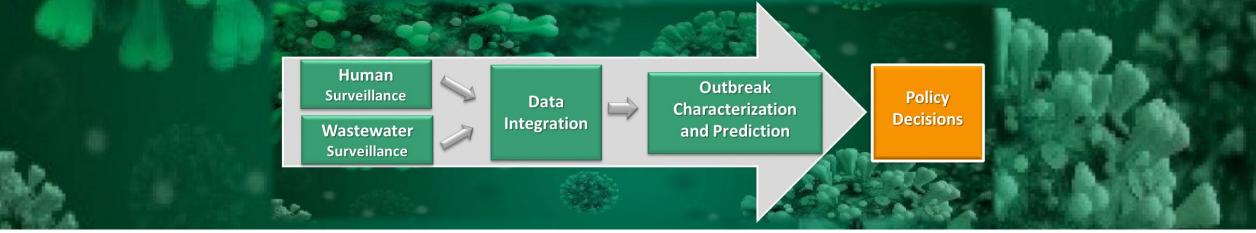
### South Florida -Rapid Acceleration of Diagnostics (SF-RAD):

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

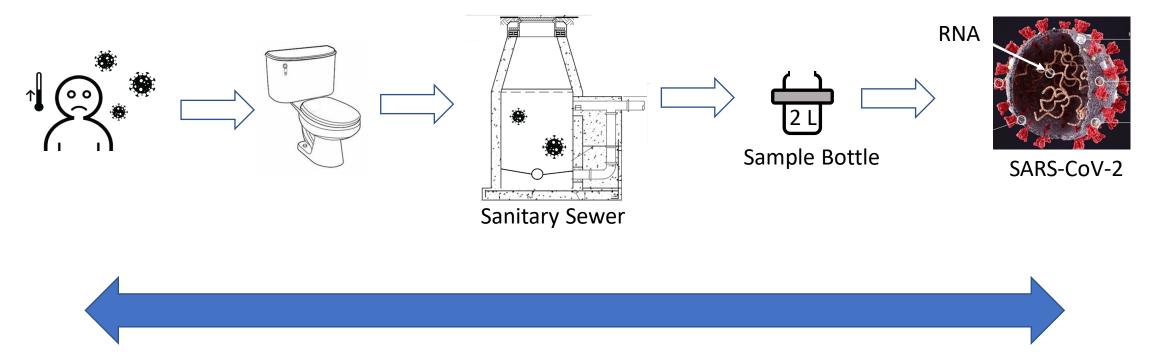






## Motivation & Objectives

Infected humans excrete COVID-19 virus in feces and urine



<u>Ultimate objective</u>: Relate wastewater measurements to predict COVID-19 cases.

