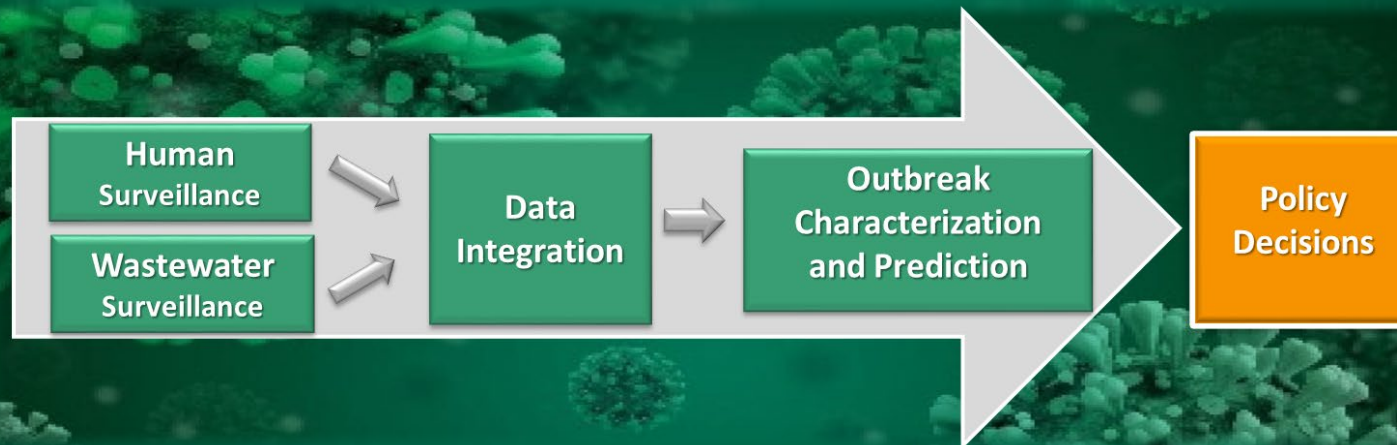


# South Florida - Rapid Acceleration of Diagnostics (SF-RAD):

Development and Proof-of-Concept  
Implementation of the South Florida Miami RADx-rad

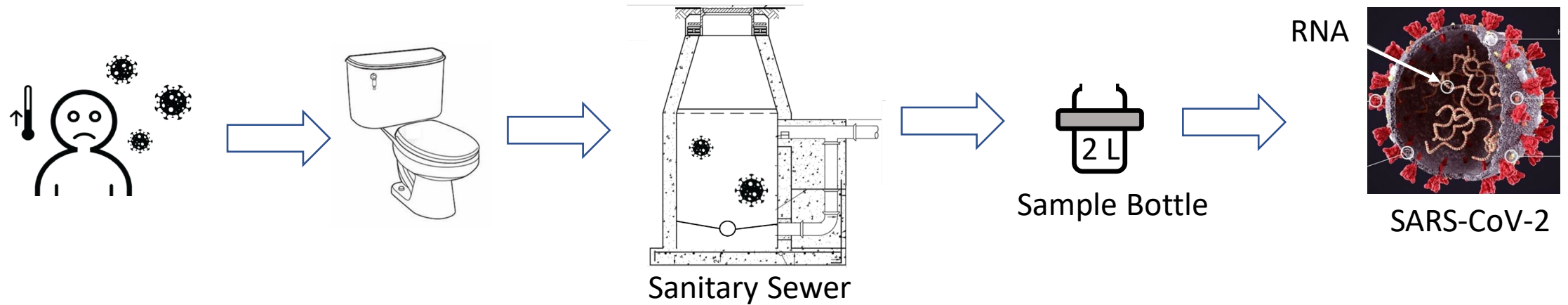
## SARS-CoV-2 Wastewater-Based Surveillance Infrastructure

Funded by  
NIH RADx-rad Grant  
1U01DA053941-01



# Motivation & Objectives

Infected humans excrete COVID-19 virus in feces and urine



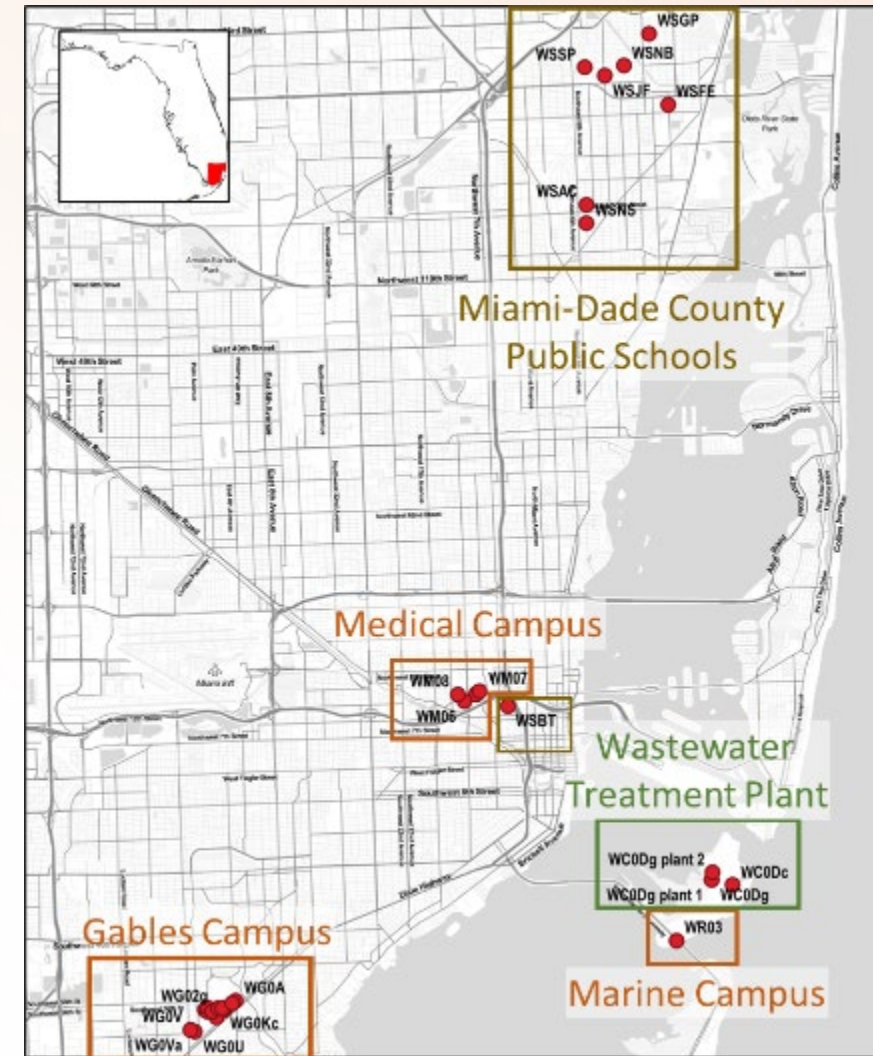
Ultimate objective: Relate wastewater measurements to predict COVID-19 cases.

