

Lessons Learned from Environmental Monitoring of SARS-CoV-2

Thursday, April 14, 2022
1:00 pm EST

Speakers



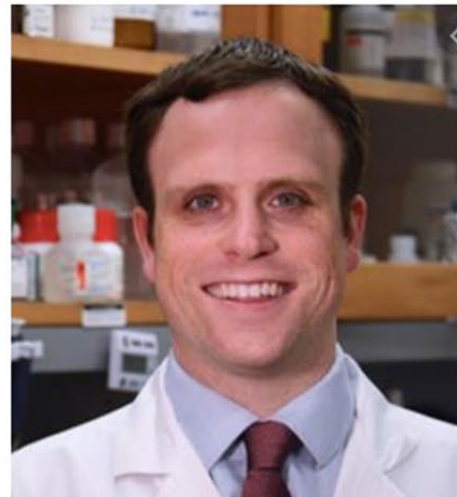
Helena Solo-Gabriele, Ph.D., P.E.

Professor, Civil, Architectural &
Environmental Engineering
Associate Dean for Research
College of Engineering, University of Miami



Stephan Schürer, Ph.D.

Professor, Pharmacology
Associate Director, Data Sciences
Sylvester Comprehensive Cancer Center
Miller School of Medicine, University of Miami



Christopher E. Mason, Ph.D.

Professor, Physiology and Biophysics
Director, WorldQuant Initiative
for Quantitative Prediction
Weill Cornell Medicine, Cornell University



George S. Grills

Associate Director, Shared Resources
Sylvester Comprehensive Cancer Center
Miller School of Medicine, University of Miami

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

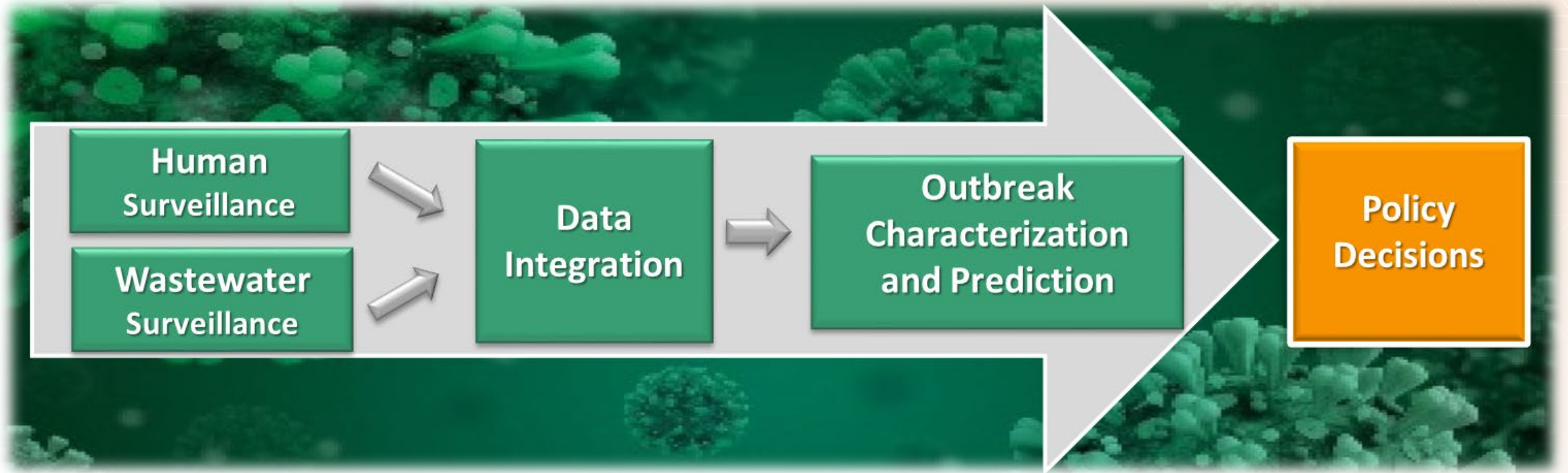
Development and Proof-of-Concept
Implementation of the South Florida Miami RADx-rad
SARS-CoV-2 Wastewater-Based Surveillance Infrastructure

Funded by
NIH RADx-rad Grant
1U01DA053941-01



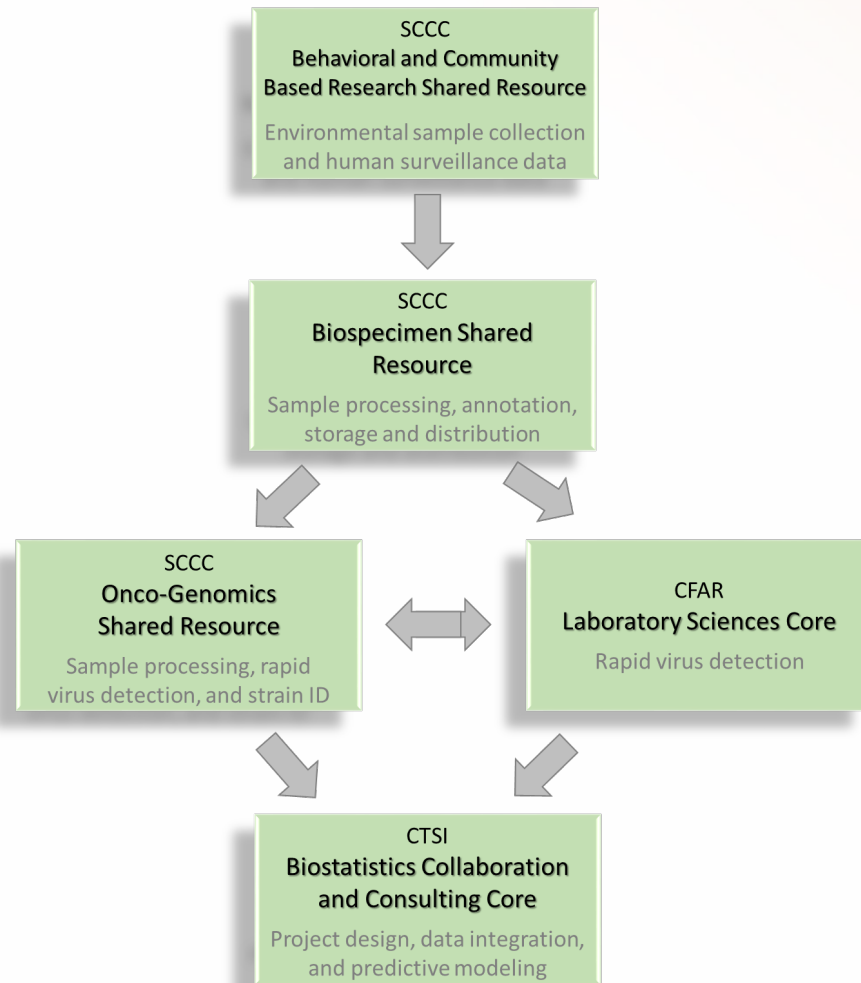
SF-RAD: SARS-CoV-2 Wastewater-Based Surveillance

Aims



SF-RAD: SARS-CoV-2 Wastewater-Based Surveillance

Integrated Multi-Shared Resources Support



Sylvester Comprehensive Cancer Center

Behavioral & Community-Based Research Shared Resource
Biospecimen Shared Resource
Onco-Genomics Shared Resource



Center for AIDS Research

Laboratory Sciences Core



Clinical & Translational Science Institute

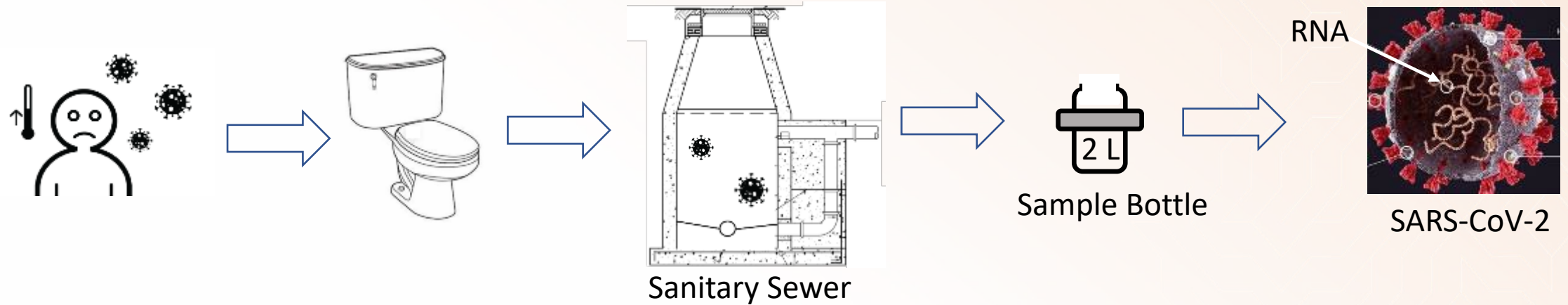
Biostatistics Collaboration and Consulting Core

Aim:

Wastewater Characterization

Motivation & Objectives

Infected humans excrete COVID-19 virus in feces and urine

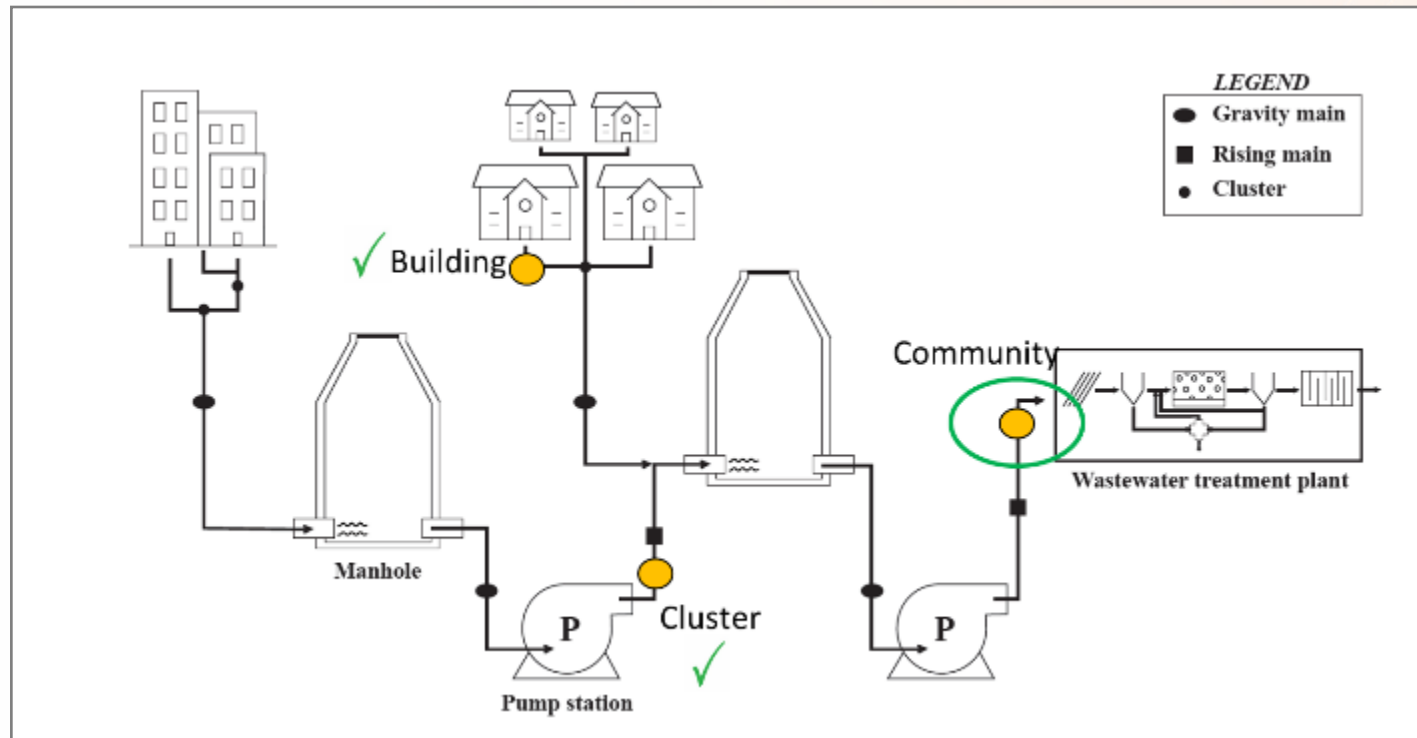


Ultimate objective: Relate wastewater measurements to predict COVID-19 cases.

