Environmental Protection

Development of NYC DEP's SARS-CoV-2 RNA Environmental Monitoring Program

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Agenda

In the beginning…
Genetic signal concentration - Method development and data
Clinical data versus wastewater data
Sequencing
Concluding thoughts

Acknowledgments

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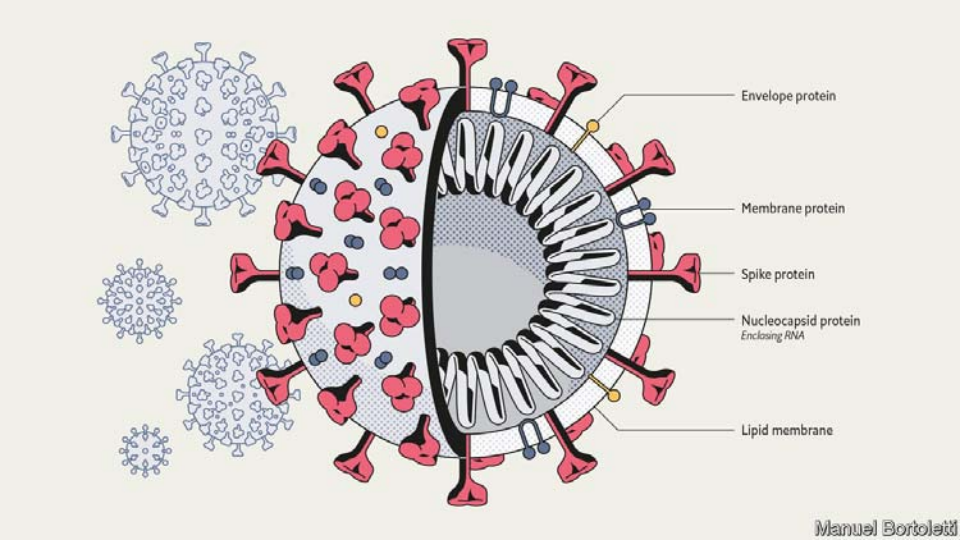
Catherine Hoar (NYU - Funded by the Alfred P. Sloan Foundation)

Overview

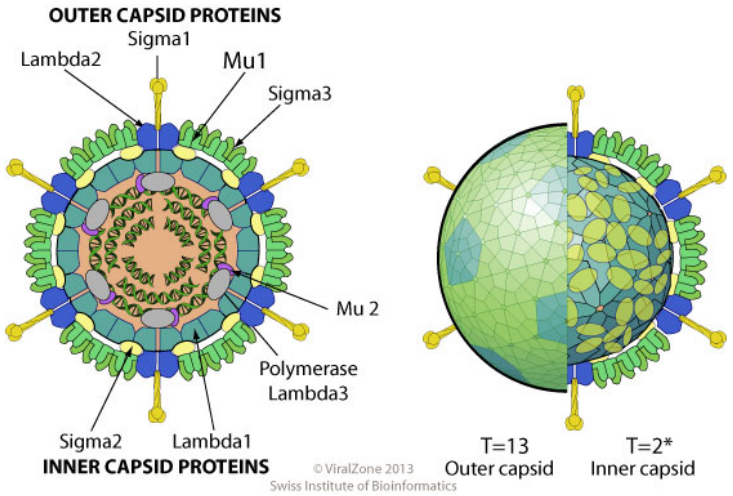
What we know:

- Viral presence in human GI tract and viral RNA (genetic material) can be shed in feces for about month
- Typically not present in urine
- Presence of Viral RNA different from Presence of Infectious Virus
  - No evidence of transmission from sewage

Coronaviruses with Lipid Bi-Layer



Typical Enteric Non-Enveloped Viruses with Protein Capsid



Overview

- Environmental Monitoring in Individual Sewersheds: a tool to identify infection trends in a population
- Examples: Samples for COVID-19 in Amsterdam and Paris using techniques developed for Polio, Norovirus and Hepatitis (capsid protected viruses)
- More research is needed to refine analytical techniques for coronavirus

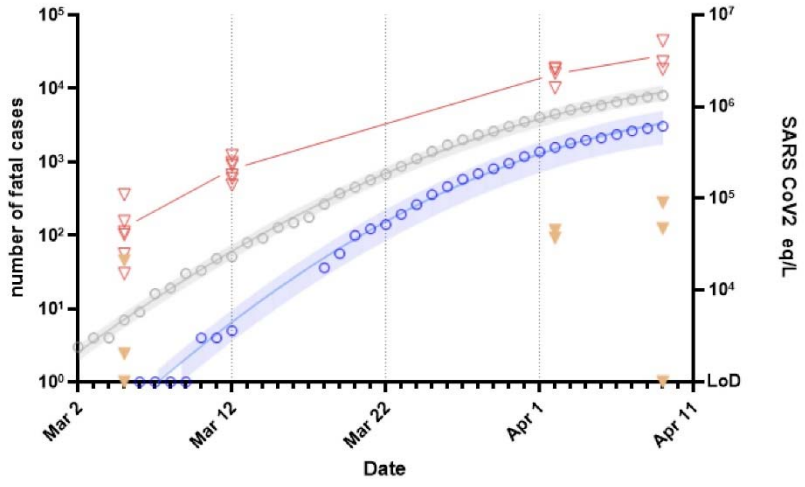
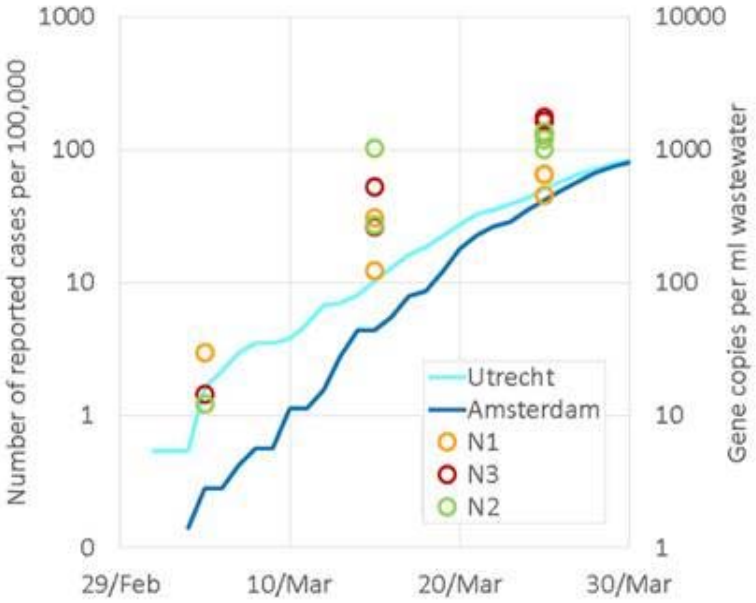


