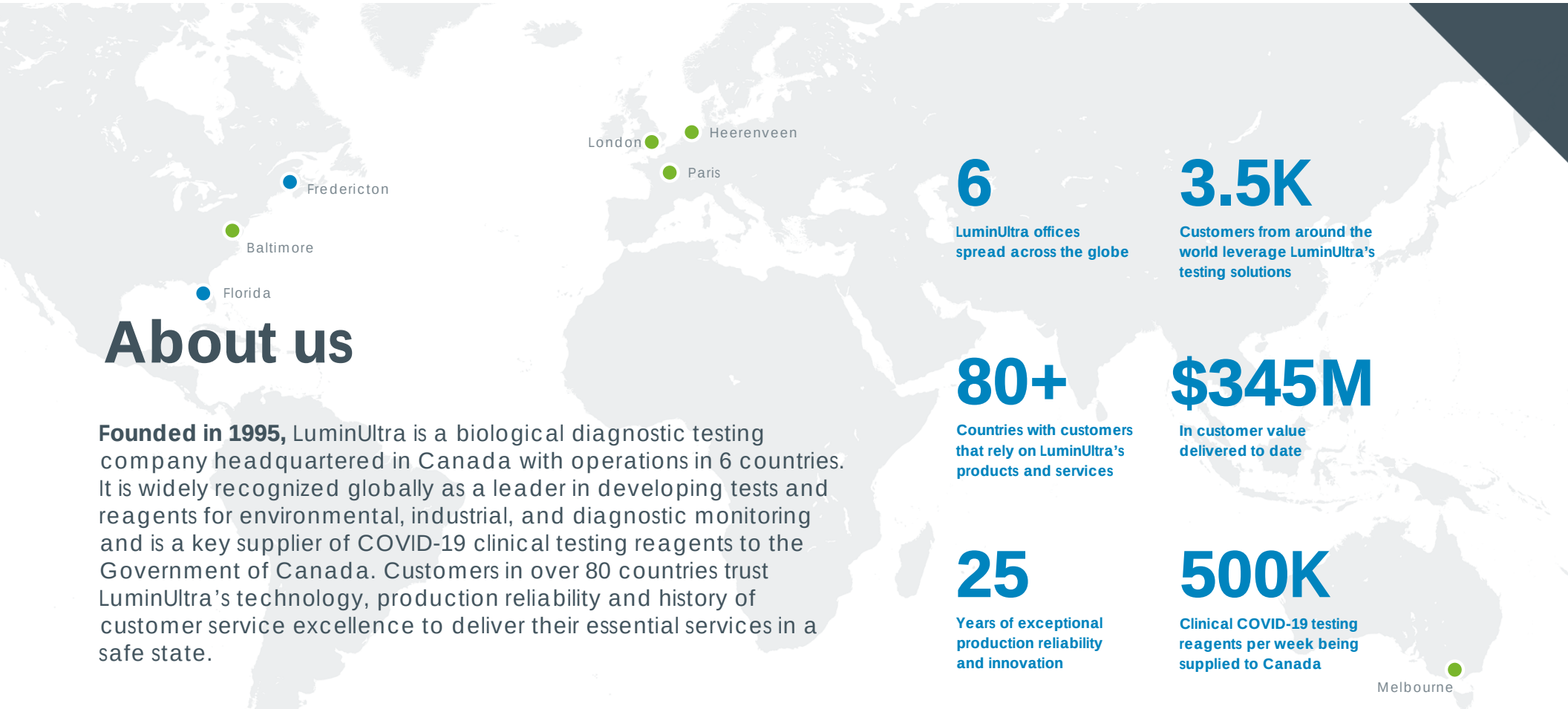
SARS-CoV-2 Quantification and Variants Detection in Wastewater Samples:

Lessons Learned from Analyzing Geographically Diverse Samples

Anda Quintero, Anusha Edupuganti, Douglas Gramajo, John Boswell, Julia Capenter, and Michael Waud

LuminUltra Technologies



About us

Founded in 1995, LuminUltra is a biological diagnostic testing company headquartered in Canada with operations in 6 countries. It is widely recognized globally as a leader in developing tests and reagents for environmental, industrial, and diagnostic monitoring and is a key supplier of COVID-19 clinical testing reagents to the Government of Canada. Customers in over 80 countries trust LuminUltra's technology, production reliability and history of customer service excellence to deliver their essential services in a safe state.

6
LuminUltra offices spread across the globe

3.5K
Customers from around the world leverage LuminUltra's testing solutions

80+
Countries with customers that rely on LuminUltra's products and services

$345M
In customer value delivered to date

25
Years of exceptional production reliability and innovation

500K
Clinical COVID-19 testing reagents per week being supplied to Canada

LuminUltra proudly serves some of the top companies in the world including:

ExxonMobil · suez · VEOLIA · DUPONT · ECOLAB · Canada

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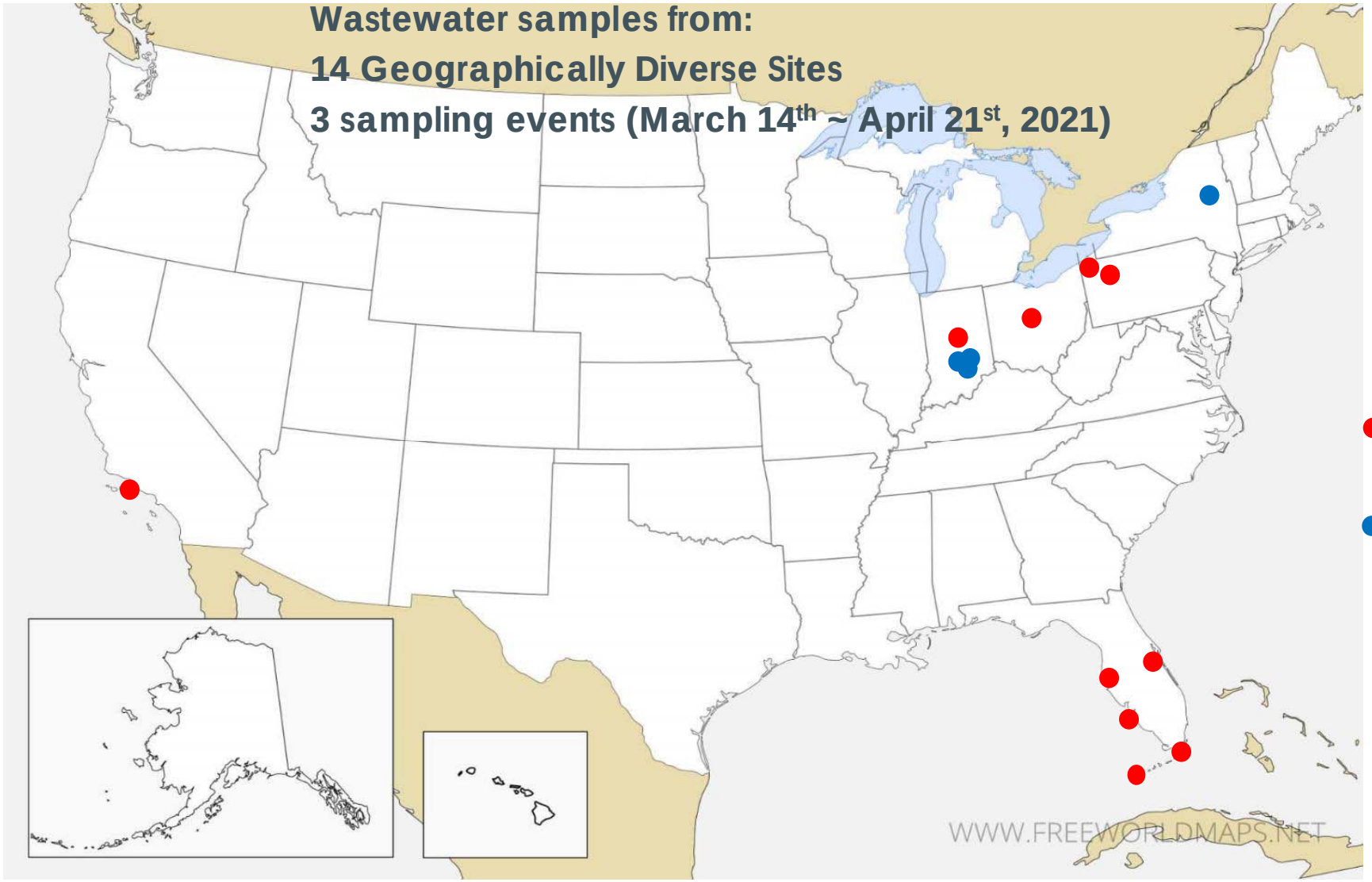
Challenges we are facing in SARS-CoV-2 wastewater monitoring since April 2020

1. Pandemic effects:
   A. High volume of samples.
   B. Short turn around time.
   C. Unstable reagents and consumables supplies.

