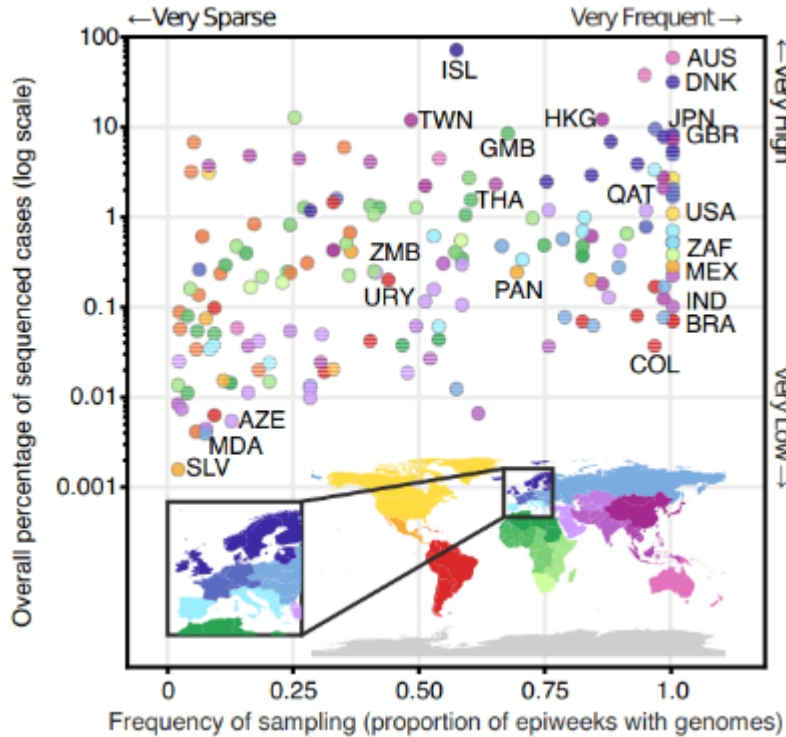


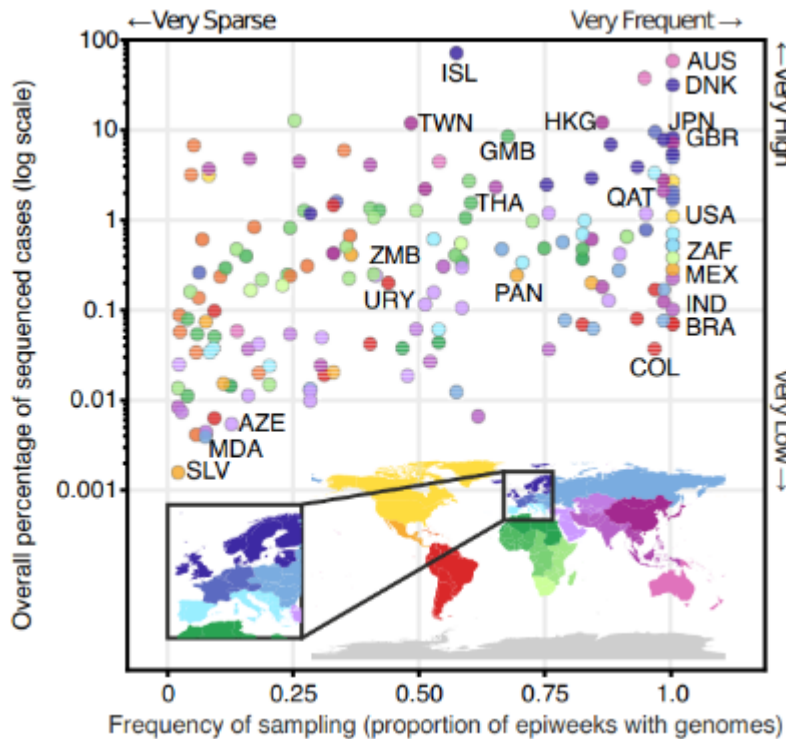
# Wastewater sequencing analyses uncover pathogen evolution and spread