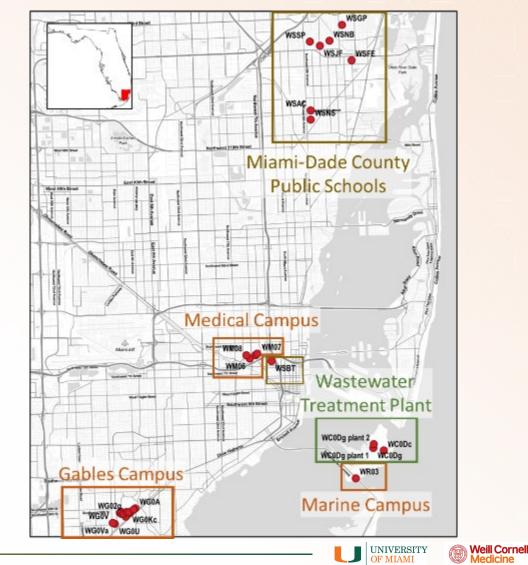


### Human & Wastewater Sample Collection

#### Human Surveillance

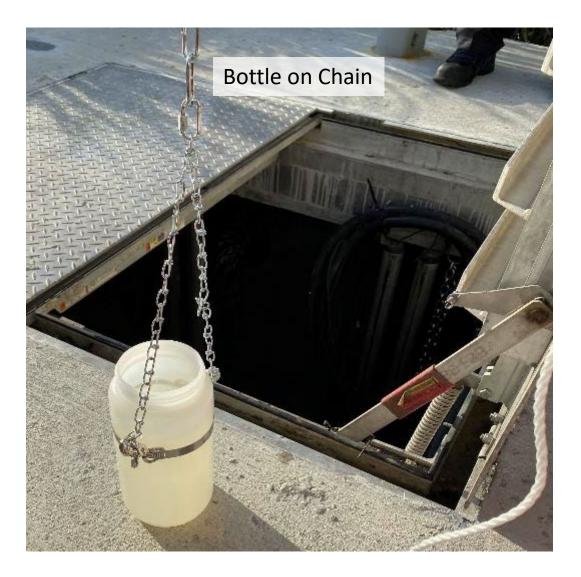
University of Miami	<ul> <li>Student Campus Residents, UMiami Gables/Marine (Sep. 2020)</li> <li>Fall'20/Spring'21 <ul> <li>Students tested weekly (nasal swab, qPCR) Supplemented by breath test</li> <li>COVID results and total tests by building/dorm room</li> </ul> </li> <li>Summer/Fall'21 <ul> <li>Unvaccinated students tested weekly</li> <li>All students tested when wastewater exceeds</li> </ul> </li> </ul>
	University Hospital, UMiami Medical (Sep. 2020)
	<ul> <li>Treat known COVID patients</li> <li>Electronic medical records pulled regularly</li> </ul>
Miami-Dade County	Miami-Dade County Residents, FDOH WWTP (Jan. 2021)
	<ul> <li>Positives by zip code</li> <li>Number of tests by zip code</li> <li>Augment with Biobot wastewater data (Apr. 2020)</li> </ul>
	Pilot, Miami-Dade County Public Schools, MDCPS (Jan. 2022)
	<ul> <li>In collaboration with RADx-UP project (Gwynn, PI)</li> <li>9 Schools (4 Elementary, 2 Middle, 3 High Schools)</li> </ul>