# Human & Wastewater Sample Collection

## Human Surveillance

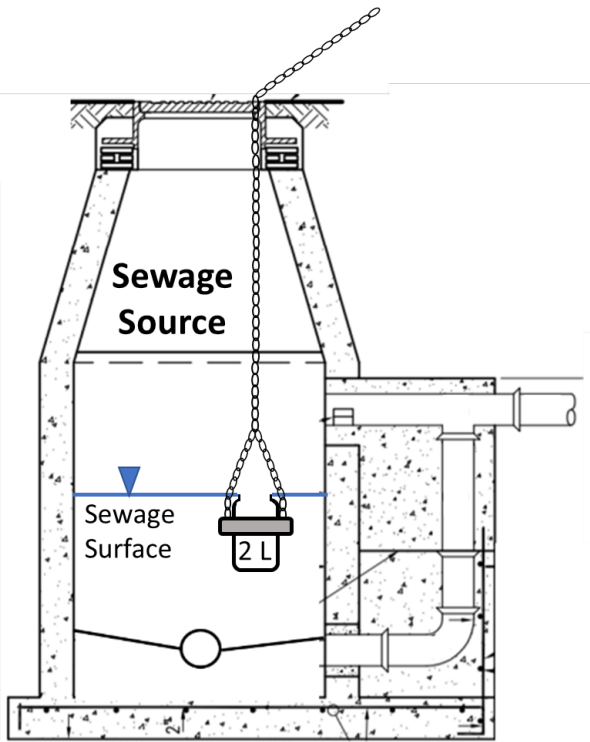
## Sample Collection Plans

University of Miami	<p><b>Student Campus Residents</b>, UMiami Gables/Marine (Sep. 2020)</p> <p><i>Fall'20/Spring'21</i></p> <ul style="list-style-type: none"> <li>• Students tested weekly (nasal swab, qPCR) Supplemented by breath test</li> <li>• COVID results and total tests by building/dorm room</li> </ul> <p><i>Summer/Fall'21</i></p> <ul style="list-style-type: none"> <li>• Unvaccinated students tested weekly</li> <li>• All students tested when wastewater exceeds</li> </ul>
	<p><b>University Hospital</b>, UMiami Medical (Sep. 2020)</p> <ul style="list-style-type: none"> <li>• Treat known COVID patients</li> <li>• Electronic medical records pulled regularly</li> </ul>
Miami-Dade County	<p><b>Miami-Dade County Residents</b>, FDOH WWTP (Jan. 2021)</p> <ul style="list-style-type: none"> <li>• Positives by zip code</li> <li>• Number of tests by zip code</li> <li>• Augment with Biobot wastewater data (Apr. 2020)</li> </ul>
	<p><i>Pilot</i>, <b>Miami-Dade County Public Schools</b>, MDCPS (Jan. 2022)</p> <ul style="list-style-type: none"> <li>• In collaboration with RADx-UP project (Gwynn, PI)</li> <li>• 9 Schools (4 Elementary, 2 Middle, 3 High Schools)</li> </ul>





# Sampling






0.5L  
Univ. Miami Lab  
(BSSR) 

Center for AIDS Research   
V2G-qPCR (quantify SARS-CoV-2)  
Quantify Fecal Indicators

Oncogenomics Share Resource   
RT-qPCR (check SARS-CoV-2)

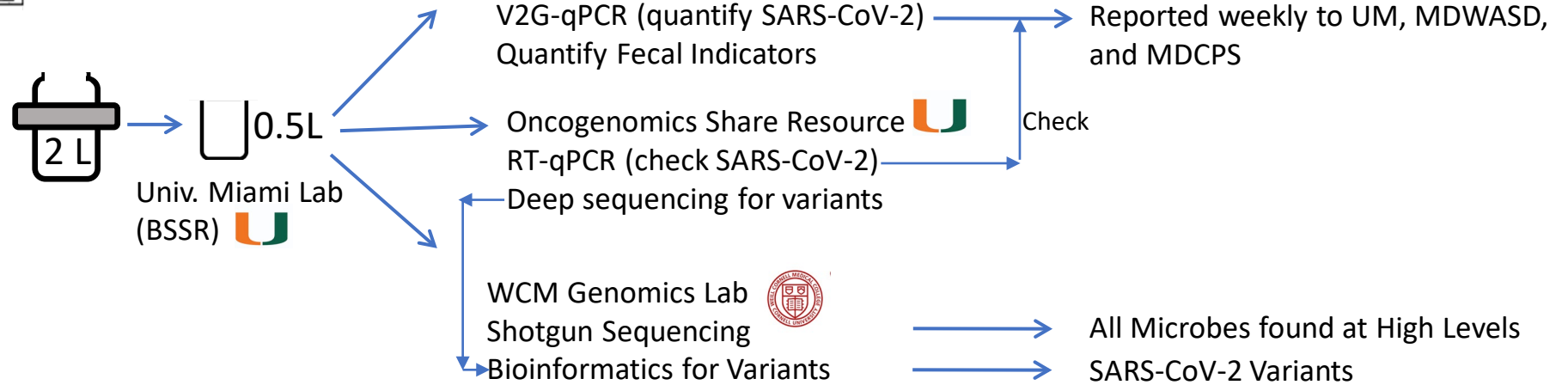
Deep sequencing for variants

WCM Genomics Lab   
Shotgun Sequencing  
Bioinformatics for Variants

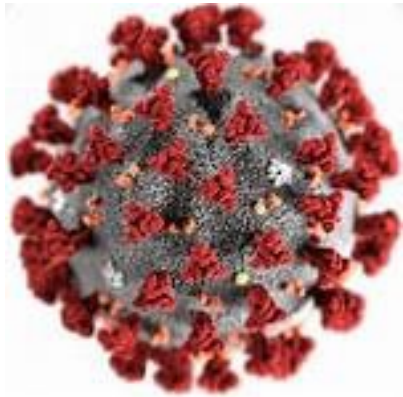
Reported weekly to UM, MDWASD,  
and MDCPS

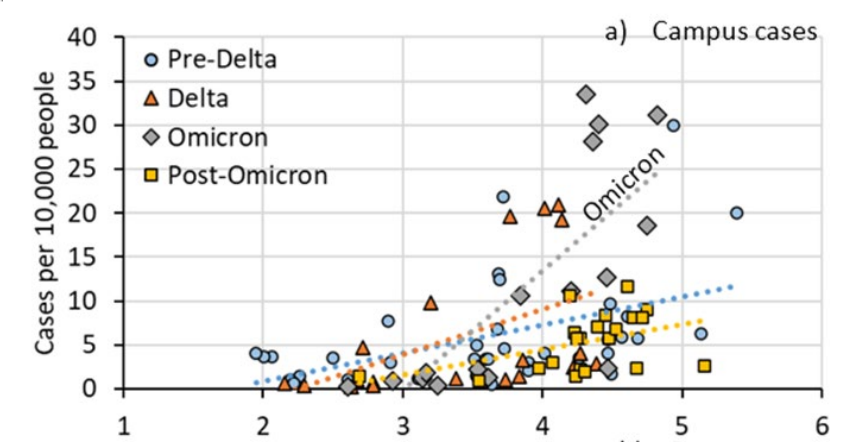
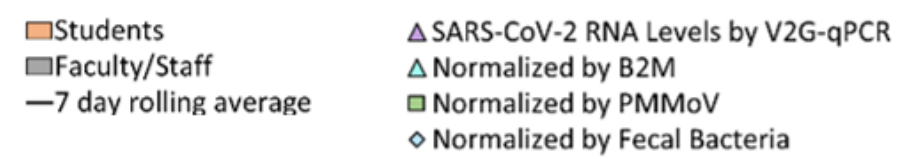
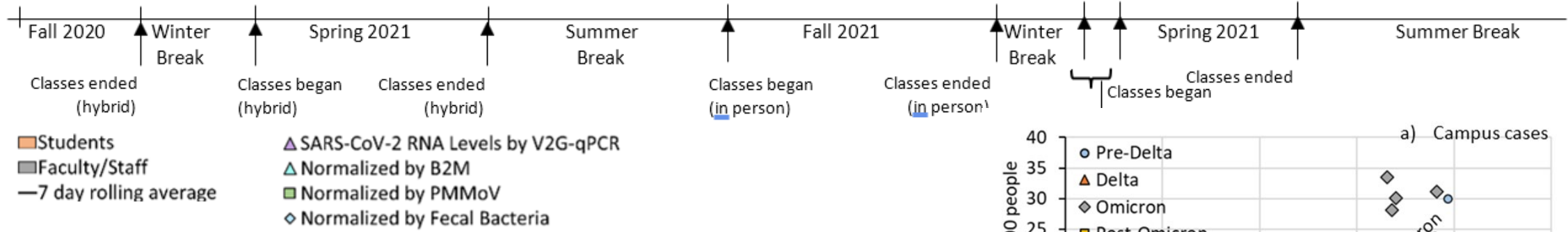
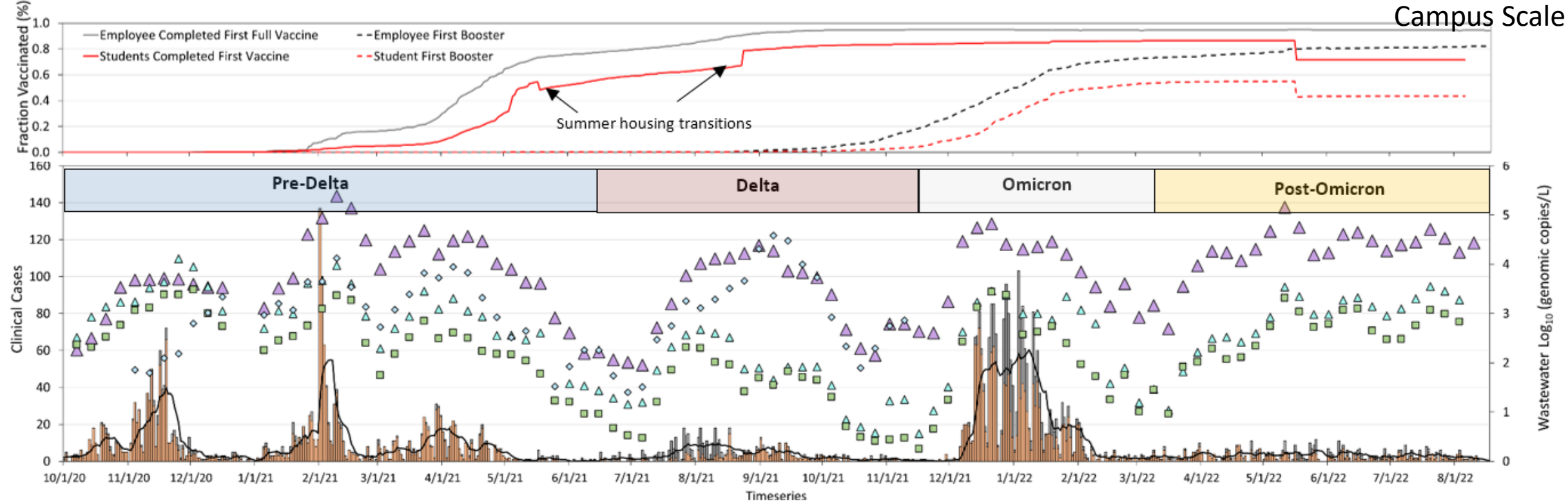
Check