Figure 1: Quantitative time-course monitoring of SARS-CoV2 in wastewater samples from Paris area

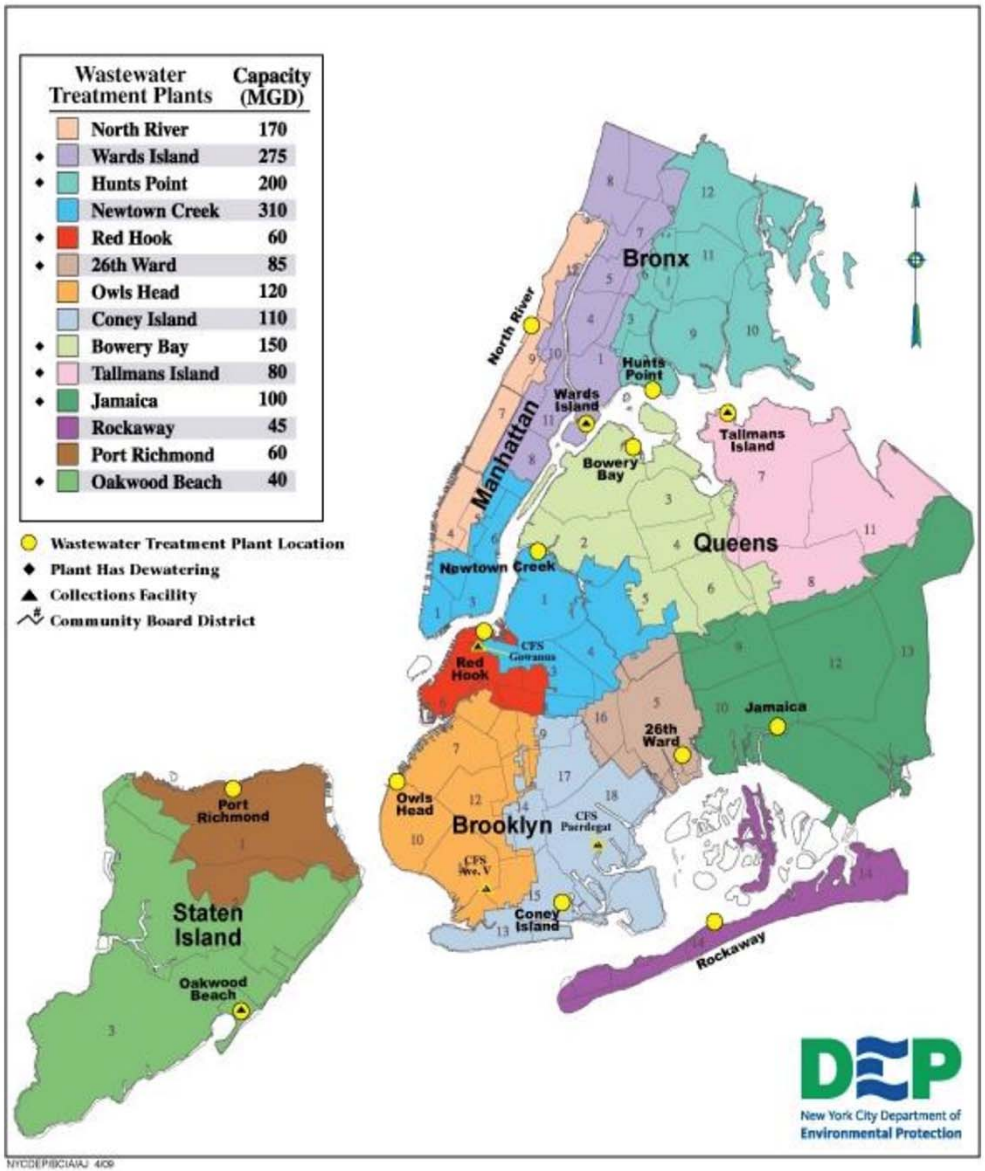


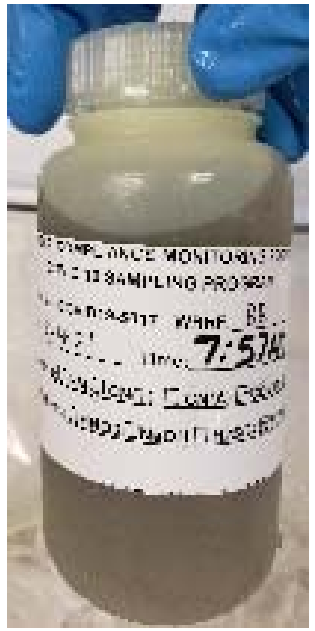
DEP Research and Development Efforts

A true partnership:

- Analytical method developed by CUNY
  - Plant influent
  - 3-day procedure
- Staff training provided by CUNY and NYU
- Procurement Support through CUNY
- Ongoing support from NYU and CUNY
  - Analytical support
  - Method extension
  - Data interpretation
  - Bridging to Microbial Source Tracking

Sampling program





Sample analysis workflow

Surrogate Addition (BCoV)

