Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Wastewater Based Epidemiology in Wisconsin:

surveillance and sequencing of wastewater for SARS-CoV-2

Kayley Janssen, PhD
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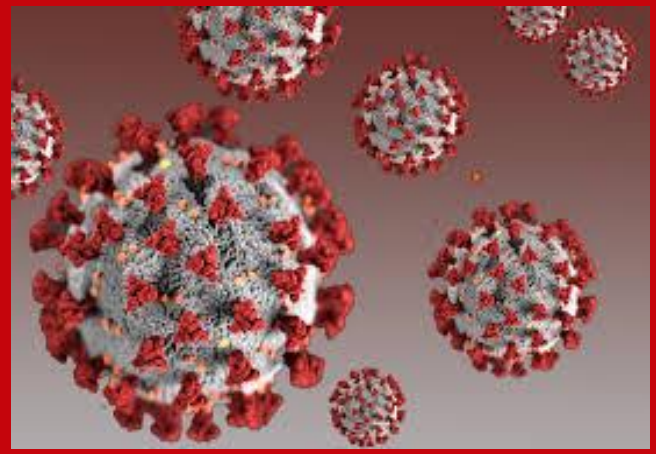
June 27th, 2022

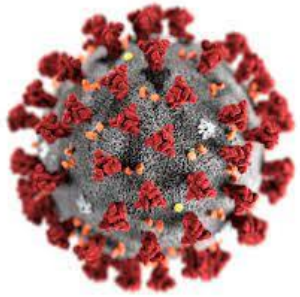
WSLH Virology Conference



Session Objectives

- What is wastewater surveillance/wastewater-based epidemiology and how is it structured and implemented in Wisconsin.
- How wastewater surveillance of SARS-CoV-2 is a useful public health tool and can inform on community prevalence and effectiveness of control measures.
- What sequencing wastewater for SARS-CoV-2 looks like in Wisconsin and the challenges.

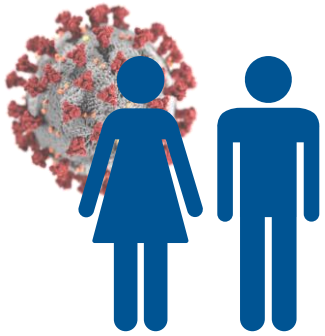
SARS-CoV-2 Pandemic



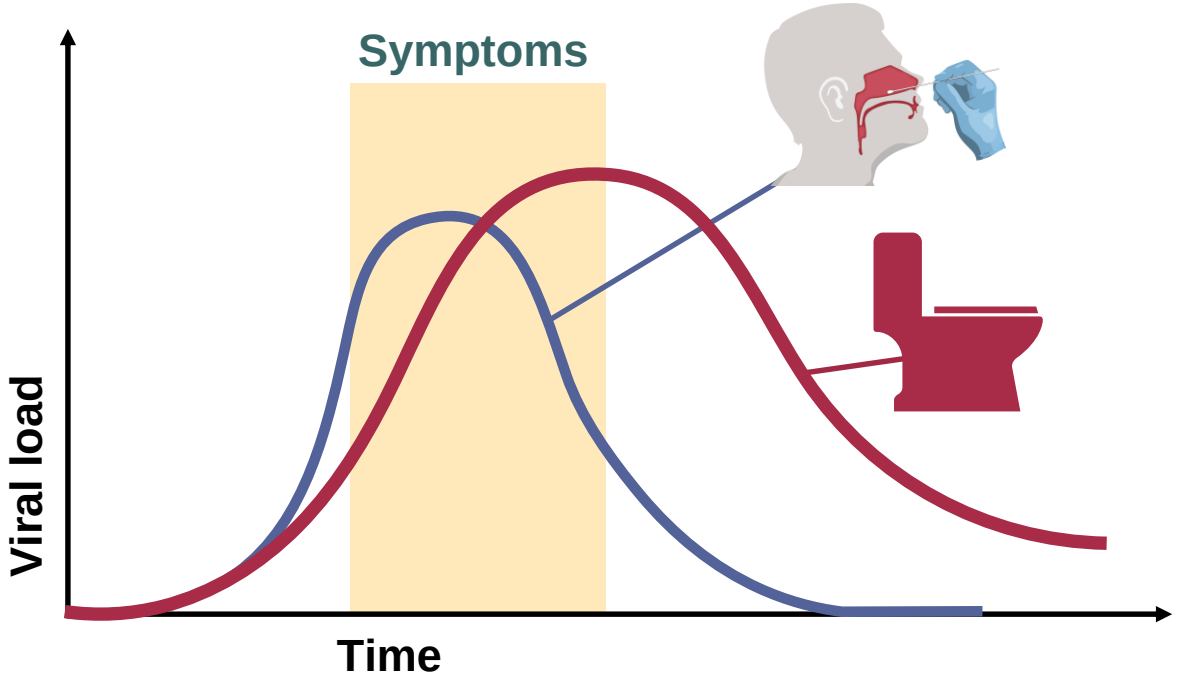
WSLH-Clinical Division started PCR tests in March of 2020

Helped to expand Wisconsin's capacity from 100 tests/day to 20,000

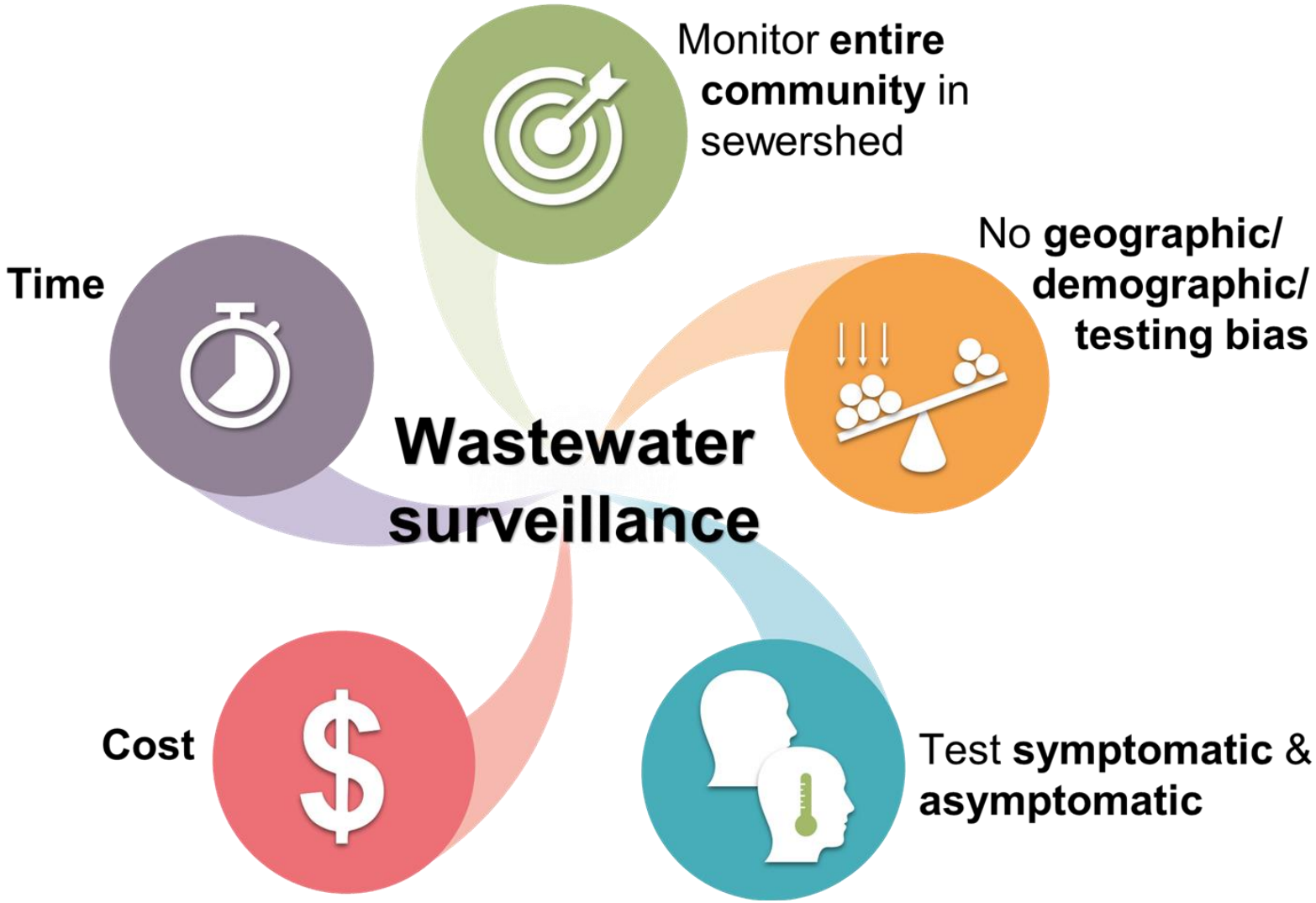
Coupling clinical surveillance with environmental surveillance



Infected persons (symptomatic and asymptomatic) shed viruses in nose/throat and stool



Why Study Wastewater?



Wastewater Based Epidemiology

A way to collect data on an entire community's health

Proposed in 1940s with use of detecting polio in urban sewage

Not popular till 2000s

-pharmaceuticals (antibiotics, antivirals)
-illicit drugs (cocaine, opiates, cannabis, amphetamines)
-flu (2011 Netherlands to assess role of entry into water cycle)
-polio (Israel outbreak 2013-2014)

Establishing Wastewater Surveillance in Wisconsin

Spring and Summer of 2020

Partnered with DHS, DNR and UW-Milwaukee to setup infrastructure and testing

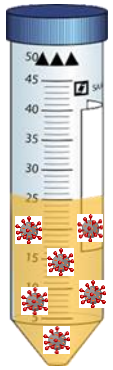
Plan was 1 year of surveillance through Summer 2021

Goal

To determine the efficacy of wastewater surveillance for monitoring SARS-CoV-2 within a community

Enroll Wastewater Treatment Plants

Develop testing for SARS-CoV-2 in wastewater



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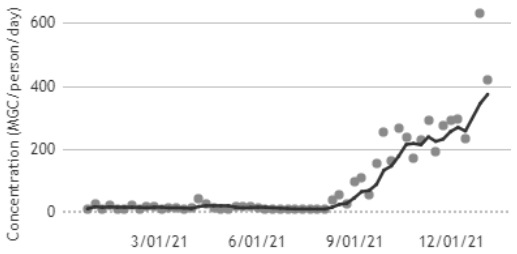
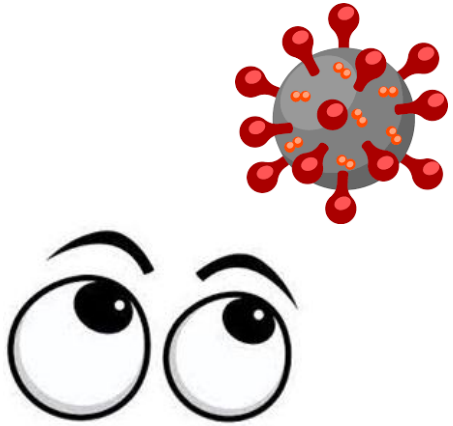
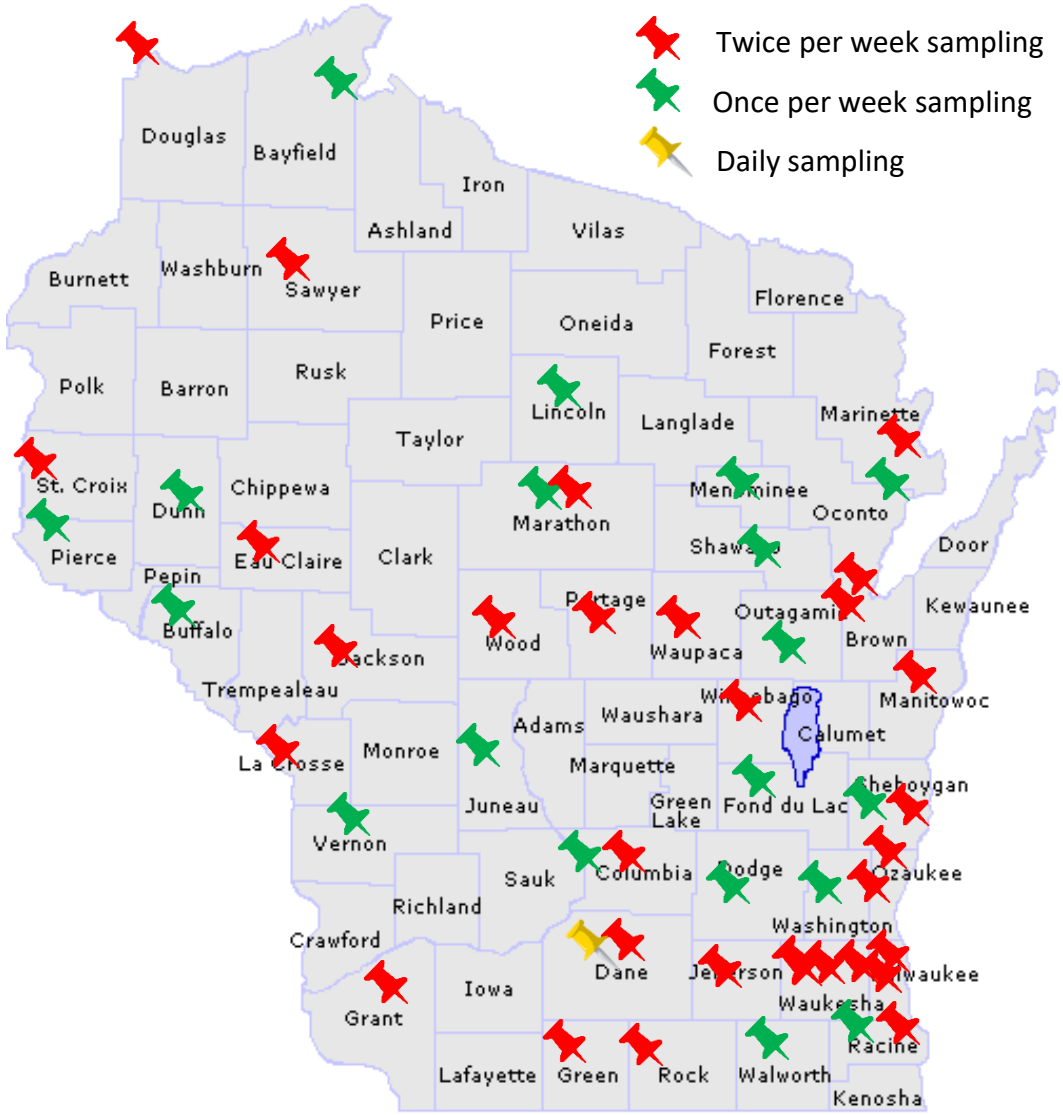