All Microbes found at High Levels  
SARS-CoV-2 Variants

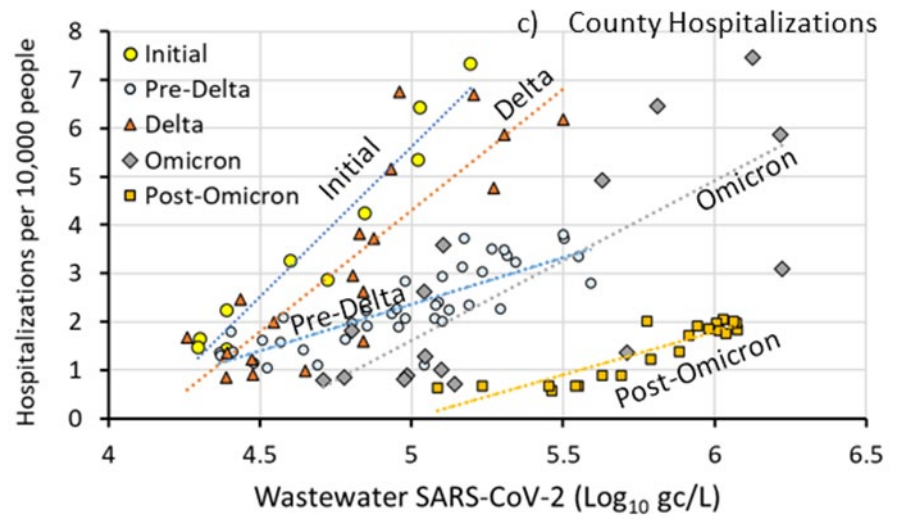
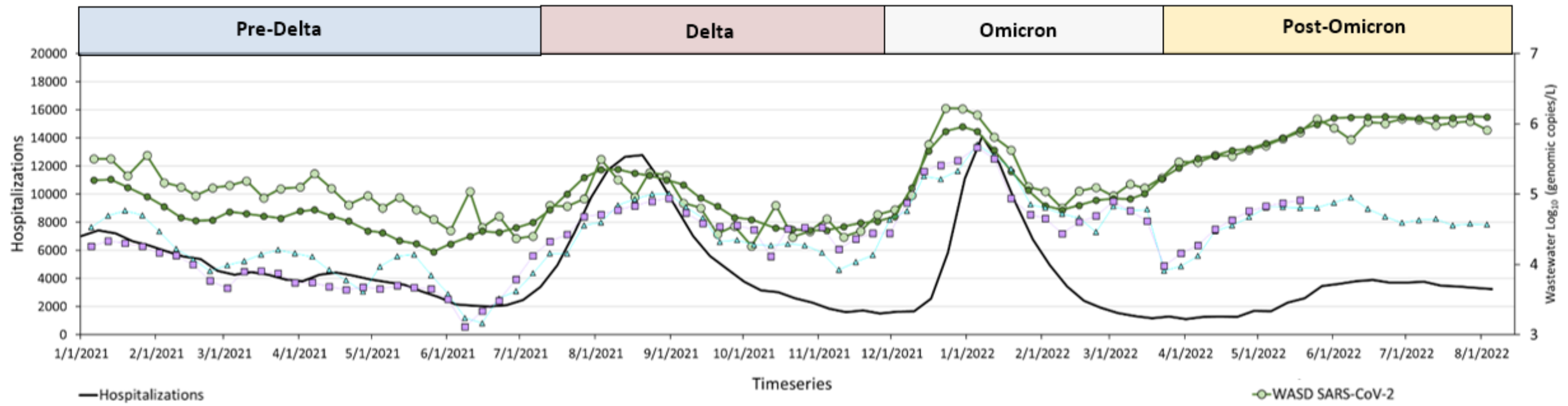