Wastewater Characterization

Aims

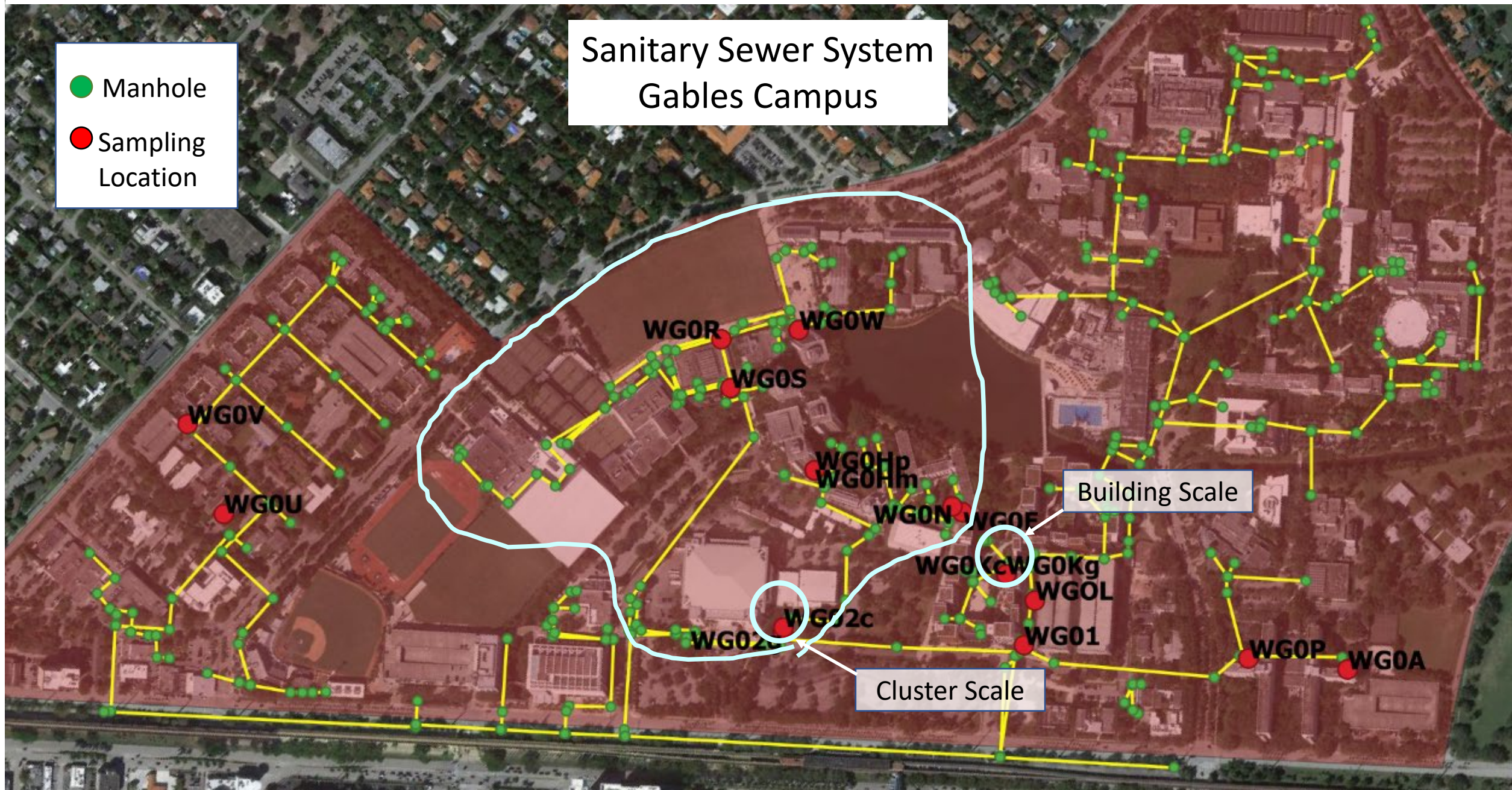
- Evaluate influence of watershed scale
- Evaluate sample concentration methods
- Evaluate sample collection method on SARS-CoV-2 measures
- Relate wastewater to human surveillance data



Evaluate Sewershed Scale

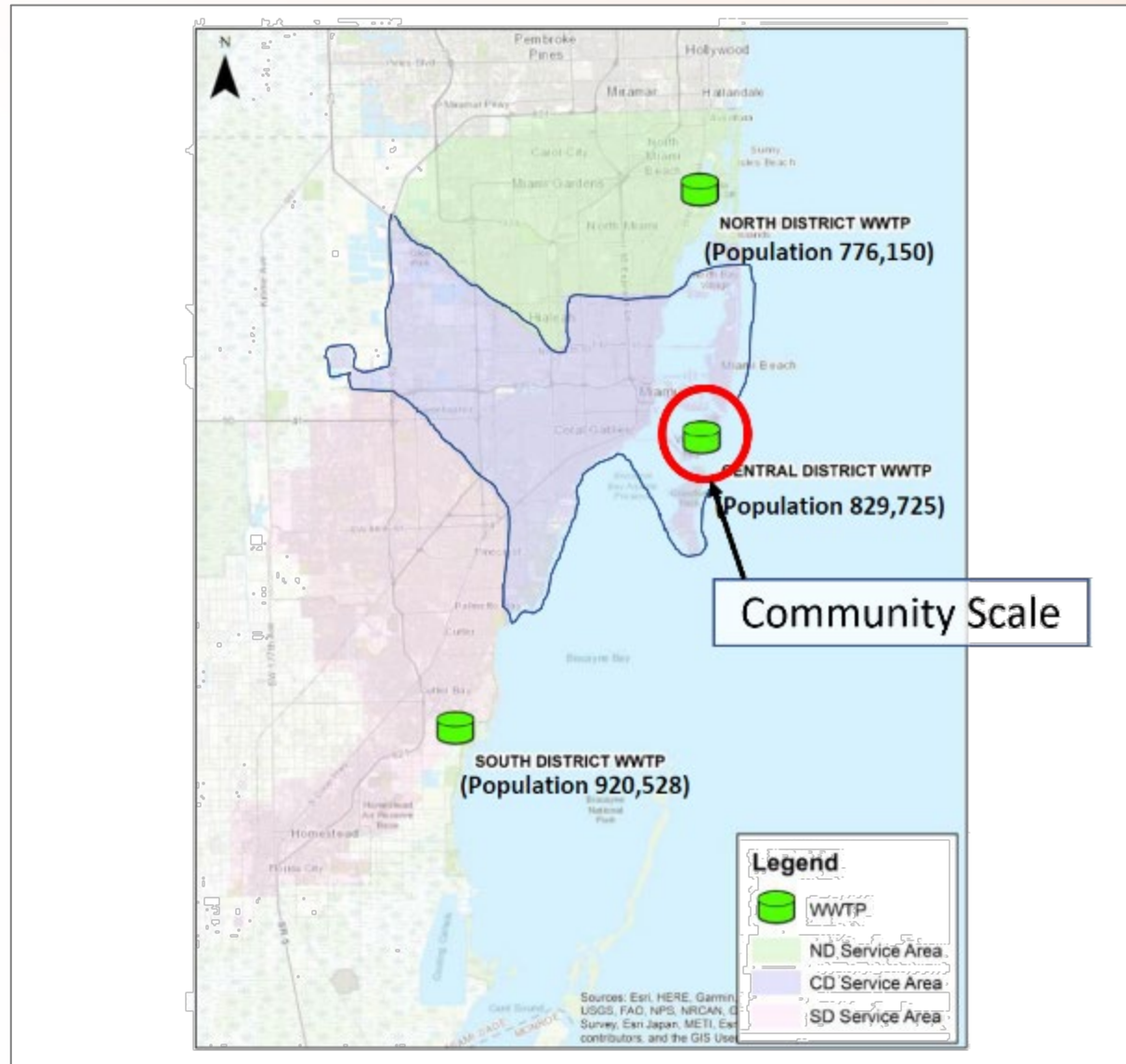
Sanitary Sewer System Gables Campus

- Manhole
- Sampling Location



Sewershed Scale

Sanitary Sewer Coverage for Miami Dade County

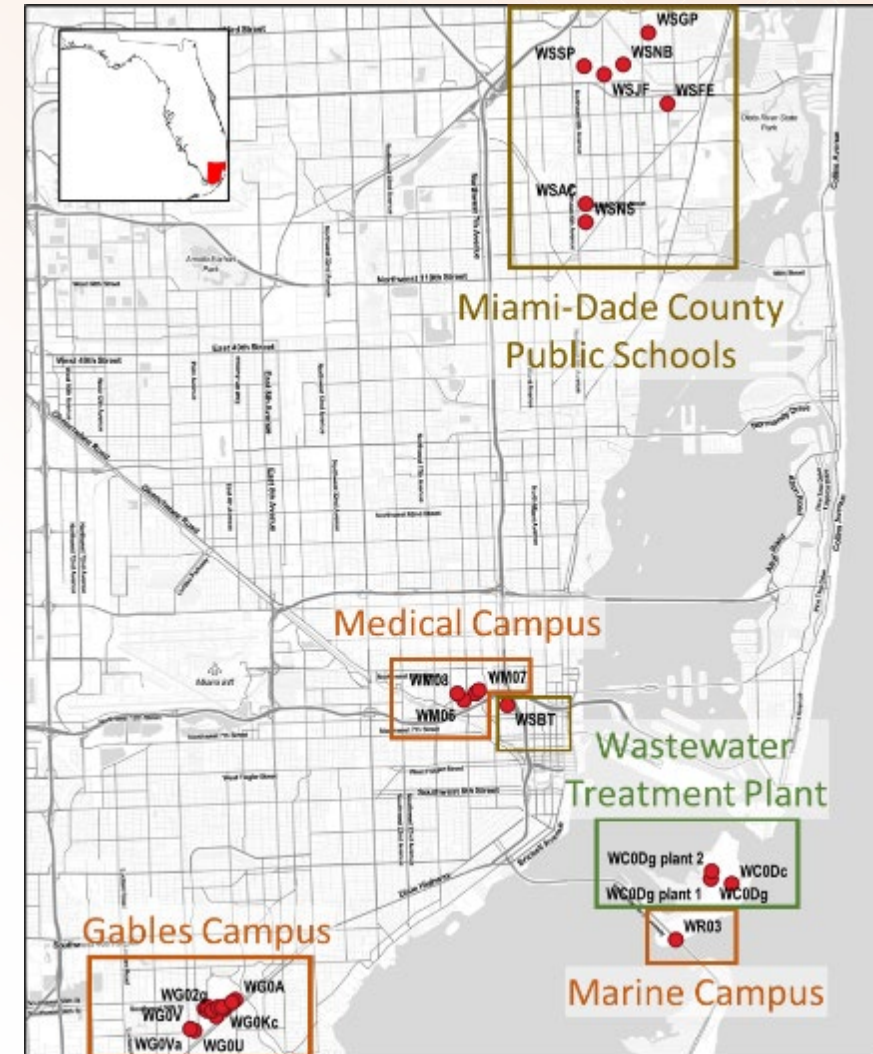


Human & Wastewater Sample Collection

Human Surveillance

Sample Collection Plans

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



Sample Collection Strategies (Composite vs Grab)



Grab



Composite

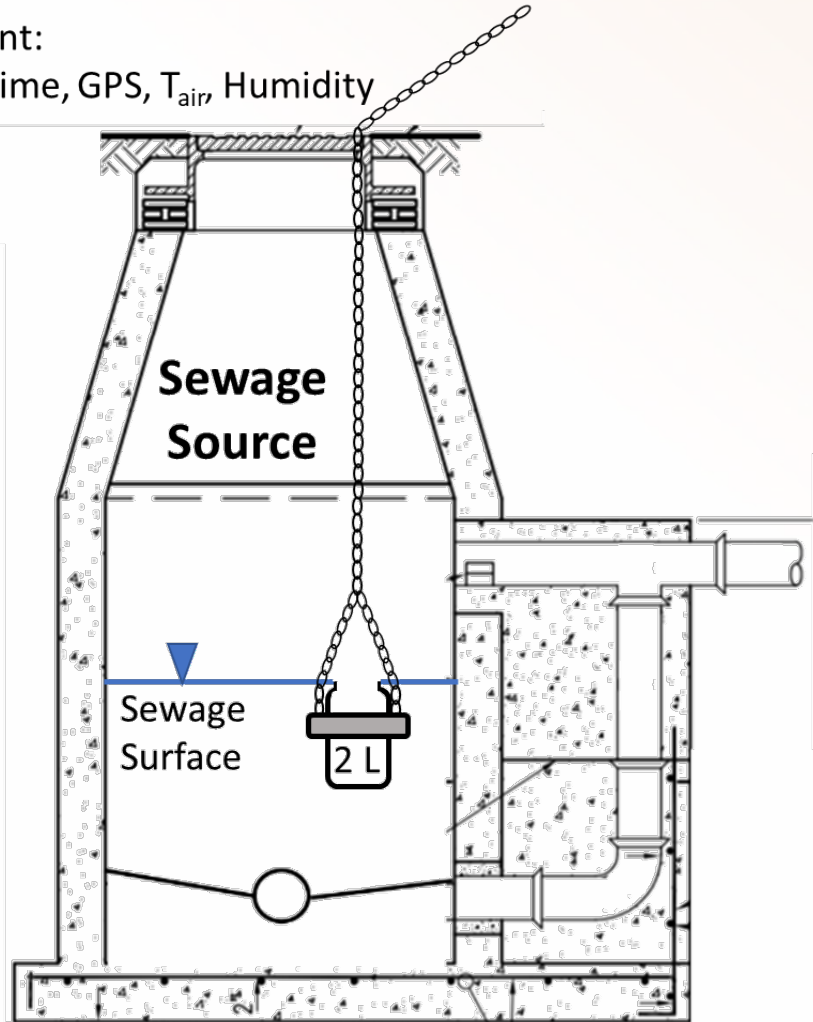


Autosampler
(Composite)

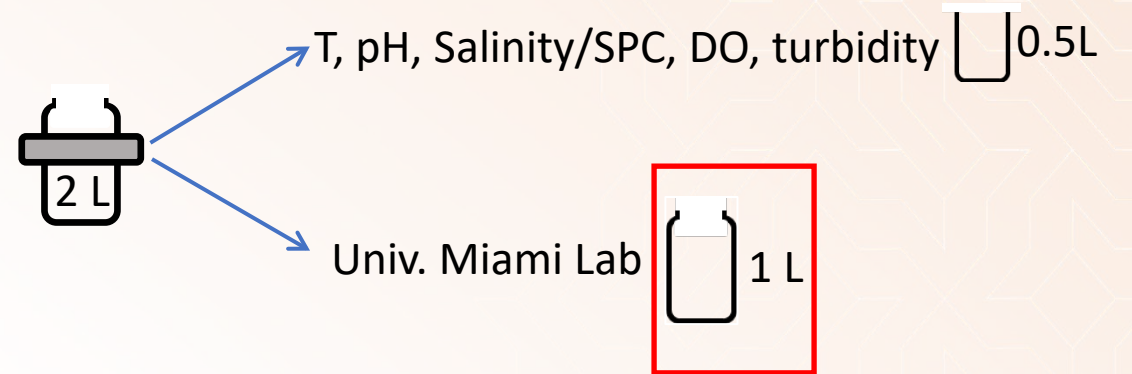
Scooper
(Grab)