Surveillance

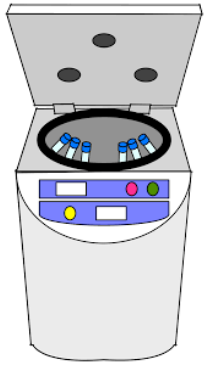


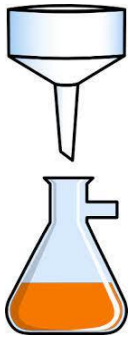
Wisconsin Statewide Sampling Plan
Enrolling Wastewater Treatment Facilities



- September 2020: 72 facilities monitored (~60% population)
- Now: 50 facilities sampled (~40% pop.)
- Facility inclusion reflects a balance of population coverage and geographic distribution

Developing a Test for SARS-CoV-2 in Wastewater

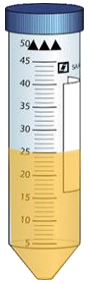


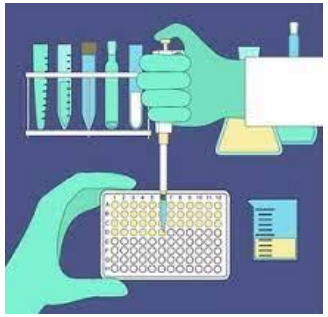


Criteria for a wastewater test

Additional factors considered

- Time to concentrate
- Equipment
- Analyst variability
- Throughput





Workflow

Developing a method for testing

WWTP

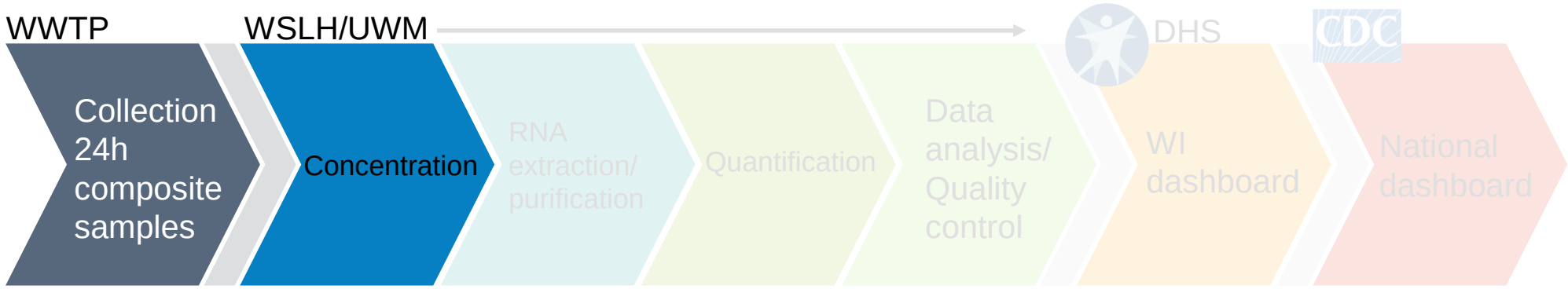
WSLH/UWM

Collection 24h composite samples

Concentration

Workflow

Selecting a Concentration Method



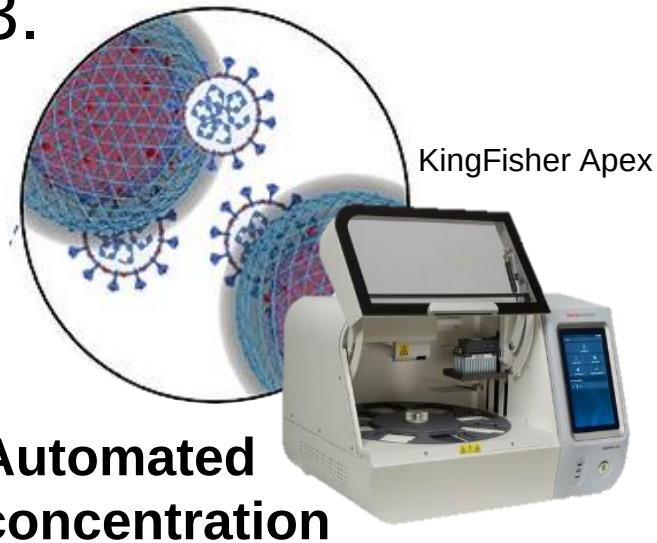
1.

PEG precipitation

2.

HA filtration

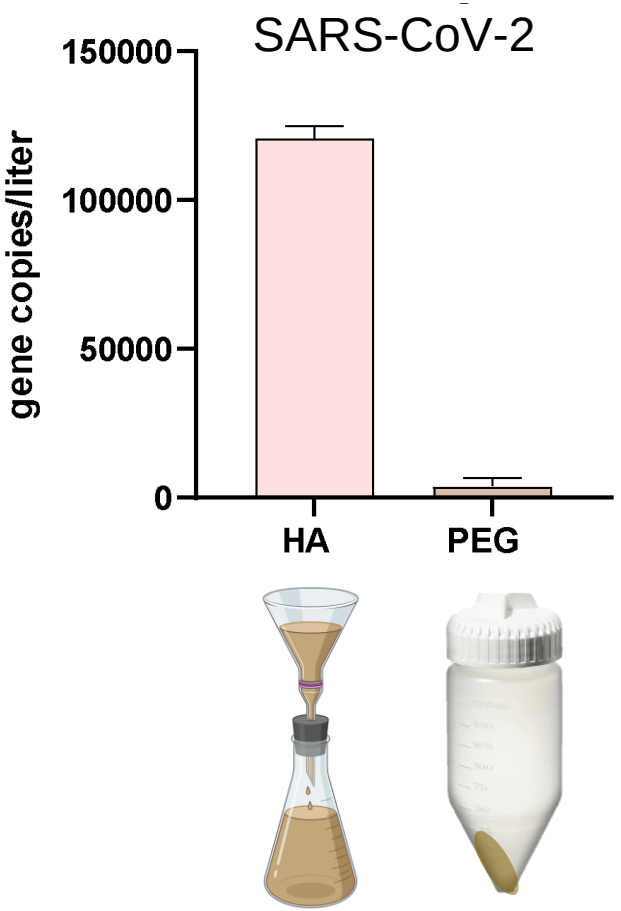
3.

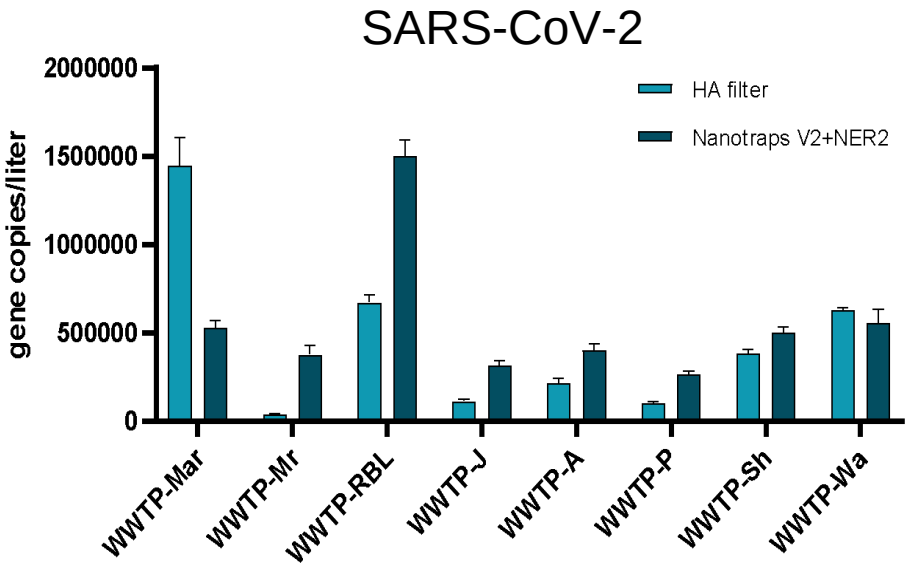


Automated concentration
Nanotrap (Ceres)
= nanoparticle capture

Selecting a Concentration Method:

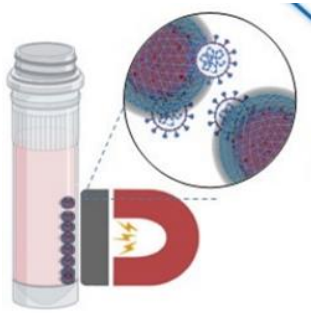
Comparisons





PEG Precipitation

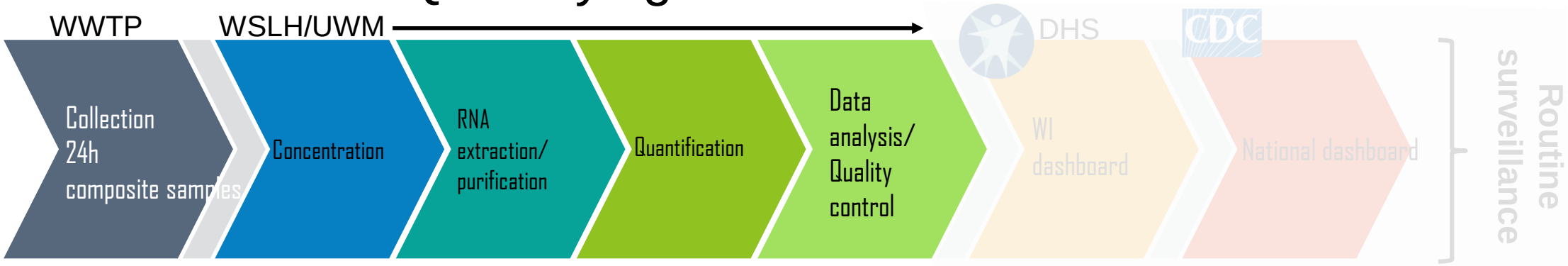
HA Filtration



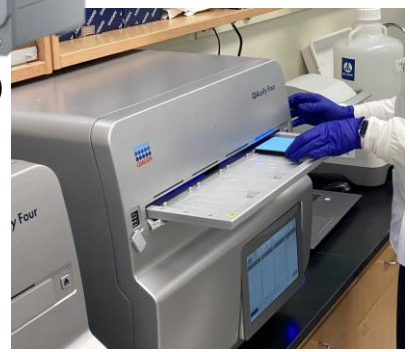
Magnetic Particles (Nanotraps)

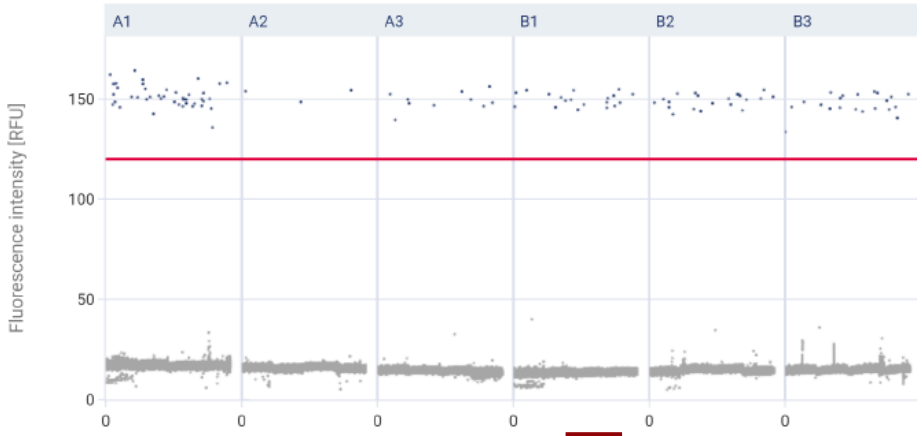
Workflow

Quantifying SARS-CoV-2



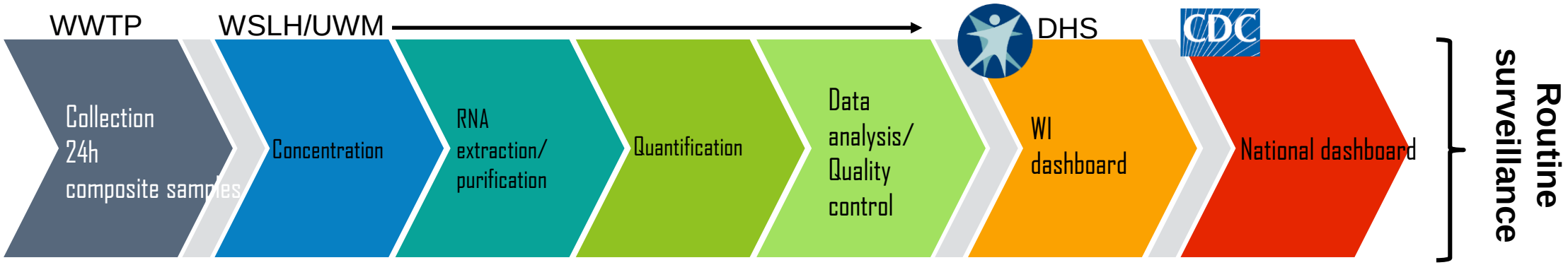
QIAGEN dPCR (dPCR)