Pasteurization

Solids separation

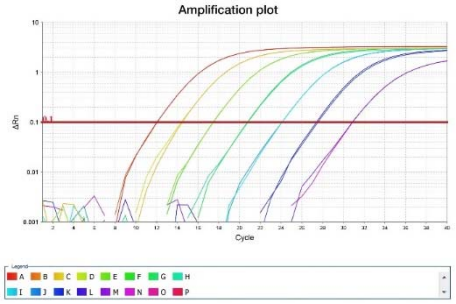
Day # 1

PEG Addition

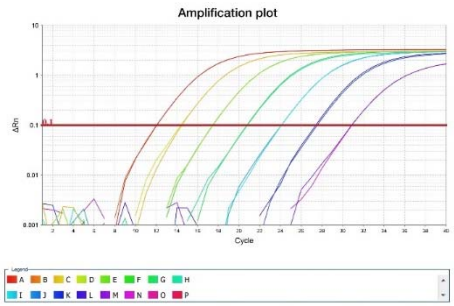
Overnight incubation

Centrifugation
12,000g 120min
No brake

Manual
RNA extraction
Day # 2



Reverse Transcription
qPCR – BCoV

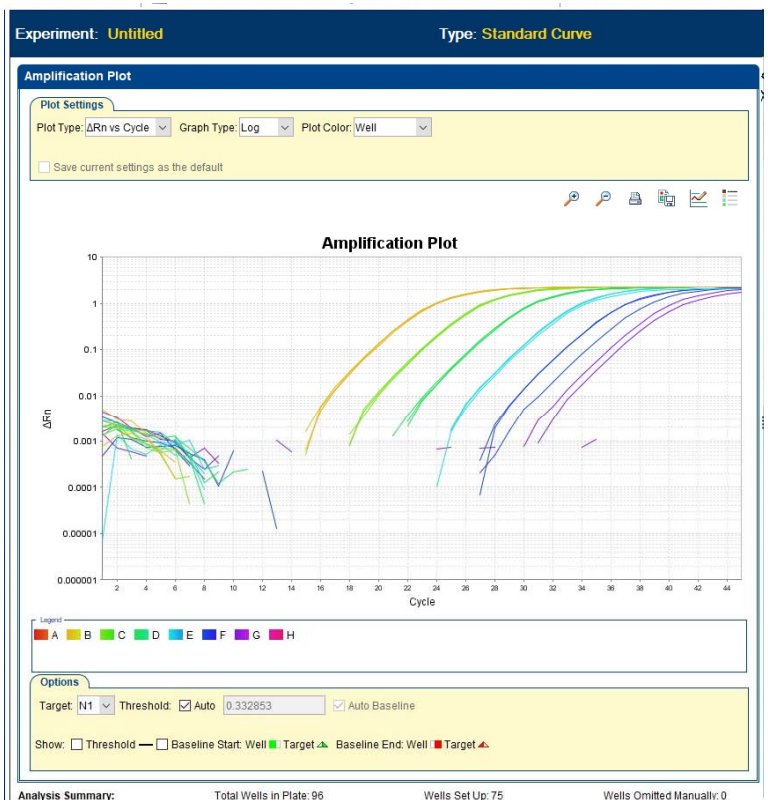


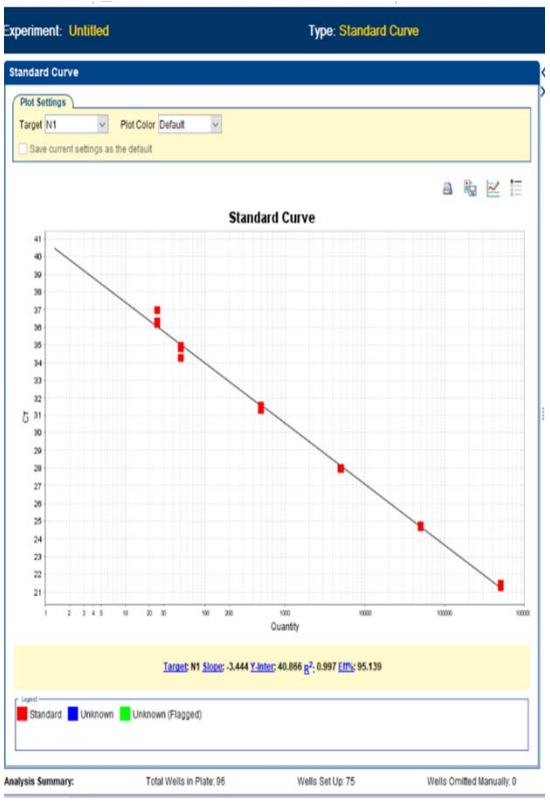
Reverse Transcription
qPCR – N1 and N2

Day # 3

Method developed by the CUNY team

RT-qPCR


Experiment: Untitled
Type: Standard Curve
Amplification Plot
Plot Settings
Plot Type: ΔRn vs Cycle
Graph Type: Log
Plot Color: Well
Save current settings as the default
Amplification Plot
ΔRn
Cycle
Options
Target: N1
Threshold:
Auto
0.332853
Auto Baseline
Show: Threshold
Baseline Start: Well
Target
Baseline End: Well
Target
Analysis Summary:
Total Wells in Plate: 96
Wells Set Up: 75
Wells Omitted Manually: 0


Experiment: Untitled
Type: Standard Curve
Standard Curve
Plot Settings
Target N1
Plot Color Default
Save current settings as the default
Standard Curve
Quantity
Standard
Unknown
Unknown (Flagged)
Analysis Summary:
Total Wells in Plate: 96
Wells Set Up: 75
Wells Omitted Manually: 0

Quality Control and Validation

- Positive control (”surrogate”):
  - Bovine coronavirus vaccine (BCoV) spiked into each sample.
  - Wide recovery range
- Negative controls:
  - Method blank = Type I water processed through the entire protocol – Acceptance criterion: non-detect
  - No Template Control = Type I water processed through RT-qPCR – Acceptance criterion: non-detect
- Duplicates:
  - One sample per batch is processed and analyzed in duplicate.
- PCR: all RNA extracts analyzed in triplicate

WRF study (2020)

Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S.

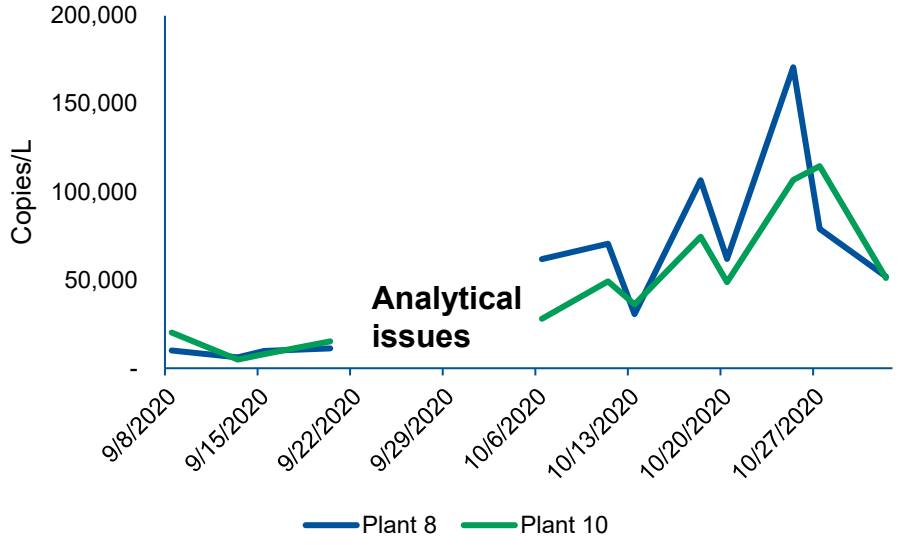
Brian M. Pecson et al. (2020) Environ. Sci.: Water Res. Technol

"A nationwide interlaboratory comparison of methods for the quantification of SARS-CoV-2 genetic signal in wastewater showed a high degree of reproducibility. 80% of the results from eight method groups (36 different methods) fell within a band of approximately +/- 1-log GC/L."