IDM  
May 23<sup>rd</sup>, 2023

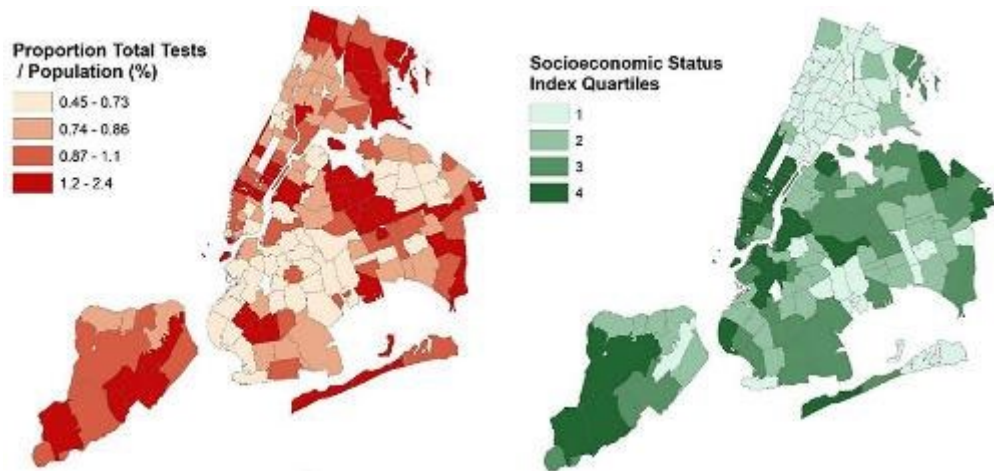
# Clinical sampling blind spots



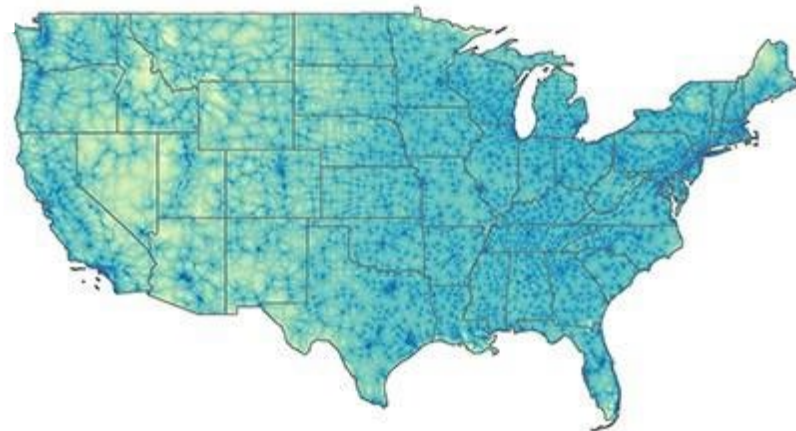
# Clinical sampling blind spots



Brito, Semenova, Dudas et al., 2021



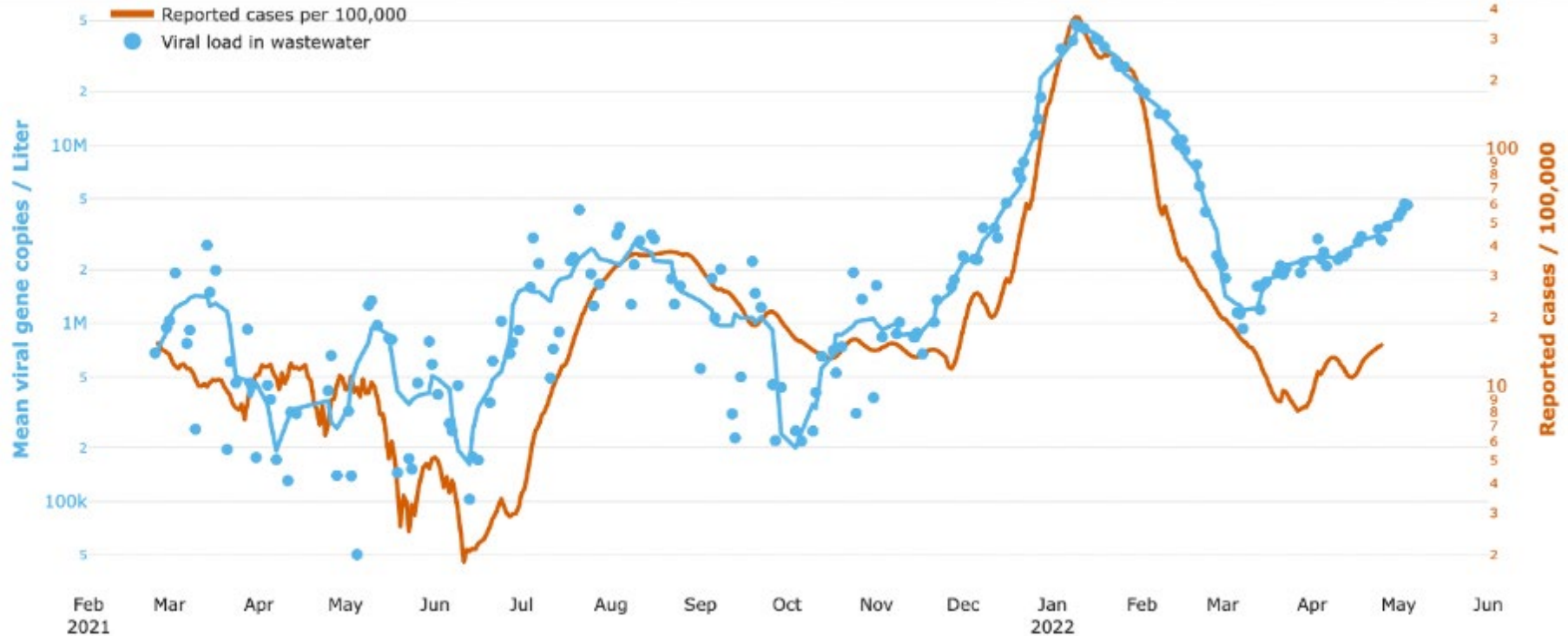
Lieberman-Cribbin et al., 2020



Rader, Astley et al., 2020

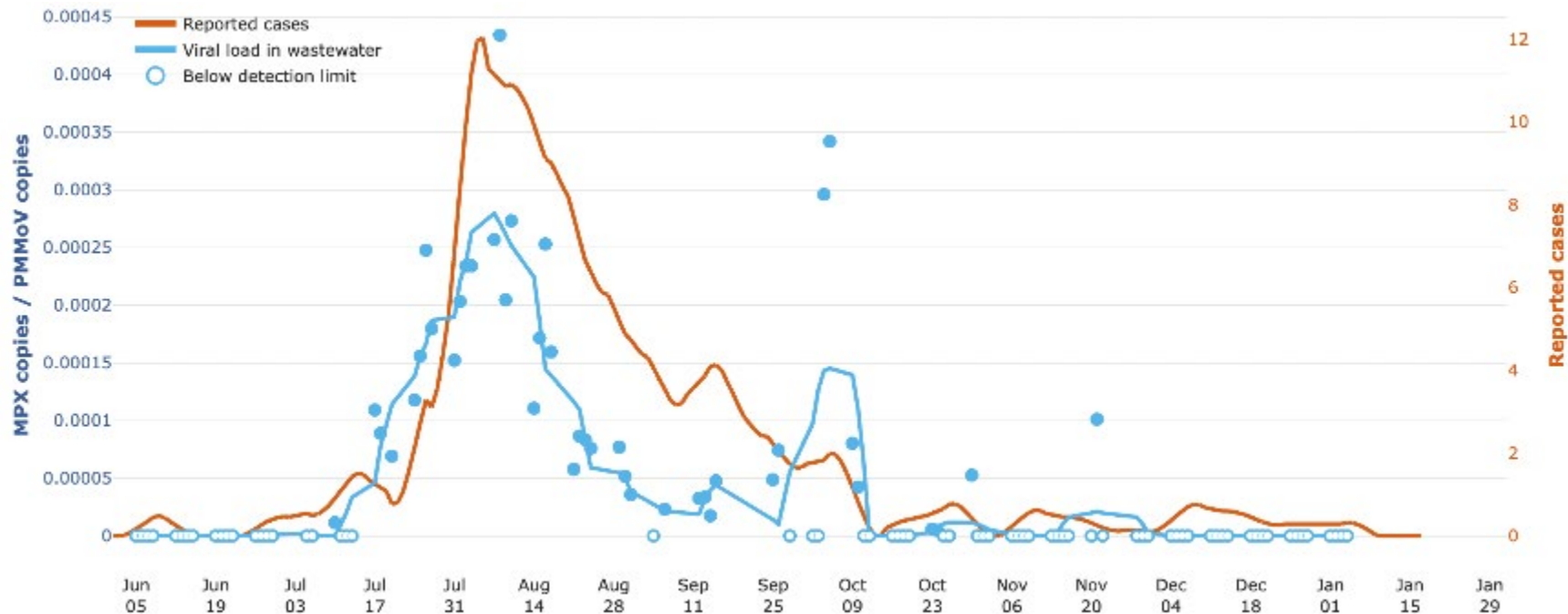
# Wastewater as a promising alternative

**SARS-CoV-2**



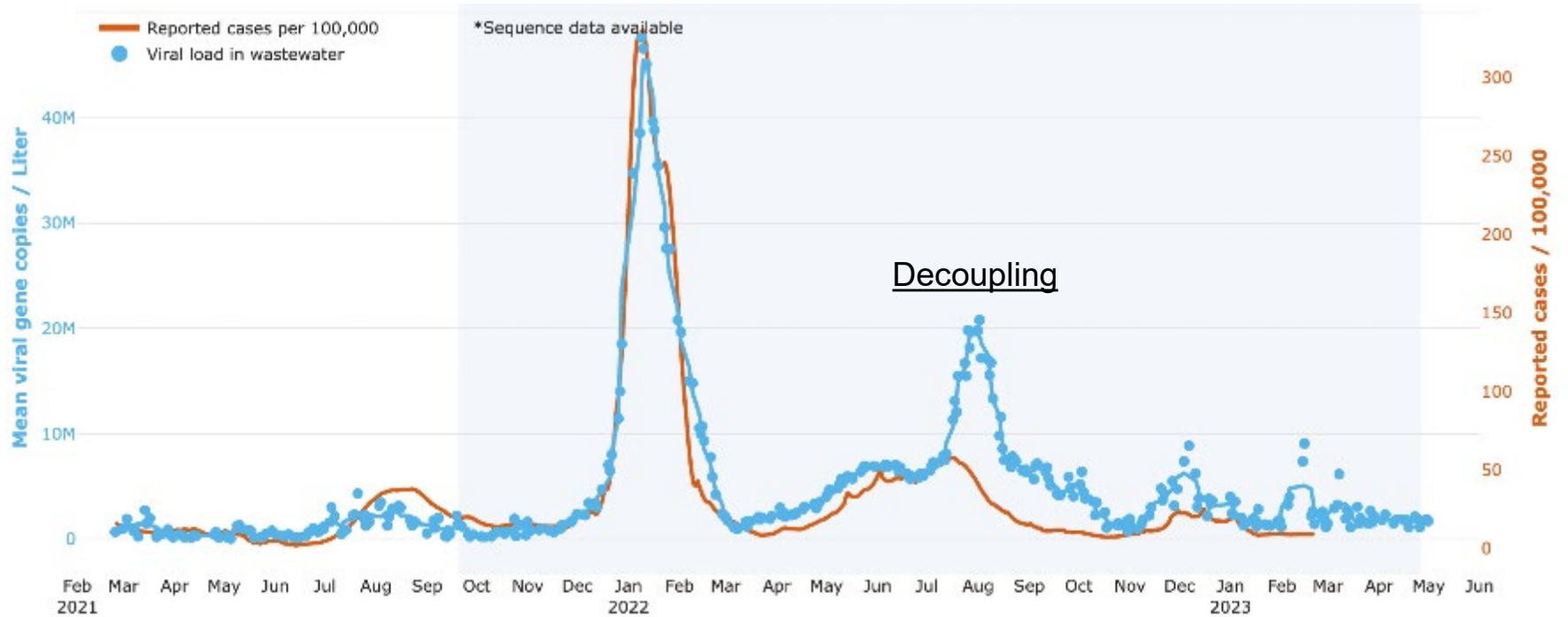