SAMPLING at UMiami

Ambient:
Date/time, GPS, T_{air} , Humidity

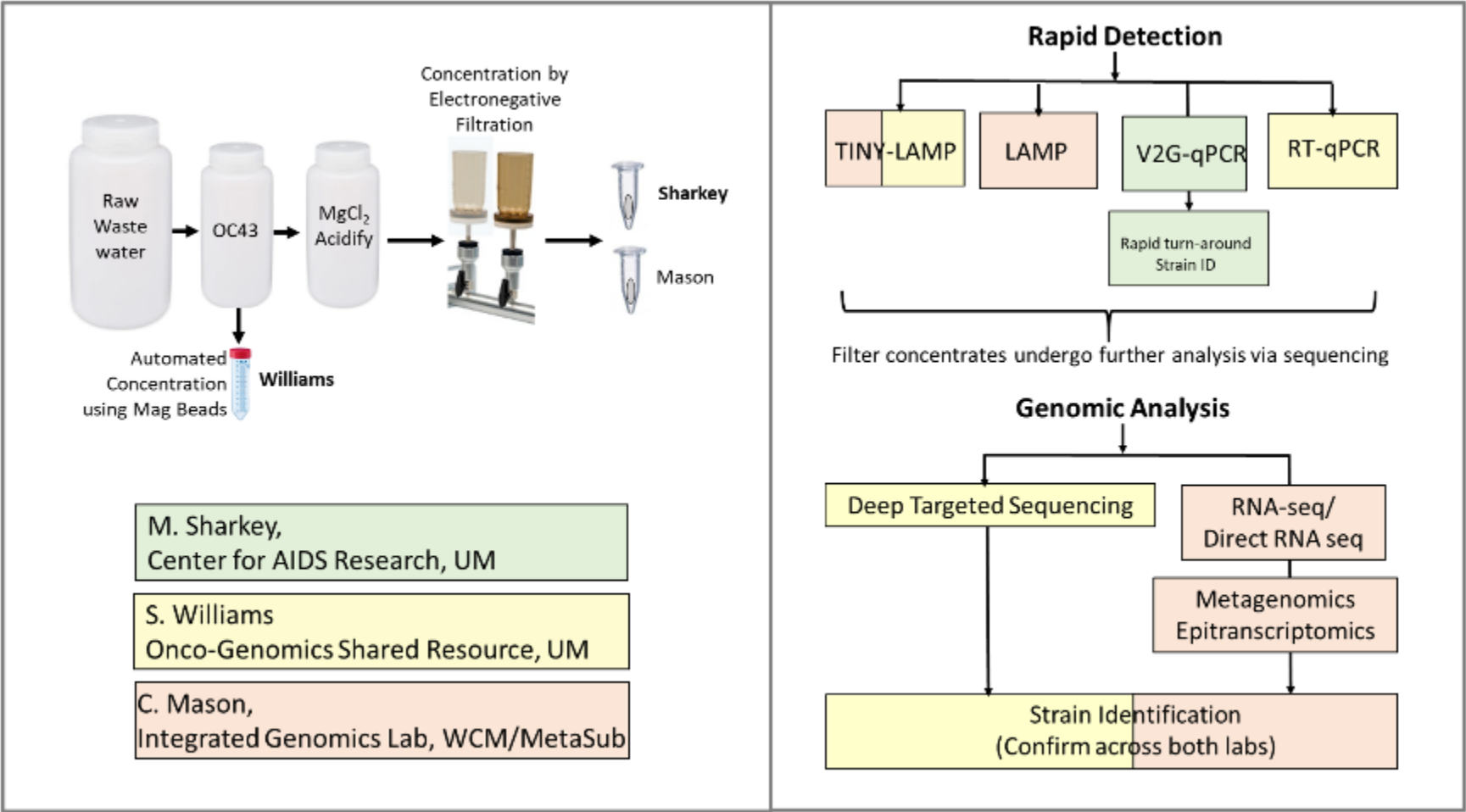


Collect Samples Weekly
Results available in 12 hours

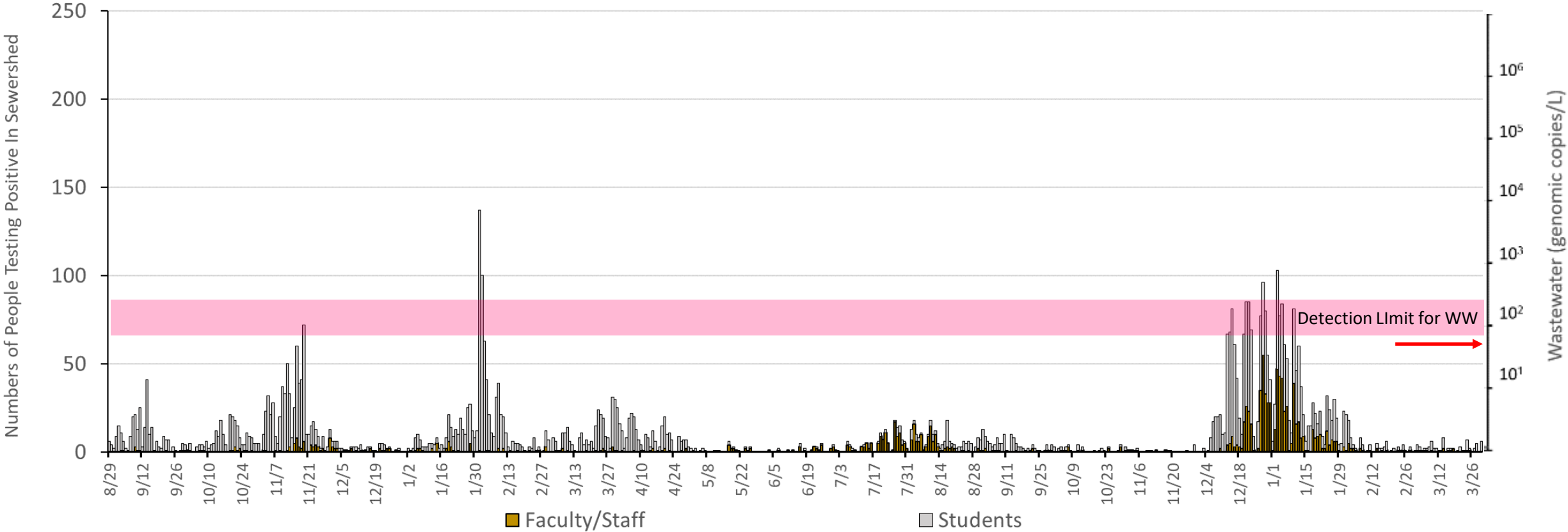


Wastewater Characterization

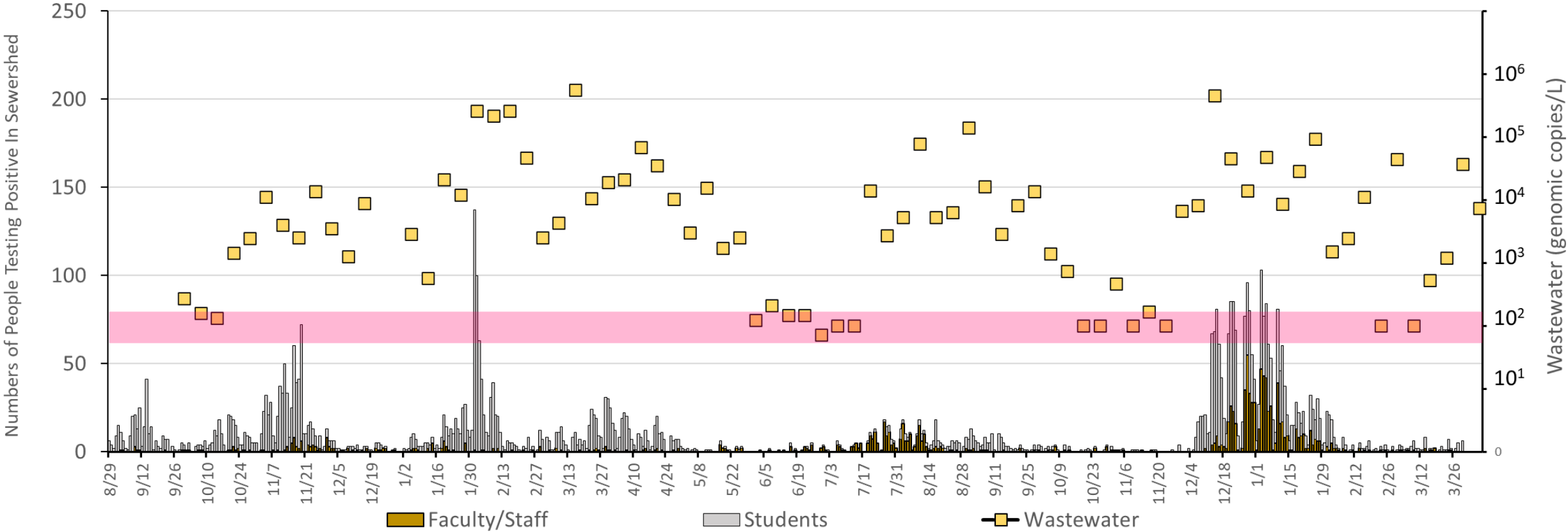
Sample Processing & Data Generation



Campus (cluster scale) time series plot

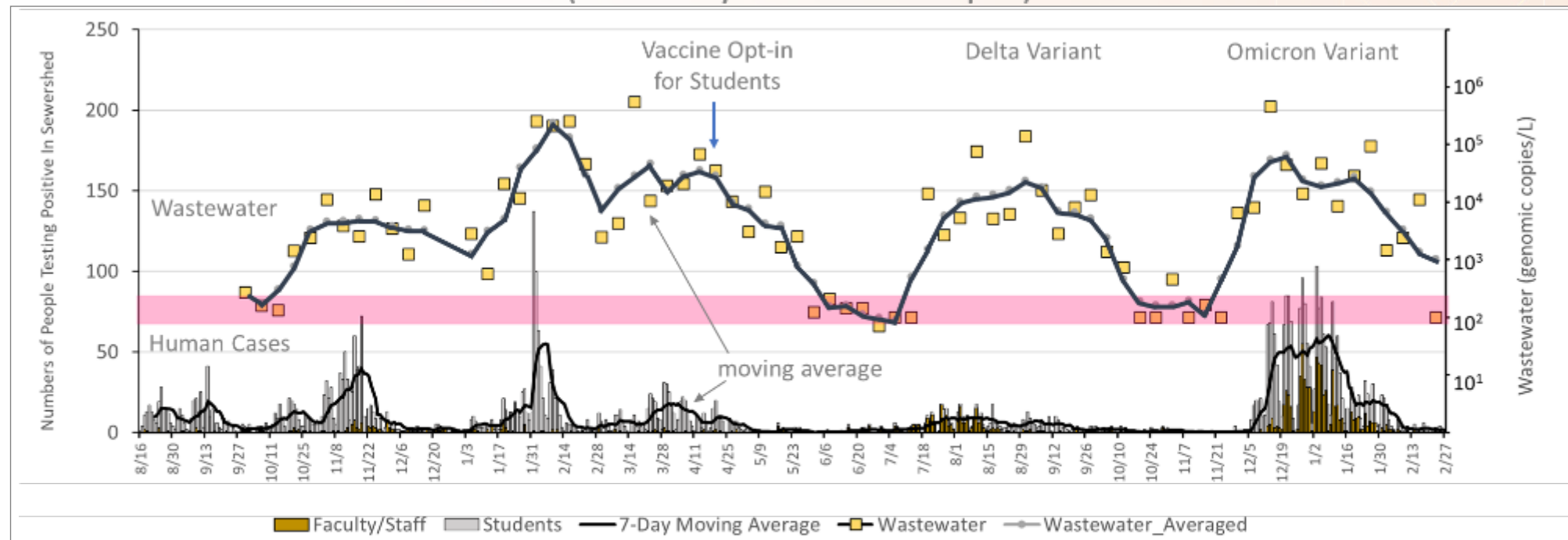


Campus (cluster scale) time series plot



Integration of Human and Environmental Surveillance

Building Cluster Scale (University of Miami Campus)



SARS-CoV-2 in wastewater was a 4-day lead indicator

$$\text{Positivity (\%)} = 9 \ln(C) / 10$$

10^2 gc/L of SARS-CoV-2 in wastewater associated with 4% positivity.