# SARS-CoV-2 Results





# Hospitalizations



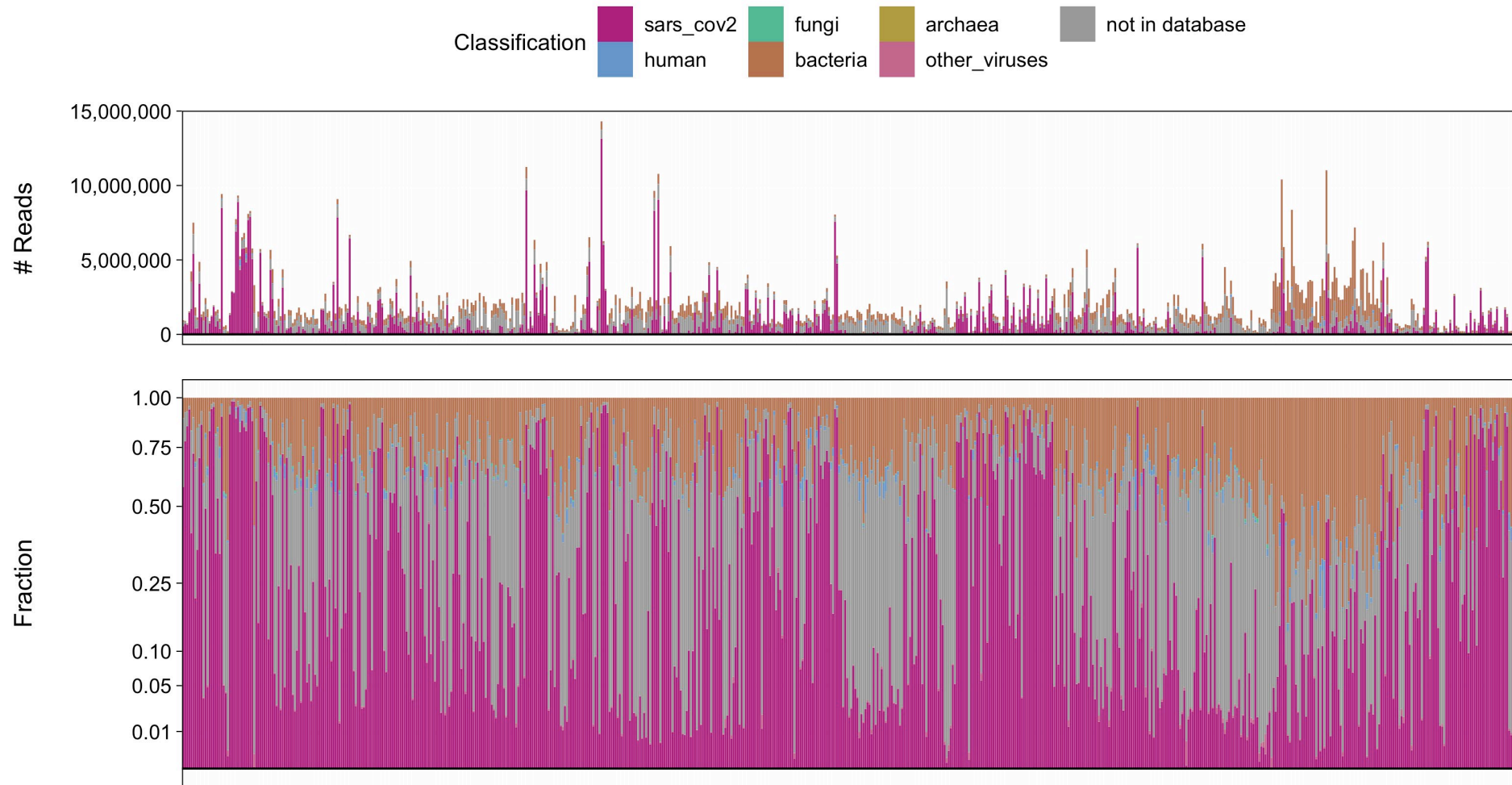


# Integration with Human Health Surveillance

# Wastewater VOC Detection Methodology

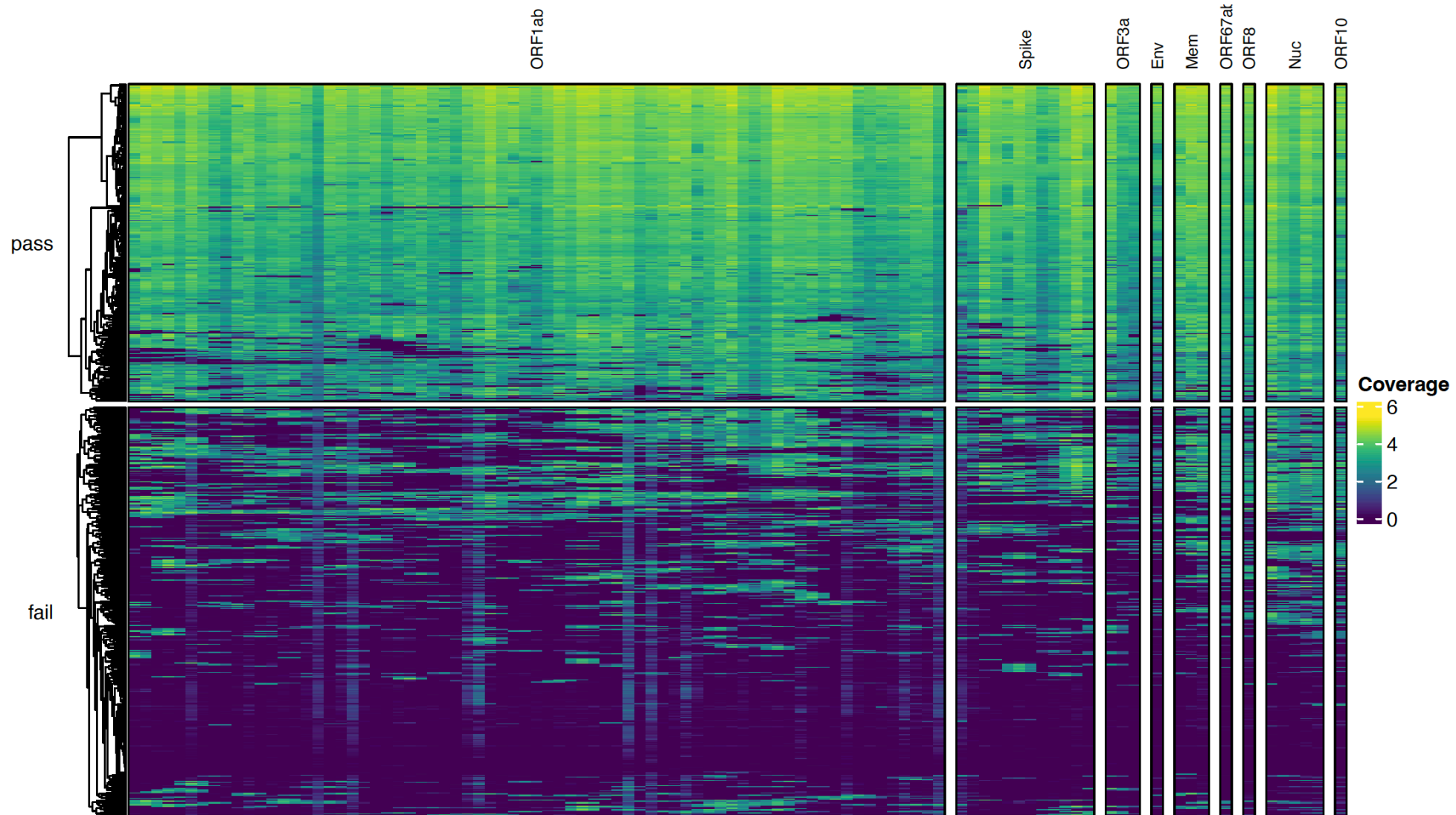
1. Assign taxonomy to all reads and filter SARS-CoV-2 matches with **kraken2**
  - Run using custom pan-kingdom DB
  - Generate SARS-CoV-2 FASTQs leveraging **seqtk**
2. Align to Wuhan reference with **bwa mem**
  - Sort and index alignment with **sambamba**
3. Trim primers with **ivar trim** using version-specific ARTIC BED file
  - Get coverage statistics on trimmed BAMs
    - **bedtools genomecov** for per-base coverage
    - **mosdepth** for per-amplicon coverage
4. Call variants using hybrid approach with **lofreq** and **ivar**
  - Take union of calls to avoid FNs and use mean DP/VAF per mutation call
5. Annotate mutations (assign gene, impact, amino acid substitutions) with **VEP**
6. Estimate relative VOC lineage abundances with **Freyja**

