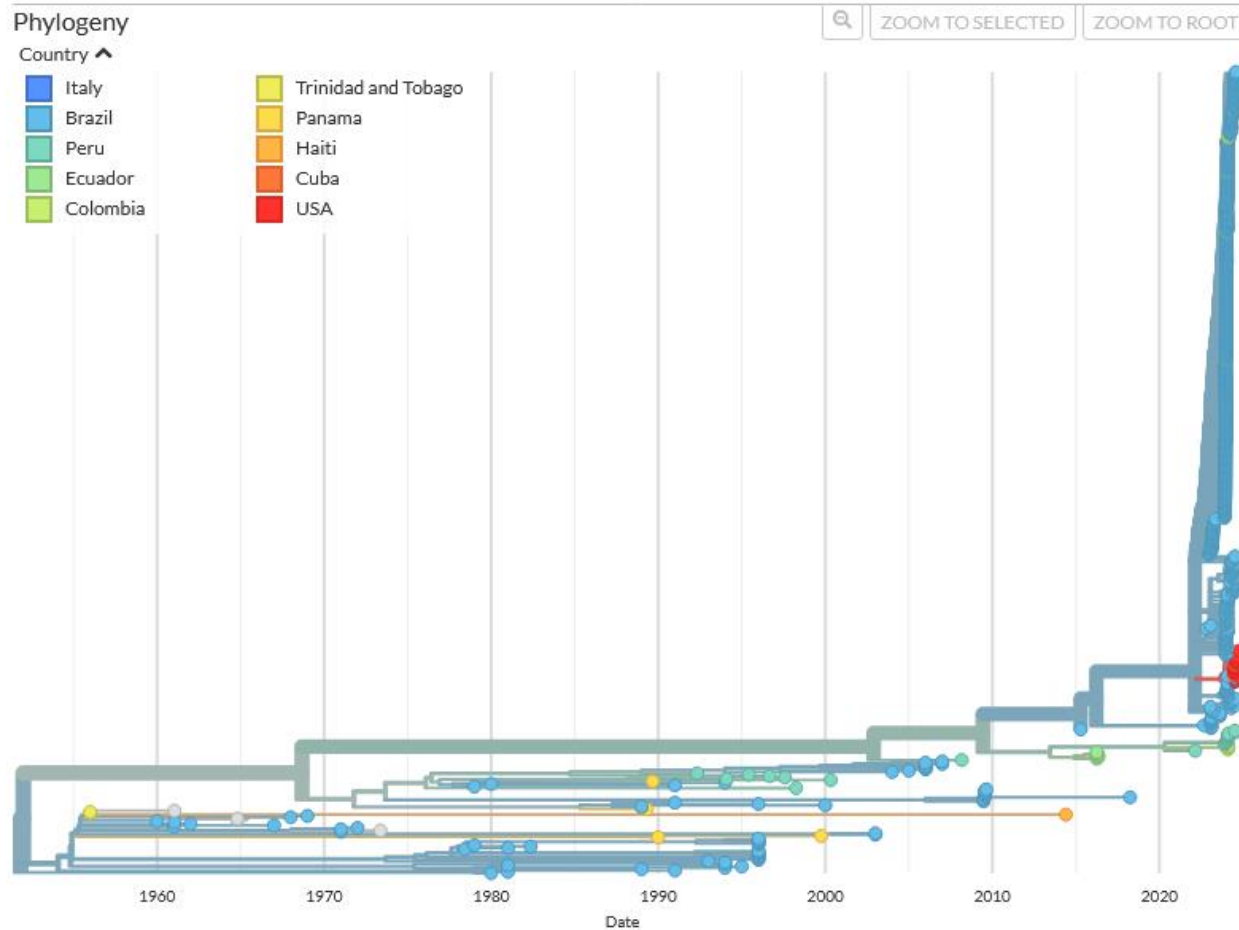
Nextstrain Groups

Datasets and narratives shared by research entities and others