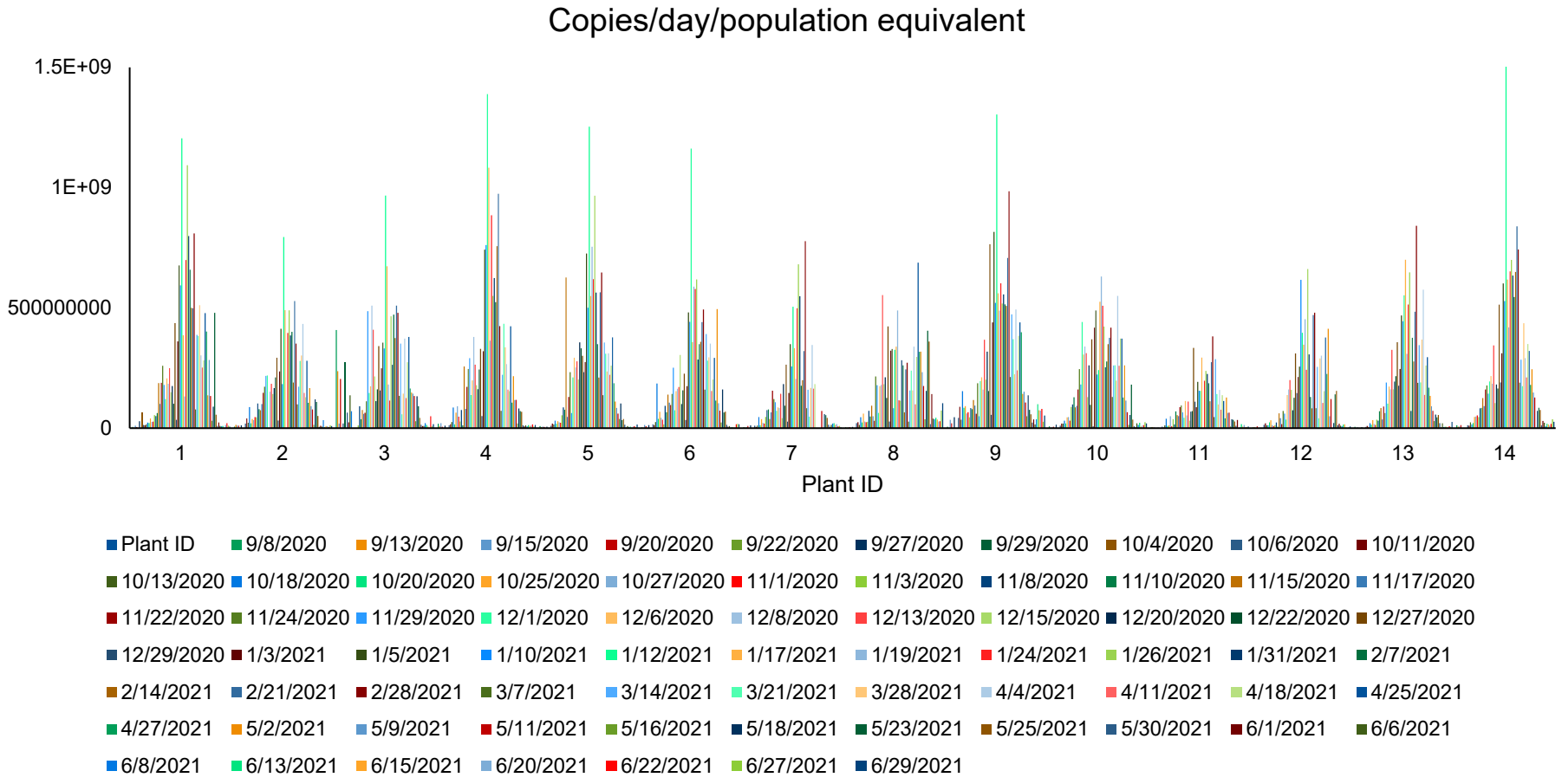
LoD and LoQ

- Limit of Detection: 4,500 copies/L
  - concentration that produces at least 95% positive replicates

- Limit of Quantitation: 15,000 copies/L
  - LoQ: lowest concentration where replicates show a CV ≤ 35% on back calculated concentrations