10^4 gc/L \rightarrow 8%

10^6 gc/L \rightarrow 12%

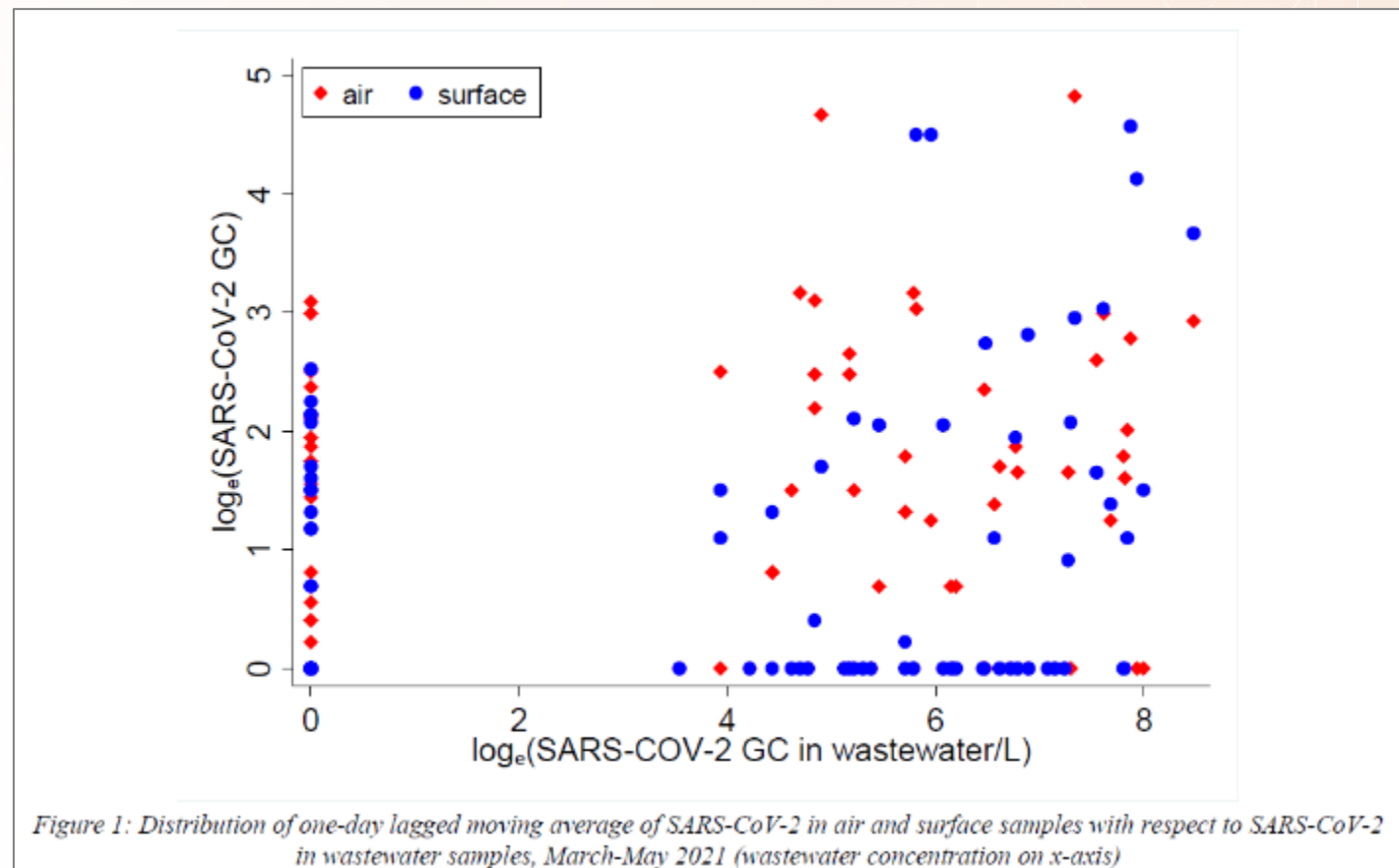
Sharkey et al. 2021, <https://doi.org/10.1016/j.scitotenv.2021.149177>

Model by Dr. Naresh Kumar

Integration of Human and Environmental Surveillance

Air and Surface Sampling Added to Wastewater Monitoring

	Prediction of COVID-19 cases (2-day lagged)
Air	100%
Surface	82%
Wastewater	73%



From Naresh Kumar

Aim:
Data Standardization

FAIR Guiding Principles for Scientific Data

Findable:

- F1. Globally unique and persistent identifiers
- F2. Rich and qualified metadata
- F3. Registered or indexed in a searchable resources

Accessible (and Attributable):

- A1. Retrievable by their identifier in an open & free protocol
- A2. Formal data citation record and provenance

Interoperable:

- I1. Formal language for knowledge representation
- I2. Vocabularies / ontologies and qualified references

Reusable:

- R1. Clear and accessible data usage license
- R2. Provenance

Replicable:

- R3. Experimental metadata (reporting guidelines)



PURL



Wilkinson et al *Nat Sci Data* 2016

Standards to Report Biomedical Data

- **Reporting guidelines (minimum information specifications):** specify what information need to be captured about an experiment for a particular purpose
- **Controlled vocabularies:** terminological resource that provides the identification and formal definition of entities - **Ontologies**
- **Data exchange formats:** specification how data are encoded to be computer-readable / -processable
- **Policies:** required data standards and experimental data quality by research consortia, funders, publishers

FAIRsharing - Resource for Data Standards and Policies

Standards

- Reporting guidelines
- Terminology artifacts
- Model / format

Policies

Journal
Funder
Society

Associated databases

Collections

[Standards](#)[Databases](#)[Policies](#)[Collections](#)[Add/Claim Content](#)[Stats](#)[Log in or Register](#)

A curated, informative and educational resource on data and metadata *standards*, inter-related to *databases* and data *policies*.

[Find](#)

Recommendations

Standards and/or databases recommended by journal or funder data policies.

[Discover](#)

Collections

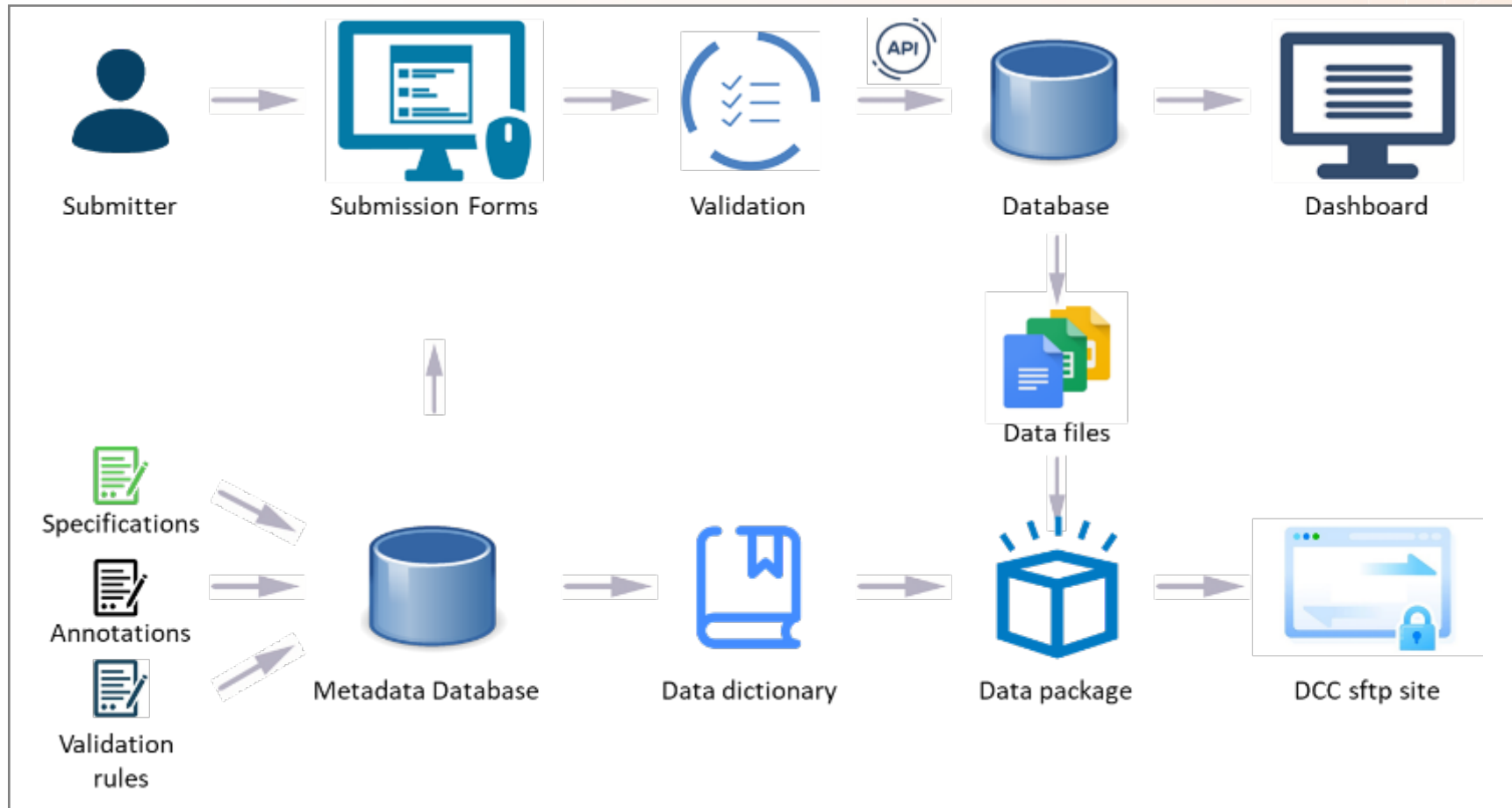
Standards and/or databases grouped by domain, species or organization.

[Learn](#)

Educational

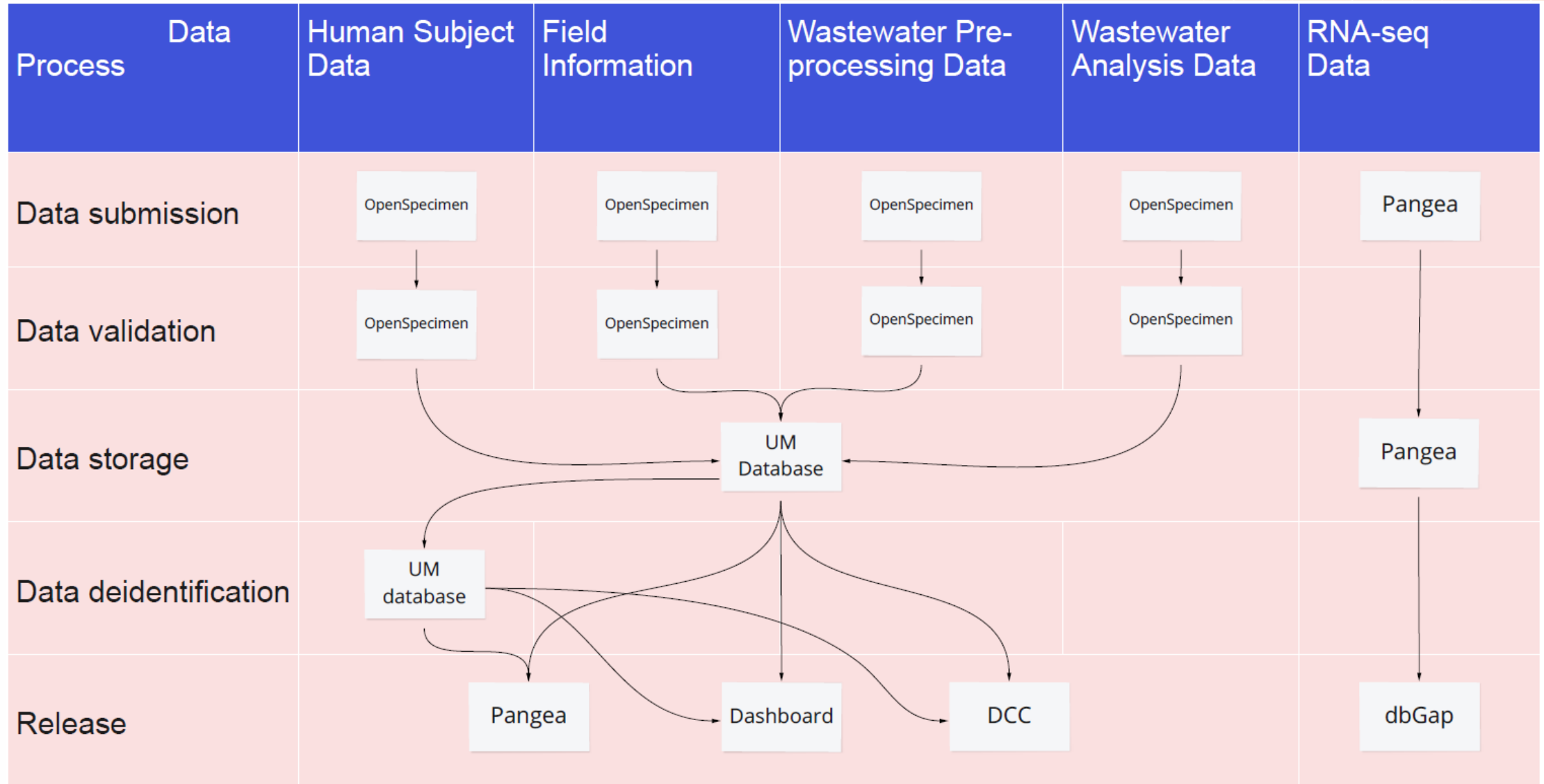
About standards, their use in databases and policies, and how we can help you.