   ---Pandemic effect put great pressure on lab personnel and quality management system.
2. Unknowns in evaluation of measurement uncertainty.
3. Market requirements of monitoring emerging variants with clinical relevance.
4. Educating clients about how to use their data.

Lessons Learned

1. Automation in the lab.
2. Properly assess sample processing spike-in recovery rates .
3. Constantly developing new assays to detect mutations and NGS solutions.
4. Knowledge sharing helps the public better understand their data and the importance of continuous monitoring effort.


Wastewater samples from:
14 Geographically Diverse Sites
3 sampling events (March 14th ~ April 21st, 2021)
Municipality (10)
Near Source (4)
WWW.FREEWORLDMAPS.NET

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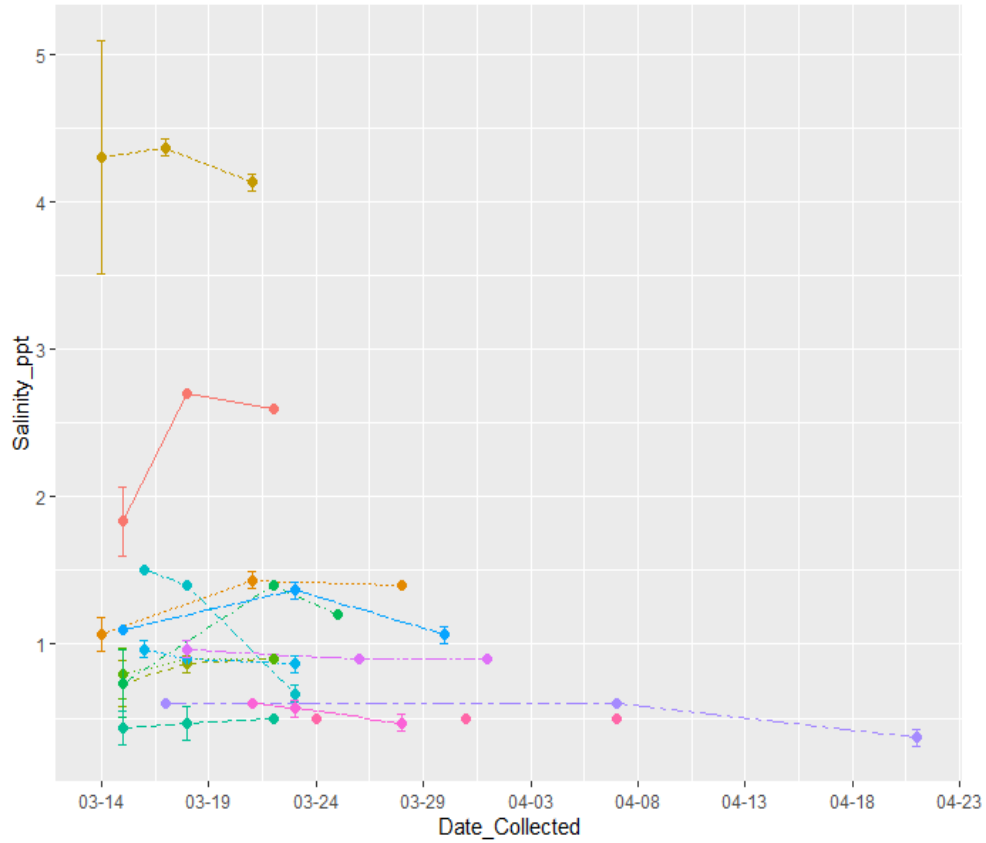
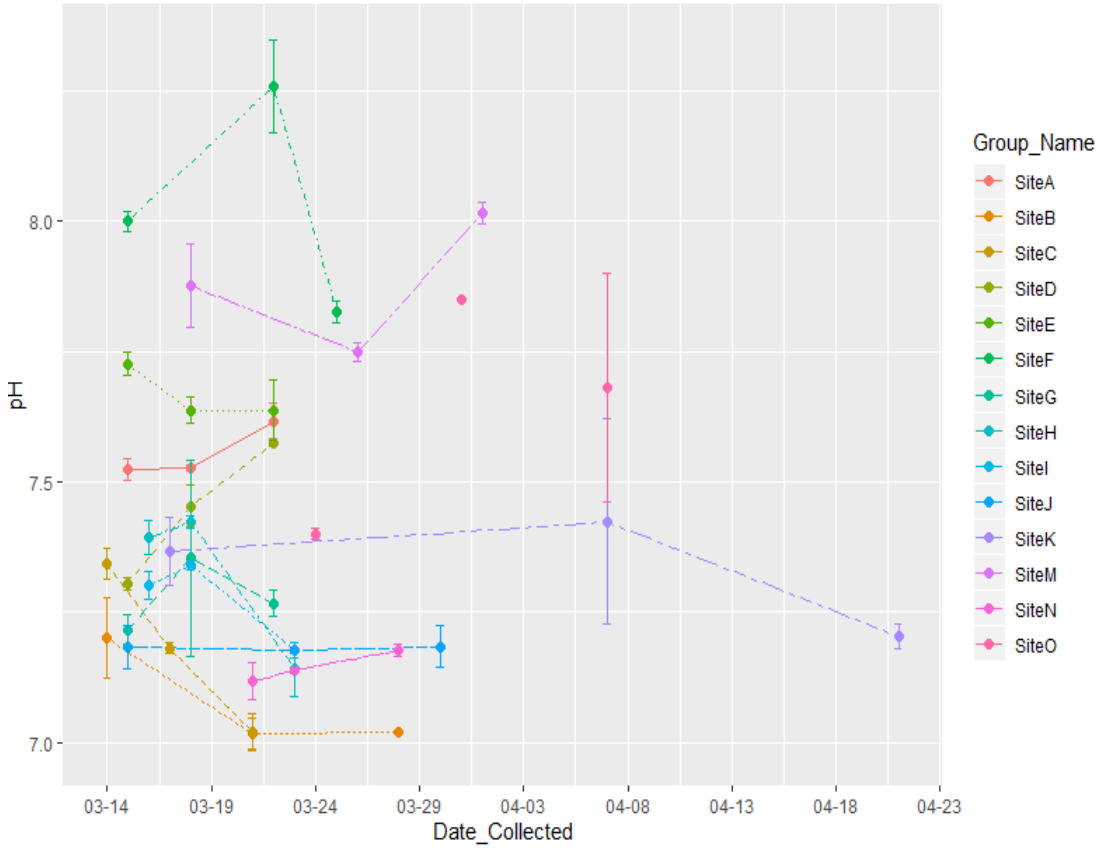
Wastewater samples characters are consistent throughout the sampling period





*Highest salinity: Florida coasts.

*Highest pH variability: A student dorm.

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SARS-CoV-2 Viral Concentration determined by two concentration methods

HA Filtration Concentration vs. Direct Extraction?