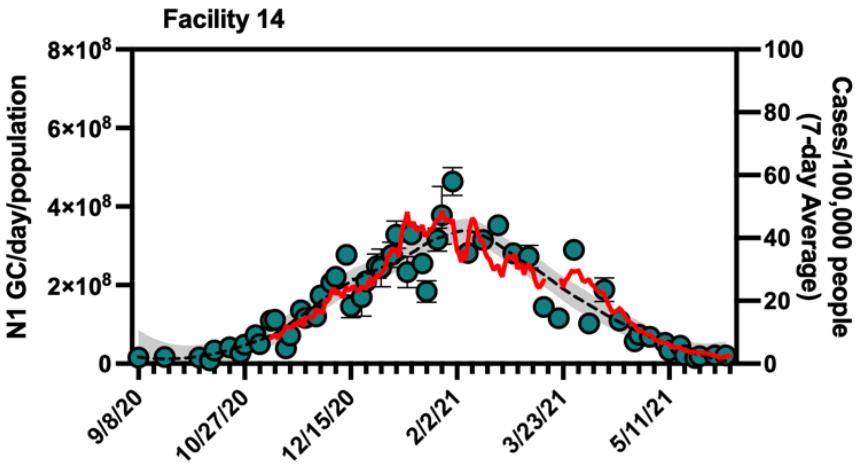


NYC WRRFs Data → NYC DOHMH



Data normalized by flow and population

Clinical data versus wastewater data

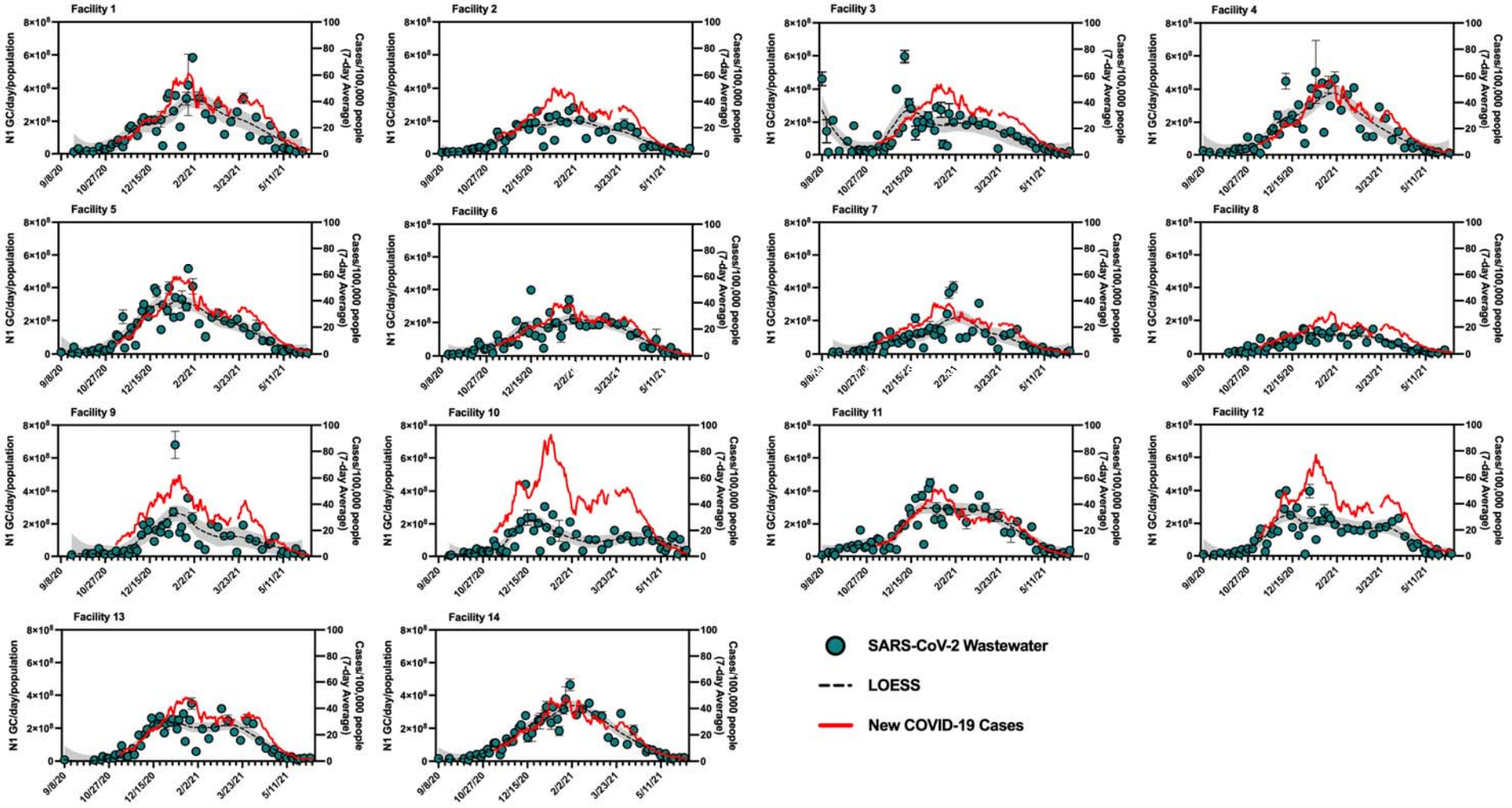


SARS-CoV-2 Wastewater

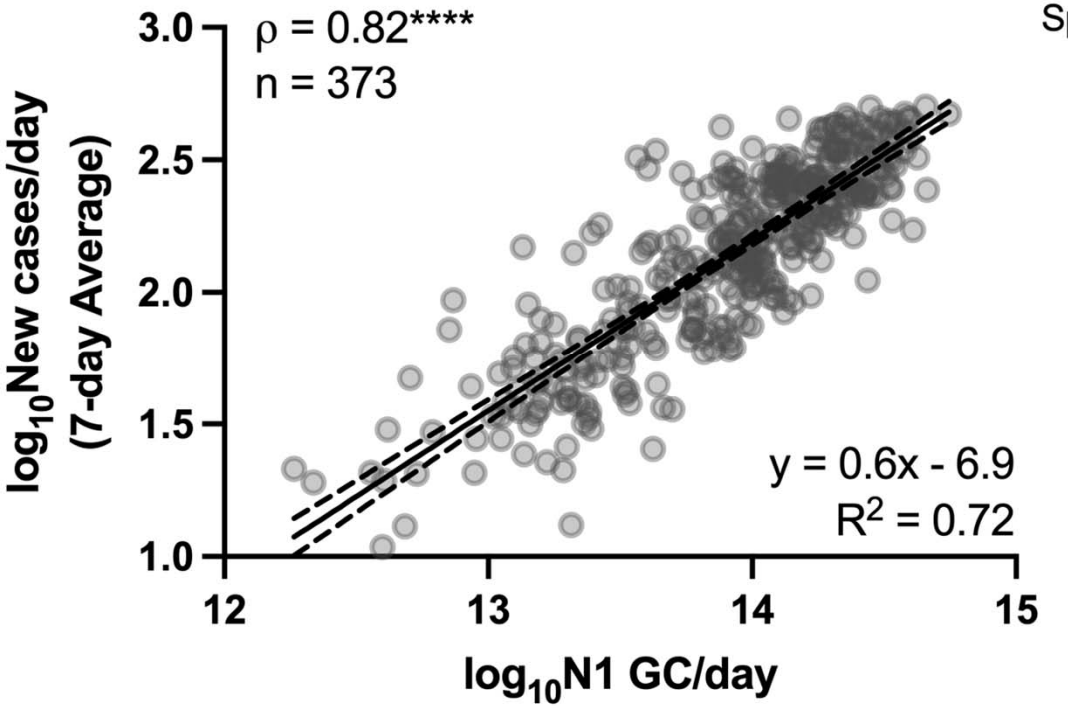
LOESS

New COVID-19 Cases

Clinical data versus wastewater data



Clinical data versus wastewater data

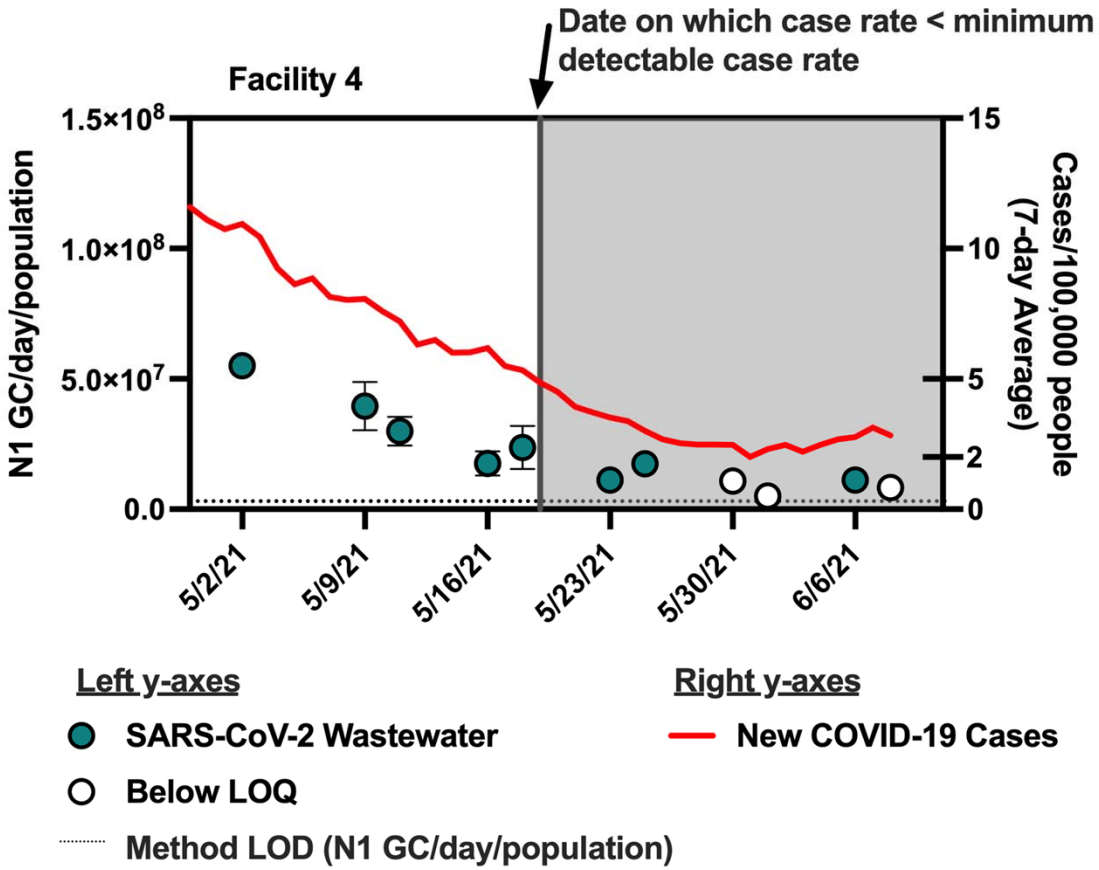


Spearman's rank correlation coefficient (ρ) significance levels

* α = 0.05
** α = 0.01
*** α = 0.001
**** α < 0.0001

NEW COVID-19 cases/day (7-day average) vs. flow-normalized SARS-CoV-2 viral loads in wastewater (N1 GC/day)

Estimating LOD – number of cases / day



Estimated minimum detectable COVID-19 case rates reached May 2021, but viral RNA still detectable in wastewater. Possibly due to:

- Decreased COVID-19 testing rates?
- Asymptomatic and mild cases of vaccinated individuals?
- Prolonged fecal shedding of the virus?
- Limitations of methodology used for estimate?

Continuous improvement