Standardized SF-RAD Data & Metadata Management



- Establish data and metadata categories and develop metadata standards
- Establish end-to-end data flow process
- Implement operational informatics infrastructure to manage data & metadata
- Implement Data Portal for data access and integration

End-to-end SF-RAD Data Flow



Status of SF-RAD Metadata Development

	Category Name	Category Description	Number of specifications	Developed
Field Info	Field Data	Sample collection information from the field	59	<input checked="" type="checkbox"/>
	Continuous Measurements of Water Quality	Continuous record of water quality measured by a sonde	19	<input checked="" type="checkbox"/>
Wastewater pre-processing	Pretreatment	Sample splitting and addition of recovery control	43	<input checked="" type="checkbox"/>
	Concentration	Sample concentration by different methods	48	<input checked="" type="checkbox"/>
	Bacterial Culture Analyses	Fecal Coliform Bacterial Results	19	<input checked="" type="checkbox"/>
Analysis sample preparation	Extraction	Nucleic acid extraction	43	<input checked="" type="checkbox"/>
Wastewater analysis	qPCR Results	V2G-qPCR, RT-qPCR, LAMP assay results	66	<input checked="" type="checkbox"/>
	RNA-seq Results	RNA-seq assay results	42	<input checked="" type="checkbox"/>
Human subject data	Clinical Patient Data	Individual deidentified patient data from hospital	56	<input checked="" type="checkbox"/>
	Students Individual Data	Individual deidentified students data from campus	-	<input type="checkbox"/>
	Students Aggregated Data	Aggregated students testing data from campus	7	<input checked="" type="checkbox"/>
	County Level Data	County level data from the FDOH	-	<input type="checkbox"/>

SF-RAD metadata are aligned across the RADx-rad research consortium (<https://www.radxrad.org>) and CDC (<https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance>)

SF-RAD Field Metadata Example

SF-RAD Metadata Specifications for:

Field Data

Importance 1: Required, 0: Optional

SF-RAD Field Name	Definition	Importance
matrix_type	What is tested: wastewater (W); air (A); surface swab (S)	1
sample_location_code	Code corresponding to the location (e.g. WG01)	1
sample_id	Format: Sample Location-Date-Time (parent ID) [XXXX-YYMMDDHH]	1
sampling_date	Date of the sampling	1
sampling_time	Time of the sampling	1
gps_latitude_degrees	GPS coordinates (latitude) of the sampling location, in degrees	0
gps_latitude_minutes	GPS coordinates (latitude) of the sampling location, in minutes	0
gps_latitude_seconds	GPS coordinates (latitude) of the sampling location, in seconds	0
gps_longitude_degrees	GPS coordinates (longitude) of the sampling location, in degrees	0
gps_longitude_minutes	GPS coordinates (longitude) of the sampling location, in minutes	0
gps_longitude_seconds	GPS coordinates (longitude) of the sampling location, in seconds	0
water_temp	Temperature of the sampled wastewater, in C	1
water_ph	pH value of the sample in the field	1
water_salinity	Salinity, quantity of dissolved salts (NaCl, Mg2SO4, KNO3, NaHCO3), in ppt (part per thousand)	0
water_conductivity	Specific Conductivity, measure of water's ability to carry an electric charge which is related to the dissolved salts in the sample ($\mu\text{S}/\text{cm}$)	1
sample_turbidity	Turbidity (light scattering property of water that results in loss of transparency) of the sample, in nephelometric turbidity units (ntu)	0
sample_dissolved_oxygen	Dissolved Oxygen, amount of oxygen dissolved in sample, in mg/L	0
location_air_temp	Temperature of the air at the location and time of sampling, in C	0
location_air_humidity	Humidity of the air at the location and time of sampling, in %	0

SF-RAD qPCR Metadata Example

SF-RAD Metadata Specifications for:

Field Data

Importance 1: Required, 0: Optional

SF-RAD Field Name	Definition	Importance
matrix_type	What is tested: wastewater (W); air (A); surface swab (S)	1
sample_location_code	Code corresponding to the location (e.g. WG01)	1
sample_id	Format: Sample Location-Date-Time (parent ID) [XXXX-YYMMDDHH]	1
sampling_date	Date of the sampling	1

sampling_time
gps_latitude_degrees
gps_latitude_minutes
gps_latitude_seconds
gps_longitude_degrees
gps_longitude_minutes
gps_longitude_seconds
water_temp
water_ph
water_salinity
water_conductivity
sample_turbidity
sample_dissolved_oxygen
location_air_temp
location_air_humidity

SF-RAD Metadata Specifications for:

qPCR Results

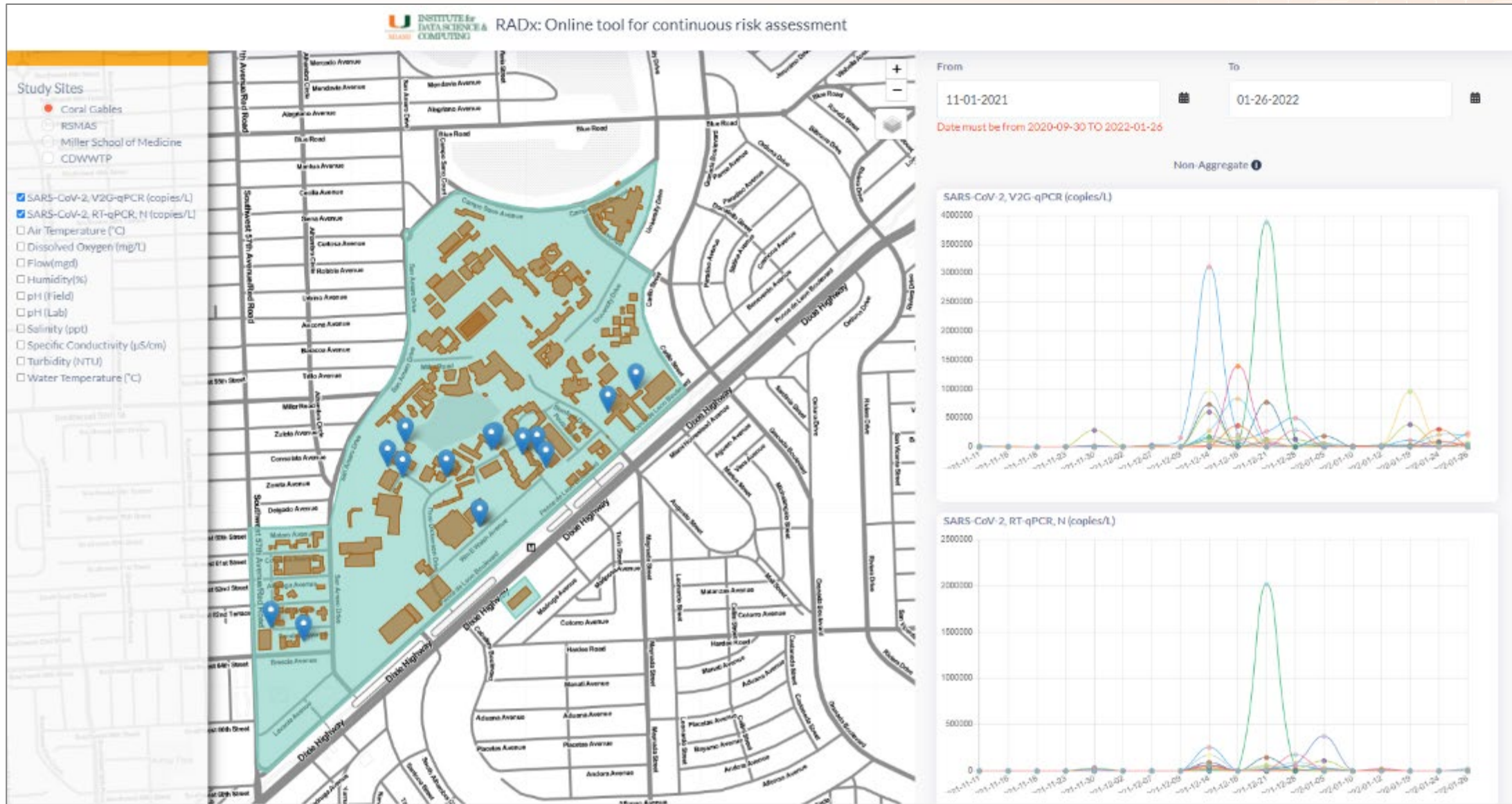
Importance 1: Required, 0: Optional

SF-RAD Field Name	Definition	Importance
qpcr_run_id	Unique identifier for the qPCR run	1
instrument_model	Maker and model of qPCR instrument used for the assay	1
instrument_serial_number	Serial number of instrument used for the assay	1
instrument_software_name	Name of software/firmware responsible for action of the instrument	1
instrument_software_version	Version of software/firmware for instrument activity	1
analysis_software_name	Software name used for performing analyses	1
analysis_software_version	Version of software used for analyses	1
plate_format	Type of plate used for assay (number of wells)	1
assay_date	Date of assay performed	1
user	User name running assay (lab name or person)	1
file_name	File (.csv or .tsv) name containing final data and calculated values	1
pcr_type	Type/molecular principle of PCR assay	1
pcr_protocol_name	Protocol name used for the experiment	1

Status of SF-RAD Data Processing and Submission

	Category Name	Submission forms	Preprocessing/QC	Preparing for DCC	Submitted to DCC
Field Info	Field Data	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
	Continuous Measurements of Water Quality	<input type="checkbox"/>	<input type="checkbox"/>	NA	NA
Wastewater pre-processing	Pretreatment	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Concentration	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Bacterial Culture Analyses	<input type="checkbox"/>	<input type="checkbox"/>	NA	NA
Analysis sample preparation	Extraction	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Wastewater analysis	qPCR Results	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
	RNA-seq Results	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Human subject data	Clinical Patient Data	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Students Individual Data	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	Students Aggregated Data	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	County Level Data	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF-RAD COVID Dashboard (alpha release)



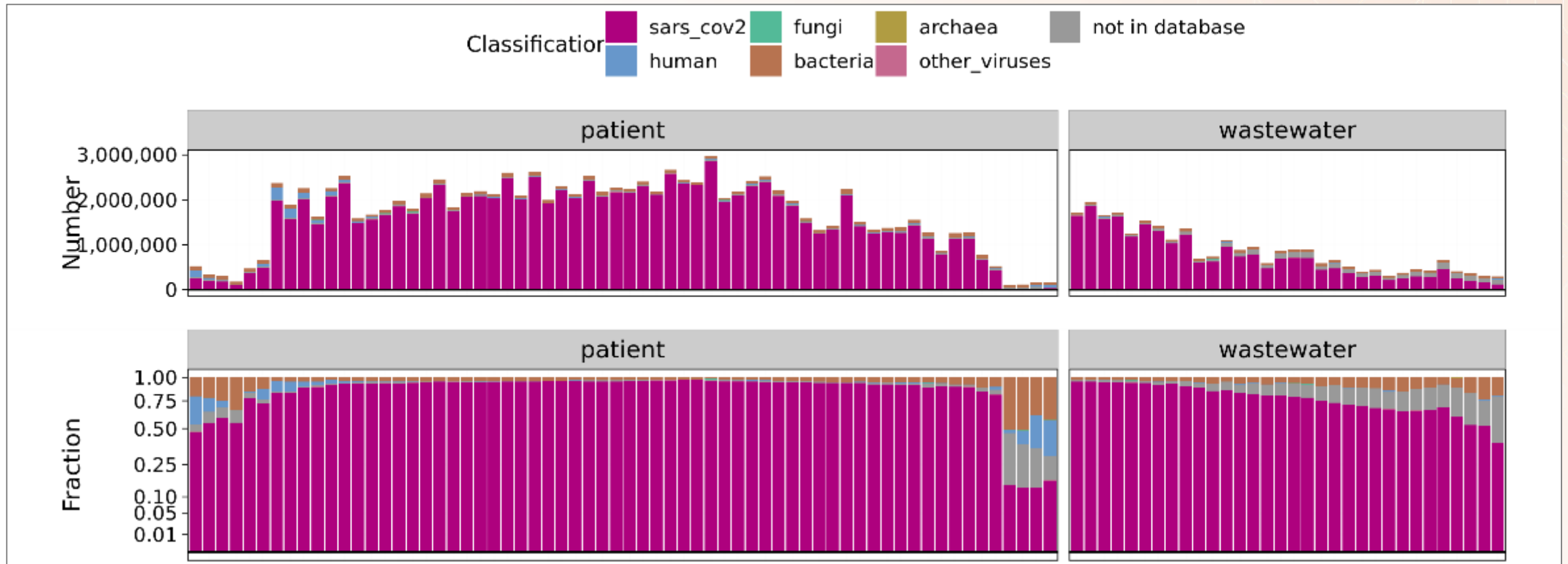
Aim:
Integration with
Human Health Surveillance

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 on clinical and wastewater samples

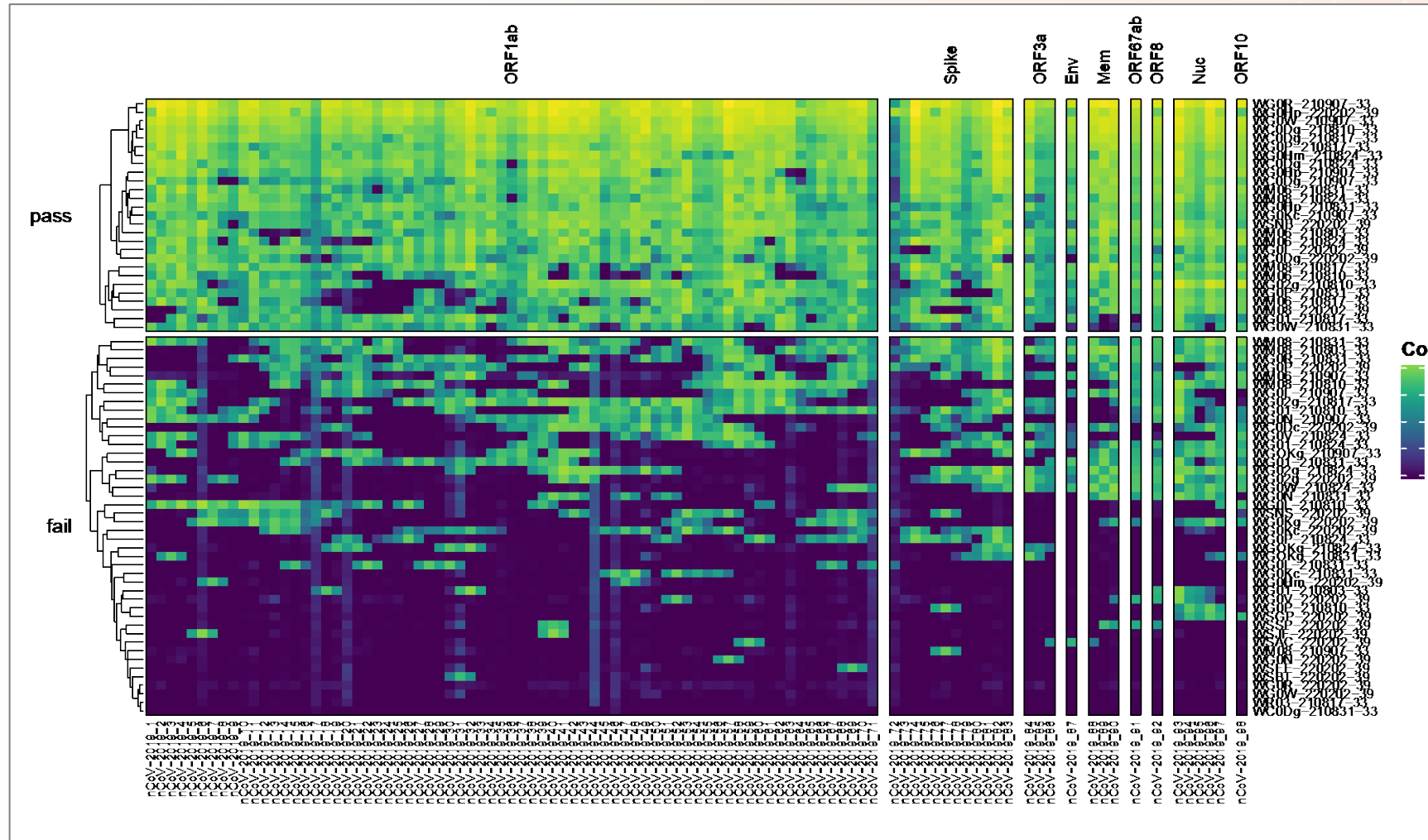
Good library efficiency to amplify SARS-CoV-2