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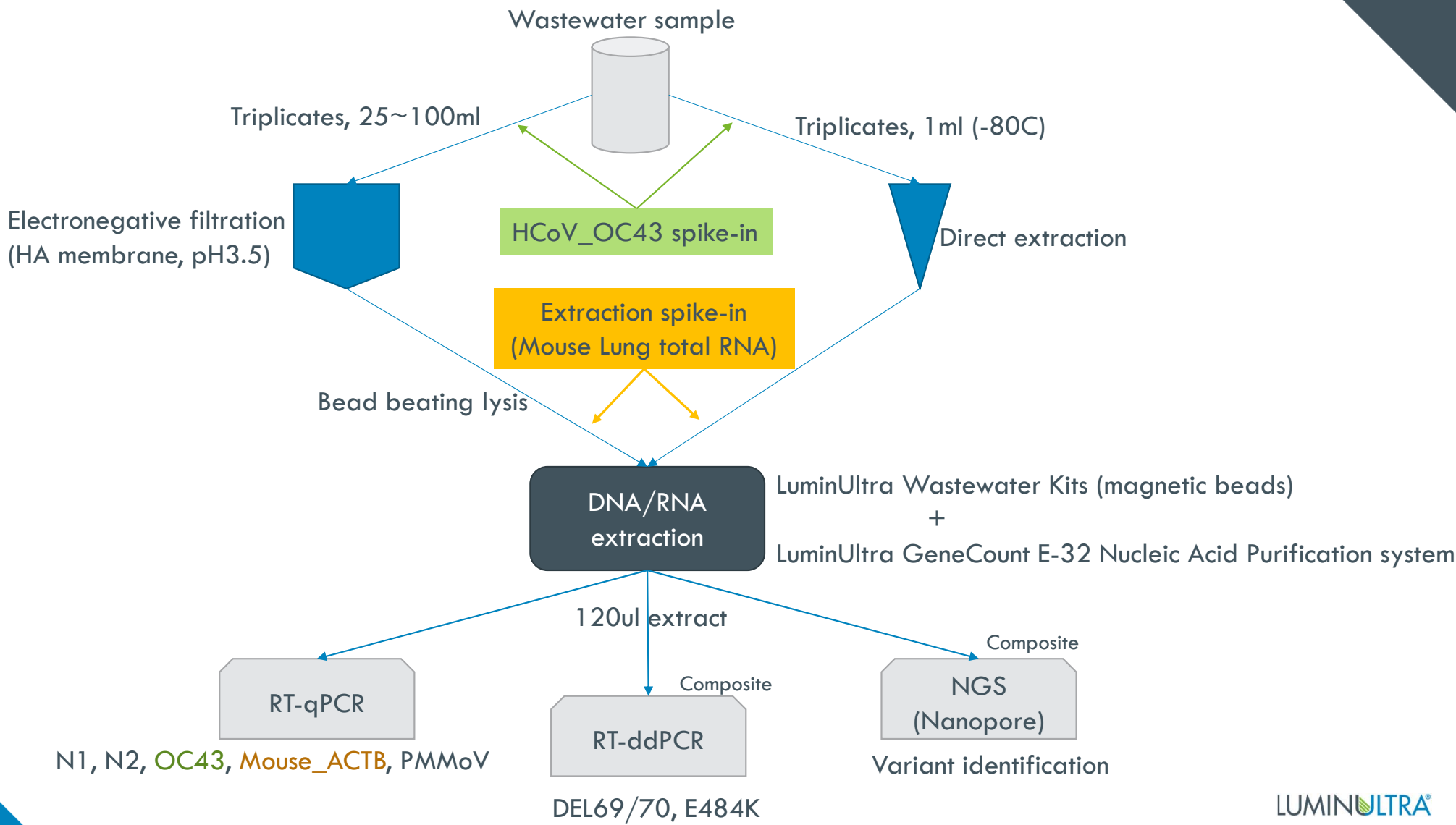
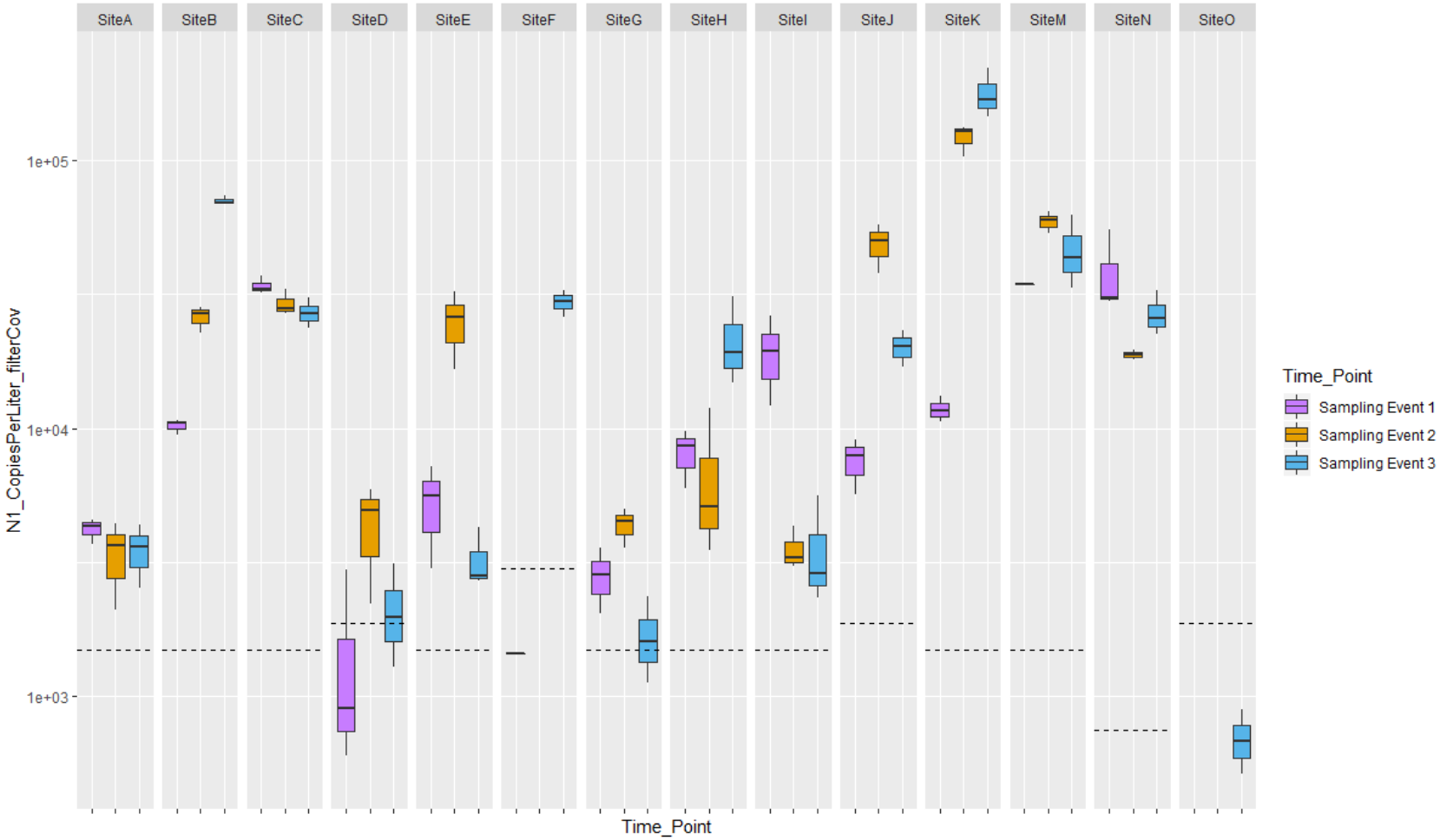

Wastewater sample
Triplicates, 25~100ml
Triplicates, 1ml (-80C)
Electronegative filtration
(HA membrane, pH3.5)
HCoV_OC43 spike-in
Direct extraction
Extraction spike-in
(Mouse Lung total RNA)
Bead beating lysis
DNA/RNA
extraction
LuminUltra Wastewater Kits (magnetic beads)
+
LuminUltra GeneCount E-32 Nucleic Acid Purification system
120ul extract
Composite
RT-qPCR
N1, N2, OC43, Mouse_ACTB, PMMoV
Composite
RT-ddPCR
DEL69/70, E484K
NGS
(Nanopore)
Variant identification

N1 (Copies/Liter) from Filter Samples



Theoretical LOD by dashed lines (3 copies/reaction, 95% detection rate, 100% recovery rate).
For a typical 50ml sample, LOD is 1.5E3 copies/liter.

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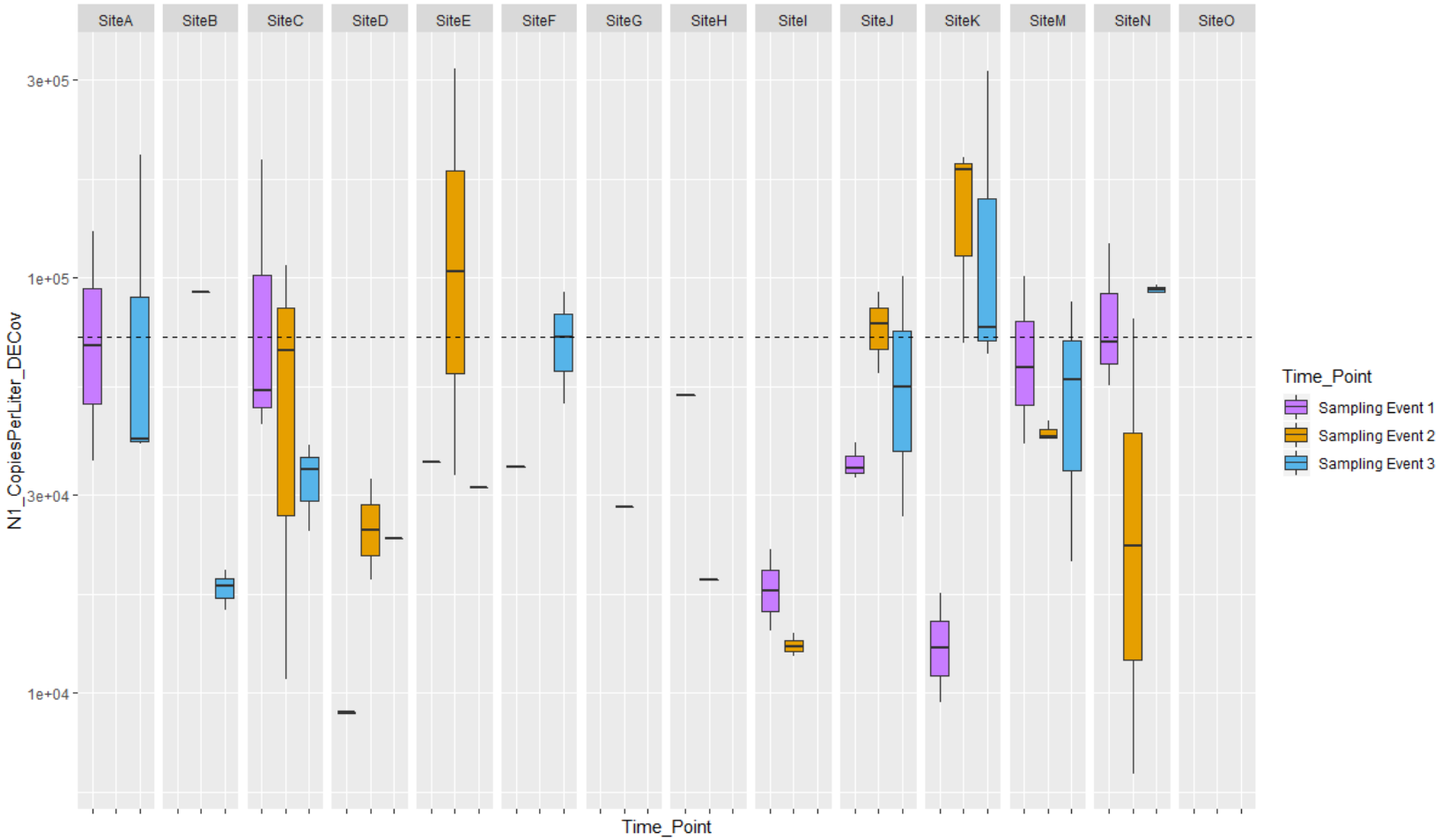
N1 (Copies/Liter) From Direct Extraction (DE) Samples