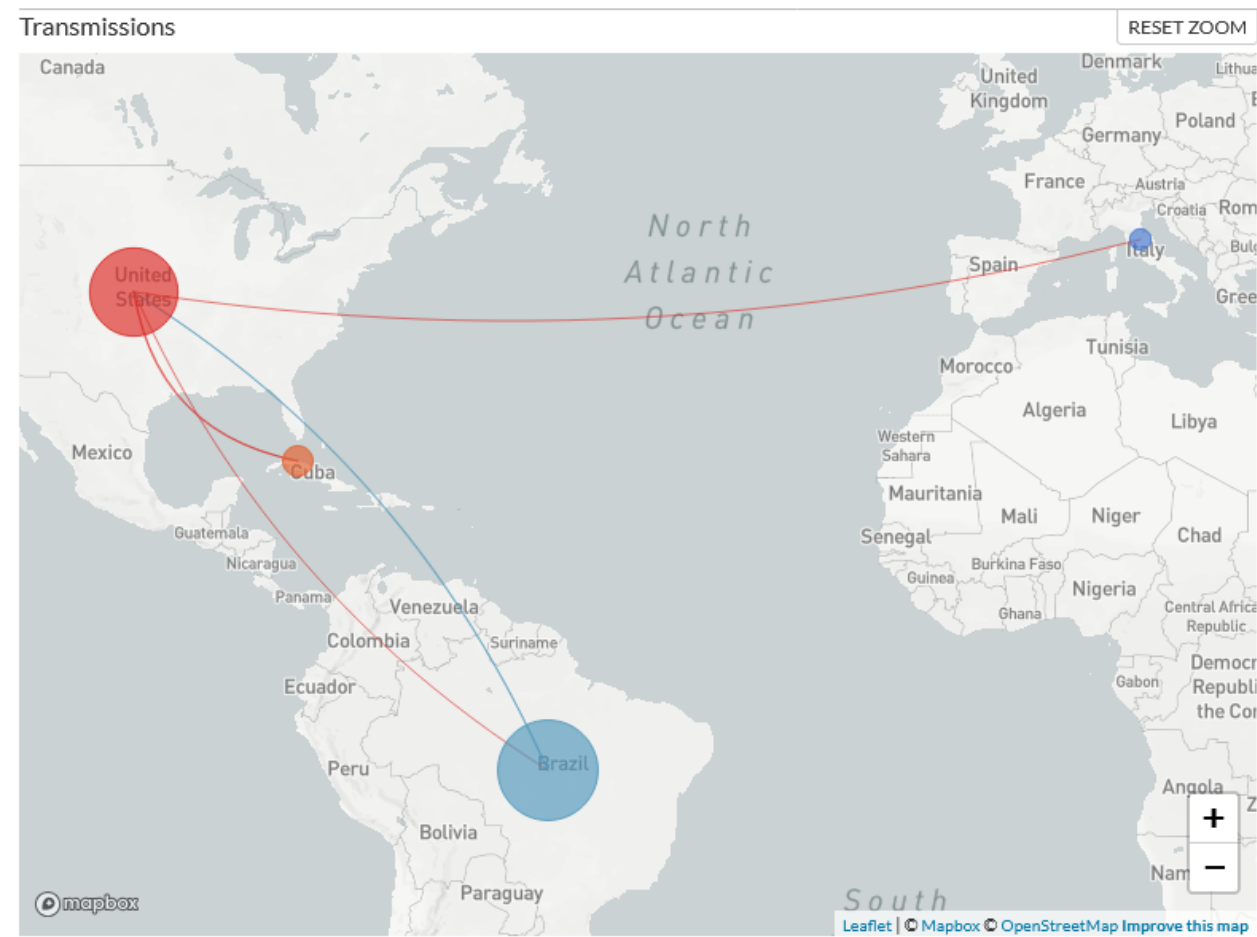
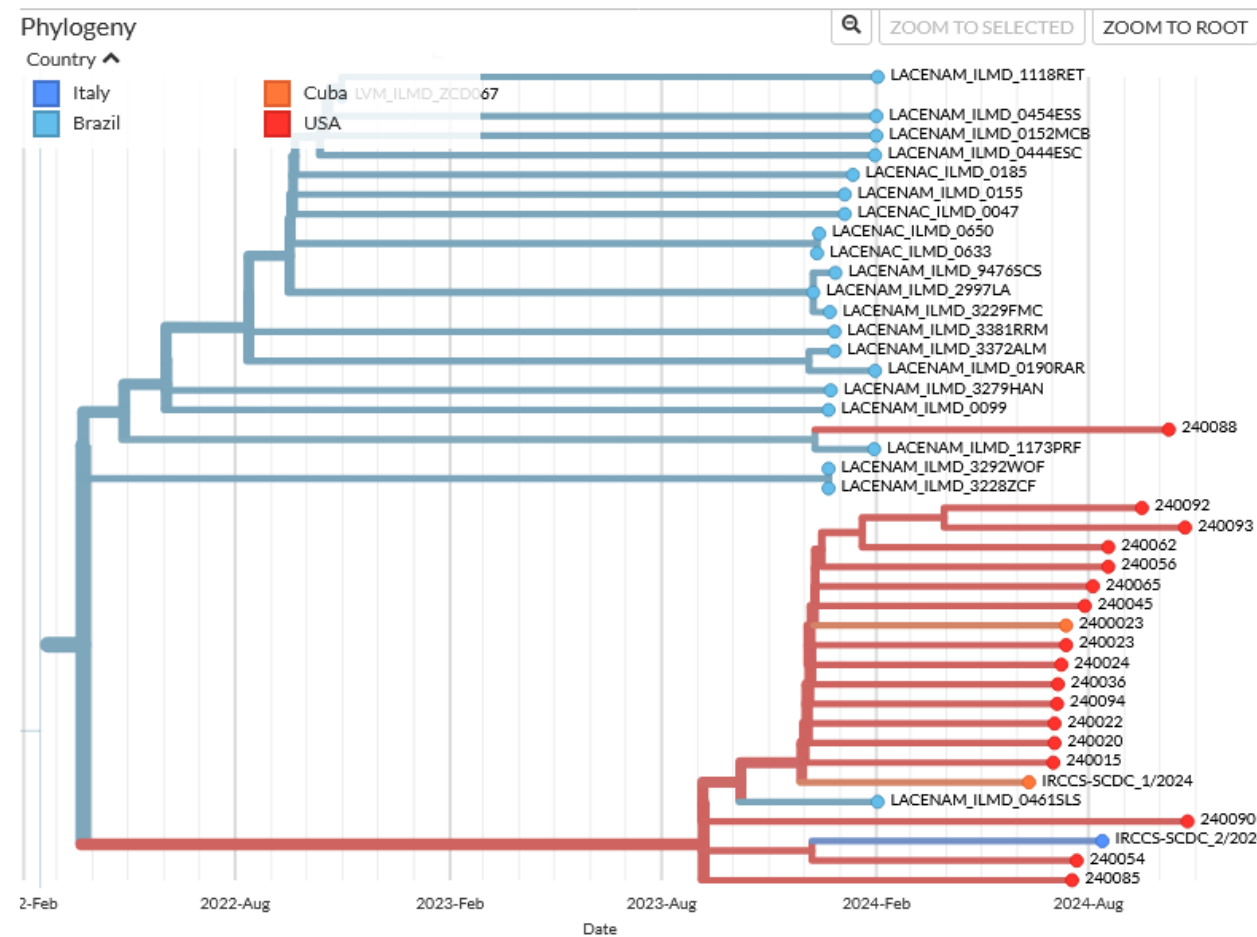
Featured analyses