#### Sample Collection Plans

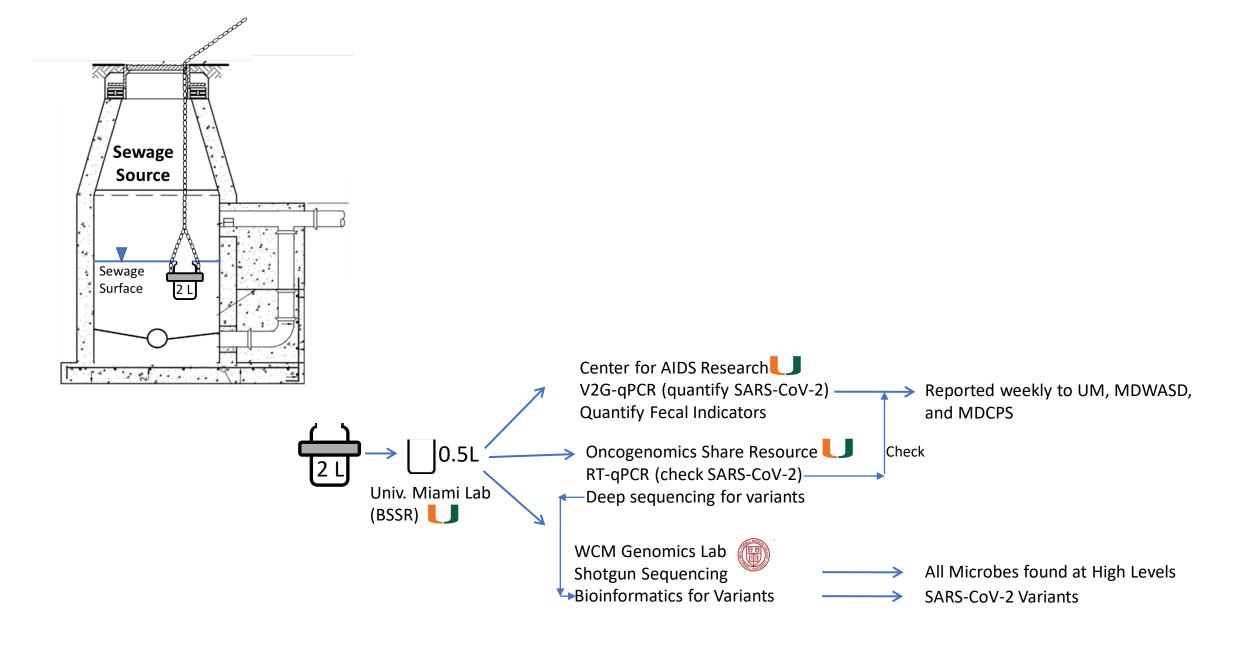


SF-RAD

## Sampling



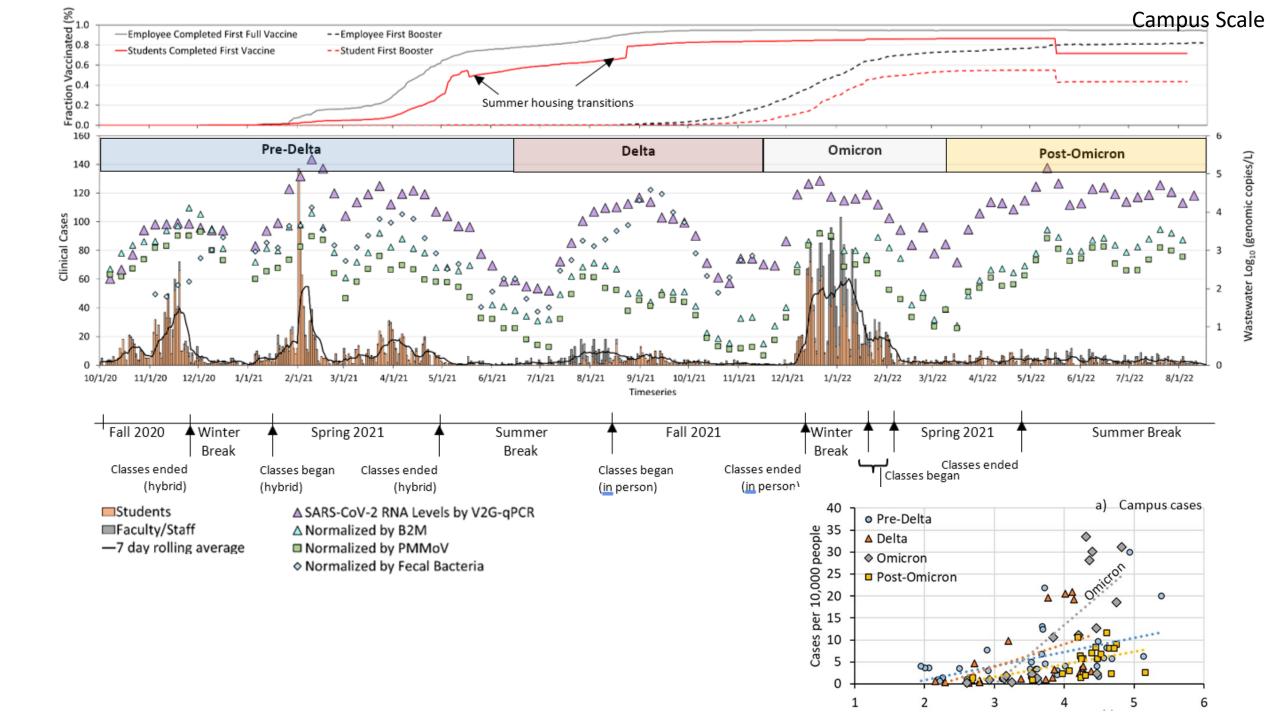




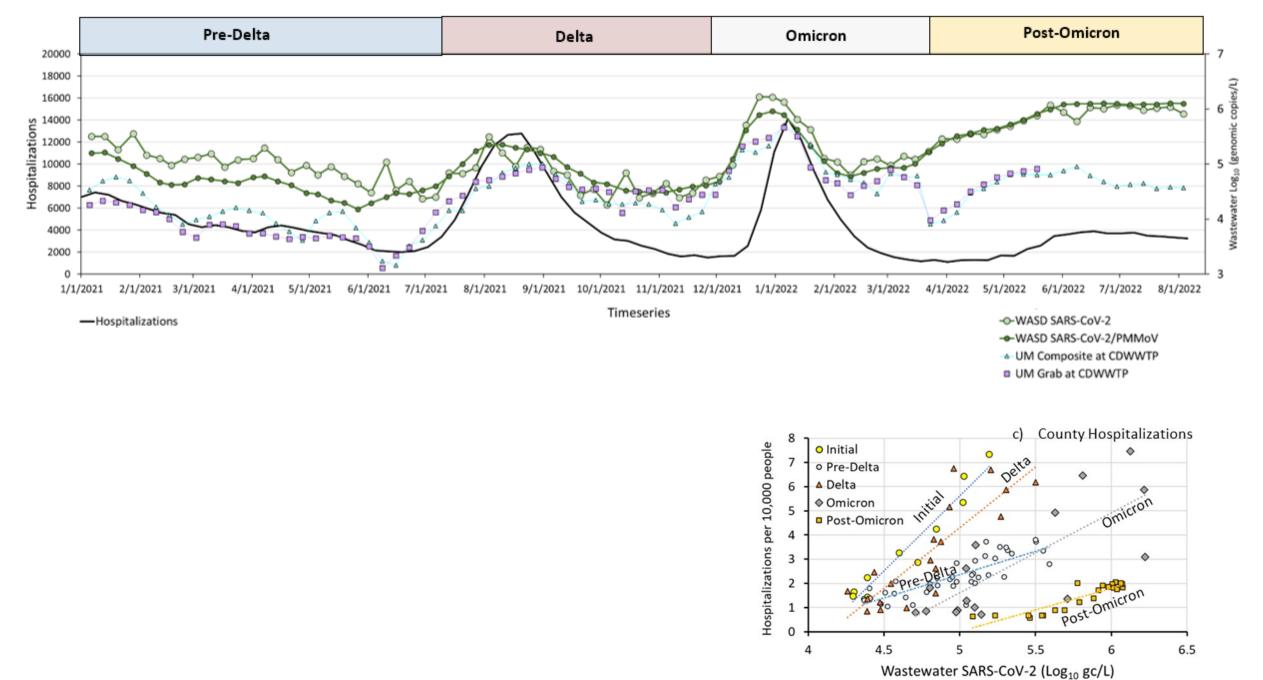