Theoretical LOD by dashed line (3 copies/reaction, 95% detection rate).
For direct extraction of 1ml water sample, LOD is 7.2E4 copies/liter.

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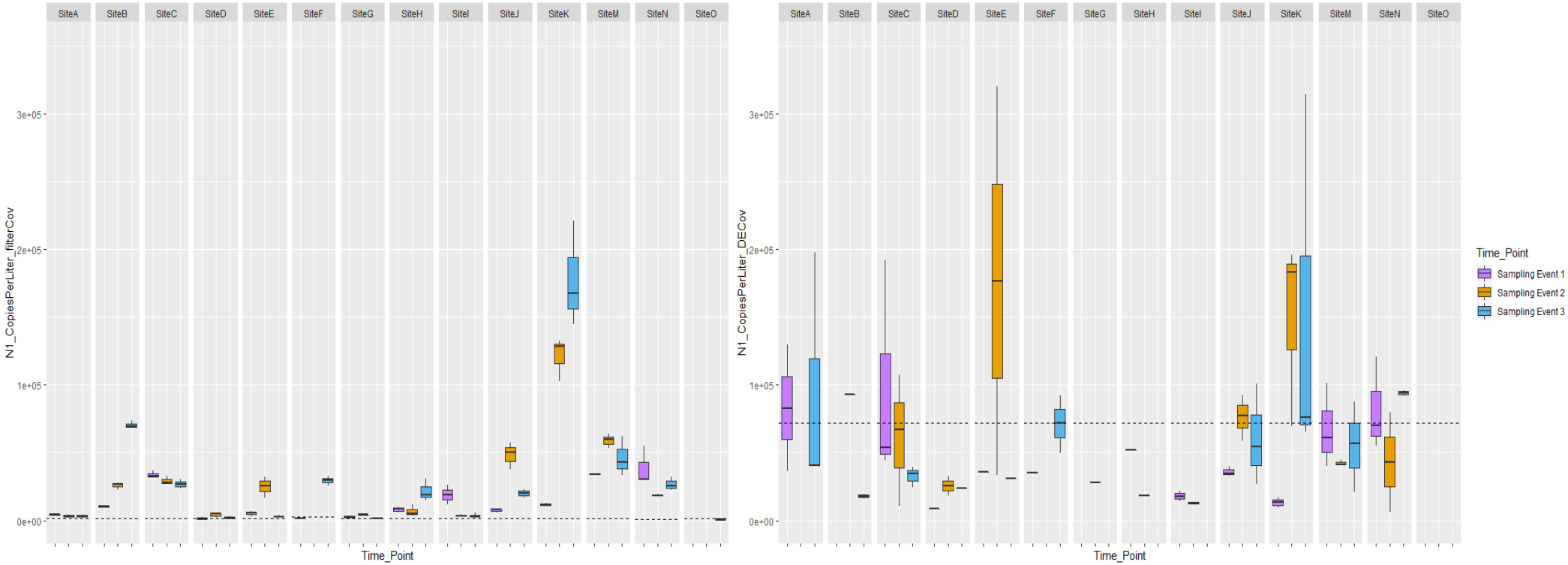
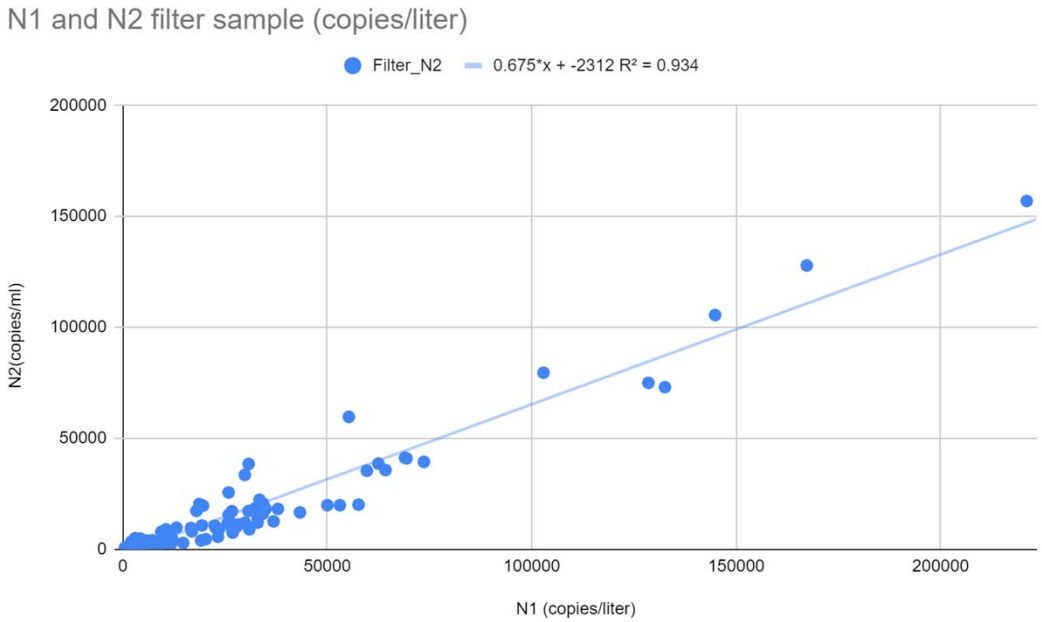
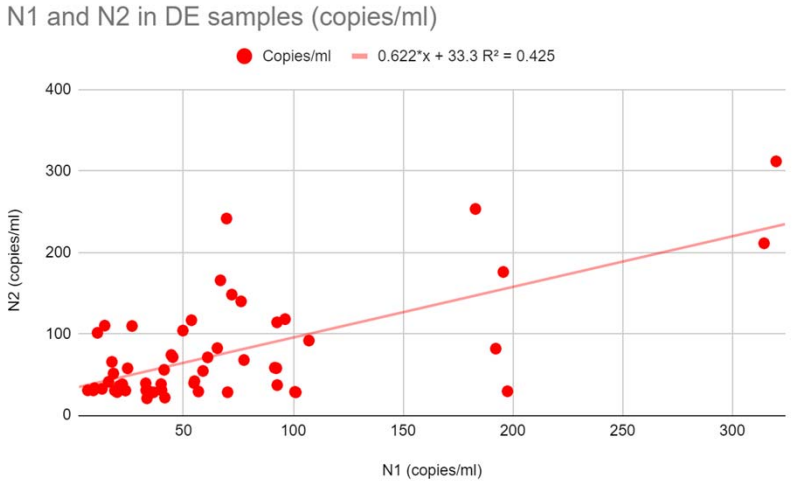
DE samples results in higher N1 concentration than filter samples, but similar trend in N1 concentration throughout 3 sampling events.