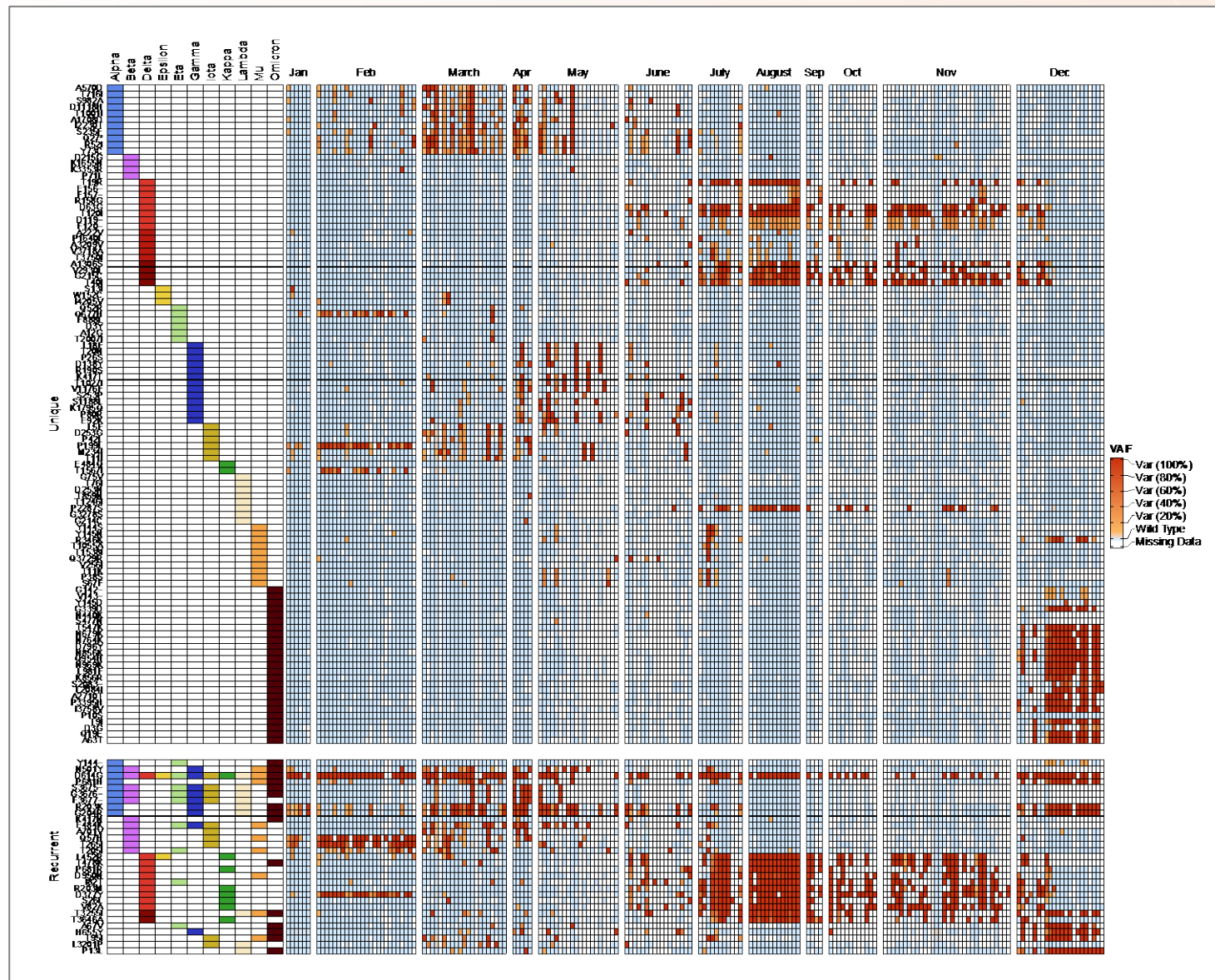
Samples are filtered by total genome coverage

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

Example shown from 2022-02-07 run



Tracking signature mutations across all 2021 WW samples

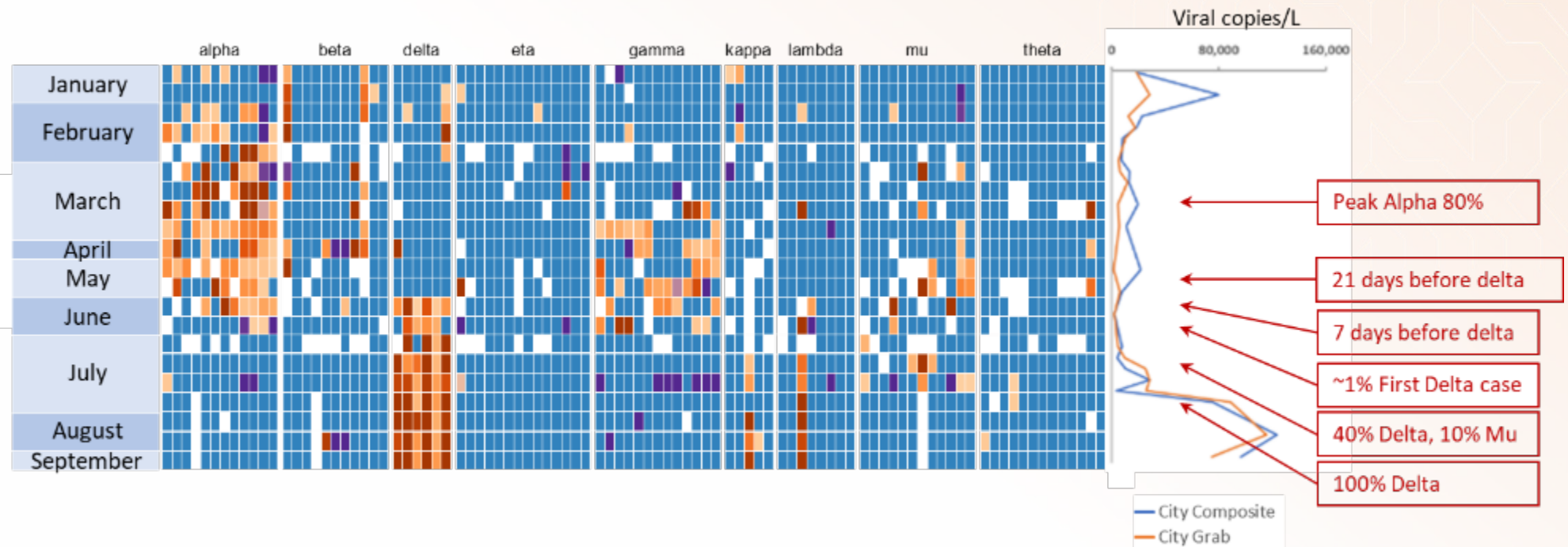


Integration of Human and Environmental Surveillance

Detection of SARS-CoV-2 lineages in wastewater

- SARS-CoV-2 lineages in City wastewater mirror patient data
- Even at low viral load lineages can be discerned
- High diversity followed by Alpha, Gamma, Mu, then Delta
- Delta detectable at -7 days before first sequenced case

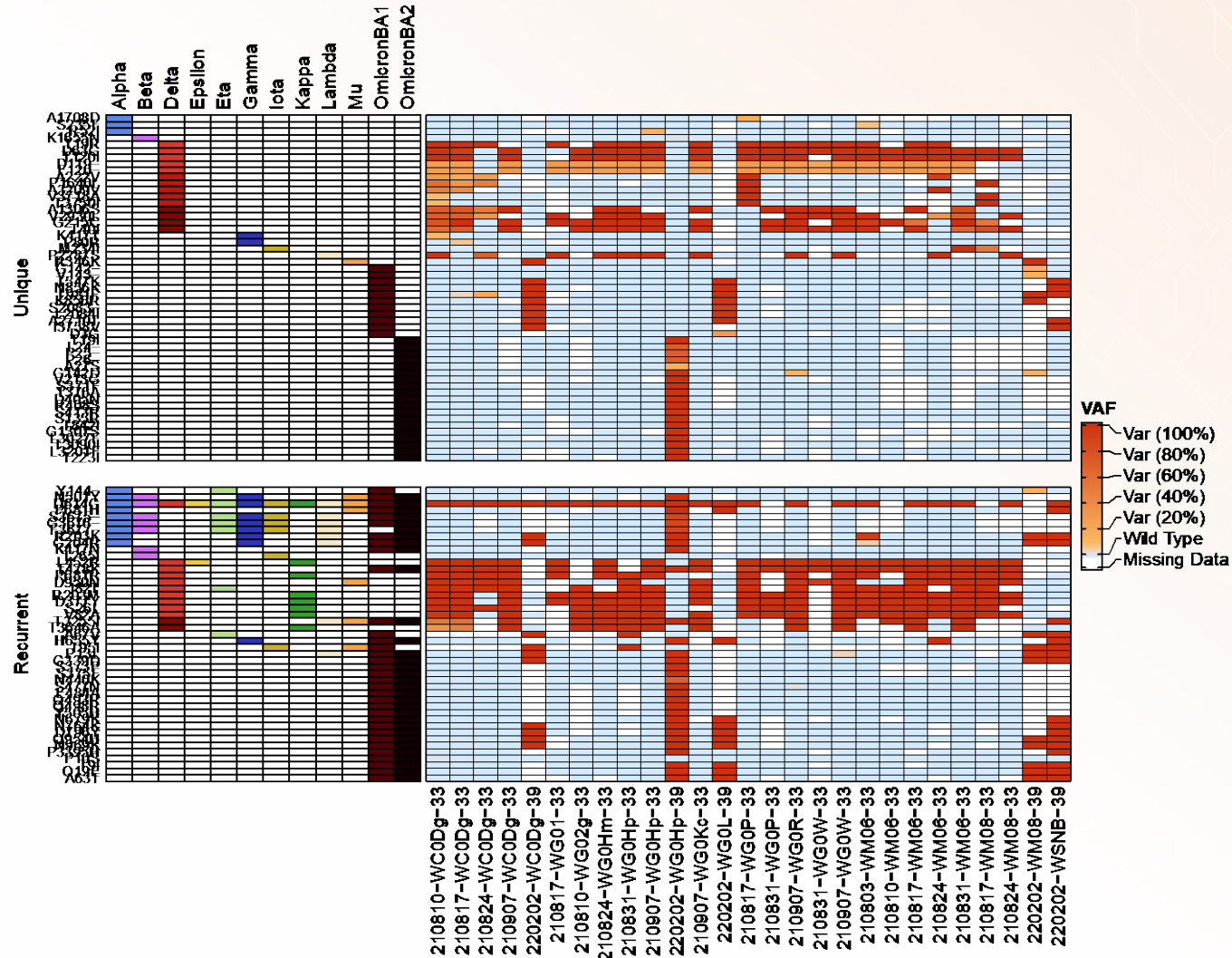
City of Miami
(Jan.-Sept. 2021)



Integration of Human and Environmental Surveillance

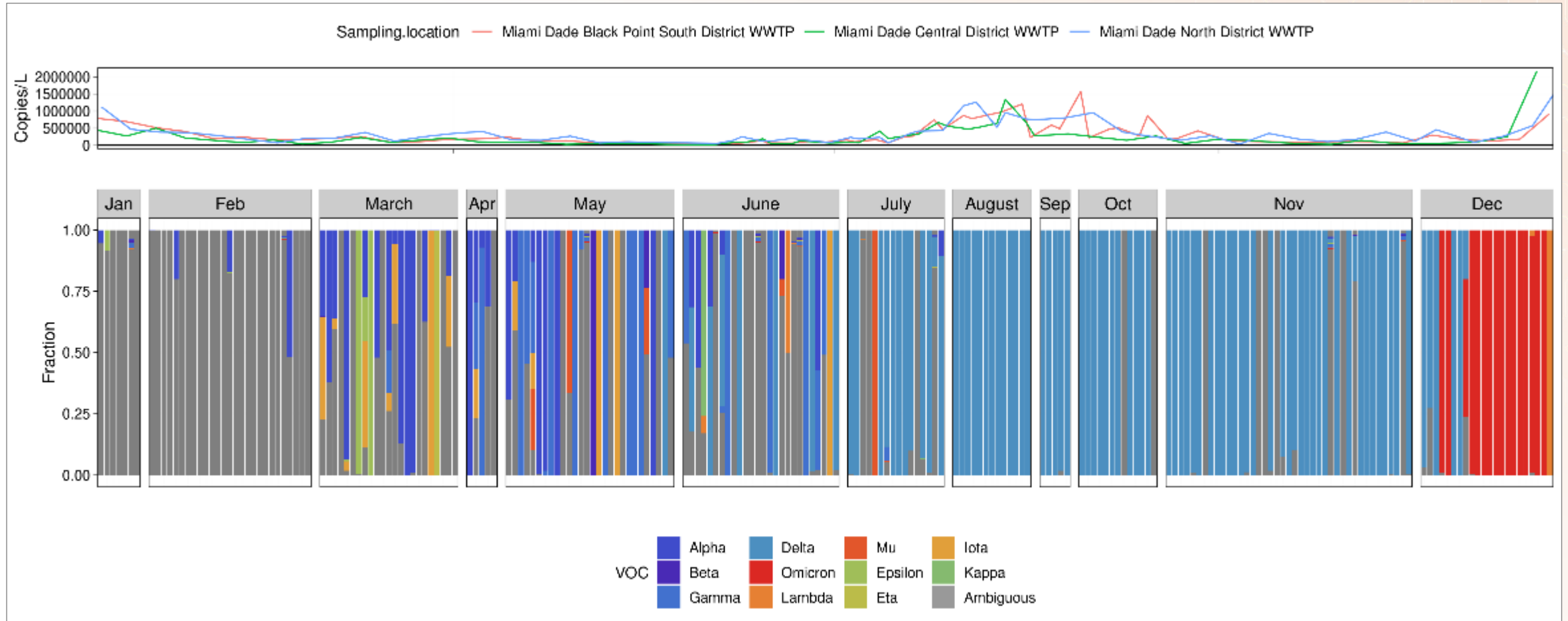
Monitoring of new variants-of-concern in wastewater

Dynamically updating VOCs to monitor: Samples shown here include Jan/Feb 2022 along with some summer 2021 samples. Omicron BA.2 signature mutations added in January 2022.