# Wastewater as a promising alternative

Mpox

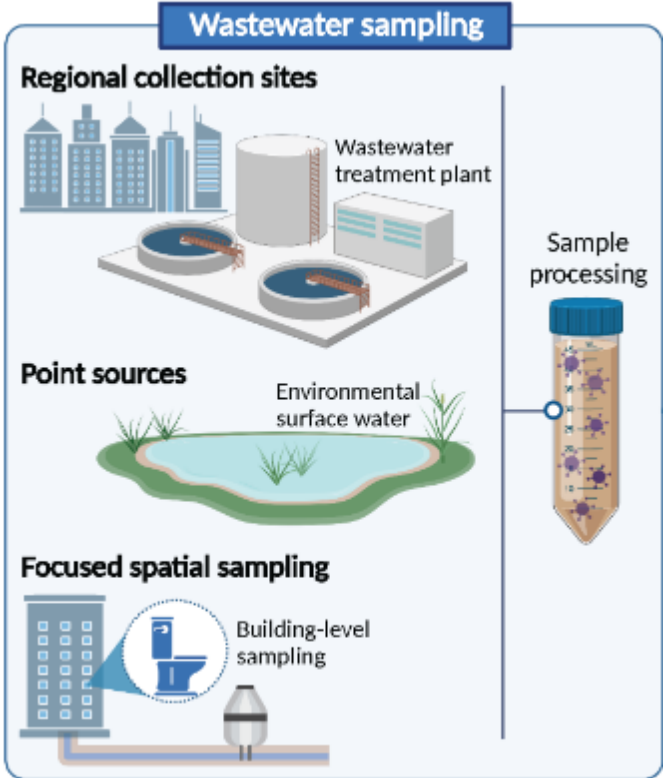


# Wastewater as a promising alternative

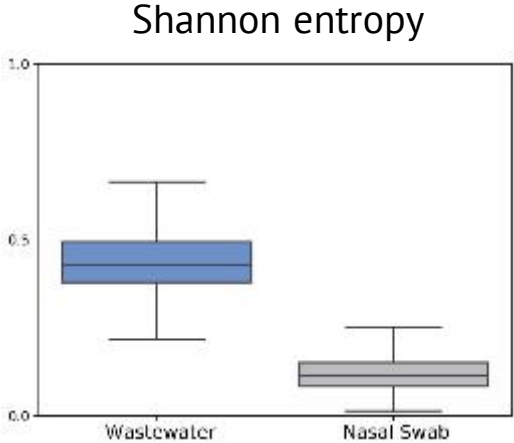
**SARS-CoV-2**



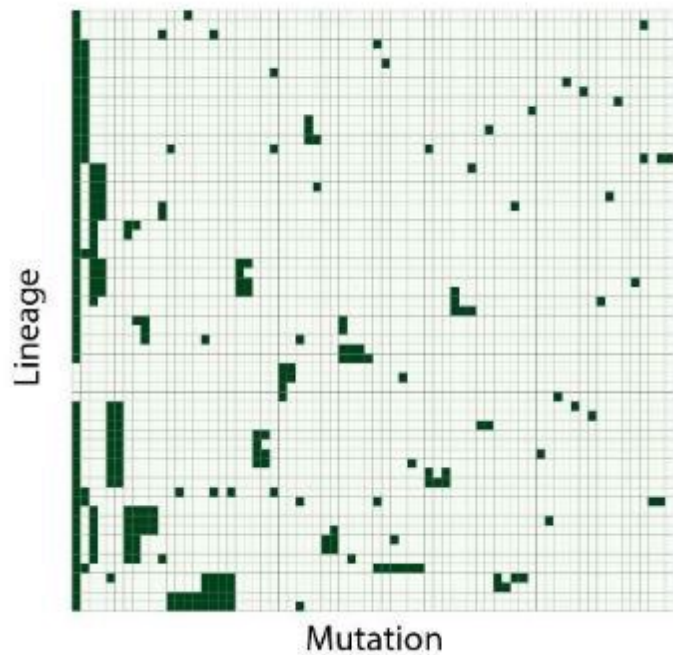
# Need a new approach for wastewater sequencing analyses



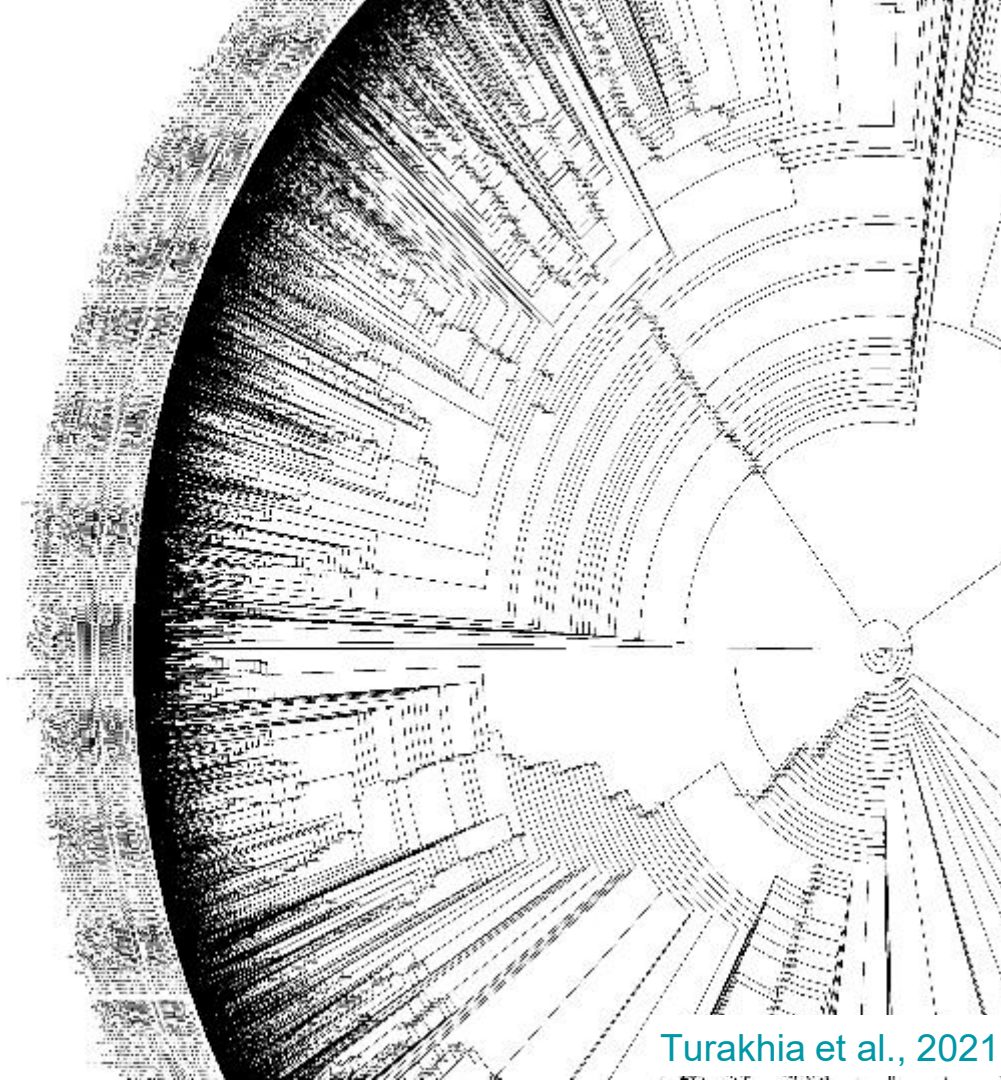
Wastewater samples are fundamentally mixtures, with mutations linked by their frequency in the sample