# SARS-CoV-2 Results







#### Hospitalizations



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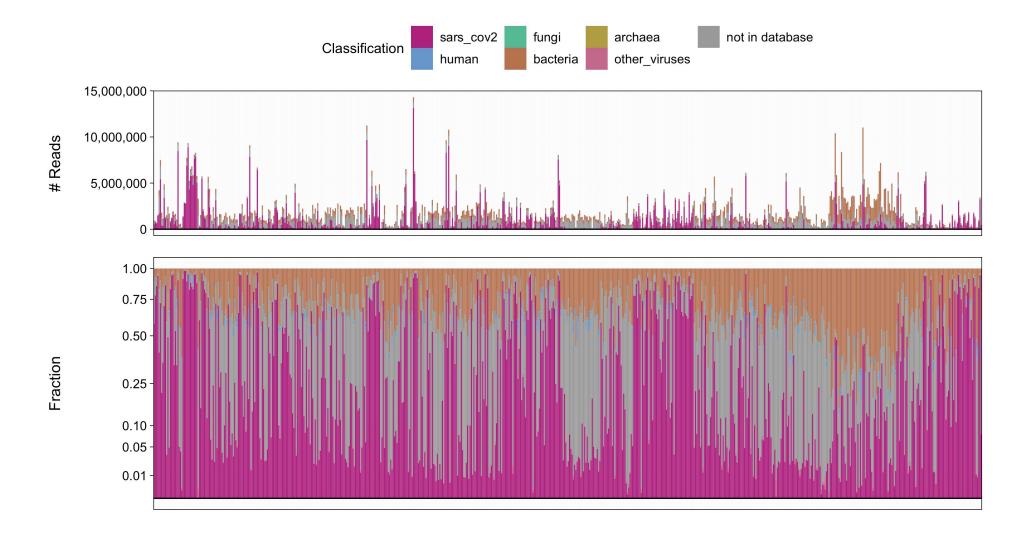
# Integration with Human Health Surveillance