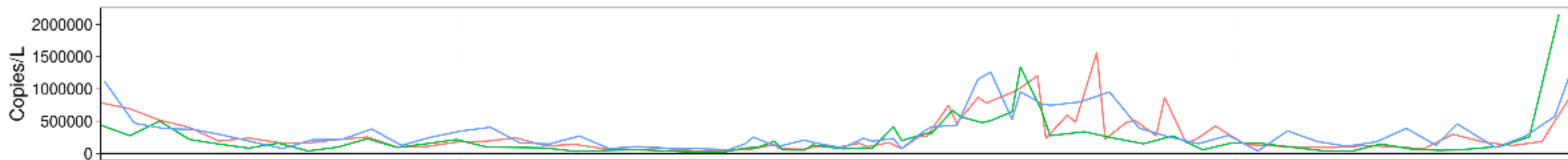
Integration of Human and Environmental Surveillance

Tracking changes in SARS-CoV-2 lineages in wastewater

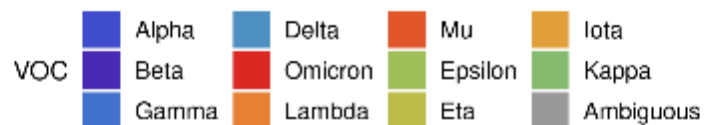
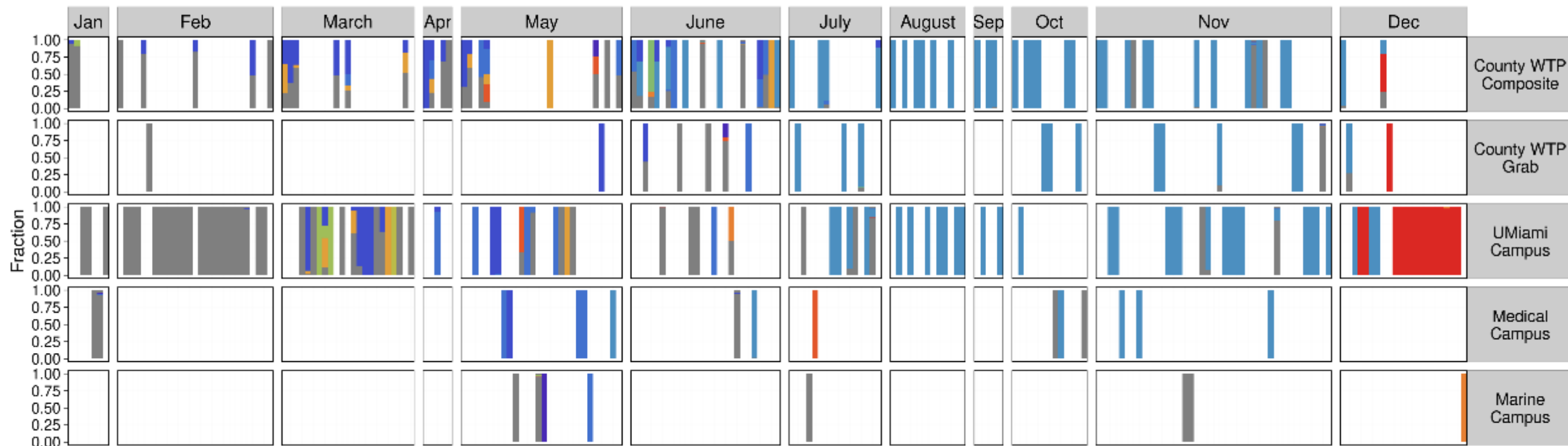


Signature mutation profiles fed into demixing model to estimate relative lineage abundances

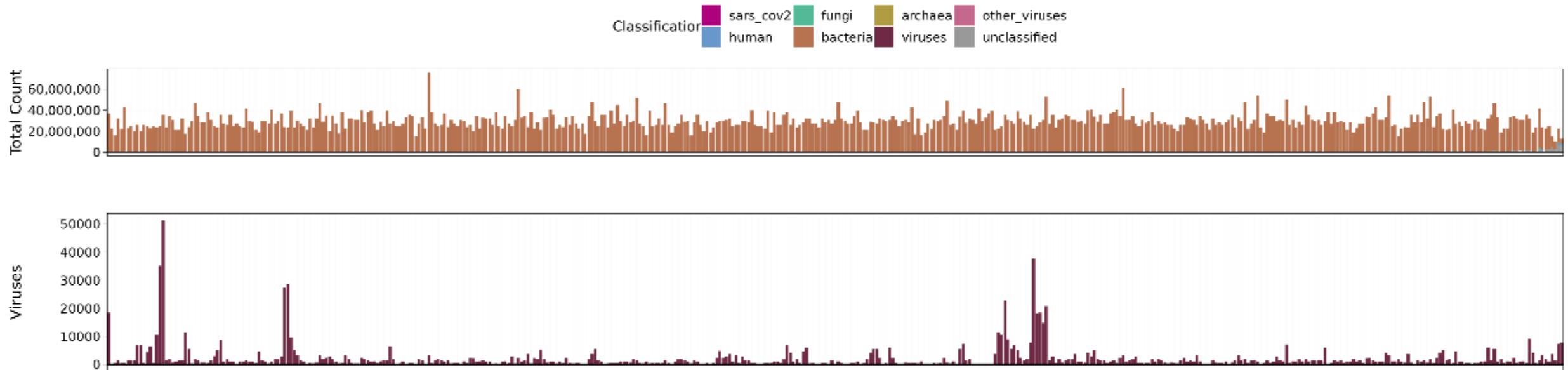
Sampling.location — Miami Dade Black Point South District WWTP — Miami Dade Central District WWTP — Miami Dade North District WWTP



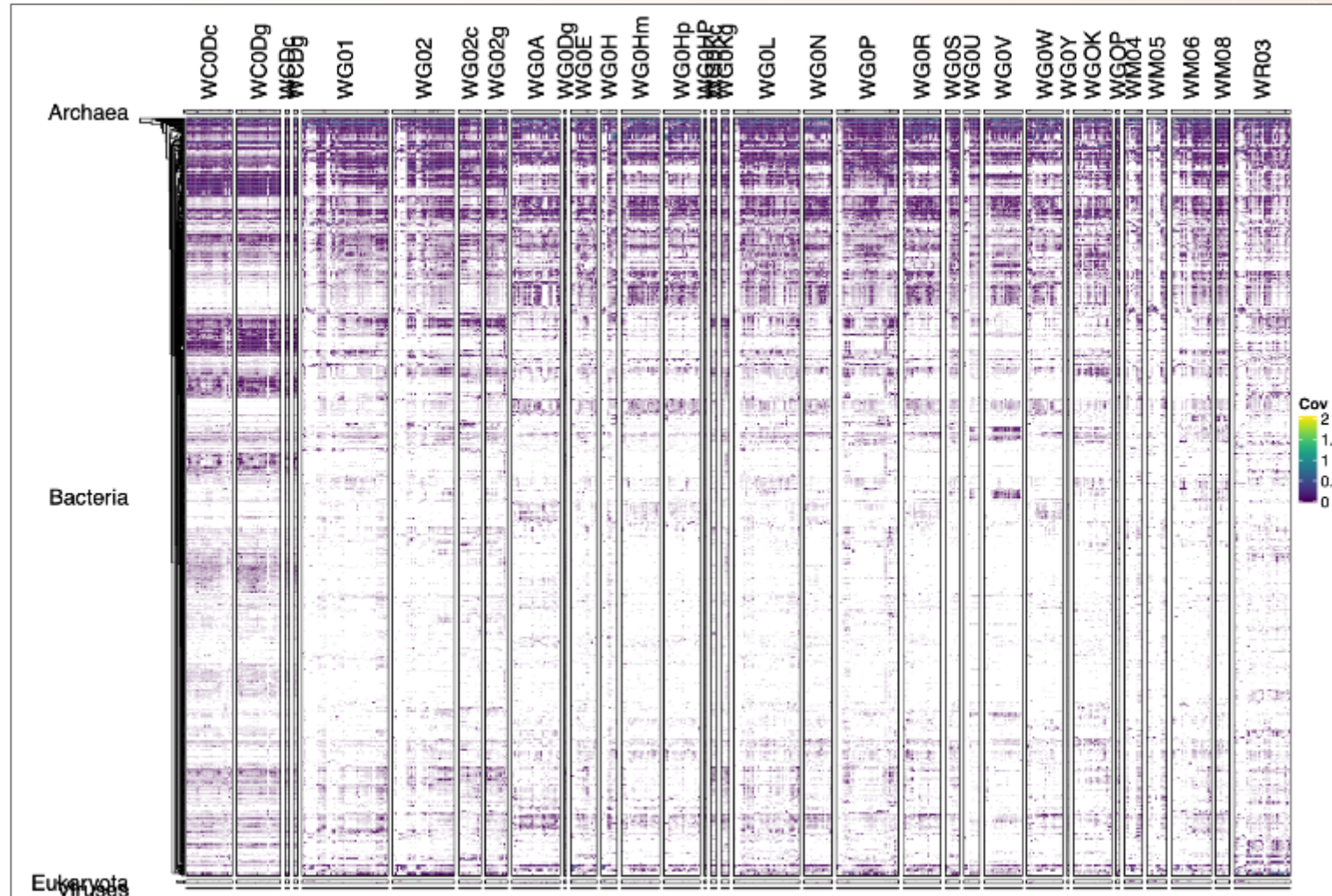
Wastewater Variant Abundance



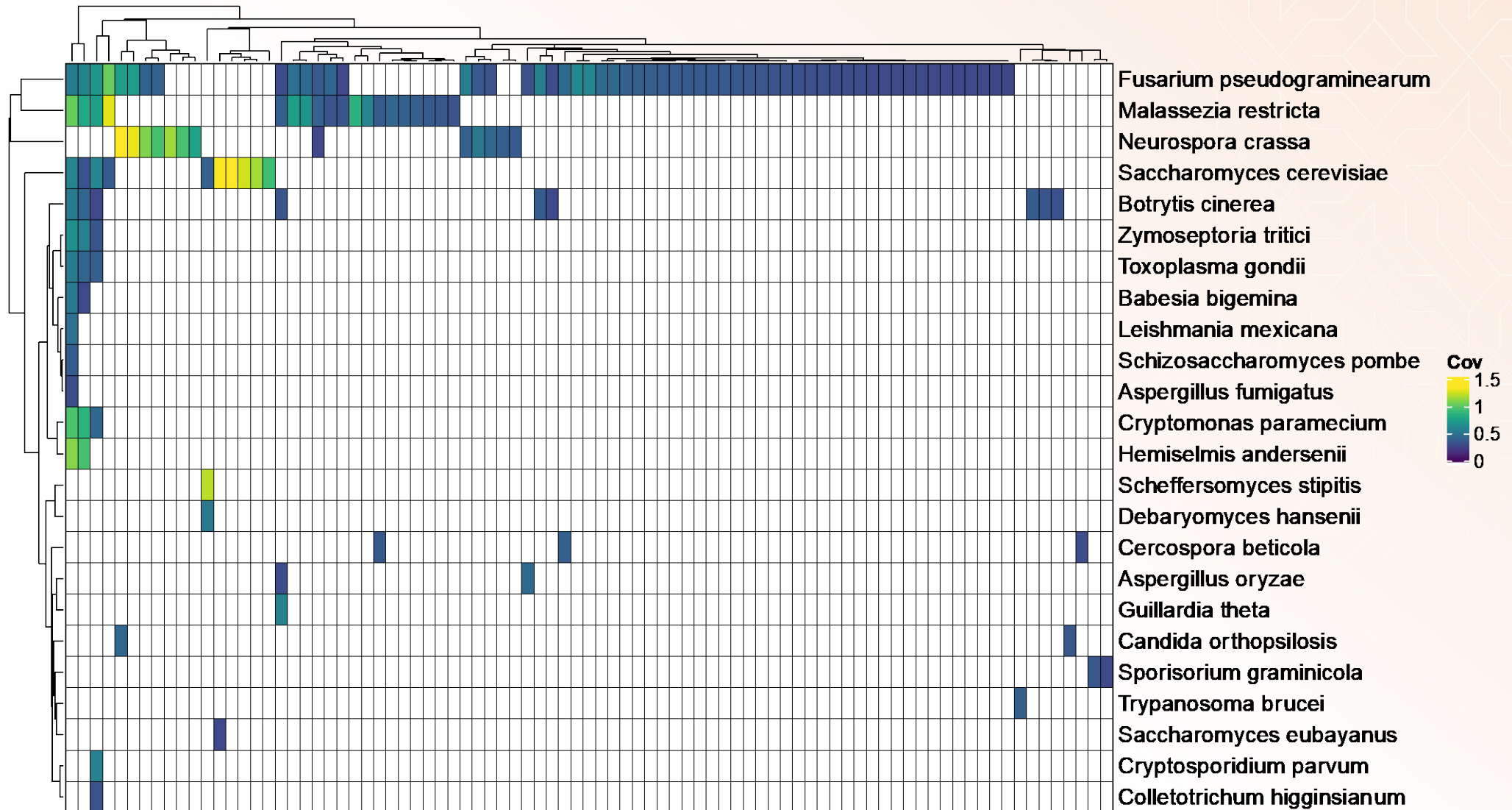
Wastewater shotgun RNAseq: untargeted monitoring of viruses/pathogens