## Lineage Barcoding

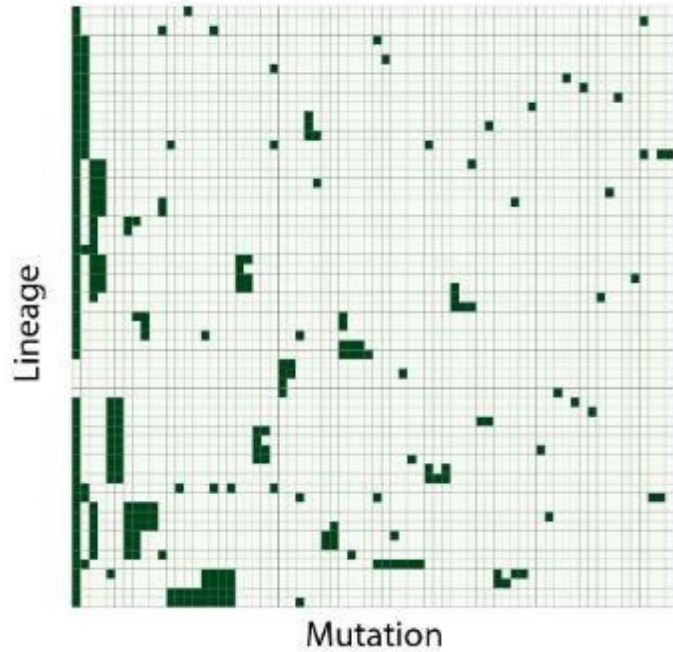


>3000 SARS-CoV-2 lineages



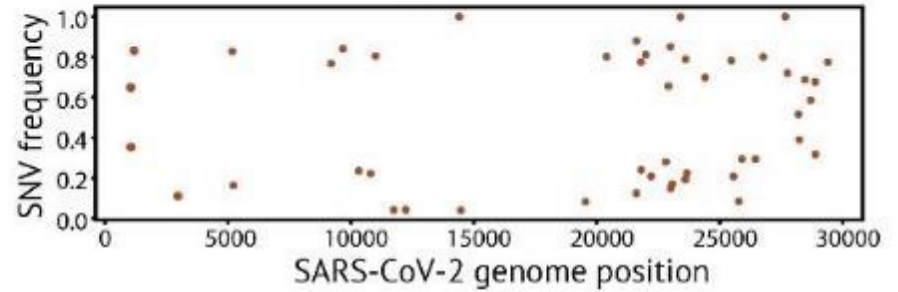


## Lineage Barcoding

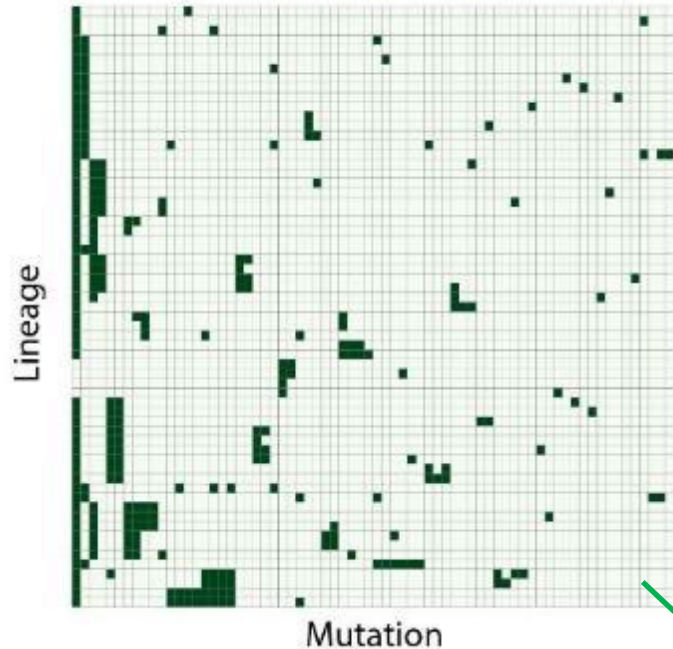


>3000 SARS-CoV-2 lineages

## Detection of Single Nucleotide Variants

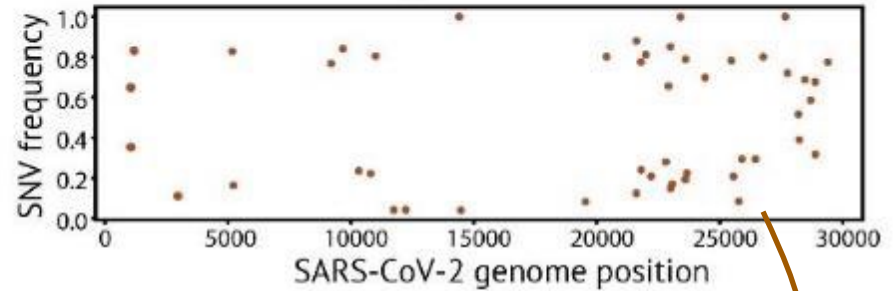


## Lineage Barcoding



>3000 SARS-CoV-2 lineages

## Detection of Single Nucleotide Variants

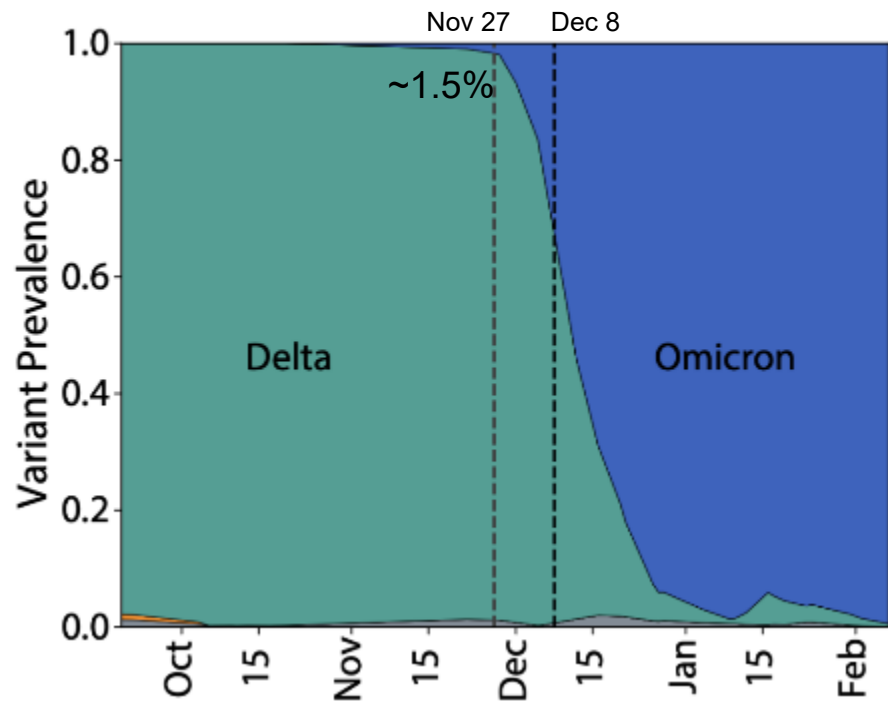


Depth-weighted de-mixing

$$\hat{x} = \underset{\substack{x \geq 0 \\ \sum_{x=1}}}{\operatorname{argmin}} \|A^T x - b\|_{1W}$$