## 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

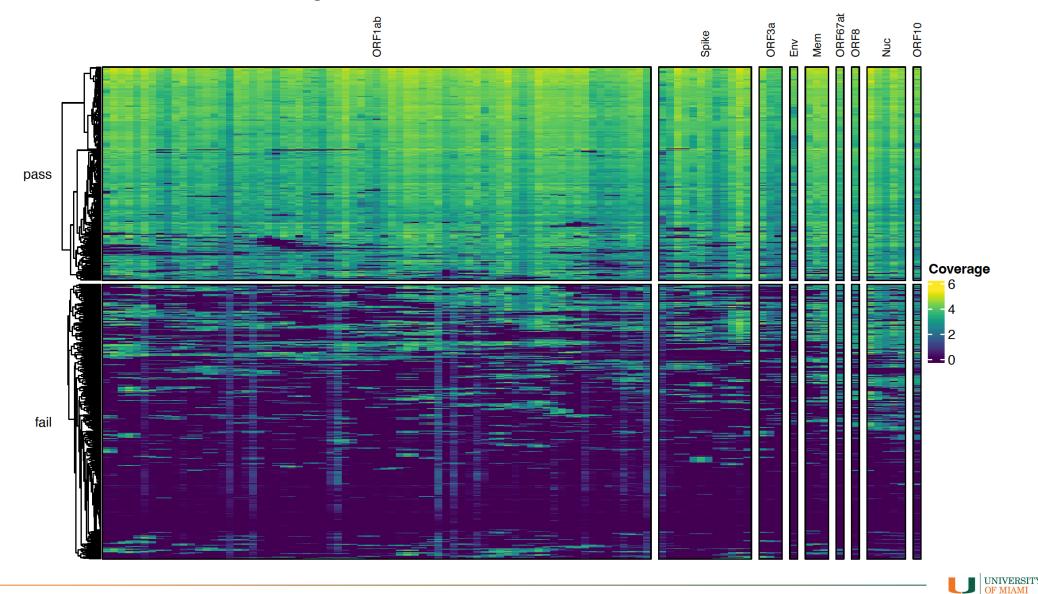
## Performance of ARTIC protocol up through June 2022