# Performance of ARTIC protocol up through June 2022

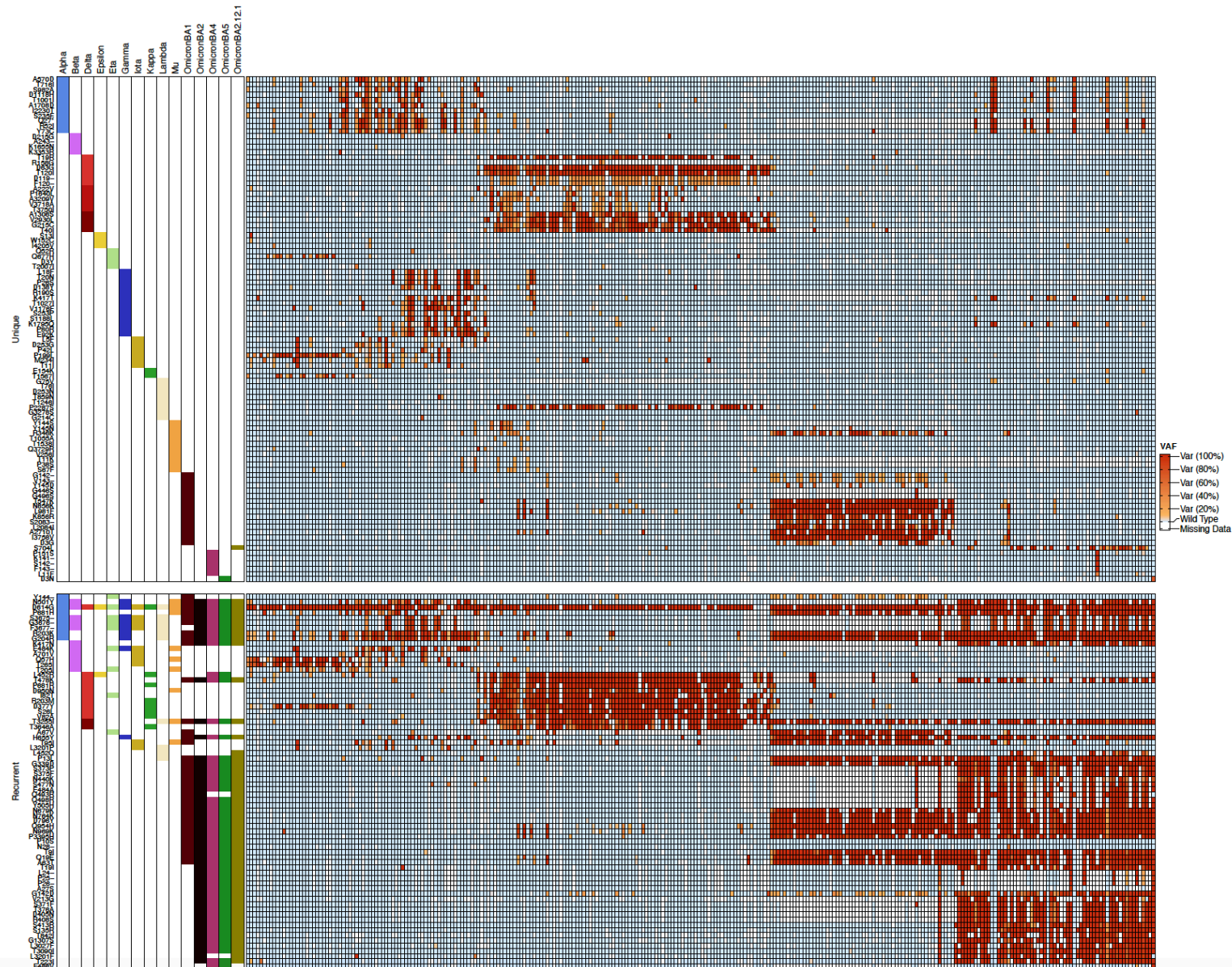


# Samples are filtered by total genome coverage

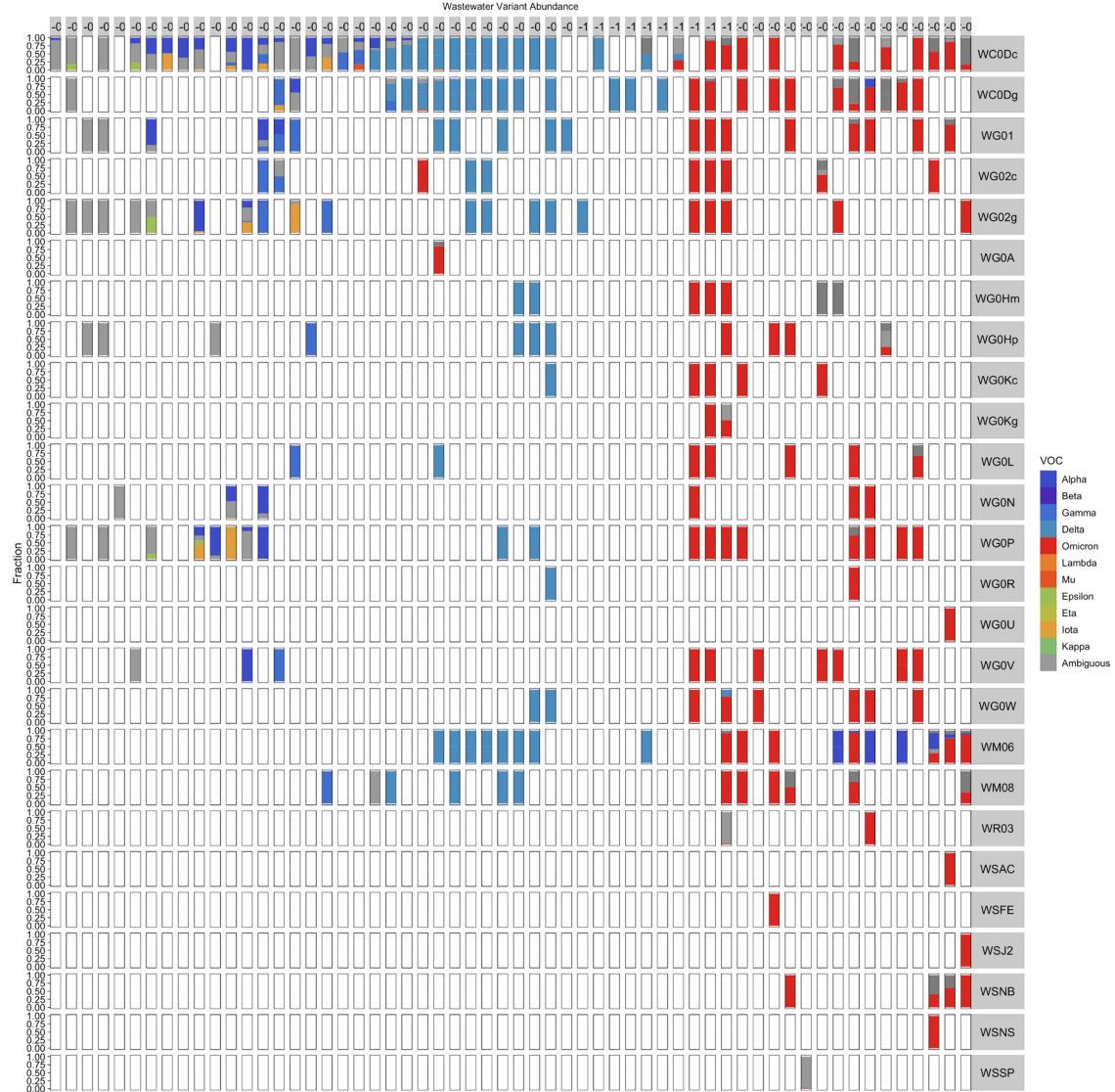
Minimum of 75% of SARS-CoV-2 genome must be covered with at least 50x



# Tracking signature mutations across all 2021-22 WW samples



# The dominance of omicron across all sites



Mapping reads to species beyond SARS-CoV-2:

The benefits of metatranscriptomics

# The bioinformatics of broad-spectrum, pathogen monitoring in wastewater

How do we leverage the ecology of wastewater to monitor bacterial, viral, and fungal pathogens?