# The BA.1 Omicron wave in San Diego

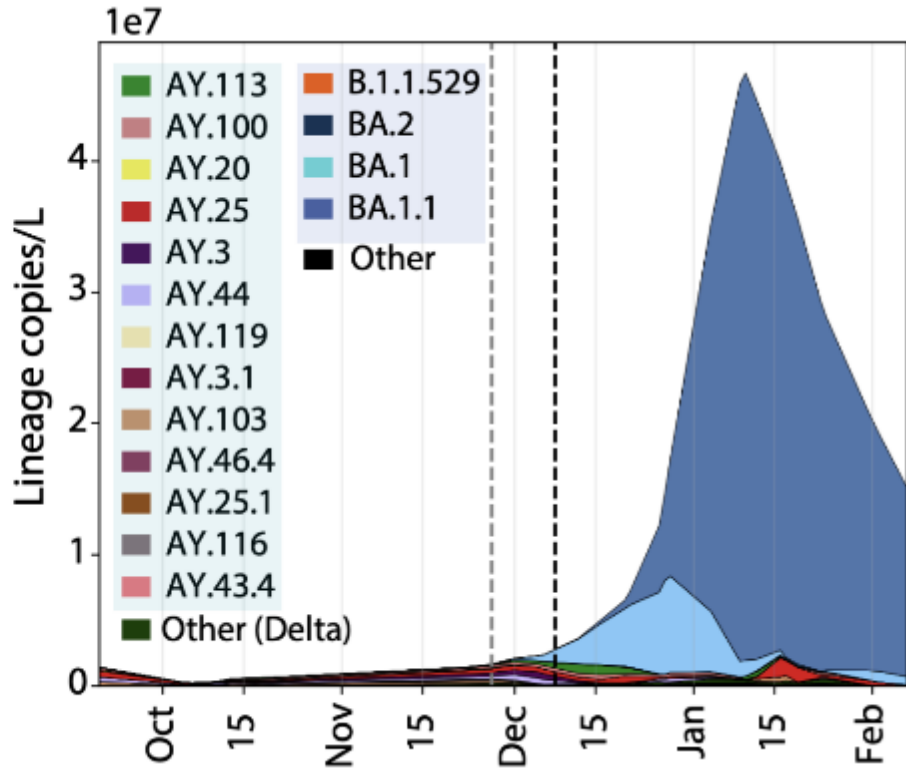
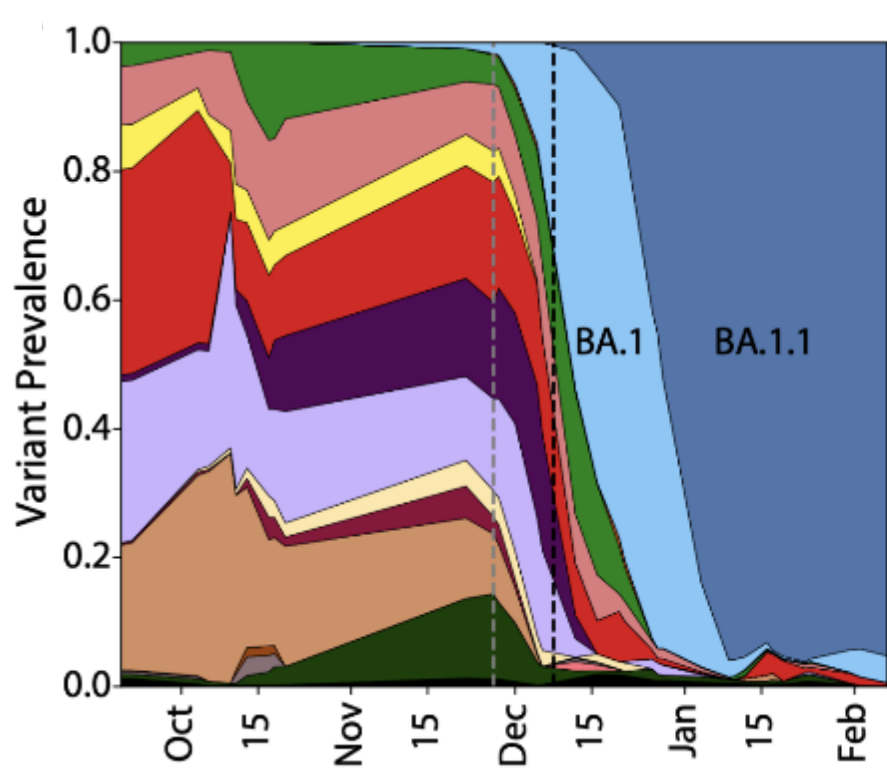


@ Point Loma

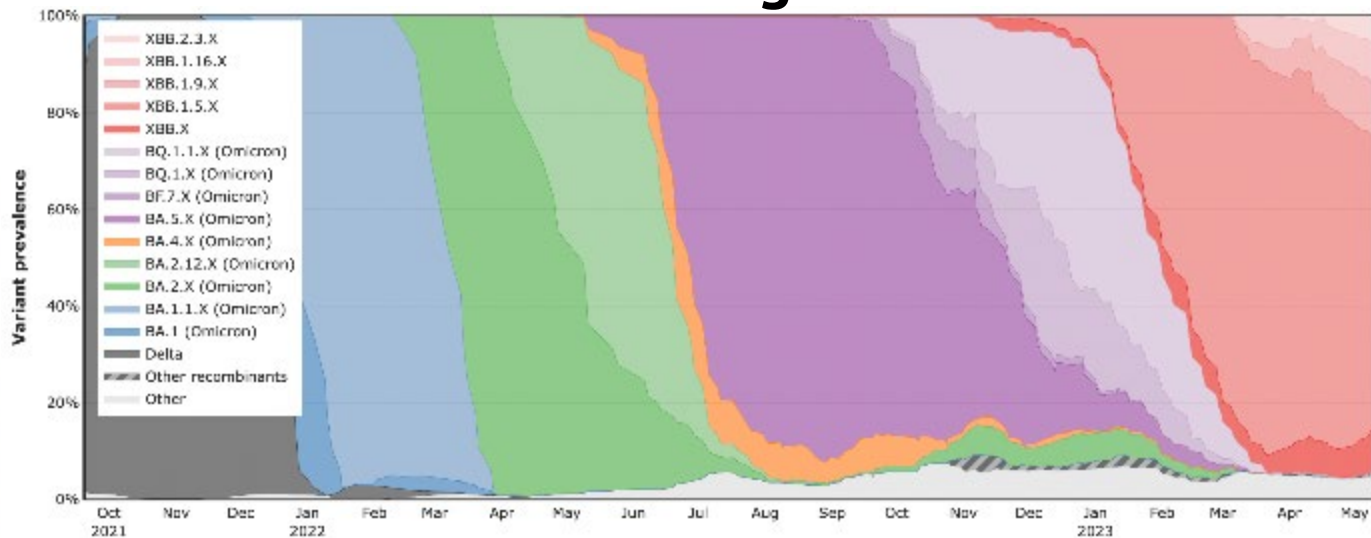


~2.3 million individuals in catchment

# The BA.1 Omicron wave in San Diego



# Ongoing real-time surveillance in San Diego



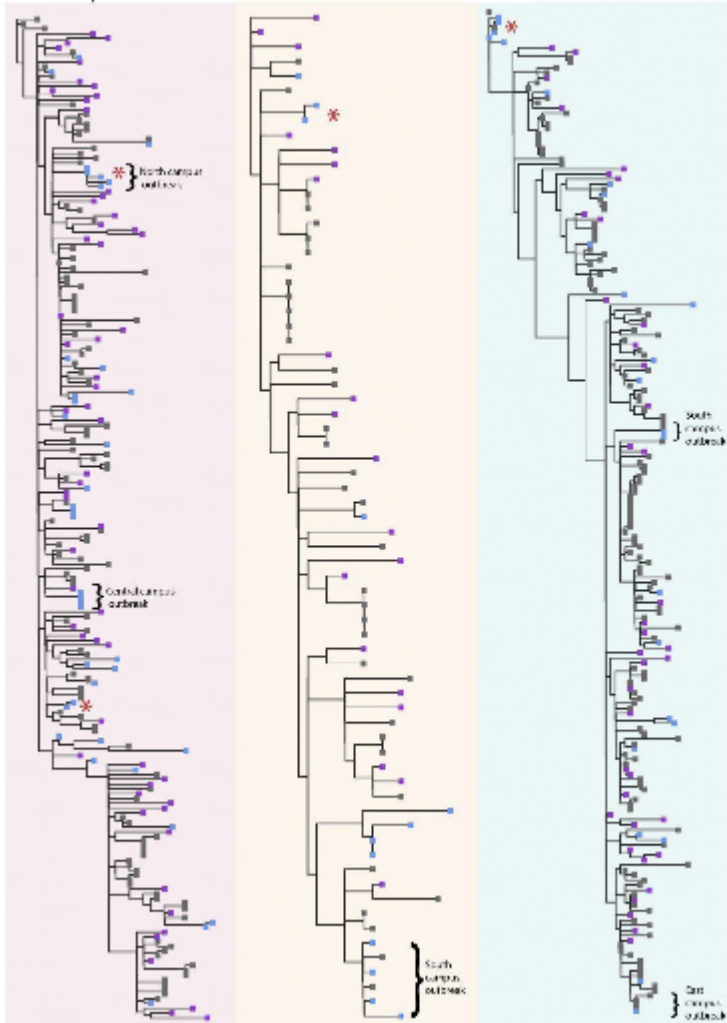
**Growth rate estimation  
from wastewater**