N1 and N2 results are highly correlated with each other in filter samples





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The Unknown: Workflow Recovery

Spike-in at sample processing vs. Spike-in at RNA extraction

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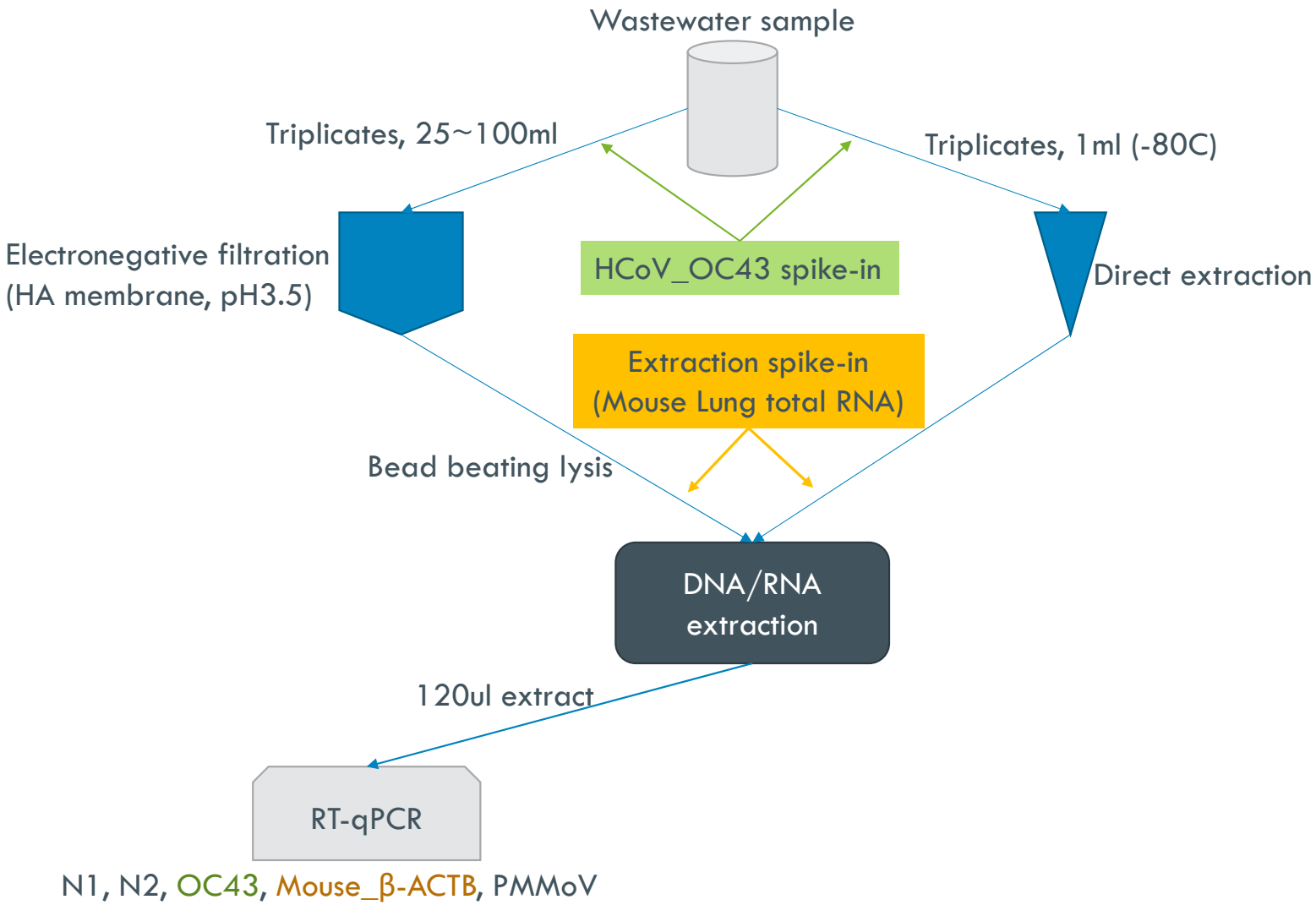
Workflow recovery is a black box

$$CF = \frac{V_{\text{sample before processing}}}{V_{\text{after concentration}}} \times \frac{V_{\text{concentrate used for RNA extraction}}}{V_{\text{after RNA extraction}}} \times \frac{1}{DF}$$

$$LOD\left(\frac{GC}{L}\right) = \frac{\text{Instrument detection limit}\left(\frac{GC}{L}\right)}{CF \times \text{Recovery}}$$

Pecson et al, Environ.Sci.: Water Res. Technol. 2021, 7, 504-520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RT-qPCR
N1, N2, OC43, Mouse_β-ACTB, PMMoV

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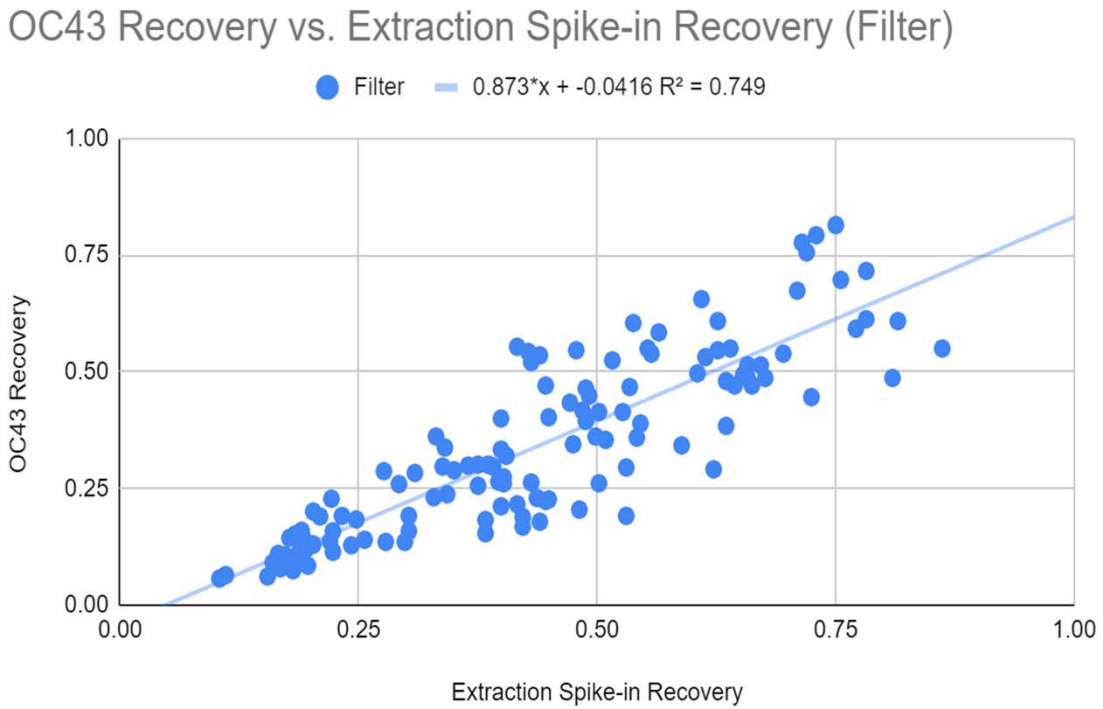
Filter Samples: Positive correlation between sample processing spike-in (OC43) and extraction spike-in (MLR) recovery rates



- OC43 recovery is determined by comparison to a pre-determined value of genomic copies of OC43 in heat-inactivated viral fluid.
- MLR recovery rate is determined by Delta Ct method with reference to Ct of 0.5ng/ul MLR per PCR reaction.

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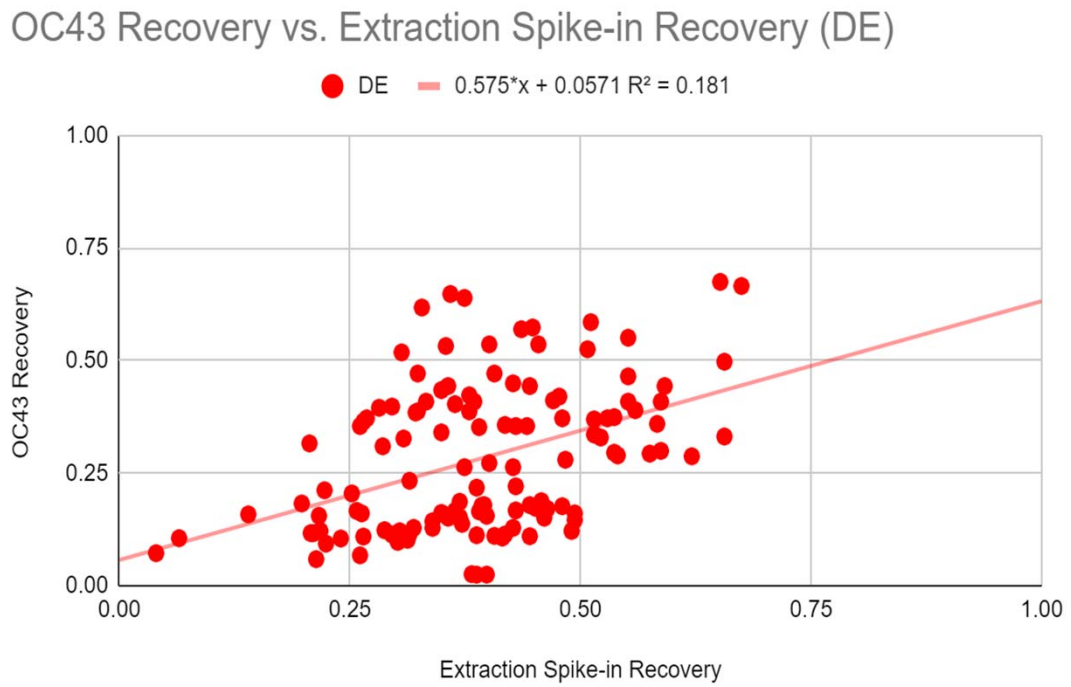
DE samples: Lack of correlation between sample processing spike-in (OC43) and extraction spike-in (MLR) recovery rates



Quantification of Clinically Important SARS-CoV-2 Mutations by RT-ddPCR

Are wastewater samples suitable for variants monitoring by RT-ddPCR?