## Samples are filtered by total genome coverage

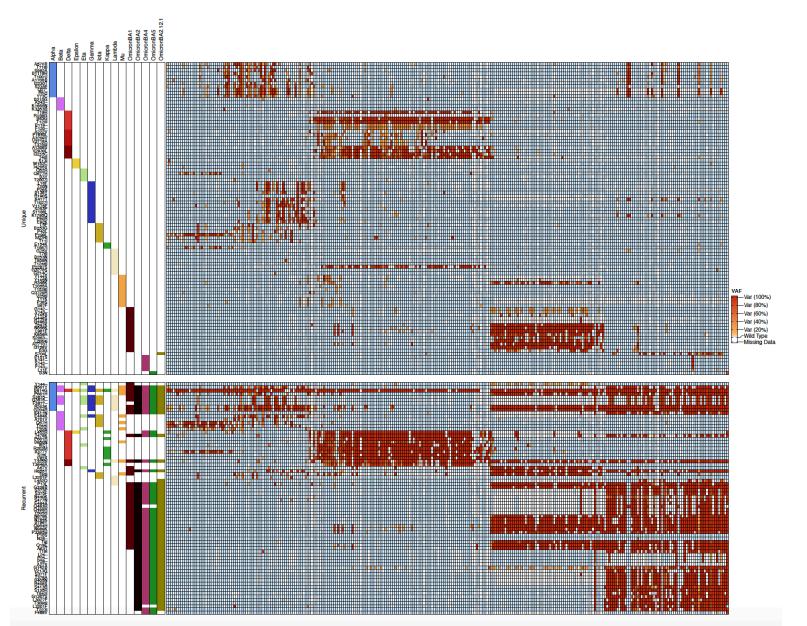
Minimum of 75% of SARS-CoV-2 genome must be covered with at least 50x





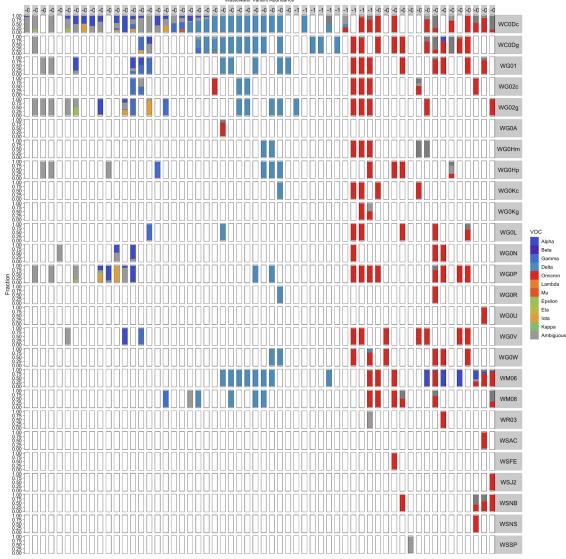
**SF-RAD** 

### Tracking signature mutations across all 2021-22 WW samples



UNIVERSITY OF MIAMI Weill Corne Medicine

## The dominance of omicron across all sites



UNIVERSITY OF MIAMI



### 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
- 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

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Aquaspirillum sp. LM1 Faecalibacterium prausnitzii Bacteroides vulgatus Bacteroides dorei Bacteroides uniformis Pseudomonas alcaligenes Laribacter hongkongensis Arcobacter cryaerophilus Cloacibacterium normanense Dechloromonas sp. HYN0024 Denitratisoma sp. DHT3 Methyloversatilis sp. RAC08 Alicycliphilus denitrificans Thauera sp. MZ1T Magnetospirillum gryphiswaldense Pseudomonas mendocina Ferriphaselus amnicola Azoarcus sp. KH32C Microvirgula aerodenitrificans Bacteroidales bacterium CF Desulfovibrio desulfuricans Ottowia oryzae Rheinheimera sp. LHK132 In(abundance) Blautia sp. SC05B48 Anaerostipes hadrus Lachnospiraceae bacterium GAM -6 Bacteroides ovatus -8 Bacteroides caccae Sutterella faecalis Sutterella megalosphaeroides Acidovorax sp. 1608163 Comamonas sp. NLF-7-7 Melaminivora sp. SC2-9 Ottowia sp. oral taxon 894 Comamonas kerstersii Burkholderiales bacterium YL45 Odoribacter splanchnicus Bacteroides sp. A1C1 Parabacteroides distasonis Bacteroides fragilis Bacteroides thetaiotaomicron Roseburia hominis [Clostridium] bolteae Bacteroides cellulosilyticus Bacteriovorax stolpii Prevotella oris Prevotella intermedia Prevotella denticola Megamonas funiformis Pseudomonas auanadonaensis