Lineage	Growth Advantage	Bootstrap 95% CI
<i>XBB.2.3.X</i>	15.4%	[3.05% , 32.75%]
<i>XBB.1.16.X</i>	9.0%	[3.29% , 17.37%]
<i>XBB.1.9.X</i>	5.9%	[1.53% , 10.31%]
<i>XBB.X</i>	3.0%	[-4.69% , 9.73%]
<i>XBB.1.5.X</i>	-7.5%	[-10.90% , -3.48%]

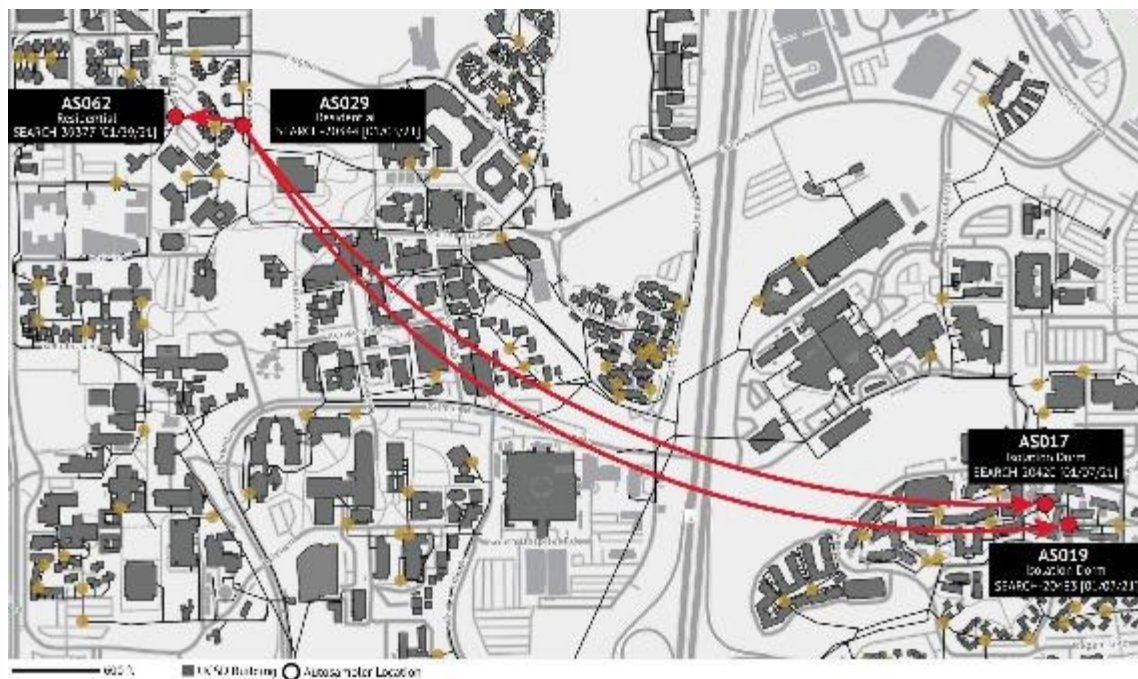
Epsilon

Alpha

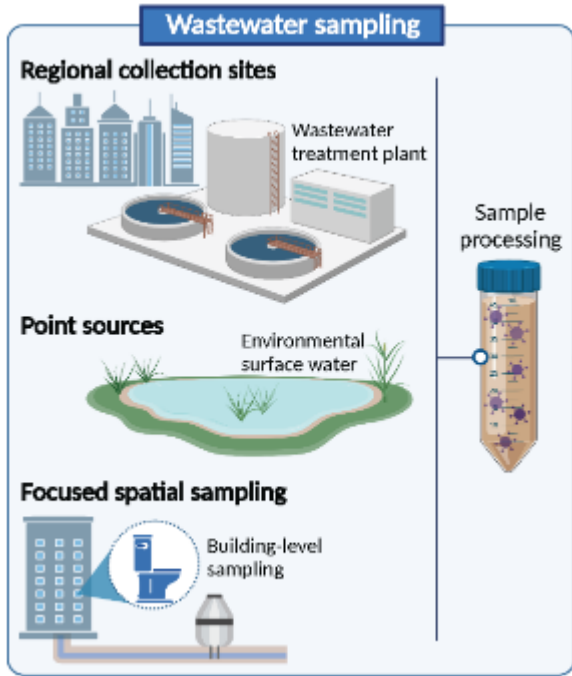
Delta



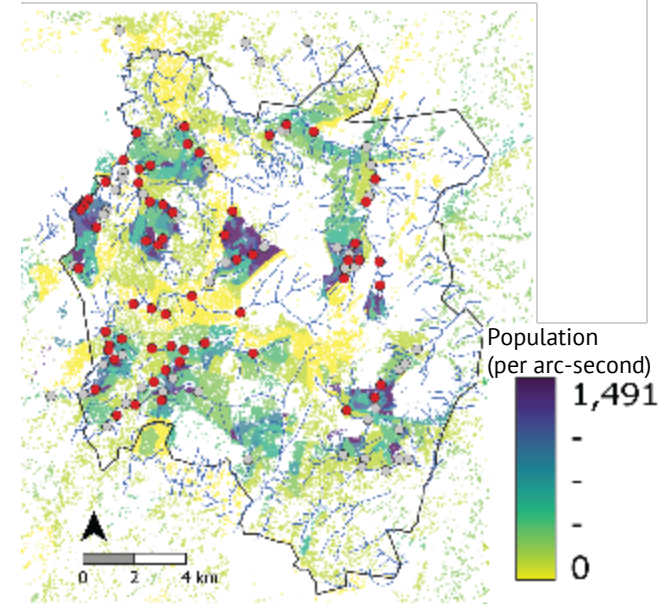
## Haplotype recovery, phylogenetics, outbreak tracking



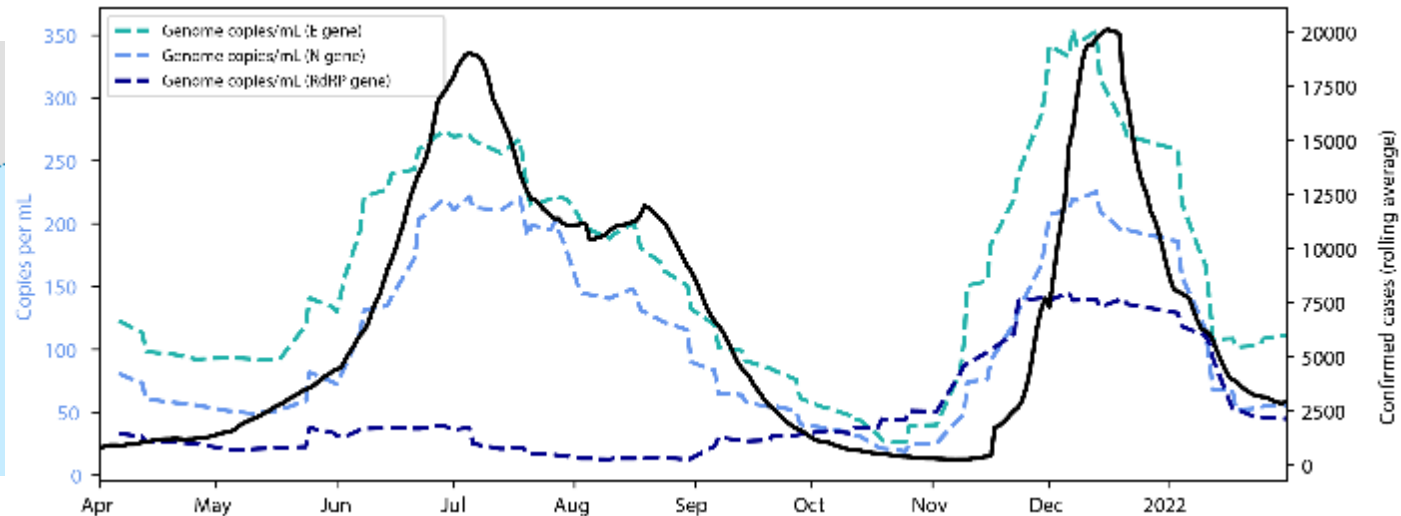
# Wastewater surveillance across the world