Shotgun RNAseq enables pan-domain surveillance of microbes

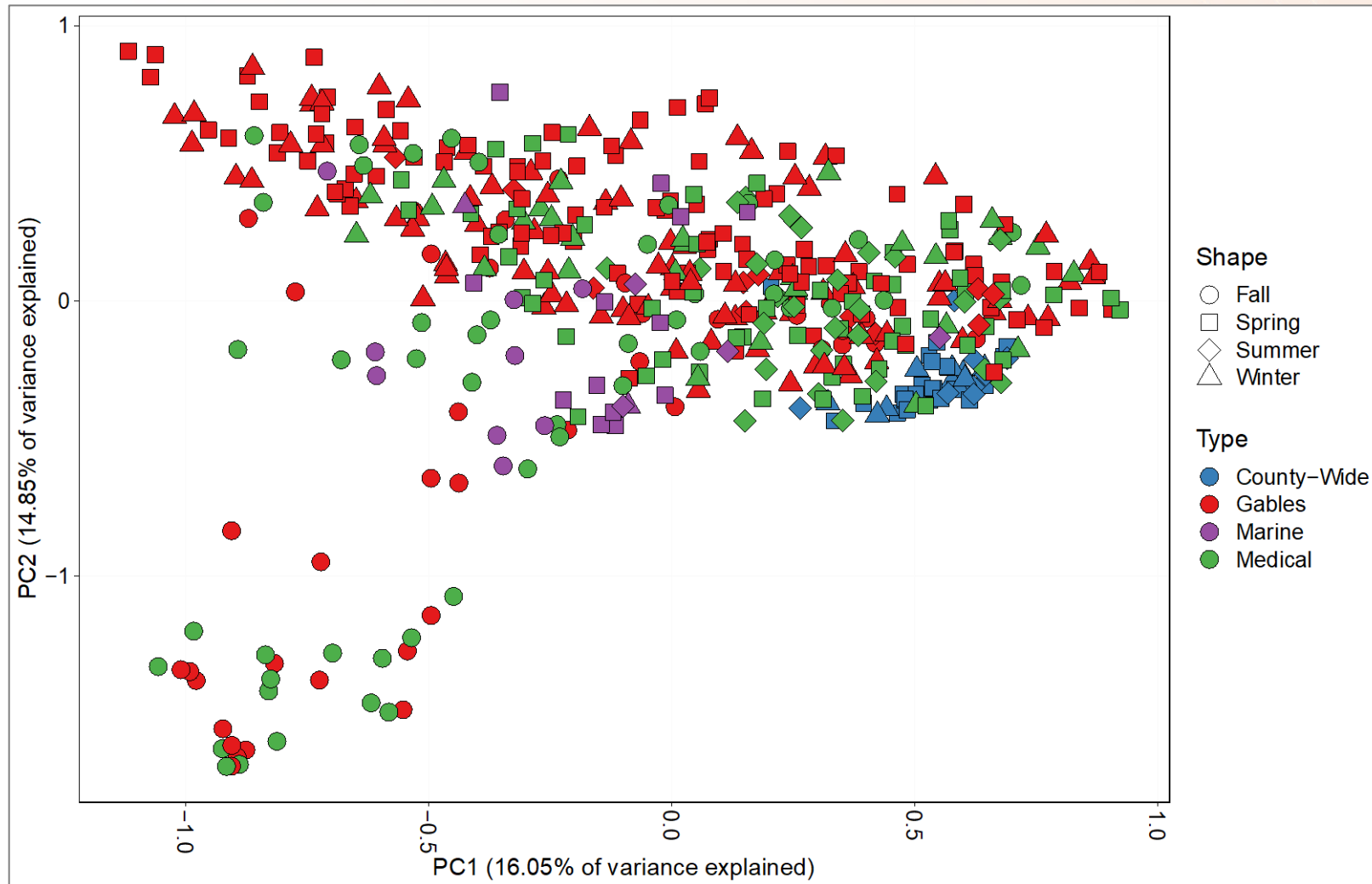


Top Fungi observed in shotgun RNA-seq





Modeling pathogen abundances over space and time



Impact and Future Directions

Ethics Related Activities

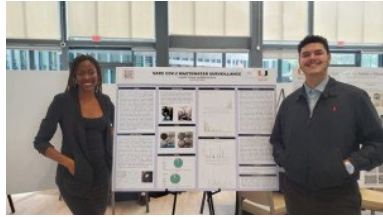
- **Ethics consultation service:** including consultation on public-facing communications, data management, and surveillance
- **Wastewater surveillance ethics project:** collaboration with colleagues at UCSD and with other stakeholders
- **Outreach:**
 - American Medical Informatics Association (AMIA) presentation
 - RADx/rad ELSI “office hours”
 - University of Miami Institute for Bioethics and Health Policy: Responsible Conduct of Research (RCR) program



From Ken Goodman, Ph.D.

Outreach

MAR
01
2022



- **Student participation:** (1) Students win first place for a poster titled “SARS-CoV-2 Wastewater Surveillance” in the University of Miami College of Engineering Research Day 2022 undergraduate poster competition. (2) Global City Sampling Day.

FEB
18
2022



- **Print and online news:** article in University of Miami newsletter: “Poop sleuths: Why researchers are tracking coronavirus in wastewater.”

JAN
19
2022



- **TV news (local):** University of Miami's student television station, UMTV, covered the SF-RAD project on their half-hour live campus news show, NewsVision.

JAN
09
2022



- **Public webinar** on “Sewers, Subways, and Space Stations: 3 Fountains for Metagenomic and Metatranscriptome Discovery” (Dr. Chris Mason)

DEC
09
2021



- **TV new (national):** Spanish language interview about the SF-RAD project on Telemundo (Dr. Helena Solo-Gabriele)

Publications

Lessons learned from SARS-CoV-2 measurements in wastewater

Sharkey ME, Kumar N, Mantero AMA, Babler KM, Boone MM, Cardentey Y, Cortizas EM, Grills GS, Herrin J, Kemper JM, Kenney R, Kobetz E, Laine J, Lamar WE, Mader CC, Mason CE, Quintero AZ, Reding BD, Roca MA, Ryon K, Solle NS, Schürer SC, Shukla B, Stevenson M, Stone T, Tallon JJ Jr, Venkatapuram SS, Vidovic D, Williams SL, Young B, Solo-Gabriele HM. **Lessons learned from SARS-CoV-2 measurements in wastewater.** *Sci Total Environ.* 2021 Dec 1;798:149177.doi.org/10.1016/j.scitotenv.2021.149177 PMID: 34375259; PMC8294117.



A rapid, isothermal, and point-of-care system for COVID-19 diagnostics

Christopher Mozsary, Duncan McCloskey, Kristina M. Babler, Juan Boza, Daniel Butler, Benjamin Currall, Sion Williams, Anne Wiley, George S. Grills, Mark E. Sharkey, Prem Premrurut, Helena Solo-Gabriele, Yoslayma Cardentey, David Erickson, Christopher E. Mason. **A Rapid, Isothermal, and Point-of-Care System for COVID-19 Diagnostics.** *J Biomol Tech.* 2021 Sep;32(3):221-227. doi: 10.7171/jbt.21-3203-019. PMID: 35136383, PMCID: PMC8802758.



A global metagenomic map of urban microbiomes and antimicrobial resistance

David Danko, Daniela Bezdán, Evan E. Afshin, Sibó Zhu, Christopher E. Mason, et al. **A global metagenomic map of urban microbiomes and antimicrobial resistance.** *Cell.* 2021 Jun 24;184(13):3376-3393. <https://doi.org/10.1016/j.cell.2021.05.002>. PMID: 34043940.



Publications

Publication Received
Journal of Biomolecular Techniques
2021 Manuscript of the Year Award



Loop-Mediated Isothermal Amplification Detection of SARS-CoV-2 and Myriad Other Applications.

J Biomol Tech. 2021 Sep;32(3):228-275. doi: 10.7171/jbt.21-3203-017.
PMID: 35136384; PMCID: PMC8802757.

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Spin-off research: Ongoing and potential pilot projects

- Wastewater surveillance of COVID-19 in public schools
 - Pilot project in collaboration with a RADx/UP funded investigator at the University of Miami (1OT2HD108111, Dr. Lisa Gwynn, principal investigator)
- Airplane and airport wastewater surveillance
 - In collaboration with the CDC-NWSS, NIST, and the Rockefeller Foundation
- Wastewater surveillance for influenza, for antibiotic resistant pathogens, and for other biomarkers of disease

National Consortium

NIH Rapid Acceleration of Diagnostics (RADx)



RADx Radical (RADx-rad)

Support new, non-traditional approaches to address current testing gaps, and non-traditional applications of existing approaches



7 awards related to wastewater detection of SARS-CoV-2, including the SF-RAD project



RADx Underserved Populations (RADx-UP)

Understand and reduce the disparities in COVID-19 morbidity and mortality for those disproportionately affected by COVID-19



Including a grant award that is funding the pilot project for wastewater monitoring in public schools



RADx Tech

Speed innovative point-of-care, home-based, and clinical laboratory tests for COVID-19



Grant application for development of a new portable technology for wastewater monitoring

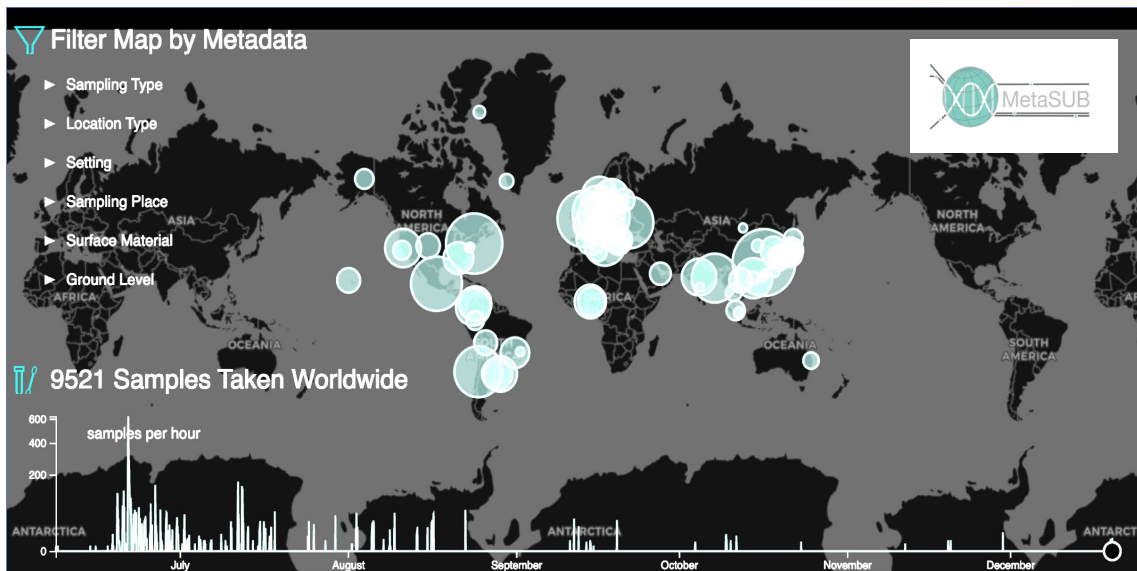


RADx Advanced Technology Platforms (RADx-ATP)

Identify testing platforms that are far enough advanced for rapid scale-up or expanded geographical placement

International Consortium

Metagenomics and Metadesign of the Subways and Urban Biomes (MetaSUB)



Ongoing MetaSUB Projects

Monumentome



- 189 surface samples
- 12 different cities
- 21 monuments/landmarks
- 47 surface samples from the ancient city of Tel Megiddo

MetaSEW



- 500+ wastewater samples collected
- 14 different cities
- Current focus on COVID-19 surveillance and variant testing

MetaCoV



- Sampling since March of 2020, beginning of the pandemic
- 27 different cities
- 3971 surface samples received
- 2256 samples on Pangea

MetaMED



- 1000+ surface samples
- Aim to collect and analyze samples from hospital/medical



Metagenomics of Urban Biomes (MetaSUB) Annual Meeting

Miami, FL, Nov. 18-21, 2022



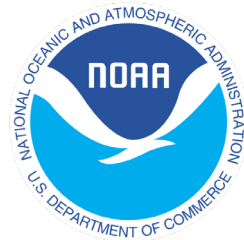
metasub.org

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**Weill Cornell
Medicine**

ABOUT

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COMMUNITY

A photograph of a large, circular wastewater treatment tank. The interior is dark and filled with water. A yellow vertical pipe or structure is visible on the right side. The concrete walls of the tank are visible on the left and right.

SF-RAD: COVID-19 PREDICTIONS FROM WASTEWATER

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<https://covidsfrad.org>