## Challenges:

1. Low abundances (especially in viruses)
2. Repeat regions (especially in fungi)

## Potential (bioinformatic) solutions:

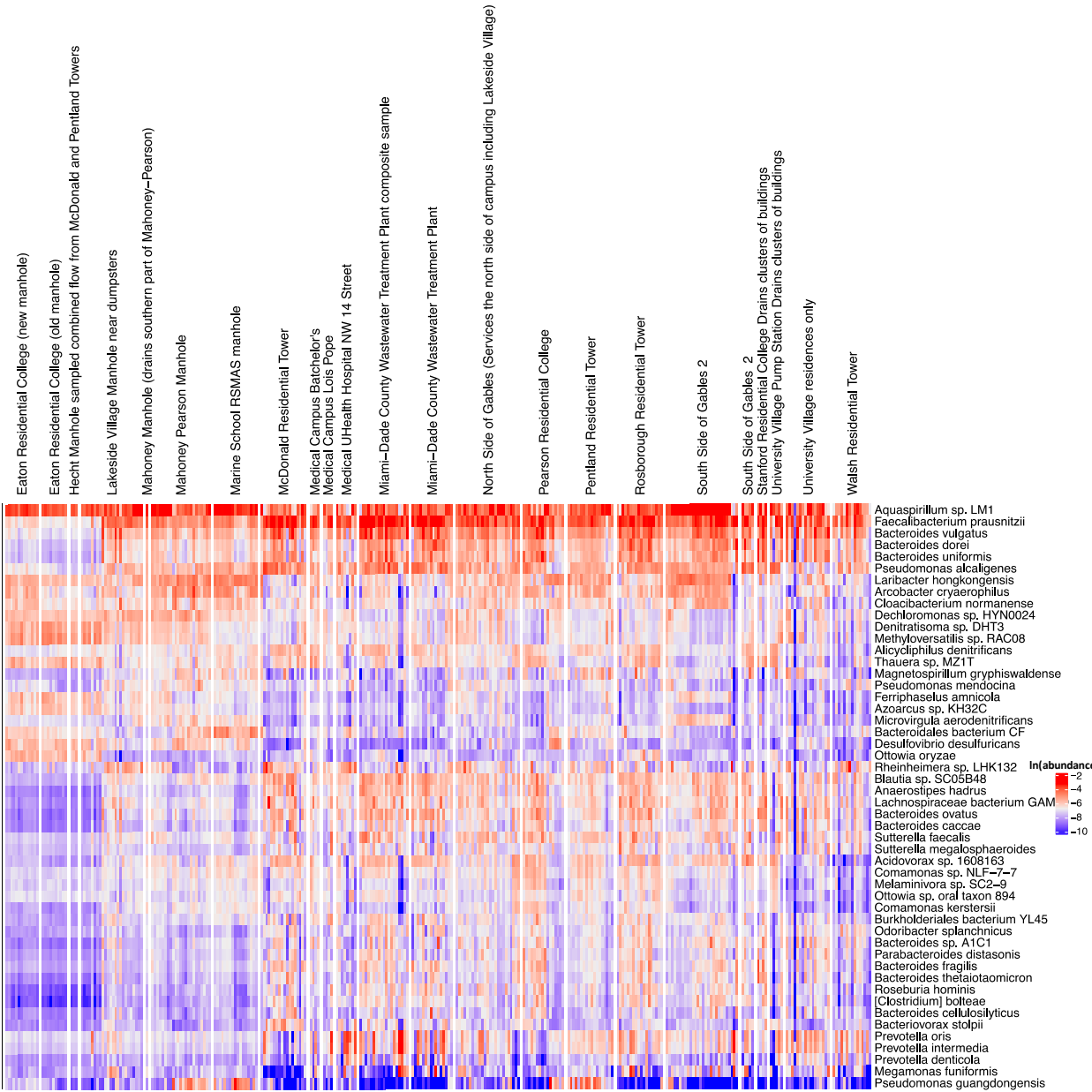
1. Masking low complexity regions
2. Integrating multiple k-mer alignment approaches
3. Integrating both coverage and abundance statistics

## Pipeline Overview:

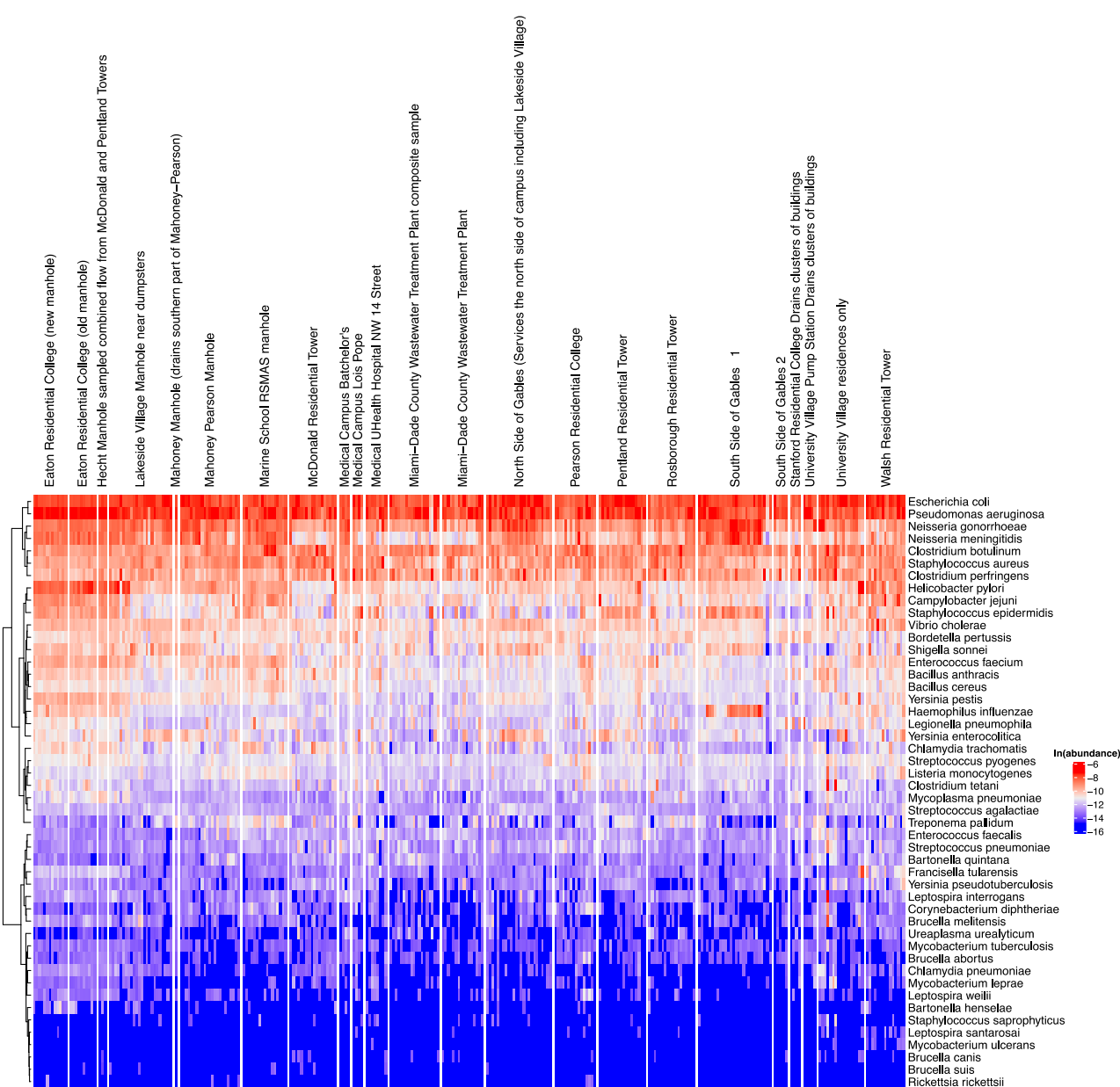
1. Read quality control + adapter removal with bbtools + optional masking
2. *De novo* assembly and contig fragment annotation to update existing databases
3. Taxonomic classification with xtree and kraken2 (aligned to RefSeq + dereplicated GenBank + assembled contigs)
4. Relative abundance calculations via a variety of methods
5. Custom taxonomic + phylogenetic viral classification