## Wastewater surveillance in Blantyre, Malawi

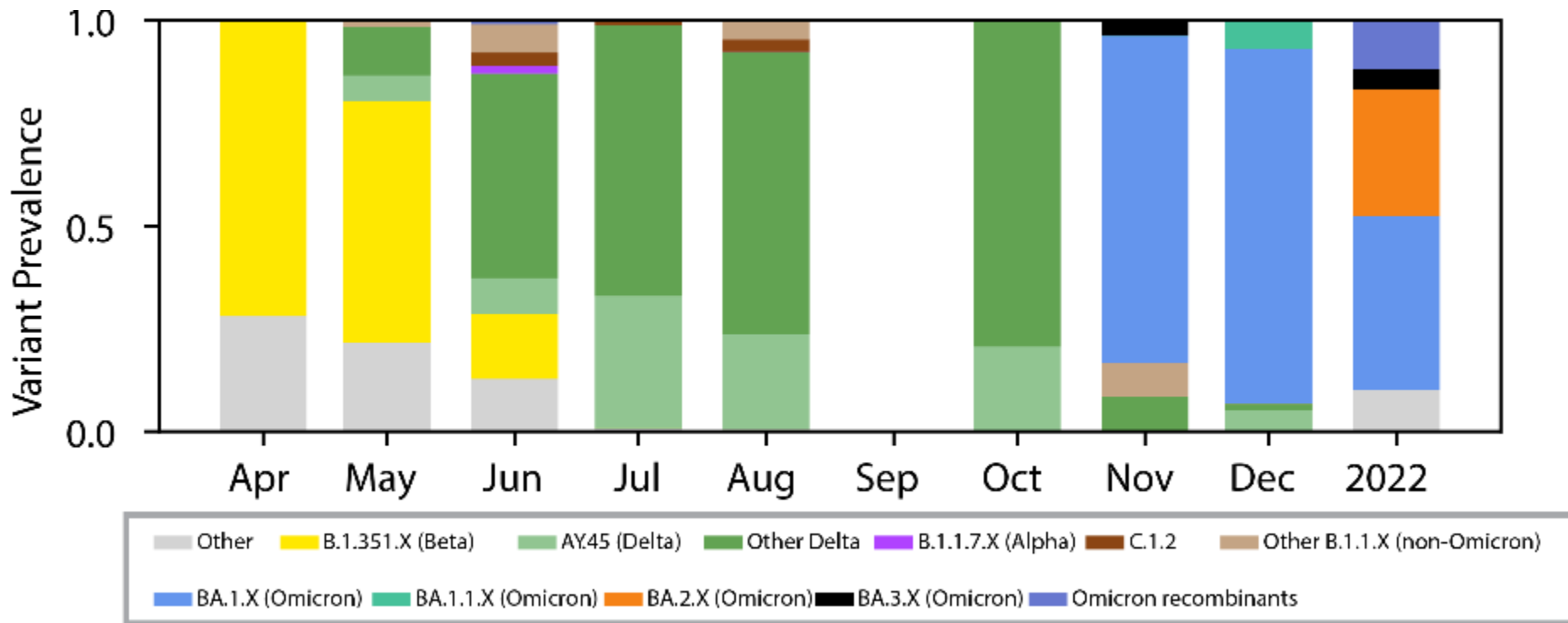


# Wastewater surveillance in South Africa with NICD

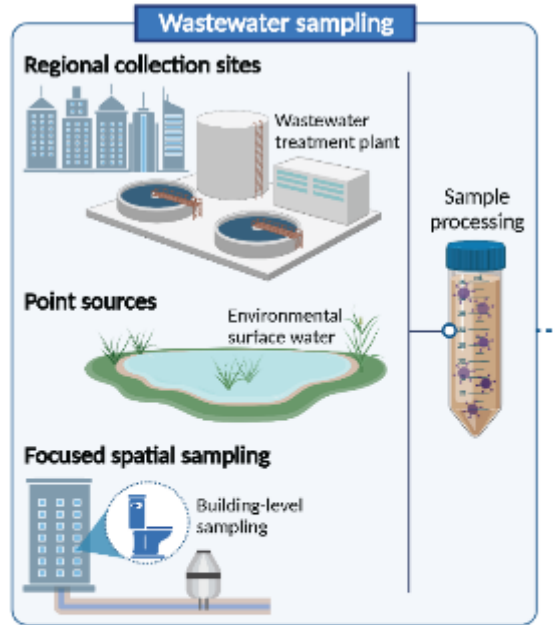




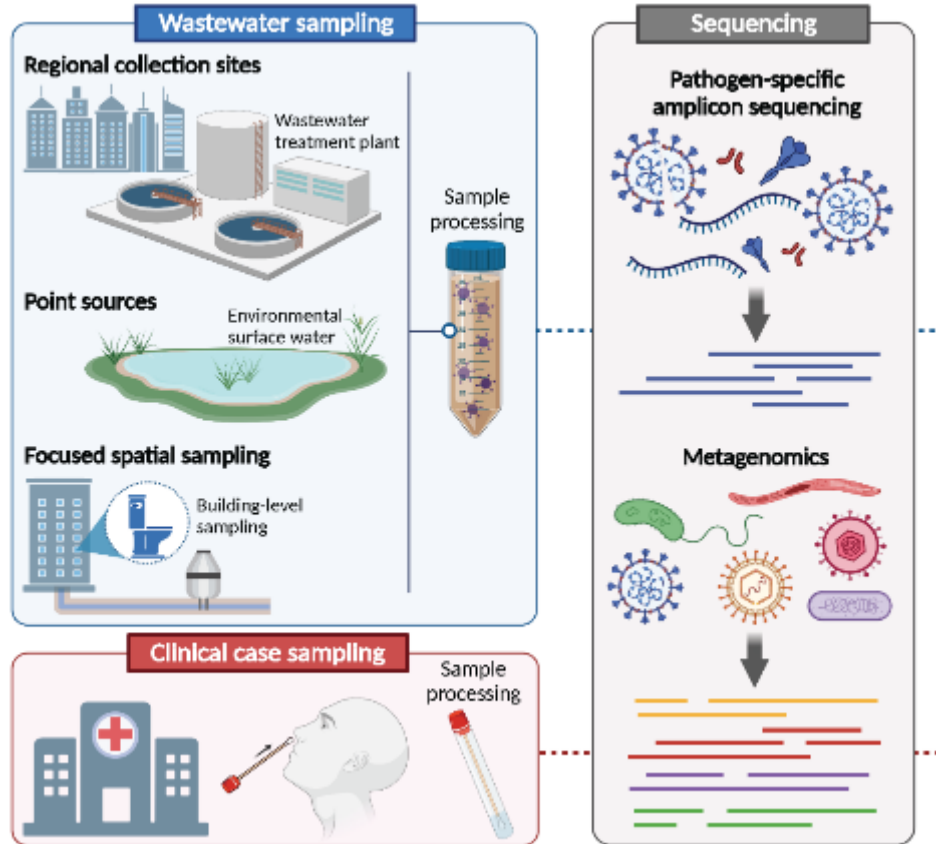
# SARS-CoV-2 waves in South Africa via wastewater