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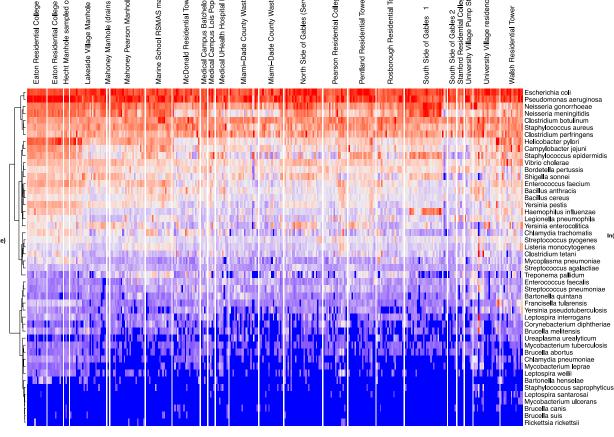
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Eremothecium sinecaudum

Pyricularia pennisetigena Besnoitia besnoiti Babesia bigemina Plasmodium relictum Sugiyamaella lignohabitans Zygosaccharomyces rouxii Plasmodium vivax Fusarium venenatum Pyricularia grisea [Candida] glabrata Saccharomyces eubayanus Plasmodium coatneyi Kluyveromyces lactis Cryptococcus neoformans Torulaspora delbrueckii

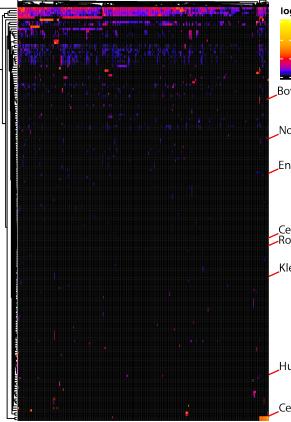
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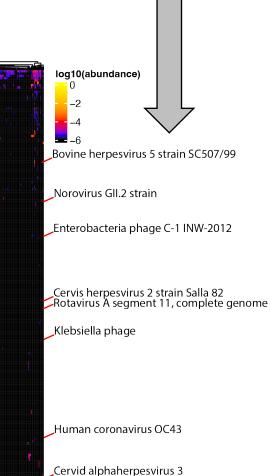
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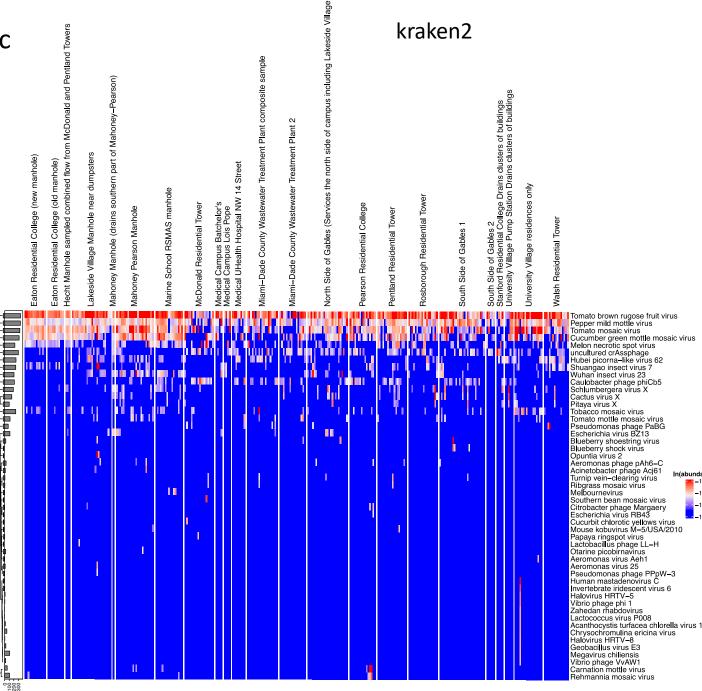
Integrating total genome coverage, abundance statistics, and multiple taxonomic classifiers

High confidence viral taxa based on unique genome coverage

Xtree







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# Thank you

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Helena Solo-Gabriele hmsolo@miami.edu