# Top bacterial taxa

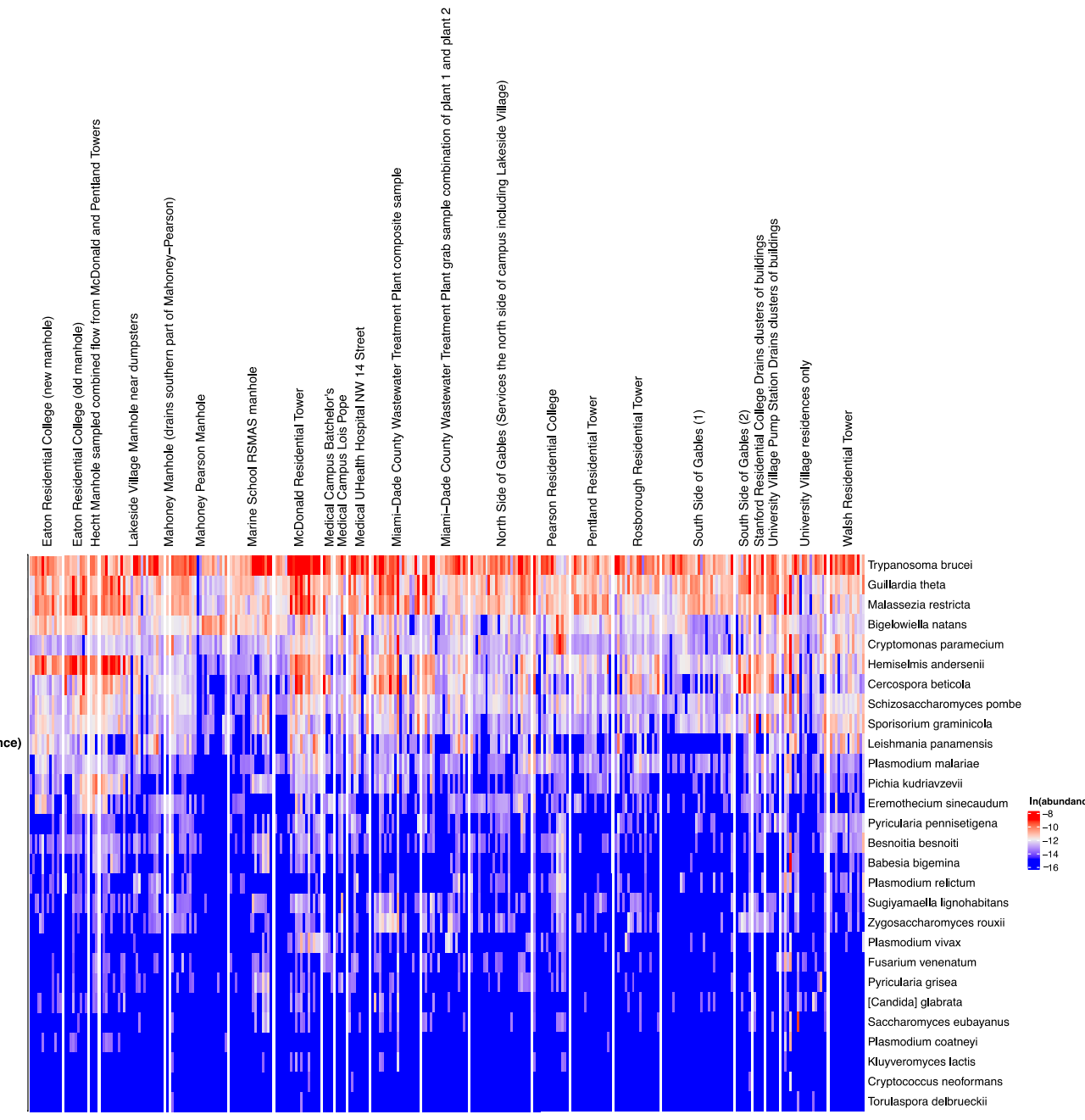
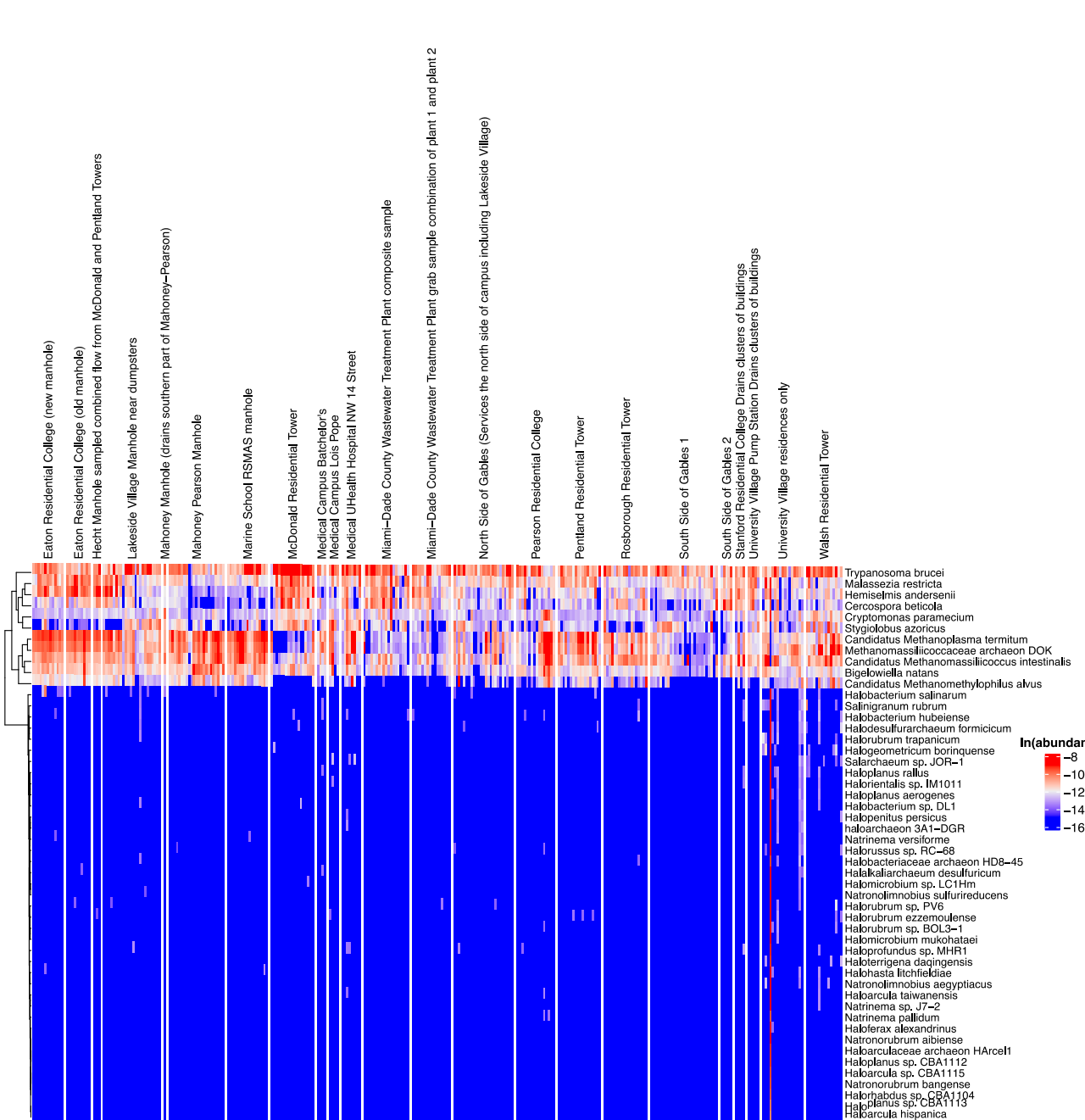