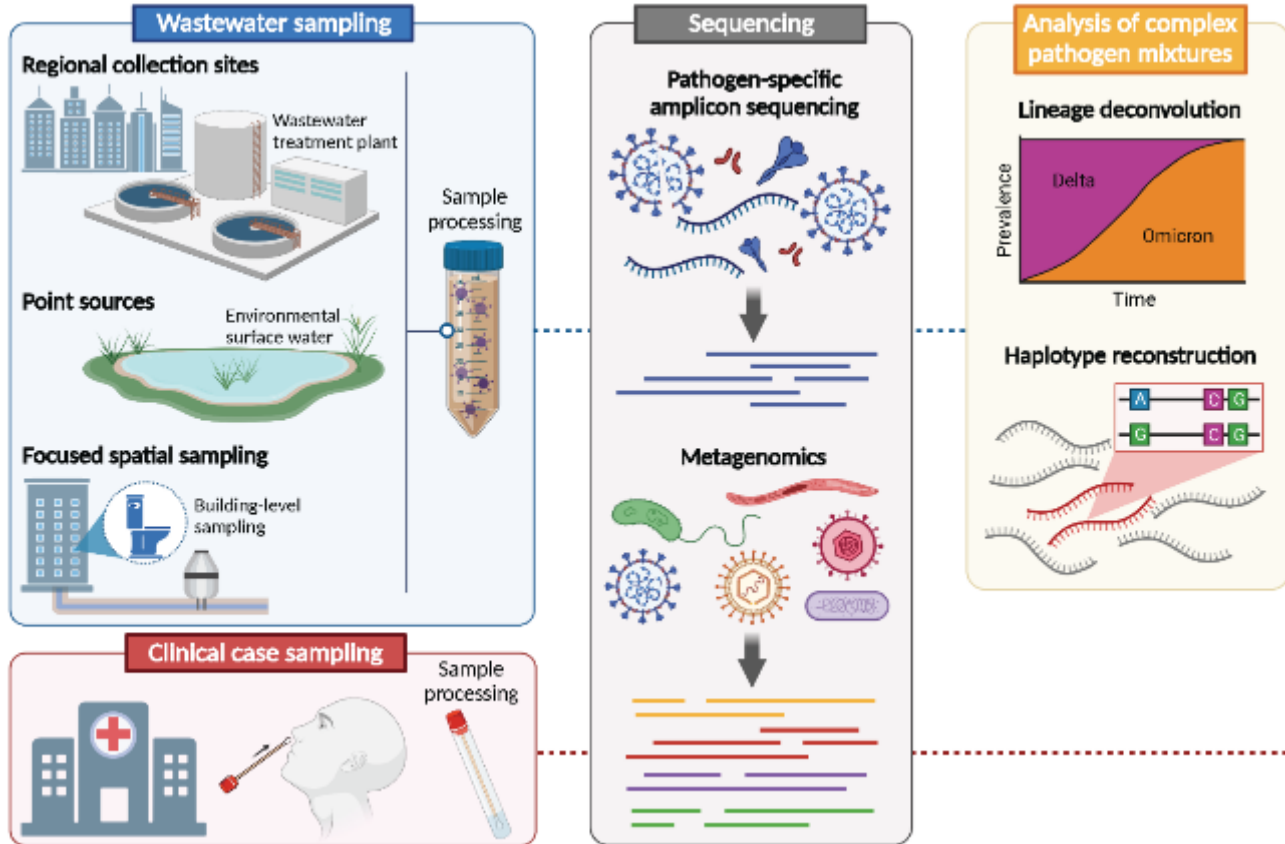
# Integrated wastewater and clinical surveillance



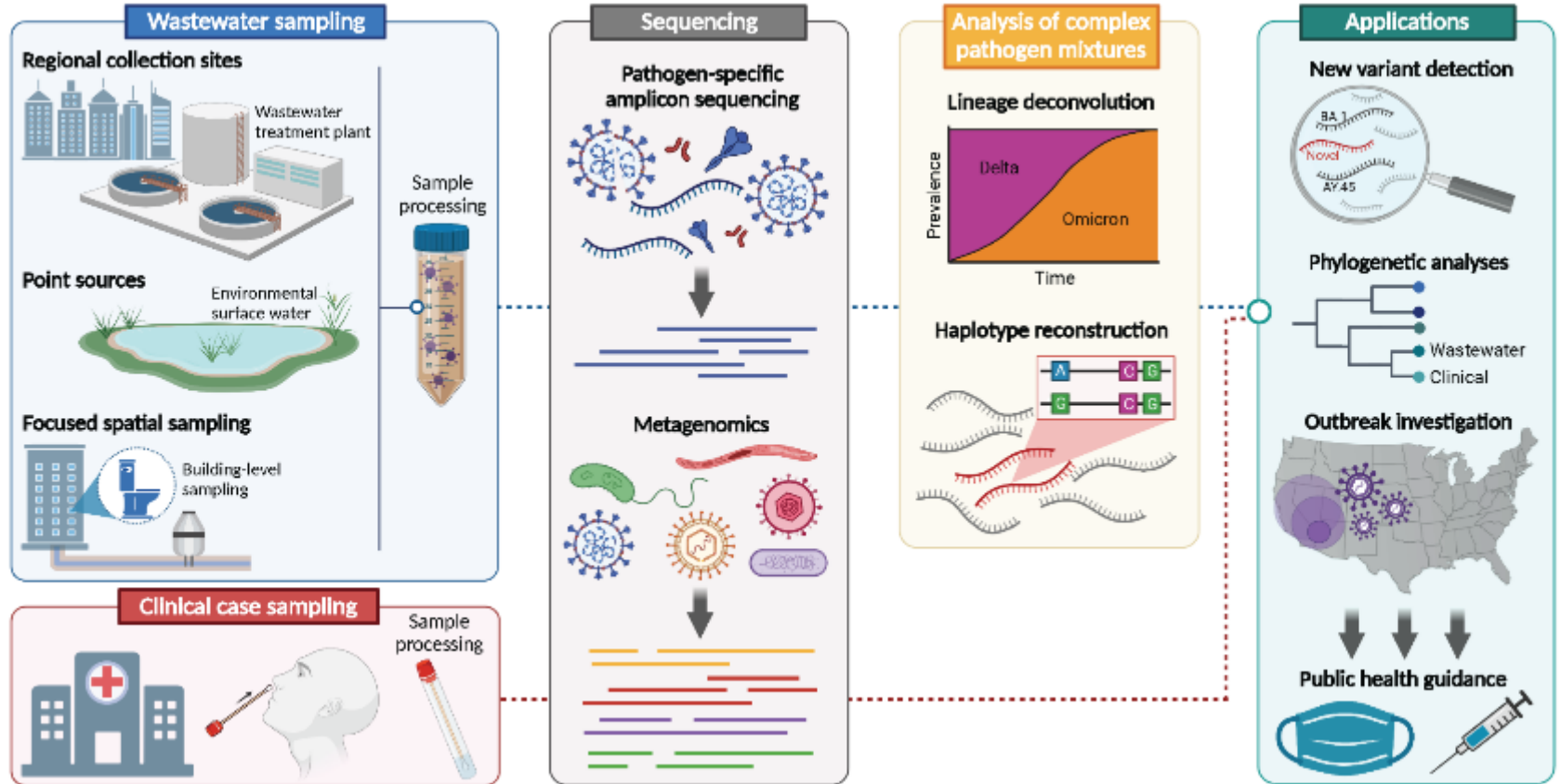
# Integrated wastewater and clinical surveillance



# Integrated wastewater and clinical surveillance


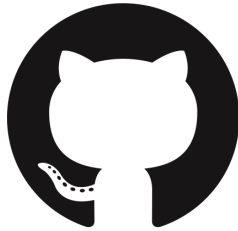


# Integrated wastewater and clinical surveillance



# Freyja availability and usage

# BIOCONDA®



dockerhub Search for great content (e.g., mysql)

explore staphb/freyja

**staphb/freyja** ☆  
By staphb • Updated 14 days ago  
Freyja: recover relative lineage abundances from mixed SARS-CoV-2 samples from a sequencing dataset.  
Container

Overview Tags

>80k downloads

# Acknowledgements



Funding



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Health and Human Services  
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KAMUZU  
UNIVERSITY  
OF HEALTH SCIENCES



National Institutes  
of Health



BILL & MELINDA  
GATES foundation