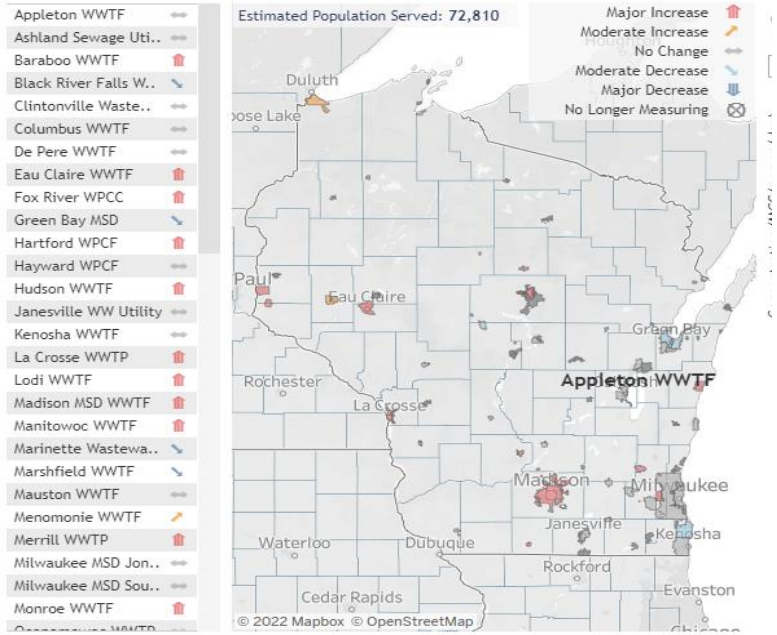
Gene copies/liter

Workflow
Data Dissemination



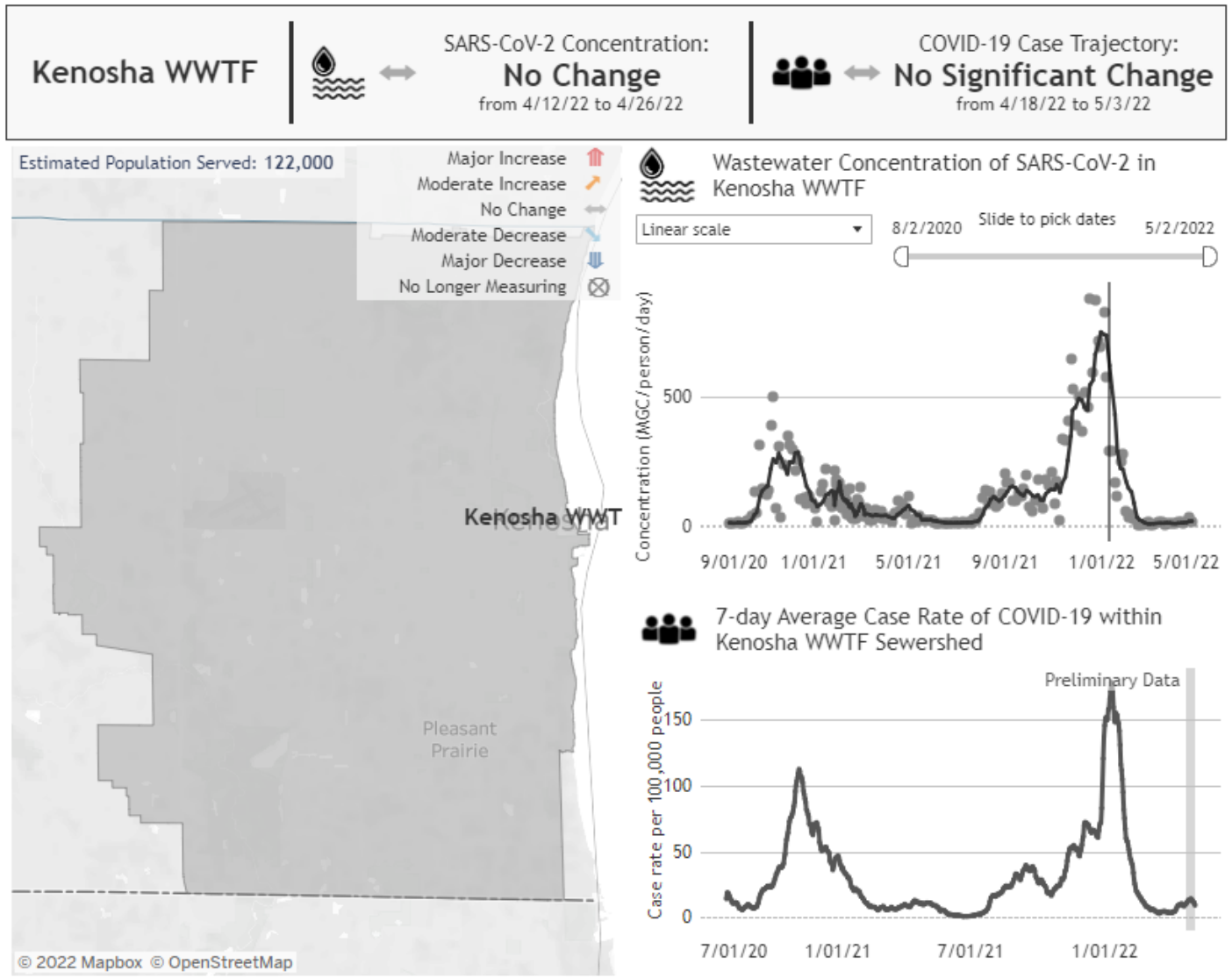
Data is sent to WWTFs and to DHS directly

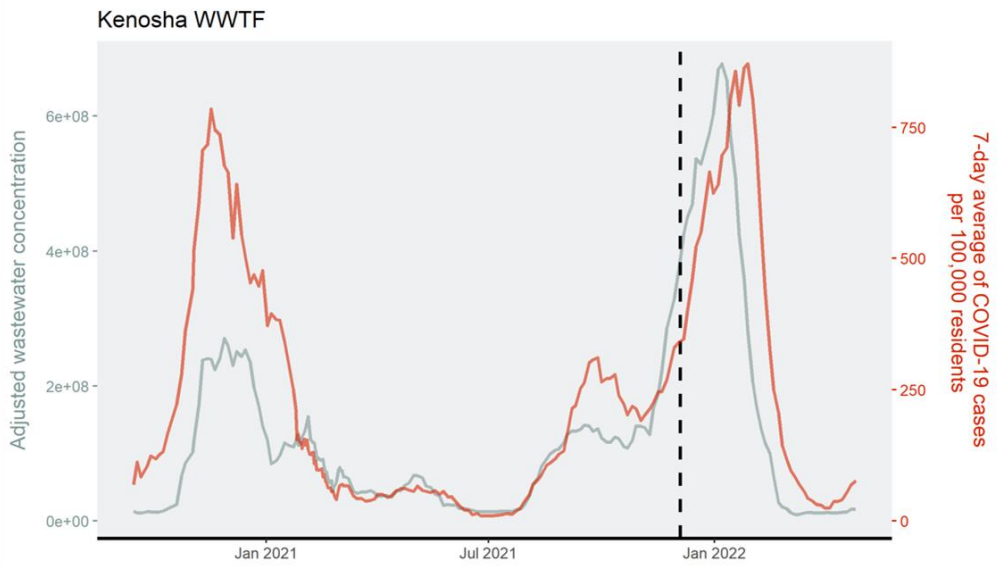
DHS maintains a public facing dashboard



https://www.dhs.wisconsin.gov/covid-19/wastewater.htm

Data Shared with Public Health Agencies



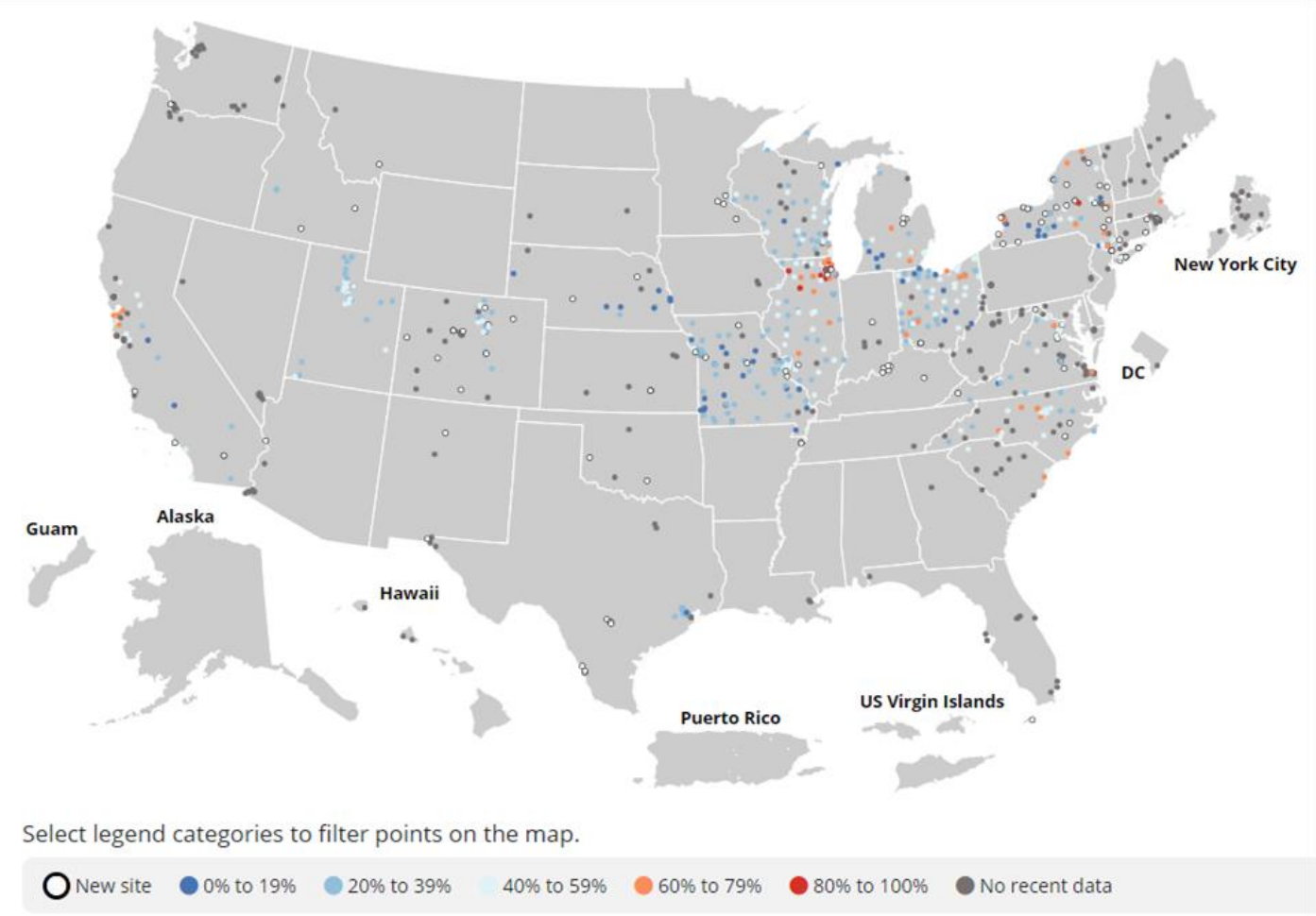


https://www.dhs.wisconsin.gov/covid-19/wastewater.htm

Nathan Kloczko and Matthew Schinwald, DHS

Data Shared with Public Health Agencies

DHS uploads data to DECIPHER for CDC



Current SARS-CoV-2 virus levels by site, United States

Current virus levels category	Num. sites	% sites	Category change in last 7 days
New Site	118	20	0%
0% to 19%	58	10	– 35%
20% to 39%	207	35	– 26%
40% to 59%	148	25	– 2%
60% to 79%	48	8	– 46%
80% to 100%	12	2	– 59%