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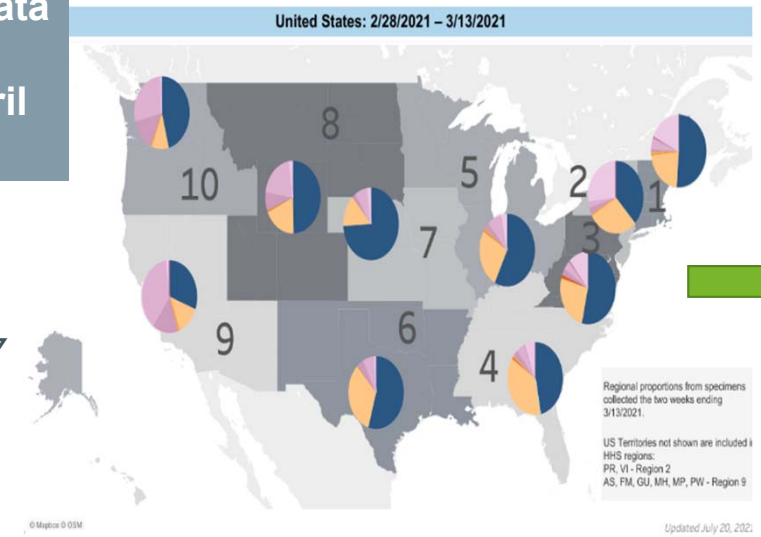
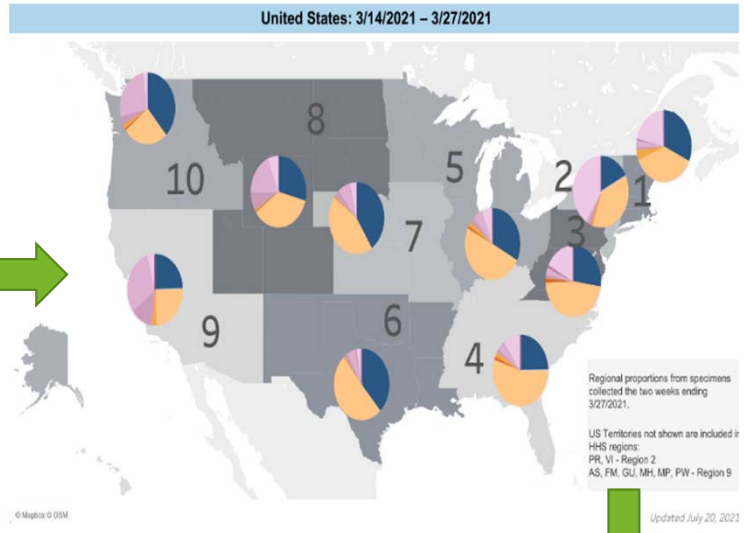
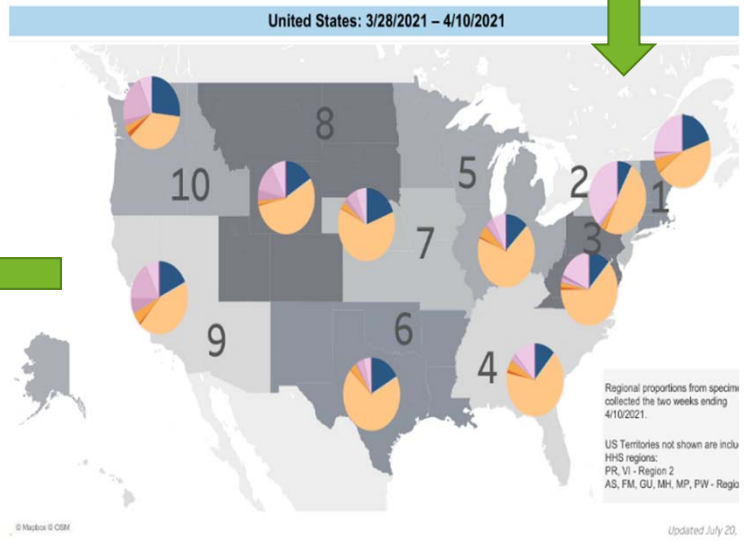
CDC COVID Data Tracker
Feb 28th ~ April 10th, 2021

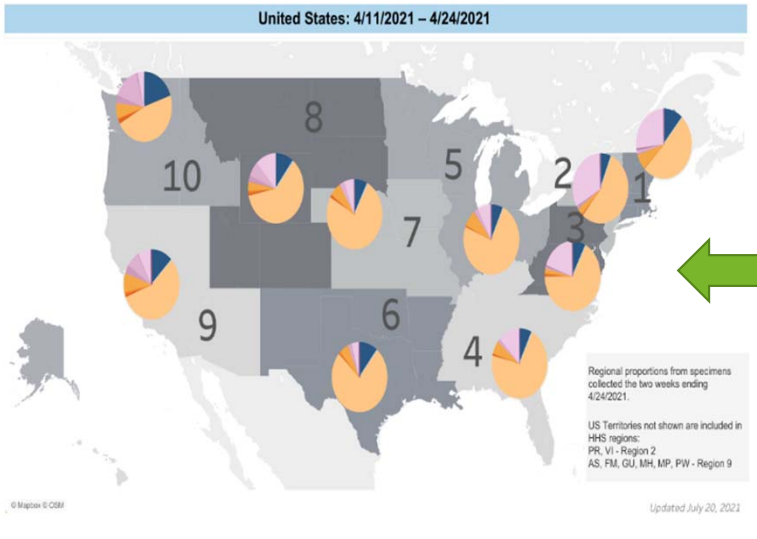
DEL69/70 – Unique to B.1.1.7 (alpha)

E484K – In B.1.351 (beta), P.1 (gamma), and B.1.526 (iota)









Lineage	
B.1.1.7	Alpha
B.1.429	Epsilon
B.1.526	Iota
B.1.427	Epsilon
B.1.621	
P.1	Gamma
B.1.351	Beta
B.1.525	Eta
B.1.617.1	Kappa
B.1.617.2	Delta
B.1.617.3	
Other	

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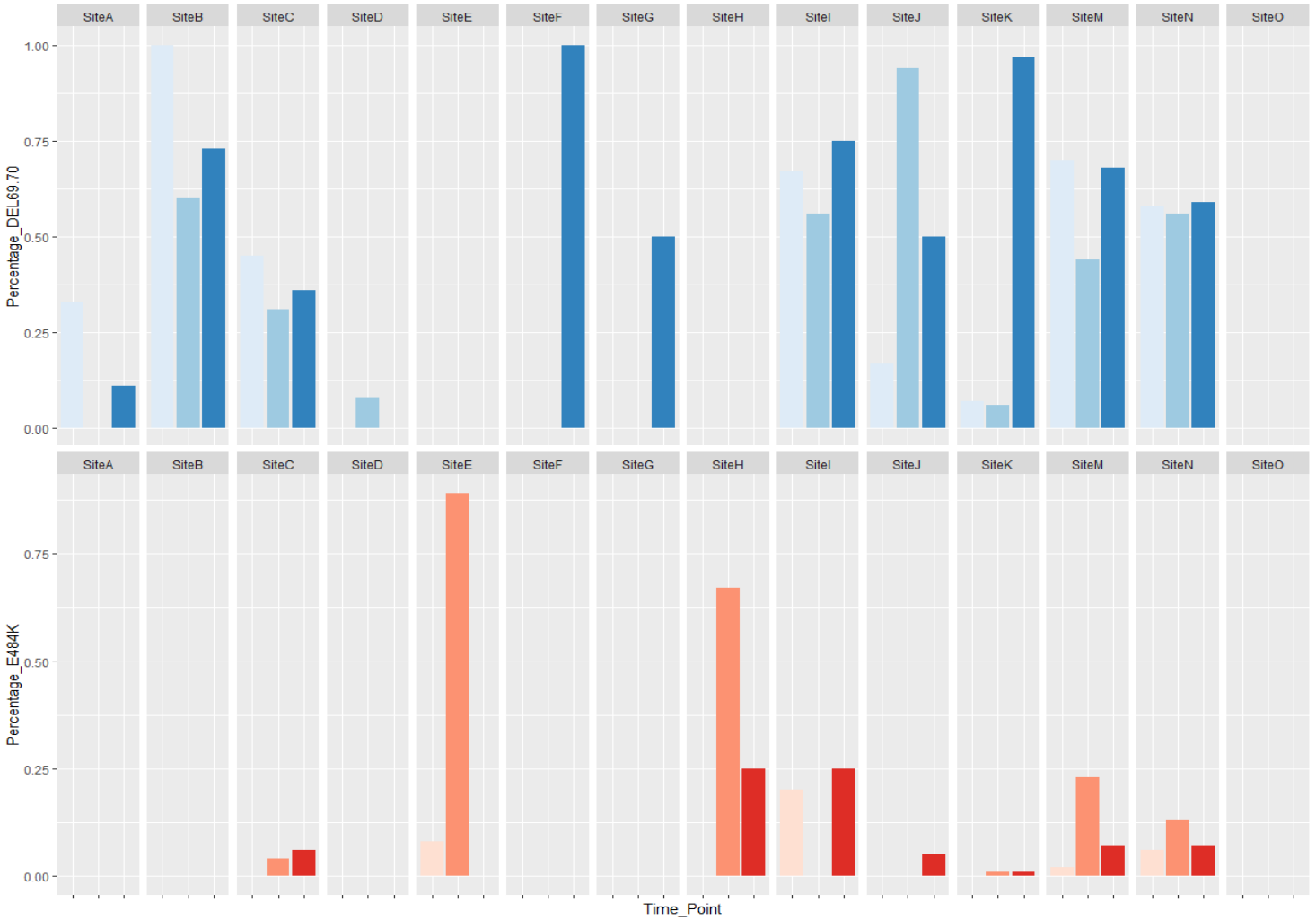
Percentage of DEL69/70 and E484K mutants in Composite Filter Samples