- QA/QC - Standard used for RT-qPCR:
  - DNA
  - DNA – Linearized plasmid
  - RNA: Twist control
  - RNA: EURM-19 standard
  - Evaluation of inactivated ATCC SARS-CoV-2
- Variability study
- Normalization
  - BCoV
  - PMMoV (fecal marker)
- Automation and Analytical Capacity
  - 14 Slot Centrifuges (2) - Started with one centrifuge (6 slots)
  - QIAcube – Automated RNA extraction - enhance analytical consistency
  - Digital PCR – Reduce analytical noise and enhance data quality and PCR system throughput
- Staffing
  - Started with a single staff member
  - Currently 3 active staff and 1 back up

"We are building the boat as we are sailing it" - (CDC)

Sample analysis protocol - N1 – Improved

Surrogate Addition (BCoV)

Pasteurization

Solids separation

PEG Addition

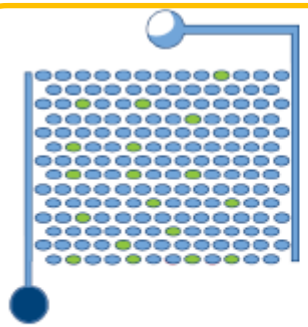
Day # 1

Overnight incubation

Centrifugation
12,000g 120min
No brake

RNA extraction
Day # 2

SOON



SOON

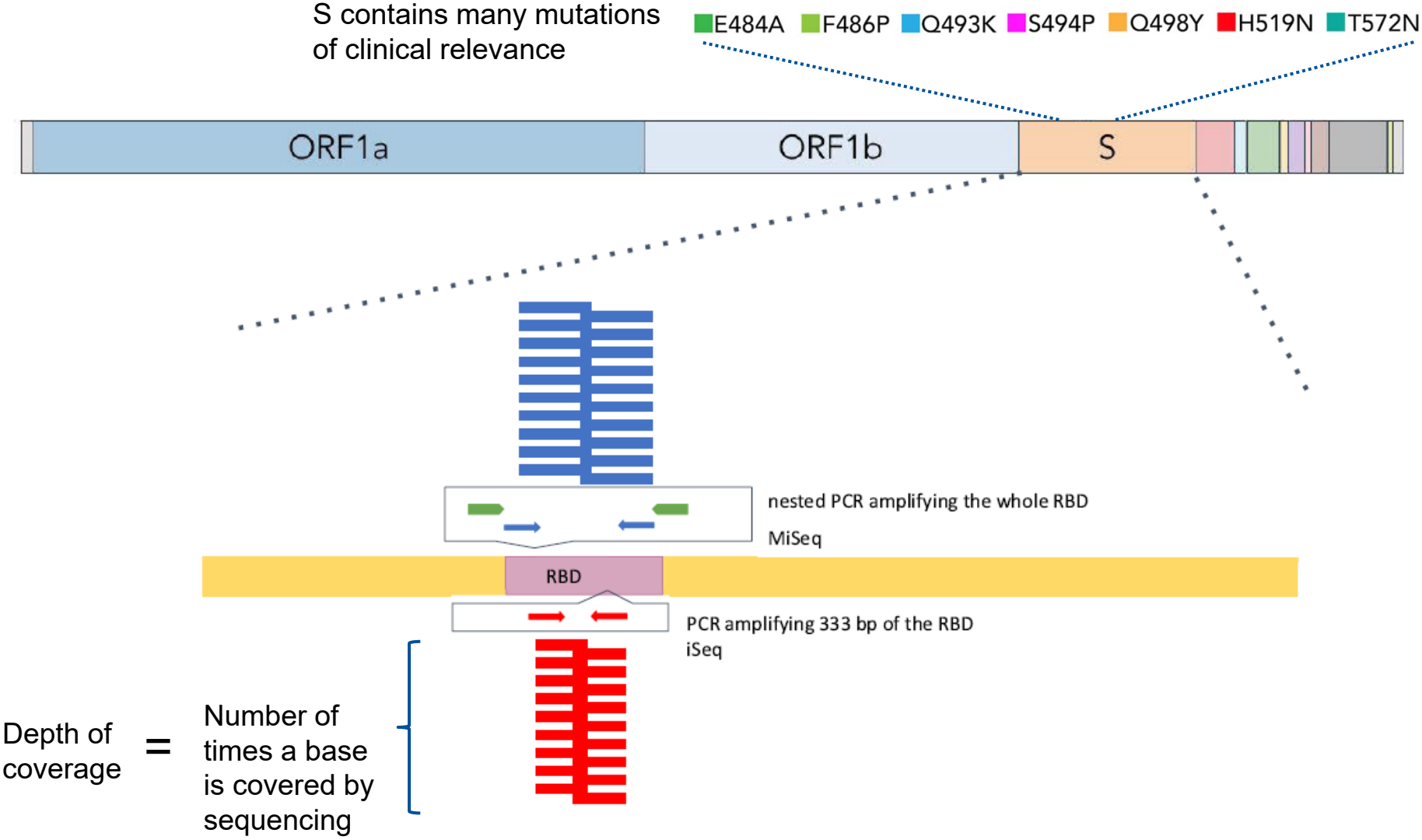
Scheme of positive (green) and negative (blue) partitions after imaging.