Total sites with current data: 591

Total number of wastewater sampling sites: 829

https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance

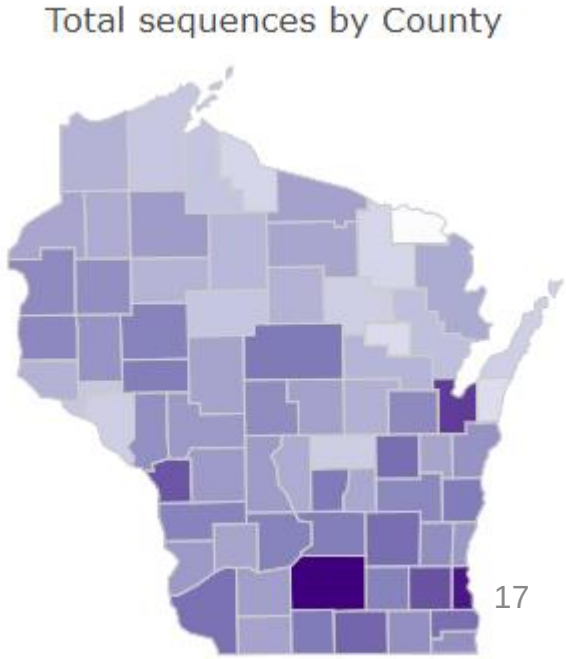
How Can Wastewater Surveillance Data Be Used

Wastewater data can be supportive to case-based surveillance

Fill in gaps of case-based surveillance

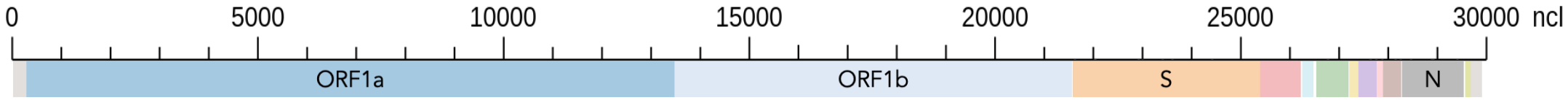
Early warning that may inform decisions in hospital preparedness

Limitation: Does not inform about circulating variants

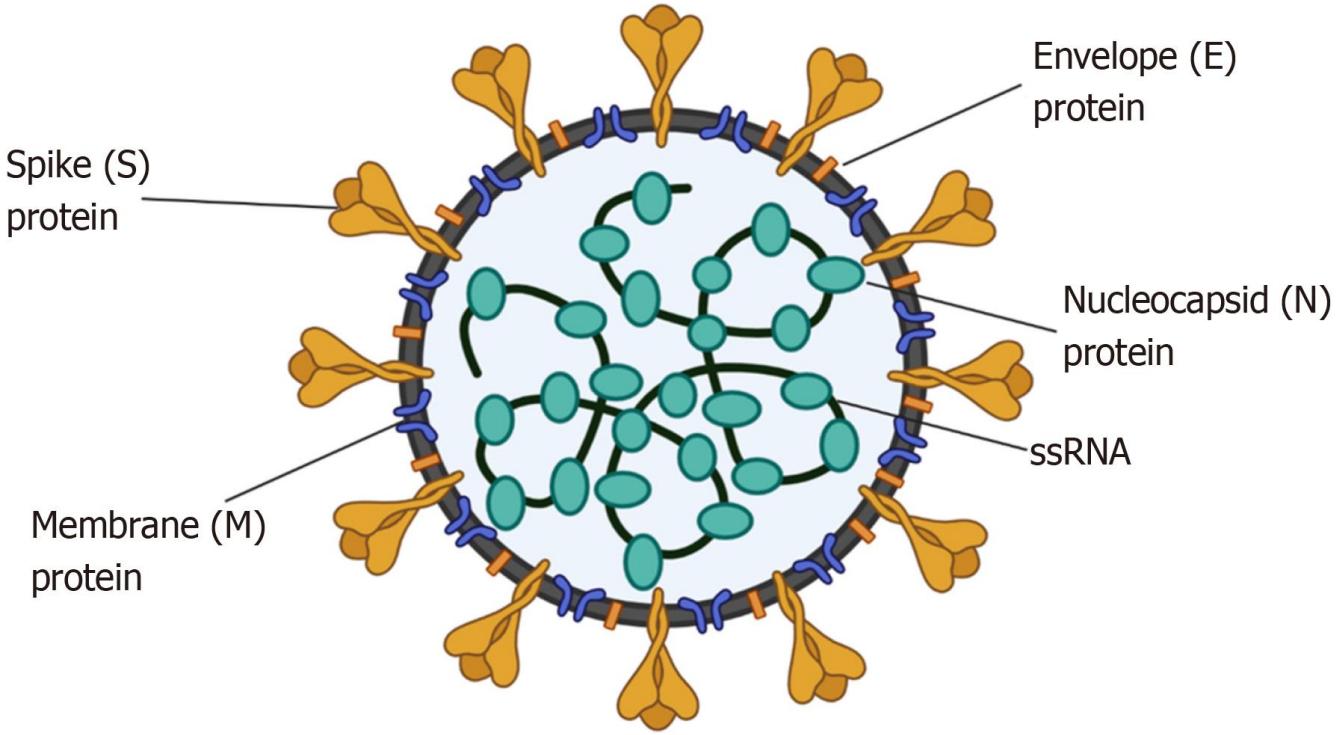


How Can We Monitor SARS-CoV-2 Variants in Wastewater?

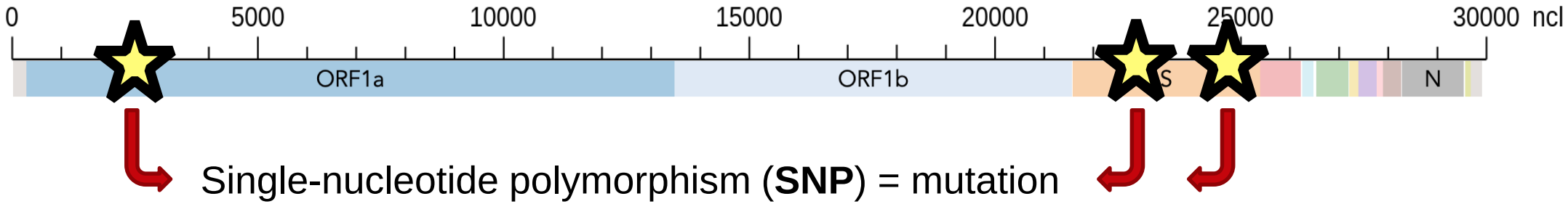
SARS-CoV-2 Genome

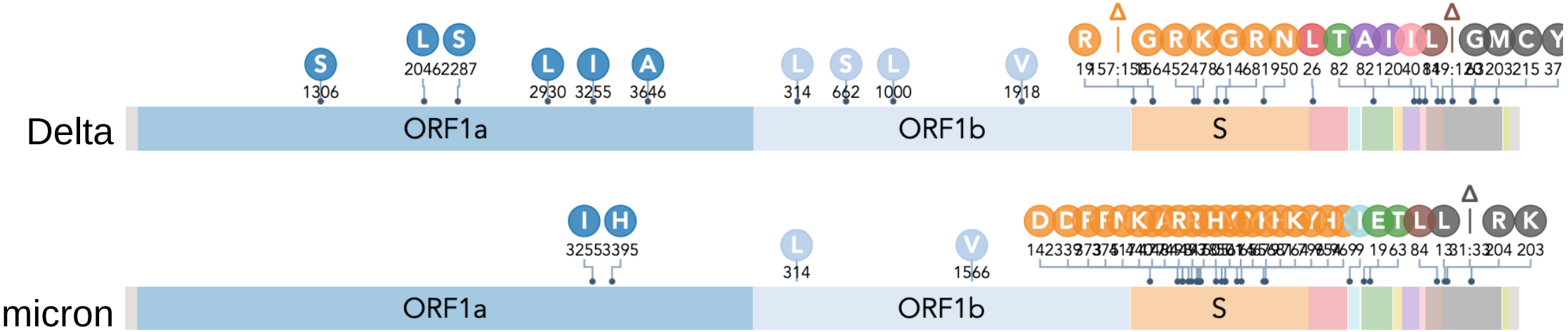


Positive-sense single-stranded RNA virus
30-kb genome

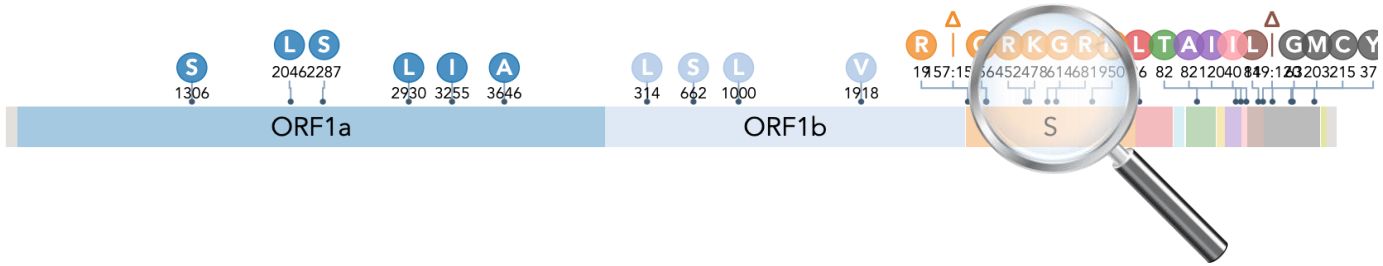


SARS-CoV-2 Genome

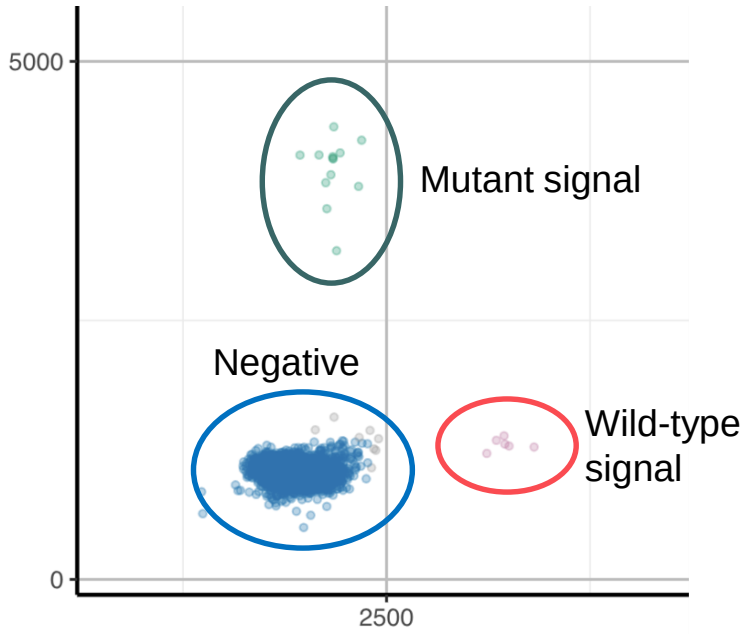


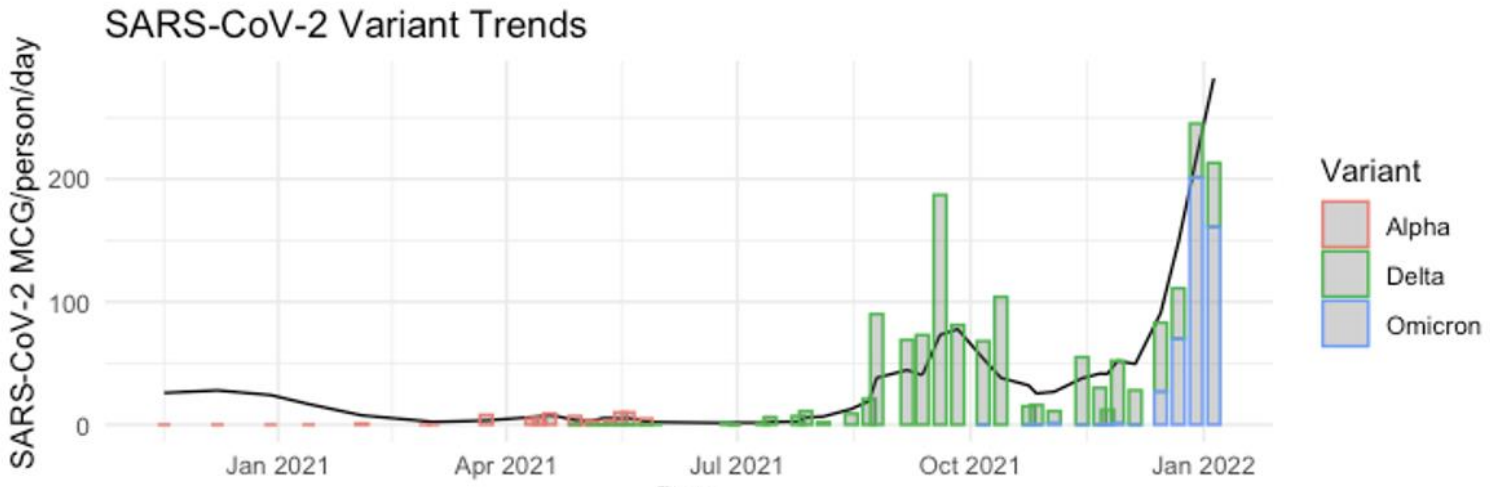


Wastewater data can track variants: PCR assays



- Variants surveillance using **PCR assays** (targeting variant-specific mutations)
  - Quantitative mutation/wild- type assays: proportions
  - Very sensitive and specific
- Monitoring since **early 2021**: Alpha, Beta, Gamma, Epsilon, Delta, Omicron





Variant trends matched timing of appearance and dominance. Each surge shifted more quickly and had higher levels than the previous

Analysis performed by: Melissa Schussman (UW-Milwaukee)