DEL69/70

Time_Point
Sampling Event 1
Sampling Event 2
Sampling Event 3

E484K

Time_Point
Sampling Event 1
Sampling Event 2
Sampling Event 3

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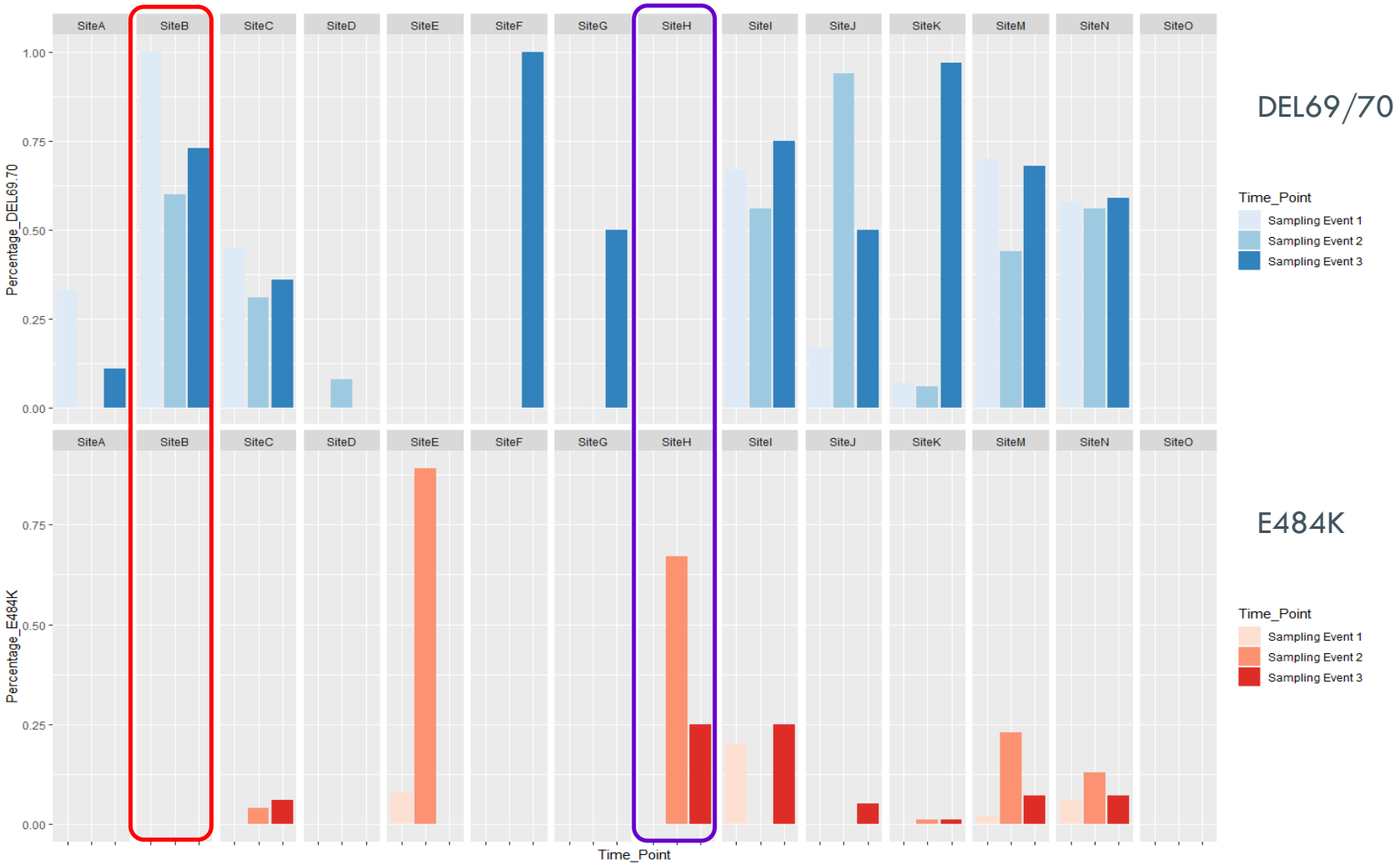
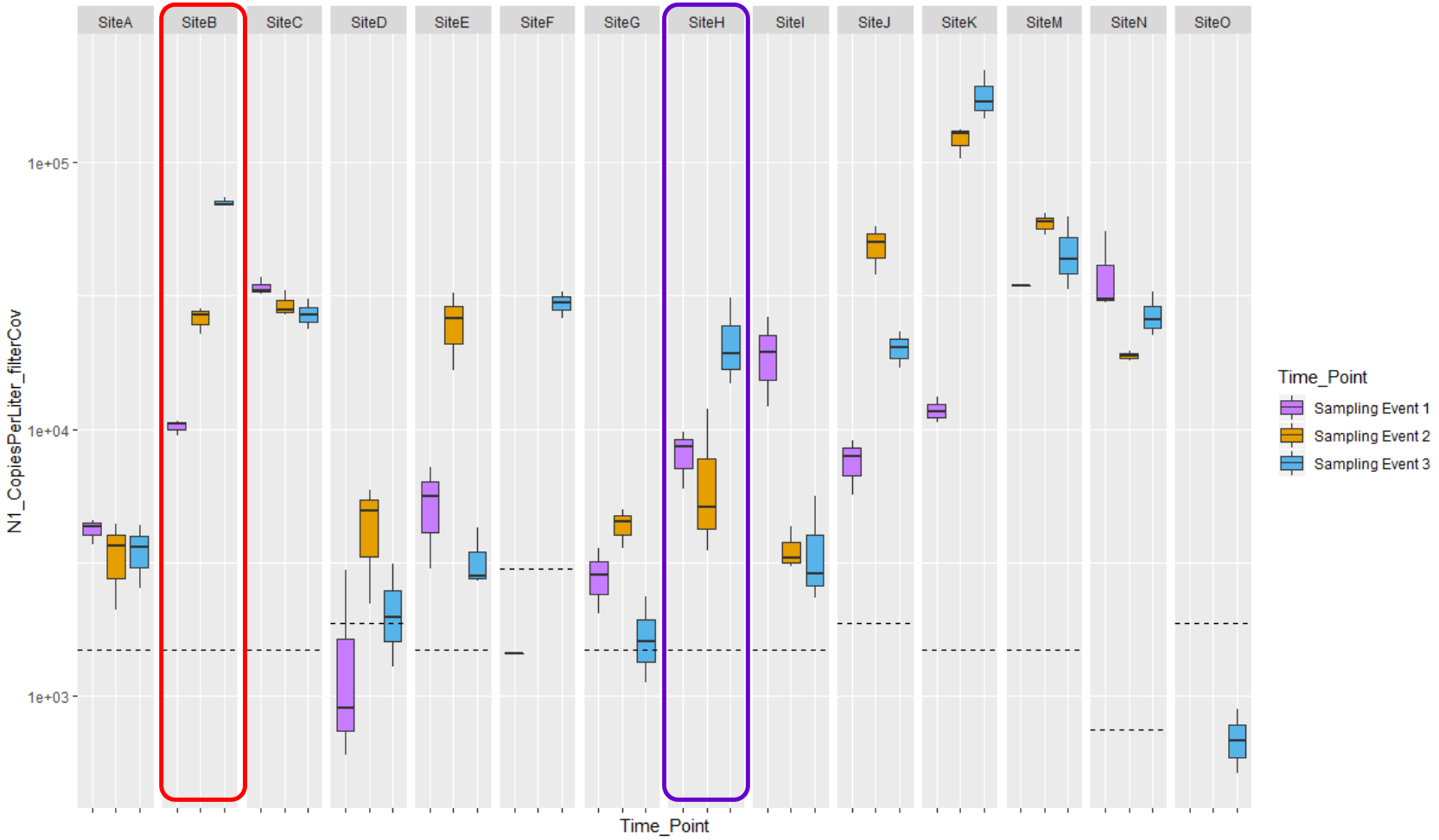
Percentage of DEL69/70 and E484K mutants in Composite Filter Samples