Reverse Transcription digital PCR – N1
Working on multiplexing

Day # 3

Targeted sequencing approach

S contains many mutations of clinical relevance

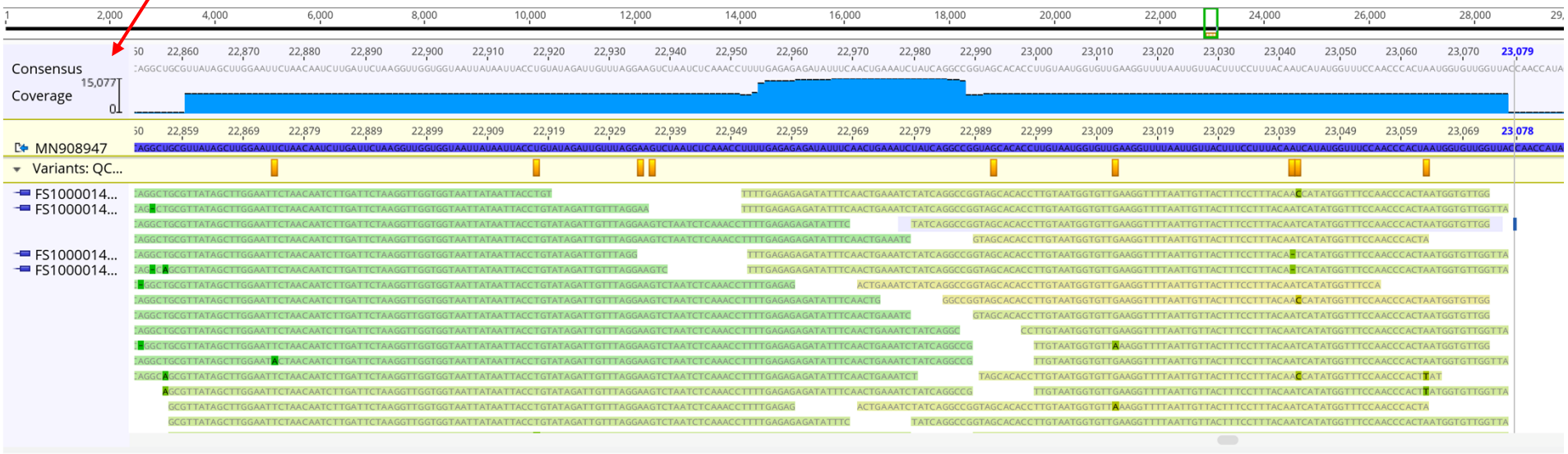


Depth of coverage = Number of times a base is covered by sequencing

The more depth of coverage you have – the more certain you can be that the sequence is real

Identifying mutations and variants

15077 depth of coverage = average 15077 sequences covering the positions



Columns | Track: Any | Export table | Pop i

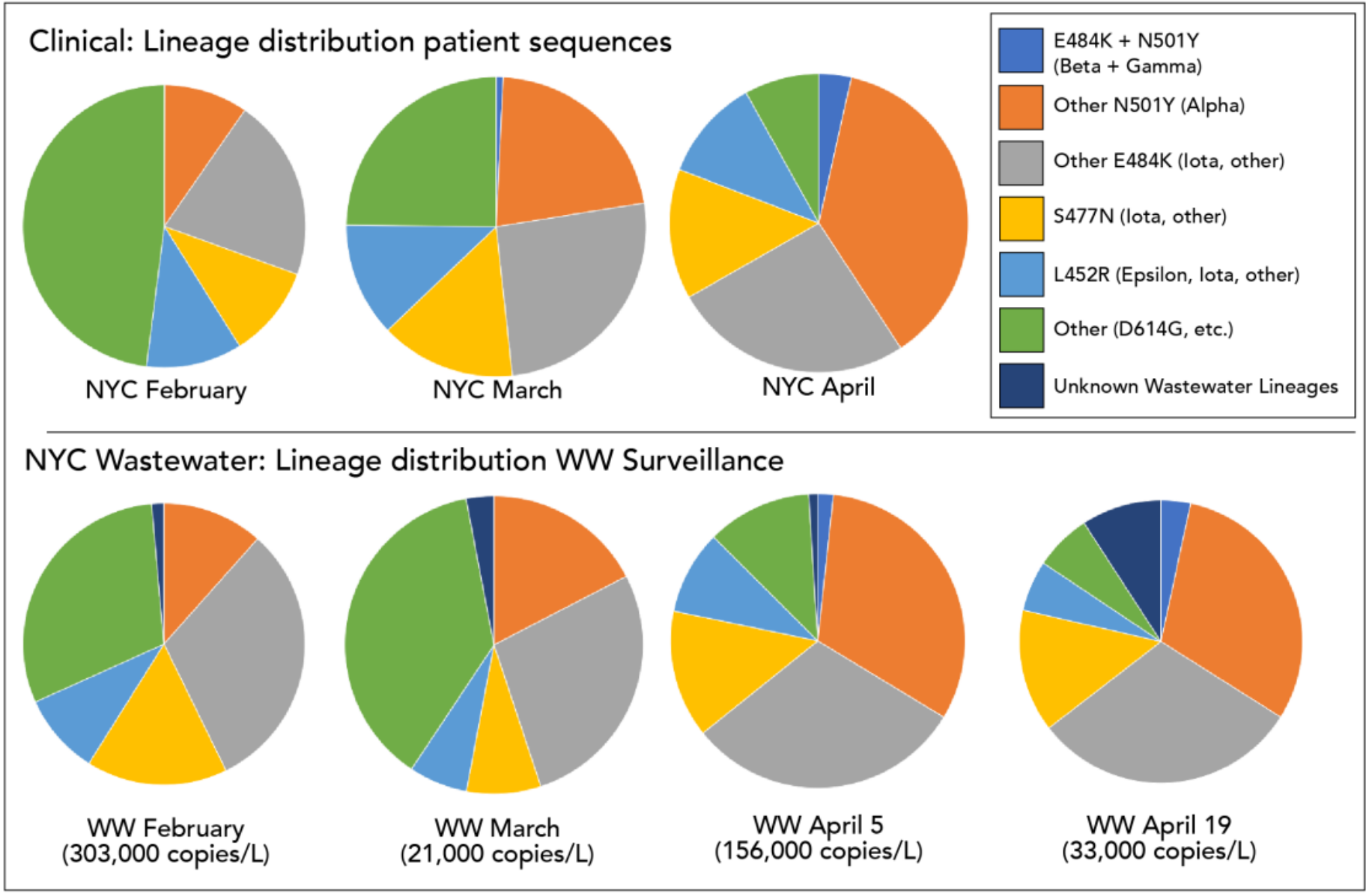
Name	Minimum	Maximum	Length	Change	Coverage	Polymorphism Type	Variant Frequency	Variant P-Value (approximate)	Amino Acid Change	CDS Position	Codon Change	Protein Effect
	23,041	23,041	1	-A	8,392	Deletion	1.4%	1.0E-85		1,479		Frame Shift
A	22,874	22,874	1	T -> A	8,380	SNP (transversion)	1.5%	4.3E-185	S -> T	1,312	UCU -> ACU	Substitution
A	22,992	22,992	1	G -> A	8,282	SNP (transition)	1.6%	1.7E-180	S -> N	1,430	AGC -> AAC	Substitution
A	23,012	23,012	1	G -> A	8,439	SNP (transition)	16.2%	0.0	E -> K	1,450	GAA -> AAA	Substitution
C	23,042	23,042	1	T -> C	8,389	SNP (transition)	8.0%	0.0	S -> P	1,480	UCA -> CCA	Substitution
G	22,917	22,917	1	T -> G	8,445	SNP (transversion)	6.0%	0.0	L -> R	1,355	CUG -> CGG	Substitution
G	22,934	22,934	1	A -> G	8,443	SNP (transition)	1.7%	3.7E-217	K -> E	1,372	AAG -> GAG	Substitution
T	22,936	22,936	1	G -> T	8,374	SNP (transversion)	1.1%	1.6E-117	K -> N	1,374	AAG -> AAT	Substitution
T	23,063	23,063	1	A -> T	8,332	SNP (transversion)	9.0%	0.0	N -> Y	1,501	AAU -> TAU	Substitution