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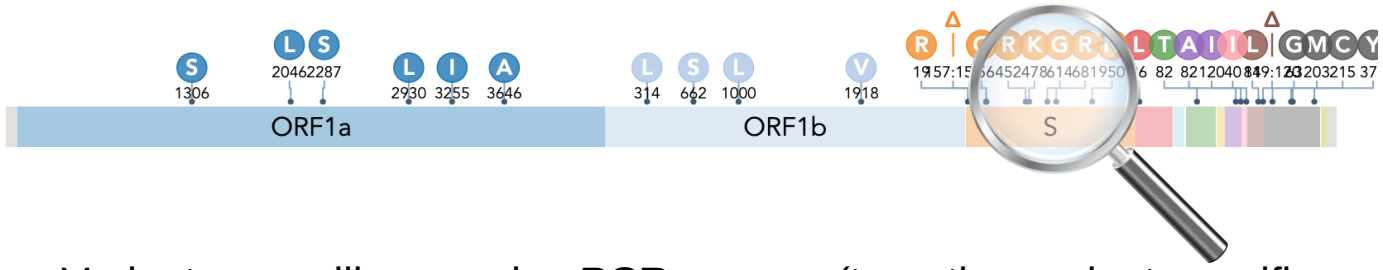
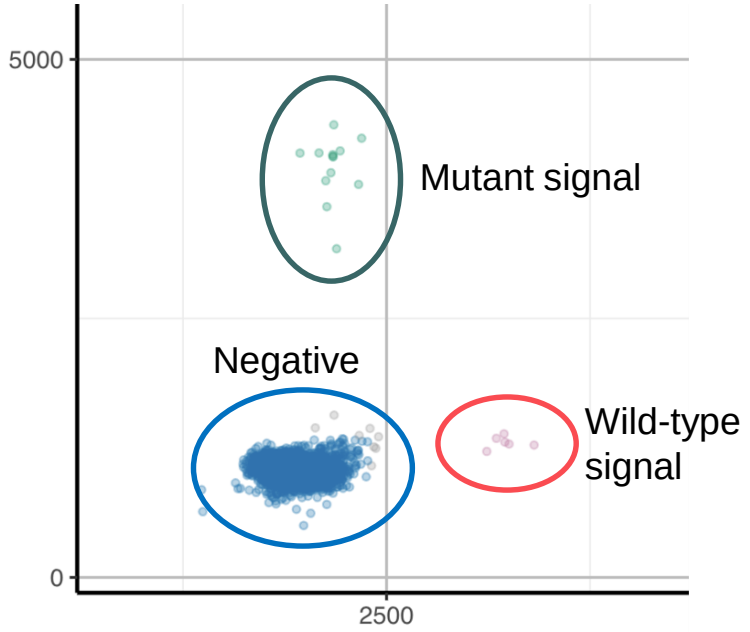
Wastewater data can track variants: PCR assays





- Variants surveillance using **PCR assays** (targeting variant-specific mutations)
  - Quantitative mutation/wild- type assays: proportions
  - Very sensitive and specific
- Monitoring since **early 2021**: Alpha, Beta, Gamma, Epsilon, Delta, Omicron

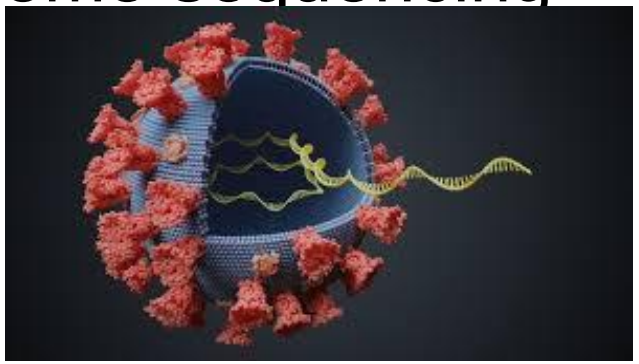
Limitations:

- Can only screen a for **specific** SNPs/deletions
  May need multiple PCR assays to identify a variant
- Delay between emergence of new variants and validated PCR assays (lag in screening)

*Limitations can be overcome with **sequencing***

Sequencing of Wastewater For SARS-CoV-2

- WSLH-CDD began sequencing clinical isolates early on in the pandemic
- Wastewater sequencing was delayed as methods were tested and developed
- Many options to select from for whole genome sequencing
  - Sequencing **platform**: **short** vs **long** reads
  - Sample **preparation**
  - Library prep kits (target amplicon)



Data analysis workflow

Unknown strain

☆ Mutations

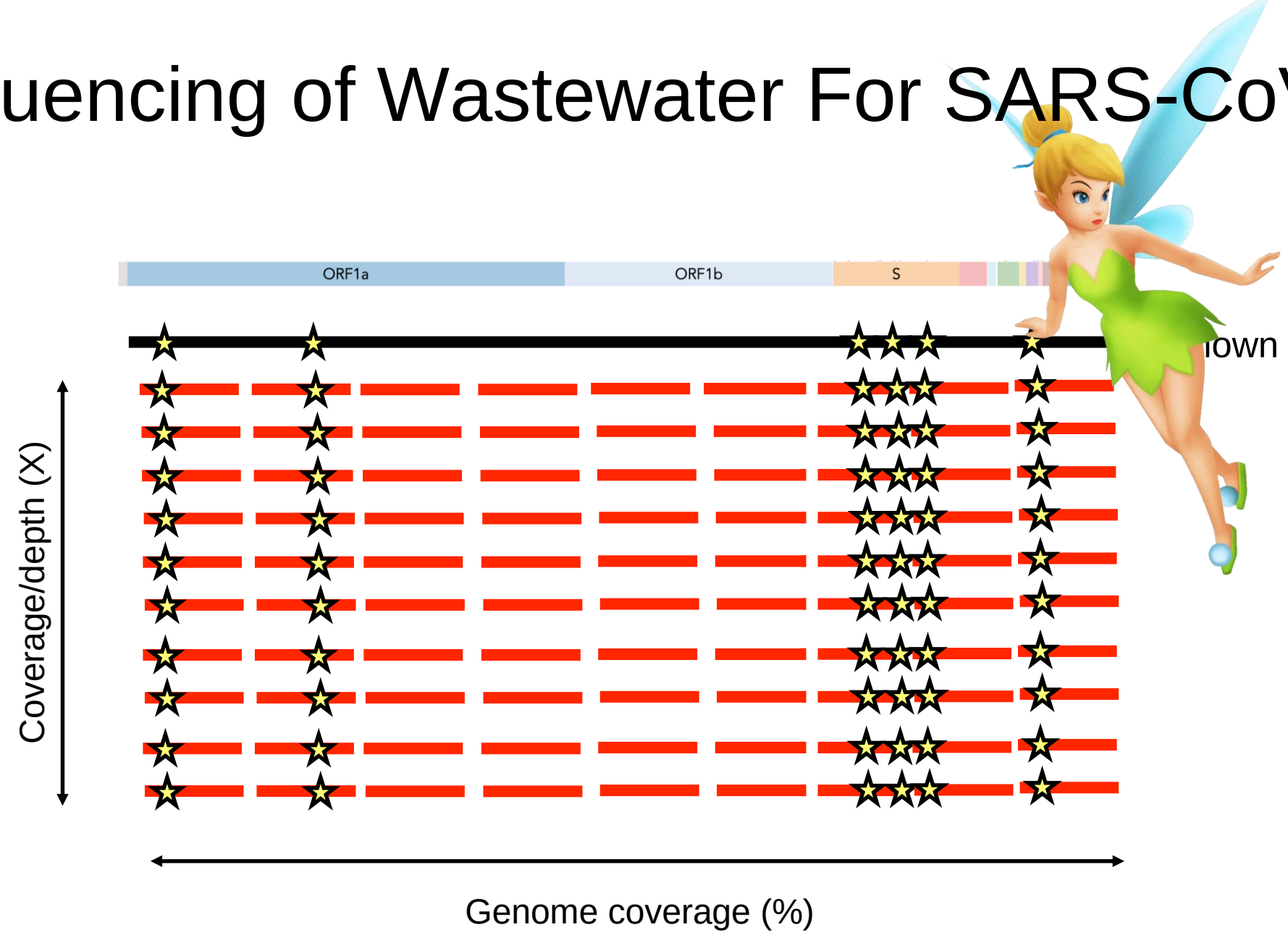
Alignment to a reference

Reference
Wuhan-1

Data analysis (comparison to databases)

Visualizations

Sequencing of Wastewater For SARS-CoV-2



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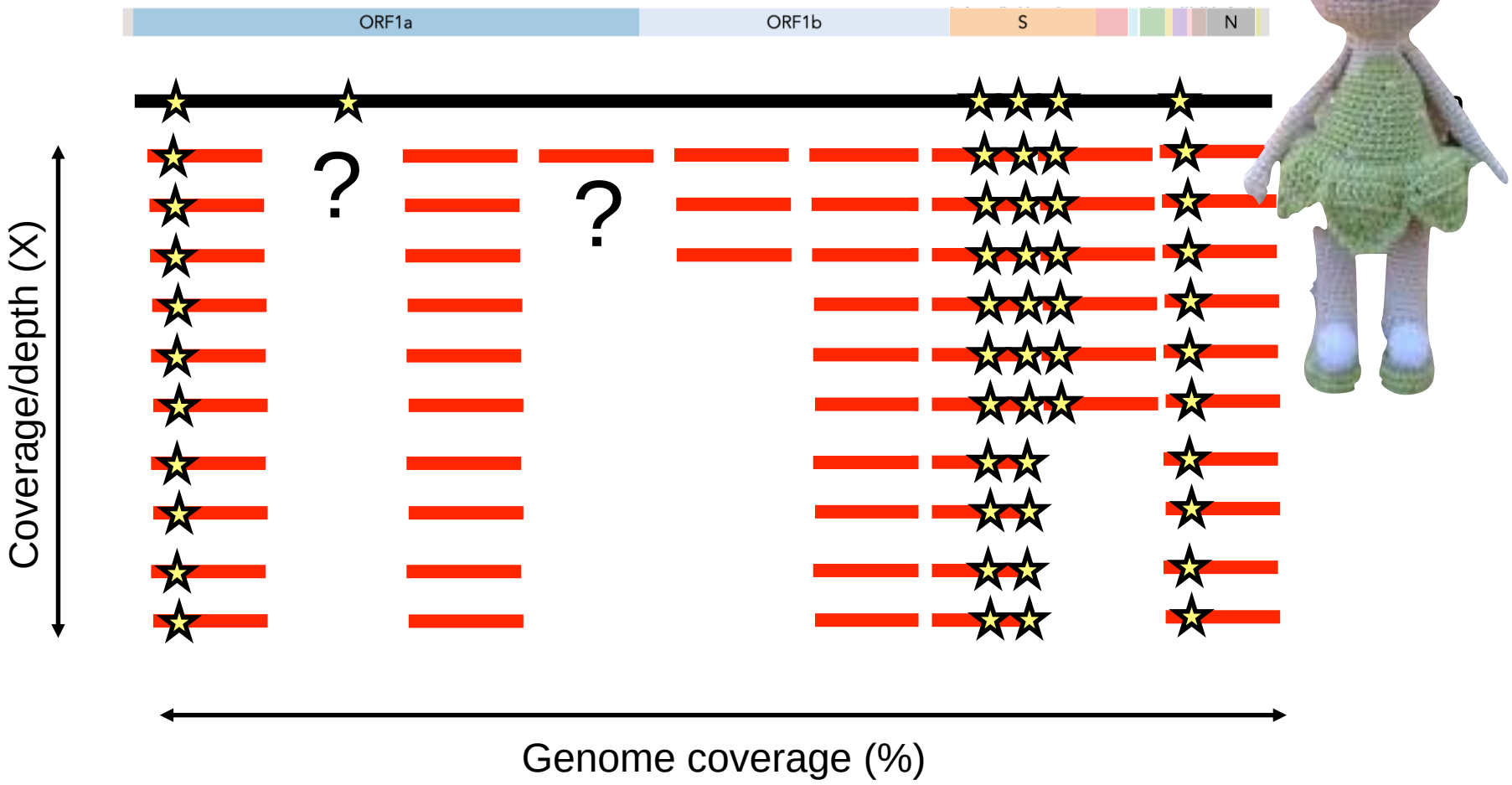