Phylogenetic Findings – Sequences Cluster



Phylogenetic Findings – Sequences Cluster



Linking Genomics to Vector Risk

EMERGING INFECTIOUS DISEASES®

Volume 31, Number 3—March 2025

Research Letter

Lack of Competence of US Mosquito Species for Circulating Oropouche Virus

Anne F. Payne, Jessica Stout, Peter Dumoulin, Timothy Locksmith, Lea A. Heberlein, Molly Mitchell, Arnold Rodriguez-Hilario, Alan P. Dupuis, and Alexander T. Ciota✉

Author affiliation: Wadsworth Center, New York State Department of Health, Slingerlands, New York, USA (A.F. Payne, J. Stout, A.P. Dupuis II, A.T. Ciota); Florida Department of Health, Tampa, Florida, USA (P. Dumoulin, T. Locksmith, L.A. Heberlein, A. Rodriguez-Hilario); Florida Department of Health, Jacksonville, Florida, USA (M. Mitchell); University at Albany School of Public Health, Rensselaer, New York, USA (A.T. Ciota)

[Cite This Article](#)

Abstract

Given recent outbreaks of Oropouche virus in Latin America and >100 confirmed travel-associated cases in the United States, we evaluated the competence of US vectors, including *Aedes albopictus*, *Culex quinquefasciatus*, *Culex pipiens*, and *Anopheles quadrimaculatus* mosquitoes. Results with historic and recent isolates suggest transmission potential for those species is low.



Linking Genomics to Vector Risk

- One of our sequences (OROV 240023) was used in a recent vector competence study published in Emerging Infectious Diseases
 - Collaboration with Wadsworth Center, New York State Department of Health
- Study tested four common US mosquito species:
 - *Aedes albopictus*, *Anopheles quadrimaculatus*, *Culex pipiens*, *Culex quinquefasciatus*

Linking Genomics to Vector Risk

| Mosquito Species | Infection | Transmission |
|---------------------------|-----------|--------------|
| Aedes albopictus | X | X |
| Anopheles quadrimaculatus | X | X |
| Culex quinquefasciatus | X | X |
| Culex pipiens | ✓ | ✓ (1/31) |

✓ = Positive result in at least one individual

X = No infection/transmission observed

- Implication: U.S. mosquitoes are unlikely to maintain OROV transmission locally, but exceptions may exist.
- Findings underscore importance of genomic surveillance to monitor evolving vector-virus interactions.

Conclusion – Key Takeaways

- **Oropouche is on the move**
 - Reassortant strains now detected in the Caribbean, U.S., and Europe.
- **Genomics is the foundation**
 - Direct-from-sample sequencing with flexible strategies enabled rapid OROV genome recovery.
- **Bioinformatics innovation is essential**
 - Automated assembly pipelines like Juno support public health response in real time.
- **Local data reshaped phylogeny**
 - Including Florida sequences in a custom Nextstrain build shifted global phylogenetic interpretations even recontextualizing cases in Europe.
- **Collaboration amplifies capacity**
 - Bringing together tools, expertise, and partners beyond the public health lab strengthens surveillance and preparedness.



Advanced Molecular Detection

Southeast Region Bioinformatics

Questions?

Group Help Inbox

Bphl-sebioinformatics@flhealth.gov

Molly Mitchell, PhD

Bioinformatics Supervisor

Molly.Mitchell@flhealth.gov

Arnold Rodriguez, MS

Bioinformatician

Arnold.RodriguezHilario@flhealth.gov