# Abundance of specific pathogens



# Masked reads (fungi)

# Unmasked reads (fungi)

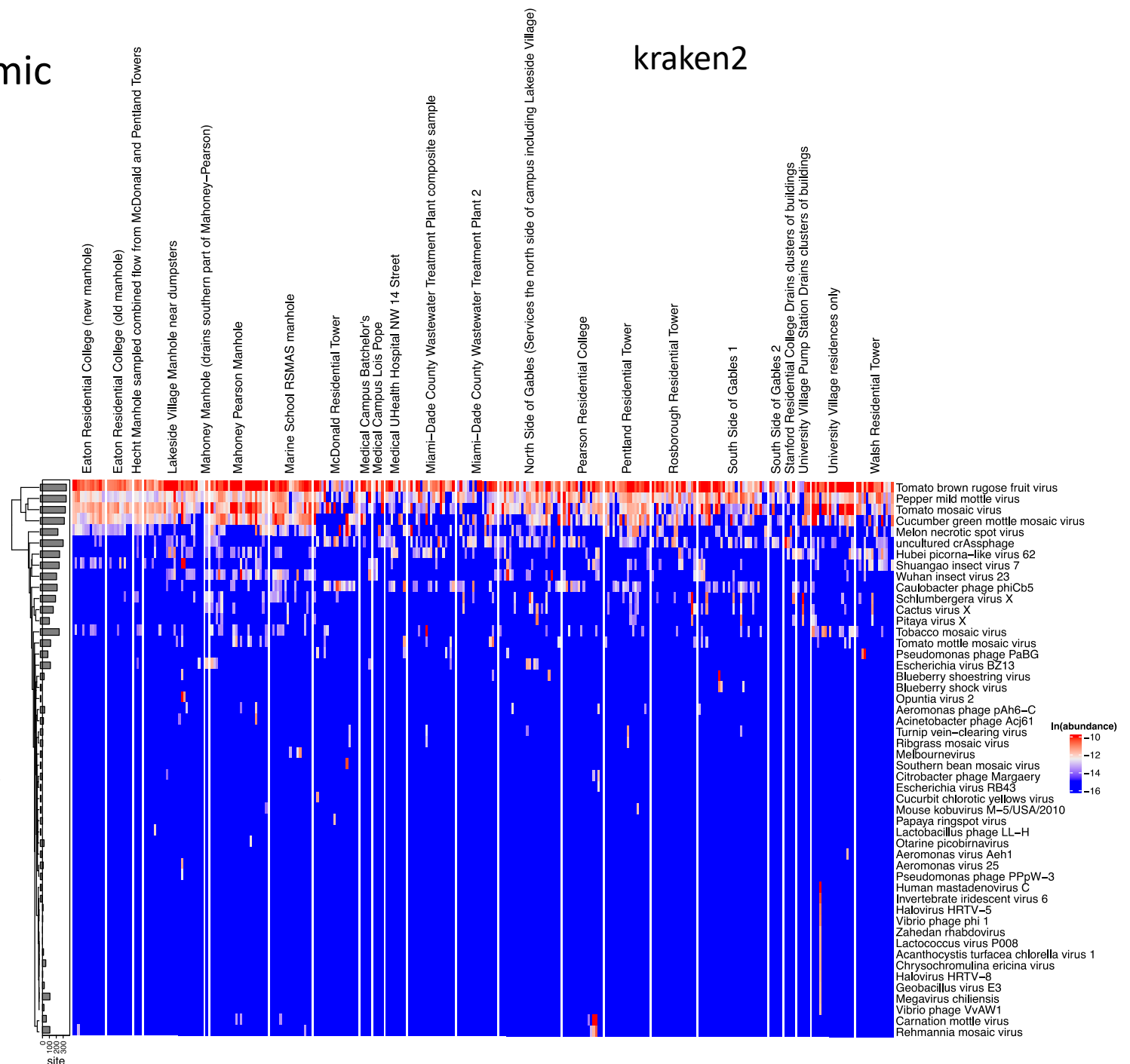
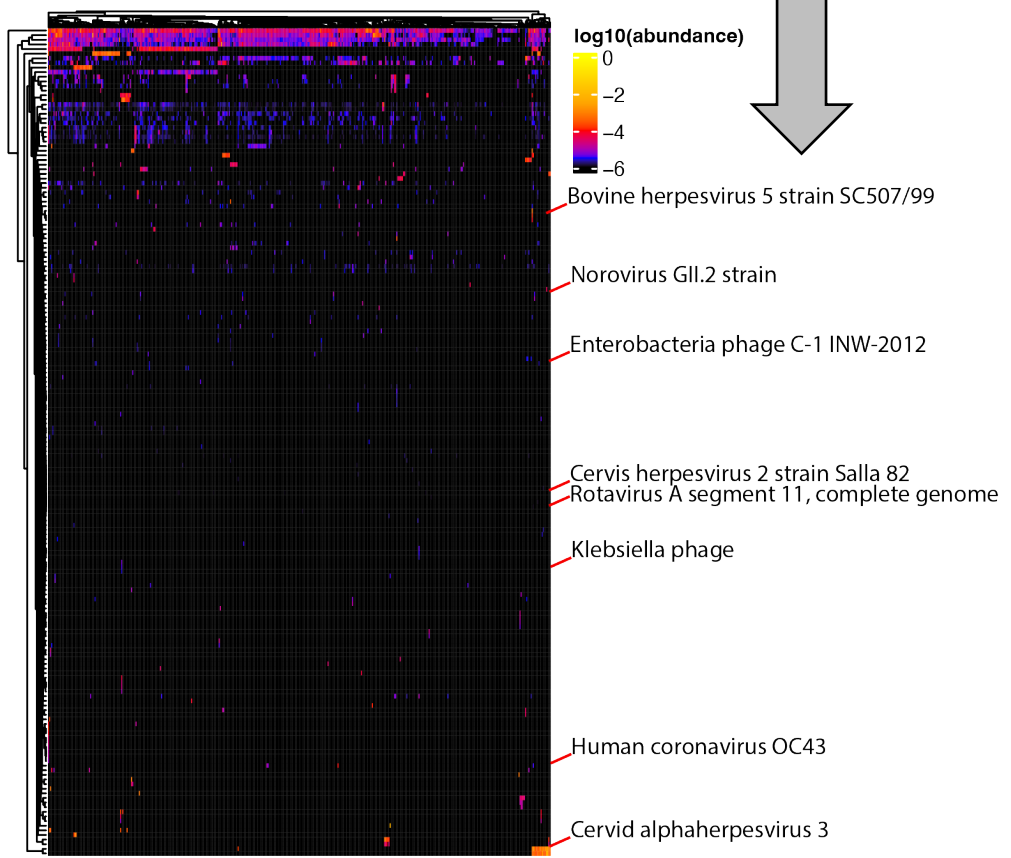


# Integrating total genome coverage, abundance statistics, and multiple taxonomic classifiers

kraken2

High confidence viral taxa based on unique genome coverage

Xtree



Eaton Residential College (new manhole)  
 Eaton Residential College (old manhole)  
 Hecht Manhole sampled combined flow from McDonald and Pentland Towers  
 Lakeside Village Manhole near dumpsters  
 Mahoney Manhole (drains southern part of Mahoney-Pearson)  
 Mahoney Pearson Manhole  
 Marine School RSMAS manhole  
 McDonald Residential Tower  
 Medical Campus Batchelor's  
 Medical Campus Lois Pope  
 Medical UHealth Hospital NW 14 Street  
 Miami-Dade County Wastewater Treatment Plant composite sample  
 Miami-Dade County Wastewater Treatment Plant 2  
 North Side of Gables (Services the north side of campus including Lakeside Village)  
 Pearson Residential College  
 Pentland Residential Tower  
 Rosborough Residential Tower  
 South Side of Gables 1  
 South Side of Gables 2  
 Stanford Residential College Drains clusters of buildings  
 University Village Pump Station Drains clusters of buildings  
 University Village residences only  
 Walsh Residential Tower

# Thank you

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