Samples considered: Genome coverage ≥80% with ≥25X coverage

RNA prep comparison for short vs long read sequencing

HA filtration → Bead beating

Direct capture

PEG precipitation → Bead beating

PEG precipitation → NO Bead beating

Library prep by Roger Wiseman

WSLH's RNA-no amplification of product

Collaborator's RNA/clinical-amplification of product

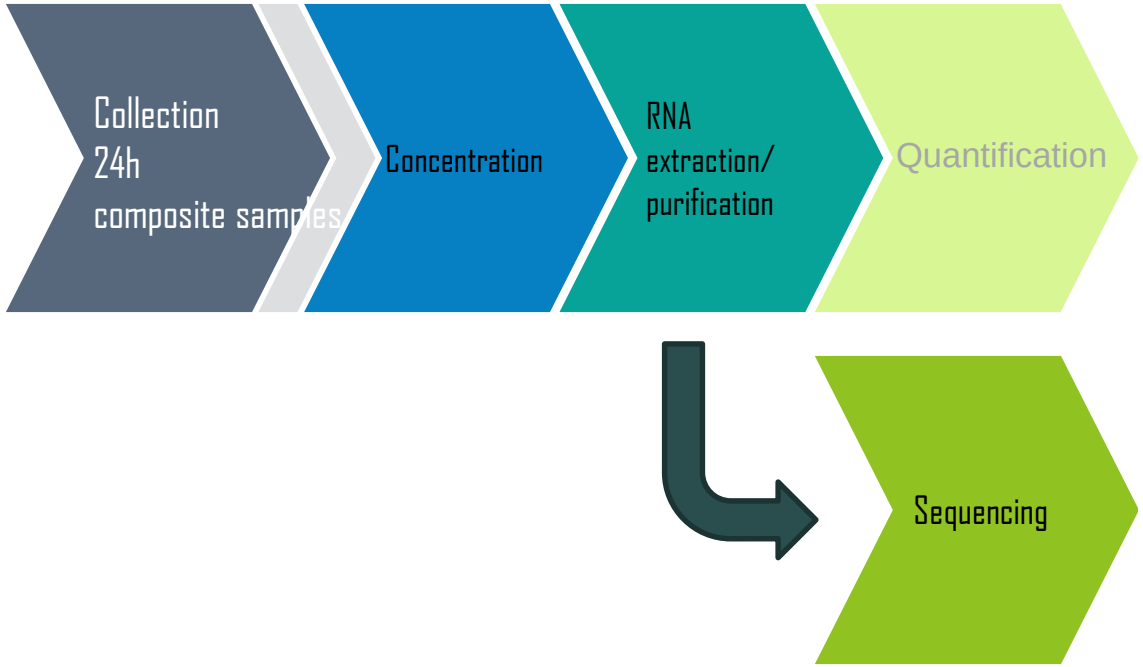
Fragmented RNA

Short reads platform (Illumina) more appropriate

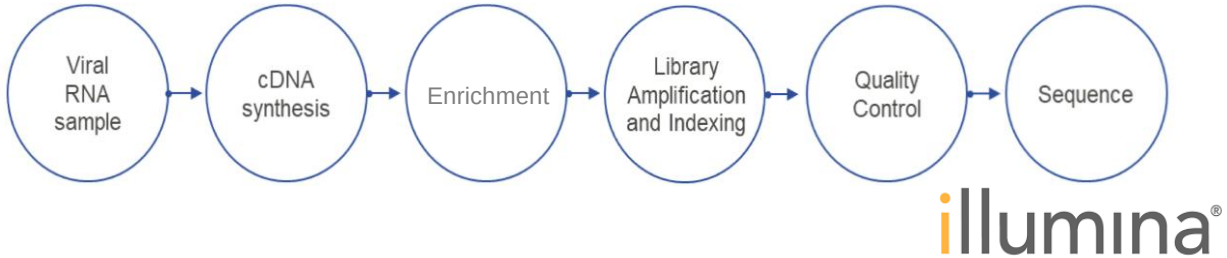
illumina®

High-throughput
Low cost
Low error rate

Library prep kit comparison



Amplify 200+ regions to cover the whole genome



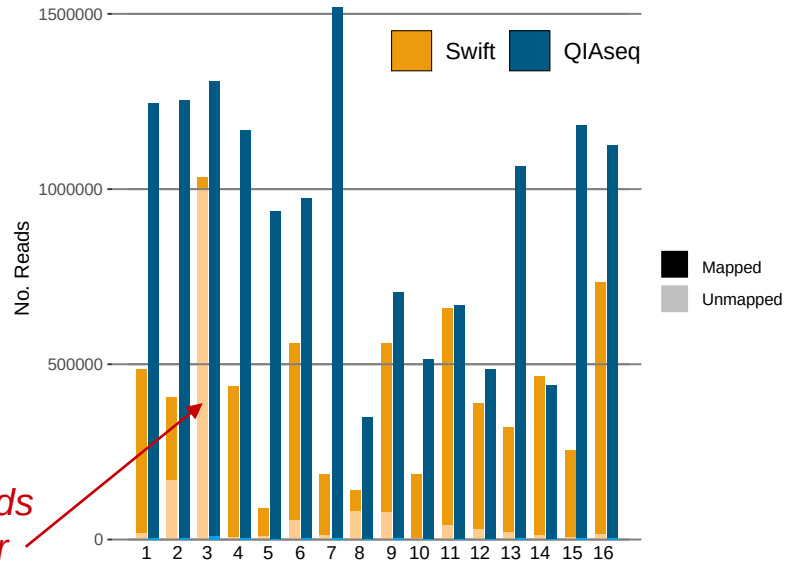
illumina®

QIAseq Direct SARS-CoV-2 Kit (Qiagen)
vs
Swift Normalase Amplicon SARS-CoV-2 kit (IDT)

Library prep kit comparison

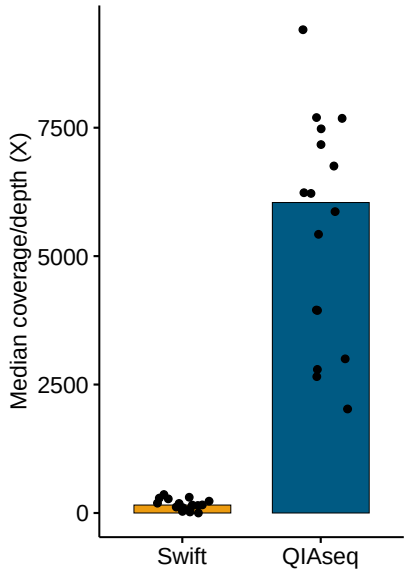
16 samples tested using **Swift Normalase Amplicon** (IDT) vs **QIAseq DIRECT** (Qiagen) SARS-CoV-2 kits

Total number of reads

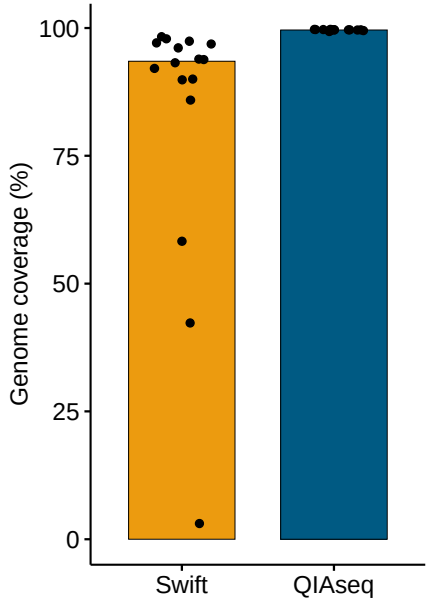


Nucleic acids from other organisms

Median depth



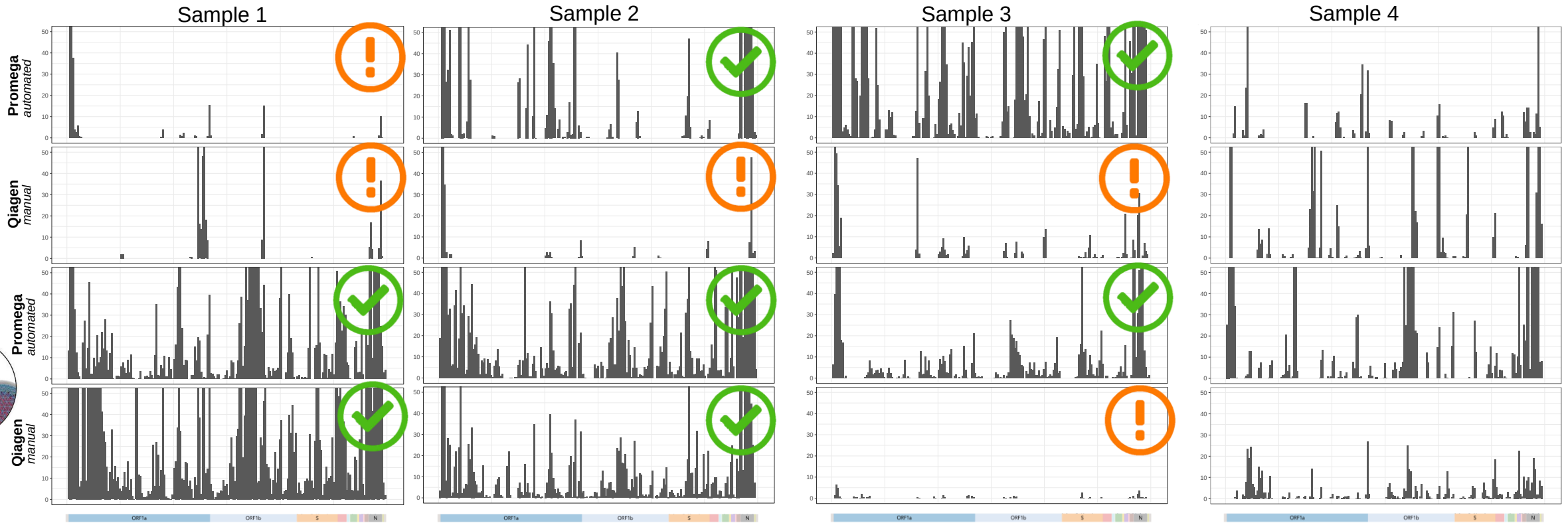
Genome coverage with at least 25X depth



Swift +	**Swift ++**	**Swift +**
QIAseq +++	**QIAseq +++**	**QIAseq +++**

Sample processing comparison

4 samples tested using **QIAseq DIRECT kit** (Qiagen)
- Concentration methods: **HA filtration** vs **NaSARS-C-2notraps**
- Extraction methods: **Promega** (automated/beads) vs **Qiagen** (manual/column) kits

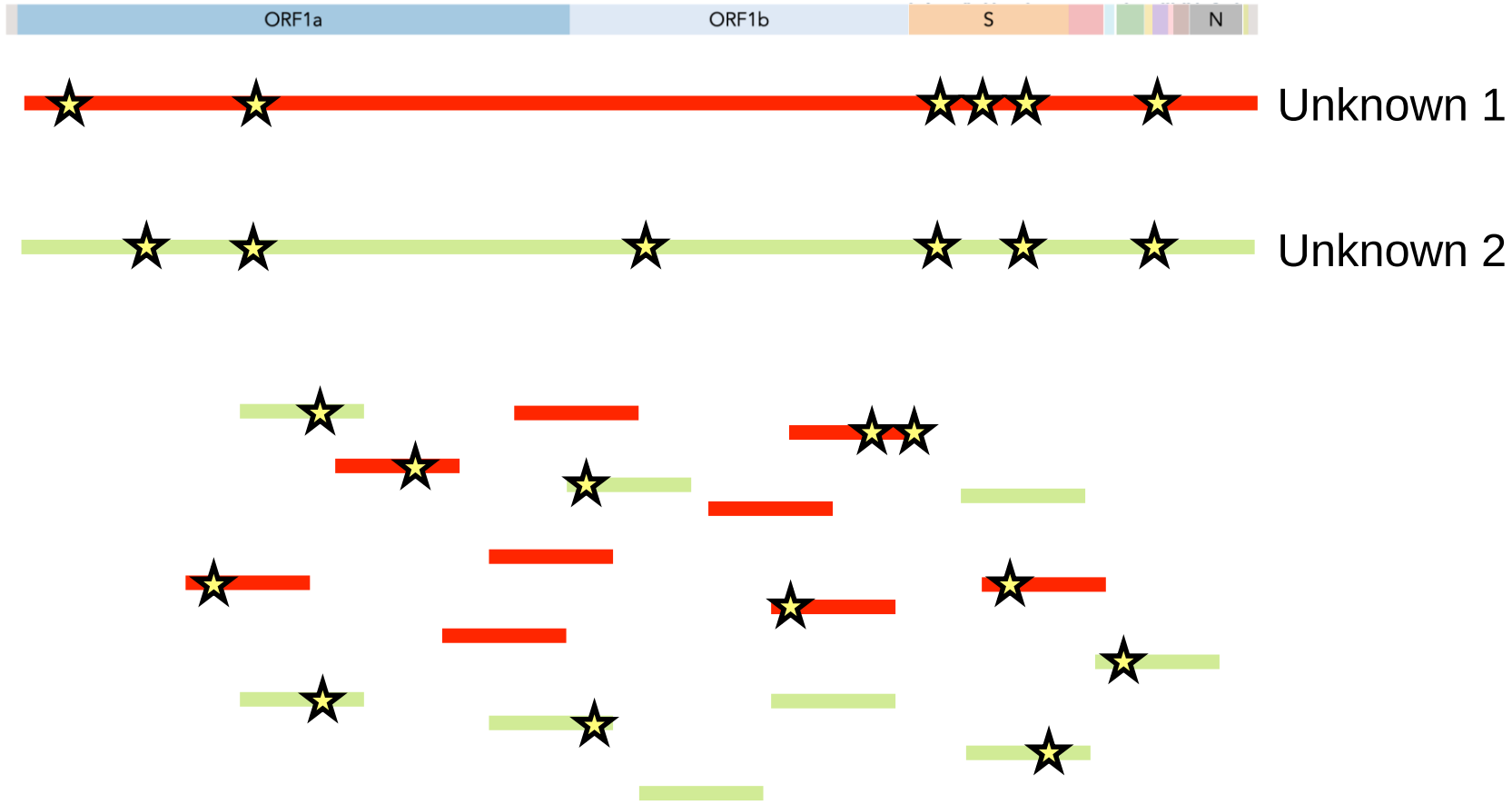


Concentration: **Nanotrap** >> Filtration (bead bashing = RNA fragmented)
Extraction: **Promega** (automated) > Qiagen (manual)

Sequencing of Wastewater For SARS-CoV-2

Limitations:

- Pool of strains



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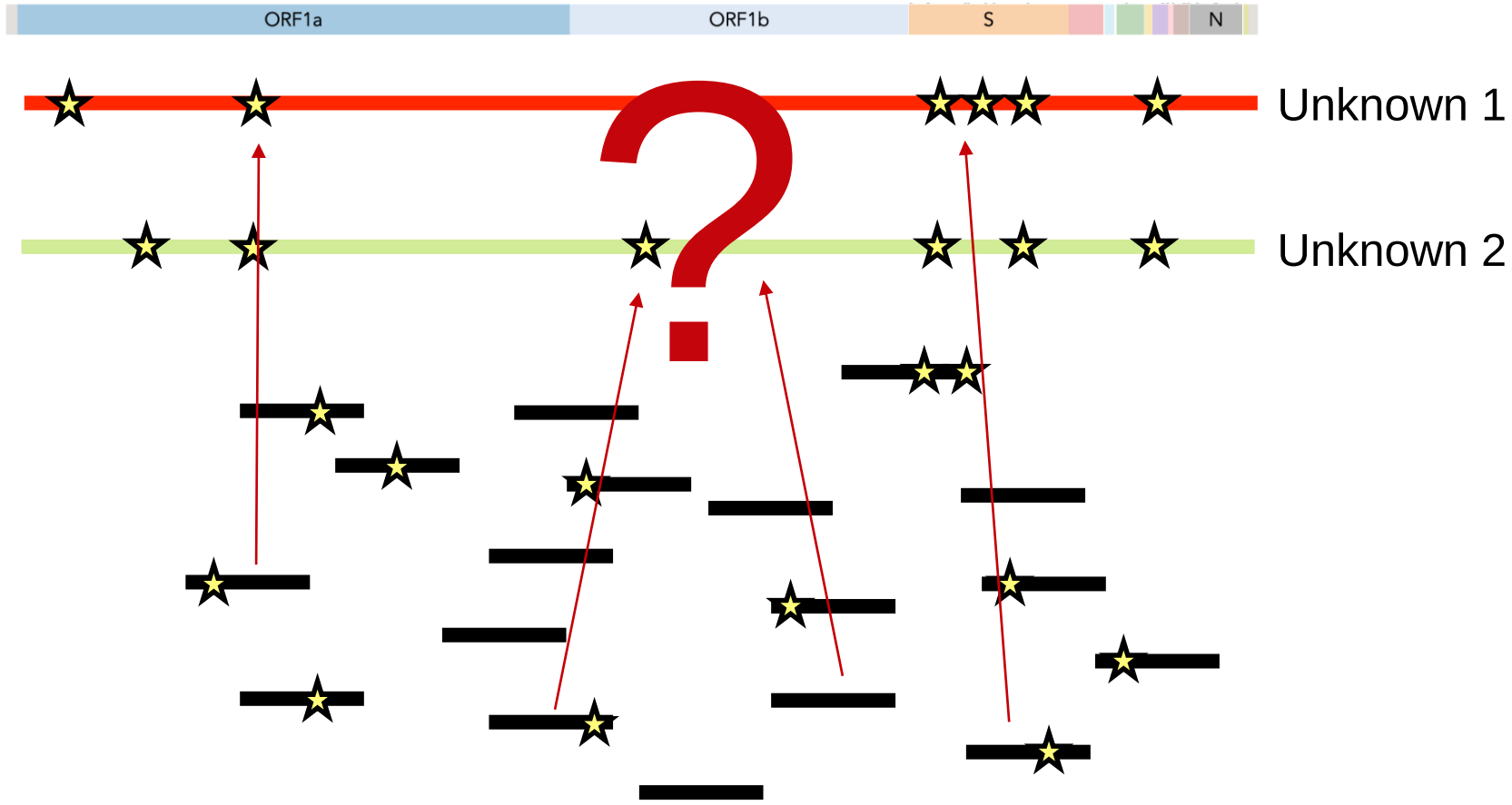


Sequencing of Wastewater For SARS-CoV-2

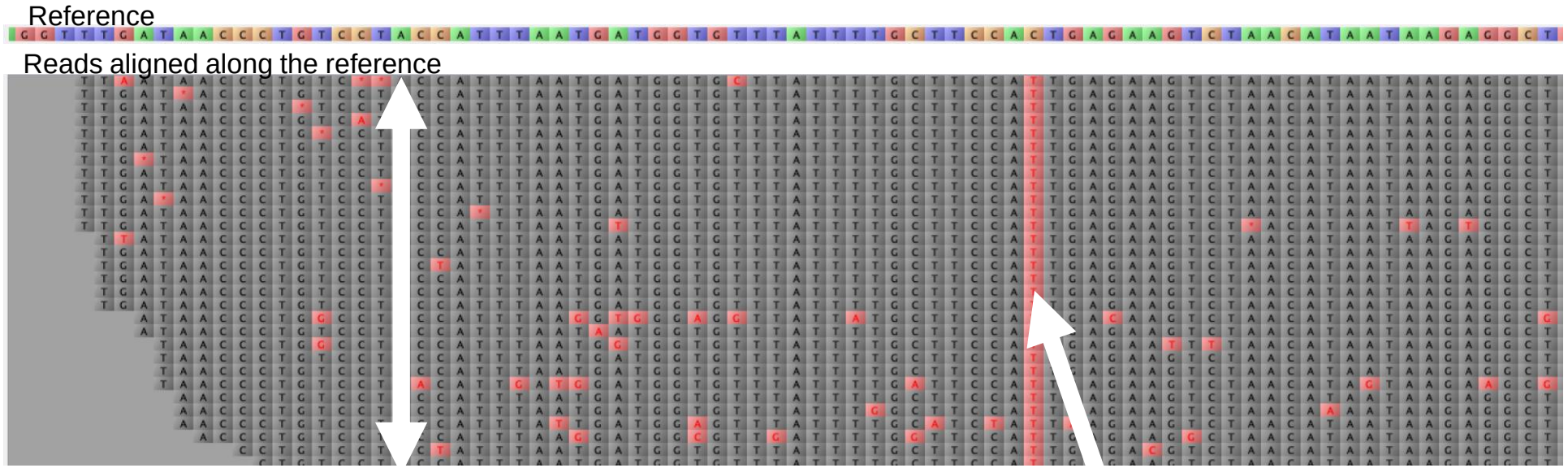
Limitations:

- Pool of strains
- Sequencing error

Base **identical** to reference

Base **different** from reference

Illumina = 0.1%



Discriminate **sequencing errors from true mutation (single nucleotide polymorphism,** SNP):

(1) minimum **depth** of **25X**

6 reads if 25 reads total

(2) minimum SNPs **frequency of detection** of **25%** (minimum >10%)

Sequencing of Wastewater For SARS-CoV-2

SNP

Limitations:

- Pool of strains
- Sequencing error
- Coverage

Unknown 1 (50%)

Unknown 2 (50%)

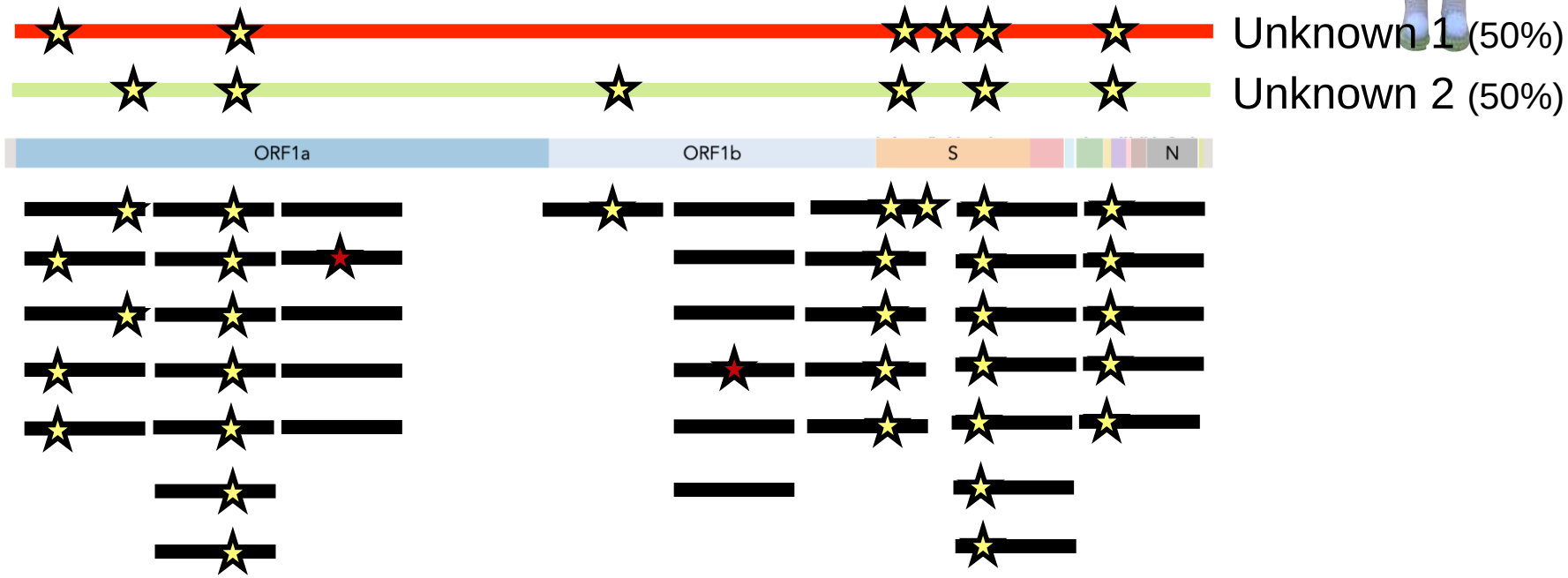
Sequencing of Wastewater For SARS-CoV-2

SNP

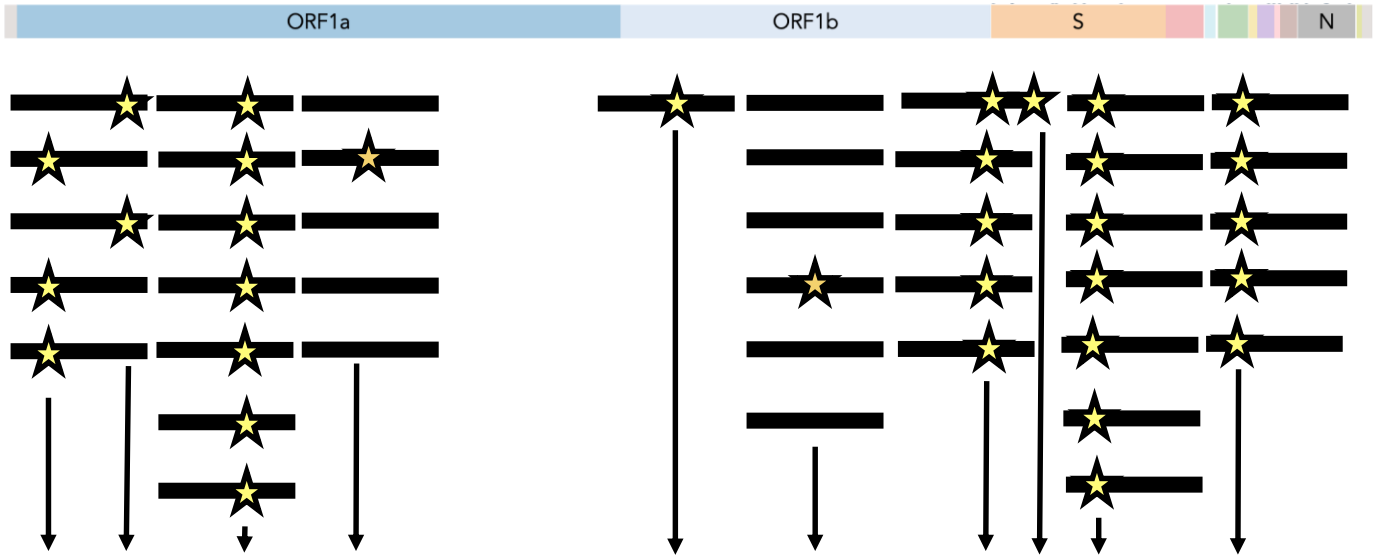
Sequencing error

Limitations:

- Pool of strains
- Sequencing error
- Coverage



Sequencing of Wastewater For SARS-CoV-2



SNP

Sequencing error

60% **40%** **100%** 20%

50X **50X** **70X** 50X

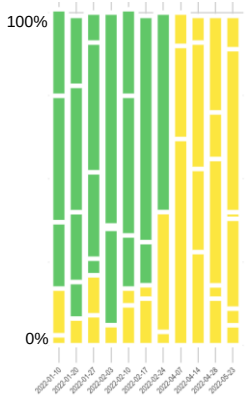
100% 15% **100%** 15% **100%** **100%**

10X **60X** **50X** **50X** **70X** **50X**

SNP frequency (criteria: >25%)

Depth (criteria: >25X)