S477N
B.1.526

S494P
B.1.177.75, B.1.1.459, B.1.1.461, B.1.1.253, B.1.595.3, B.1.1.451, B.1.476, B.1.1.174, B.1.486, B.1.575, B.1.575.1

N501Y
B.1.351, B.1.1.7

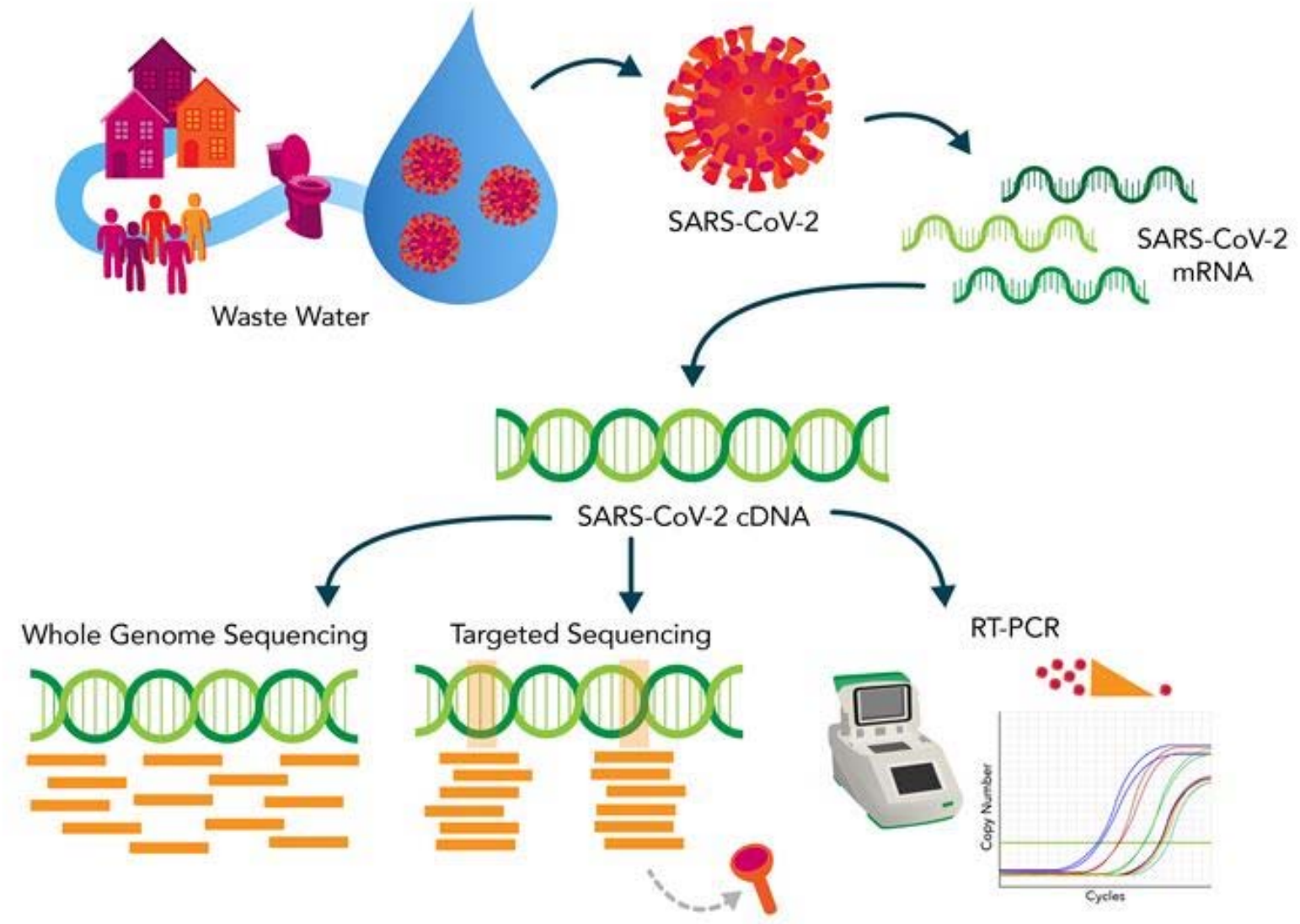
Tracking variants with Wastewater



Concluding Thoughts

- Environmental Surveillance: An opportunity to convert applied research to a public health tool
- The toolkit is still in its infancy – although many years of development have been condensed into the past 18 months
  - Significant progress in the analytical components of the toolkit
  - Still unclear how it will be fully leveraged by public health specialists
  - How strong of a predictive tool is it?
  - Sequencing for detection of variants being developed (CUNY/ Queens College /The New School)
  - Epidemiology (NYU, DOHMH)
- Opportunities to apply it in other public health emergencies, including non-pandemic conditions

Overview


Waste Water
SARS-CoV-2
SARS-CoV-2
mRNA
SARS-CoV-2 cDNA
Whole Genome Sequencing
Targeted Sequencing
RT-PCR
Copy Number
Cycles
Molly Metz, March 2021