FREYJA

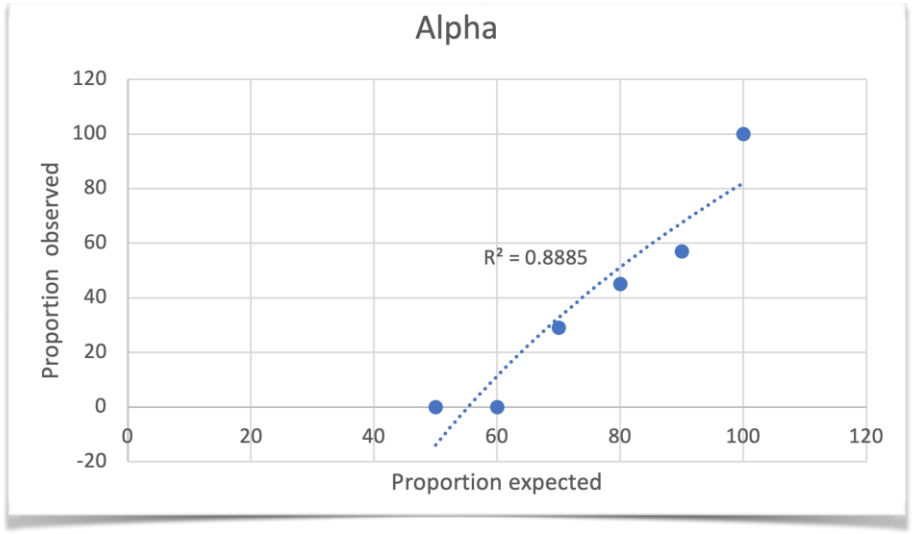


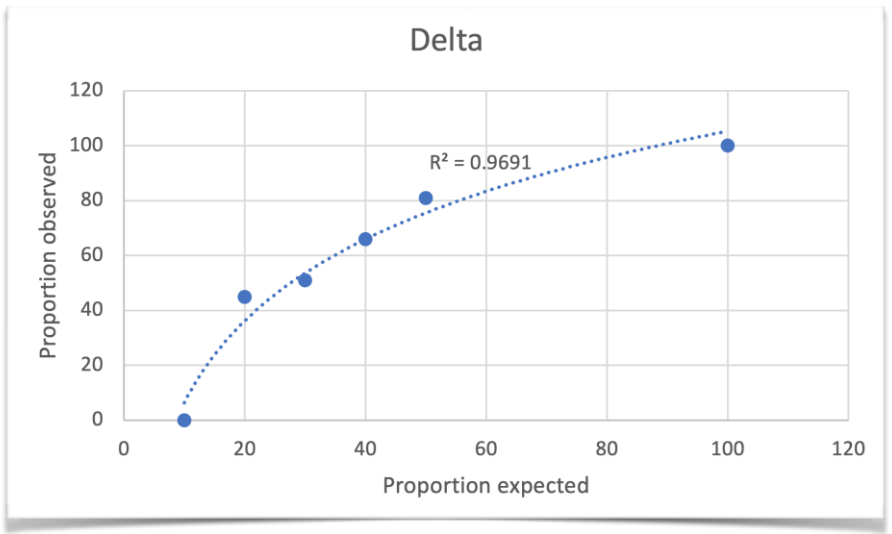
Proportion of Variant of Concerns

SARS-CoV-2 in Wastewater: Results

Variant of Concern	WHO	Pangolin	Variant proportions within SARS-CoV-2 signal
Alpha 100%	Alpha	Q.1	100 %
Delta 100%	Delta	AY.103	100 %

Mix with different ratio





Positive relationship between observed and expected proportion
Difference of sensitivity between VoC

SARS-CoV-2 in Wastewater: Results

Variant of Concerns	WHO	Pangolin	Proportion variants within SARS-CoV-2 signal
Alpha 100%	Alpha	Q.1	100 %
Delta 100%	Delta	AY.103	100 %

Mix with different ratio

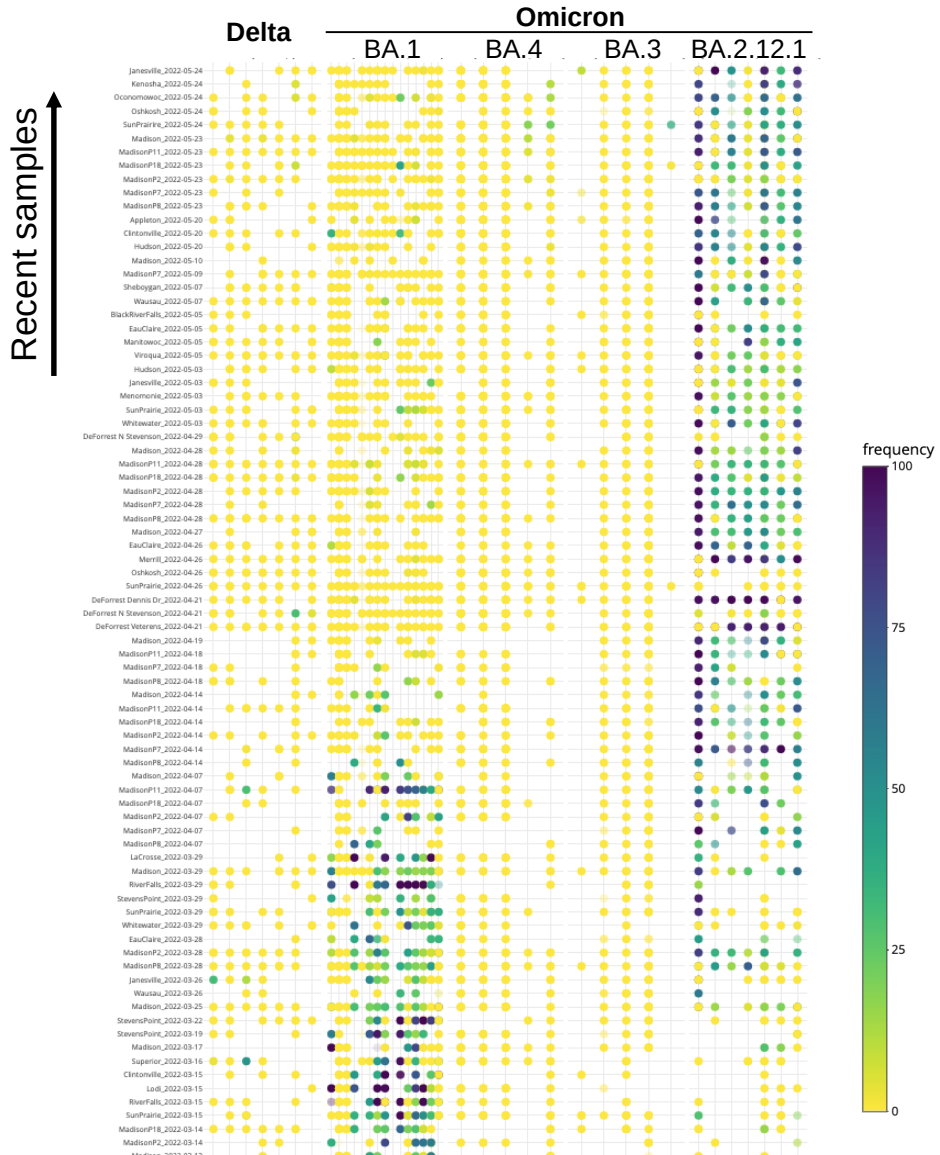
Variant of Concerns	WHO	Pangolin	Proportion variants within SARS-CoV-2 signal
Alpha 70% **+** **Delta 30%**	**Alpha**	**Q.1**	**29 %**
	Delta	**AY.103**	**51 %**
	Other	B.1.1	11 %
	Alpha	B.1.1.7	15 %

Alpha 44%
Delta 51%

Difficulties in interpreting Freyja's outputs

(misleading ratios, classifications)

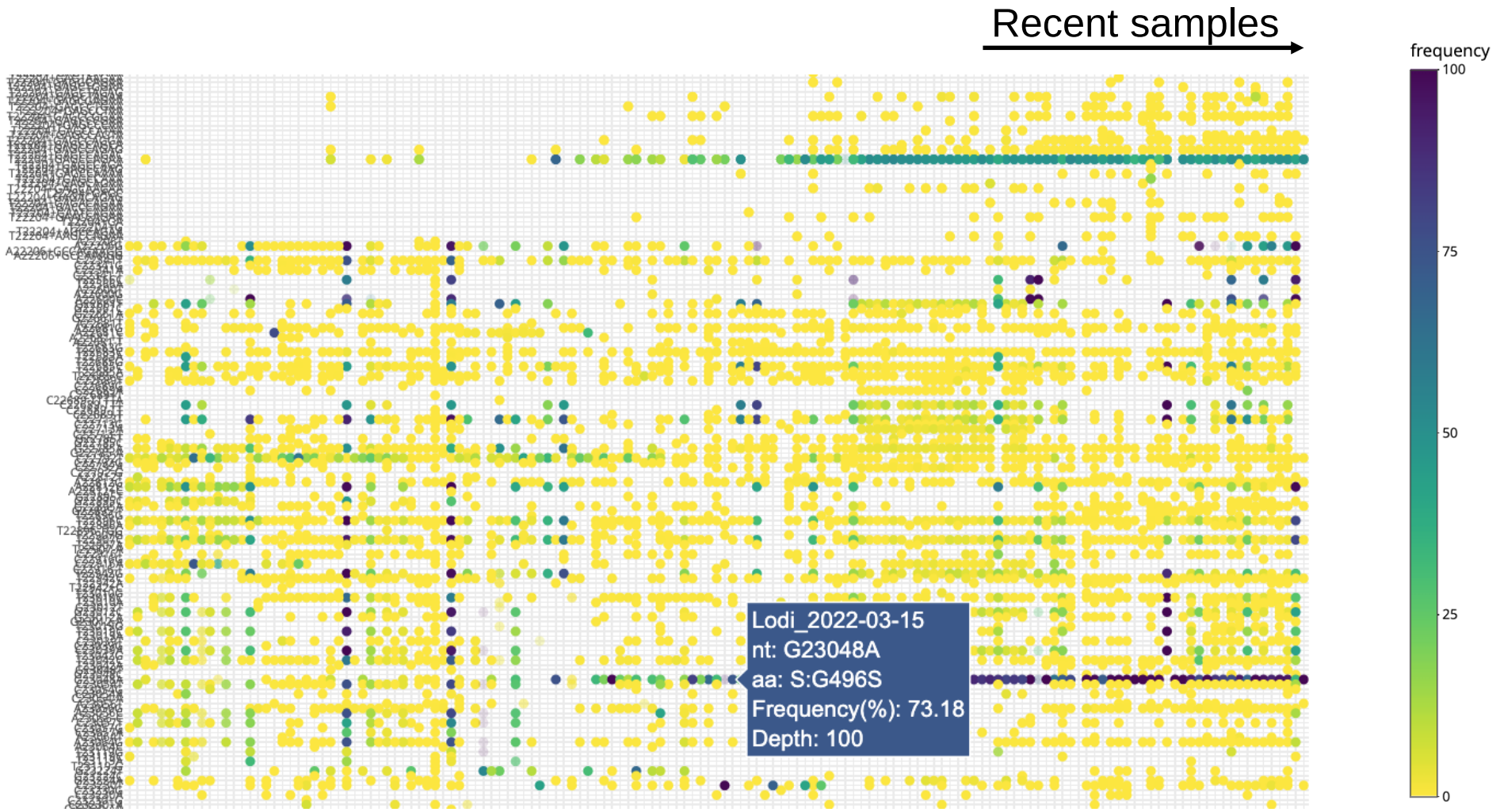
SARS-CoV-2 in Wastewater: Results



Display **only specific Variant of Concern's SNPs**

SARS-CoV-2 in Wastewater: Results

Display **SNPs** not detected in Variant of Concern: detection **cryptic variants**



Challenges of Sequencing Wastewater

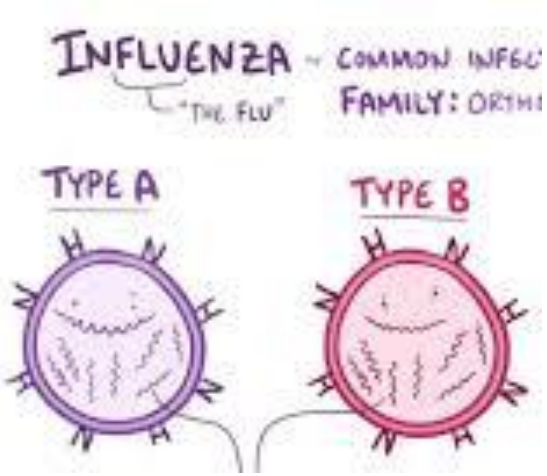
Library preparation

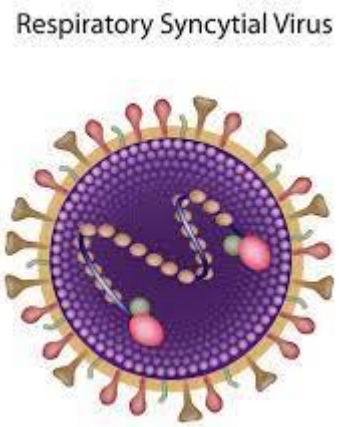
- Lower viral concentrations compared to clinical samples
- Inhibitors
- A lot of nucleic acids from other organisms
- Fragmented RNA
- Sequencing depth varies across genome/incomplete coverage

Bioinformatics

- Low coverage
- Sequencing errors
- Short-reads and mix of variants

What Else Could Wastewater Monitor





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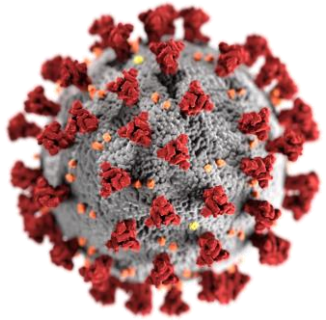
NATIONAL WASTEWATER SURVEILLANCE SYSTEM

WWTP operators



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