High N1 (Copies/Liter) from Site B and Site H Filter Samples



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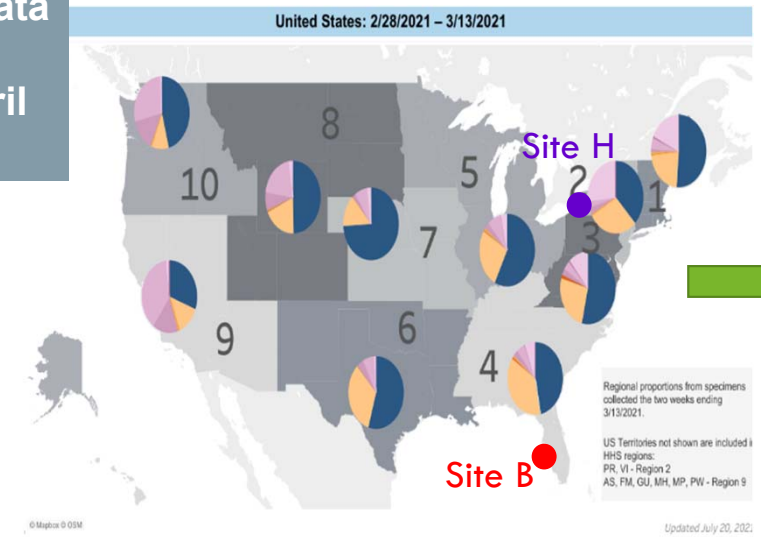

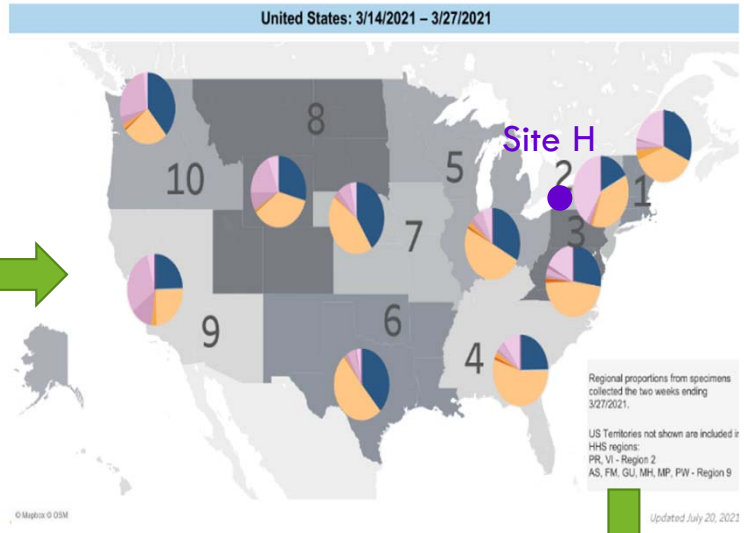

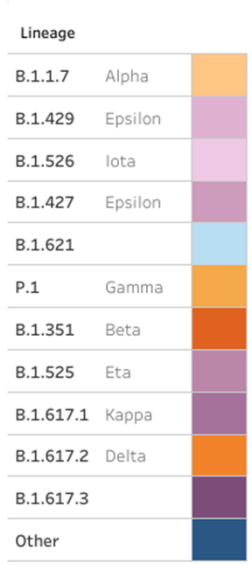

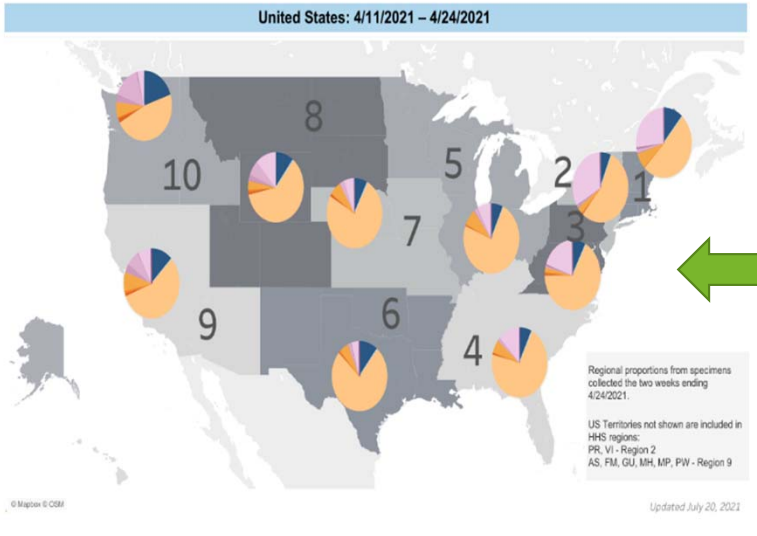

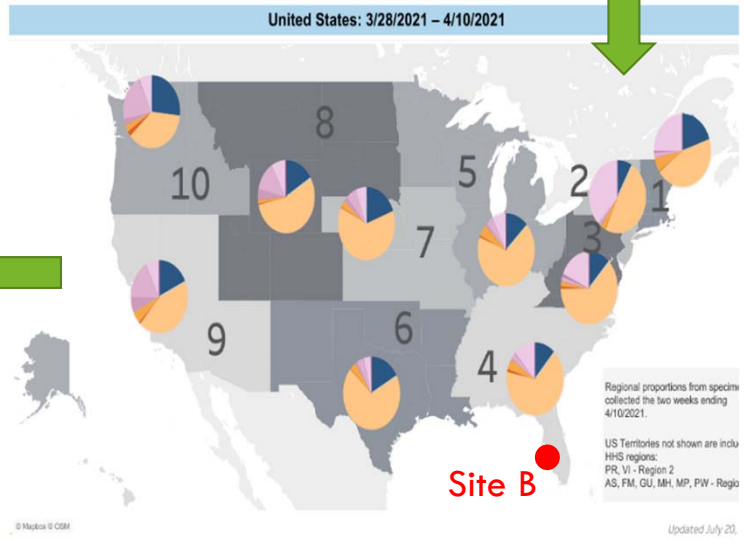

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SARS-CoV-2 Variants Identification by Whole Genome Sequencing

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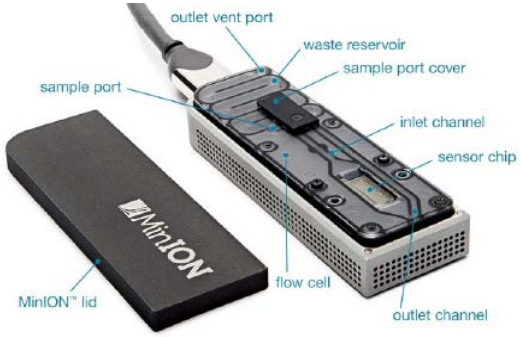
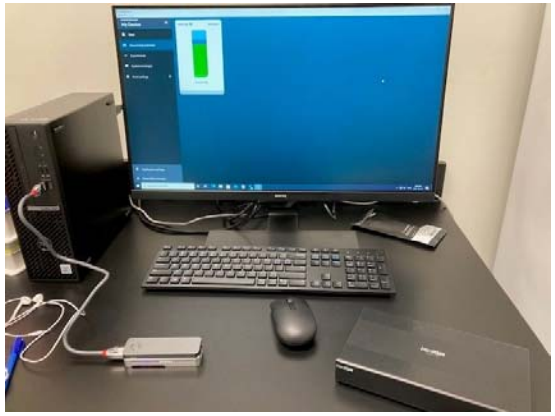
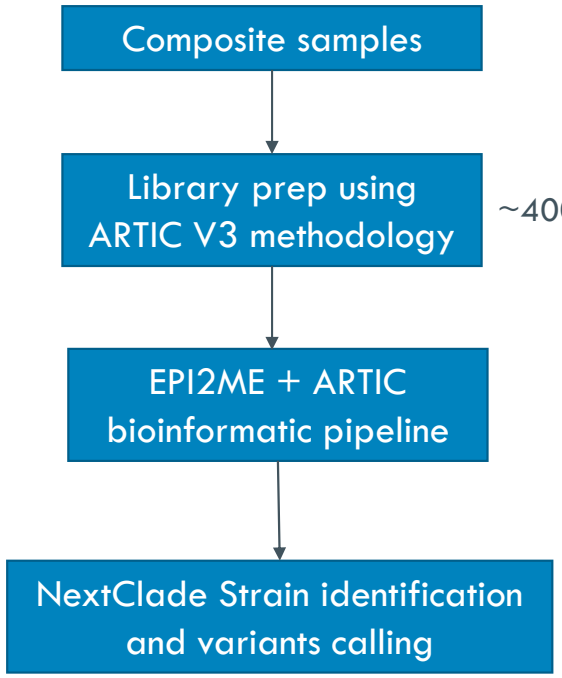
NGS Workflow





Oxford Nanopore MinION Mk 1B device and sequencing workspace



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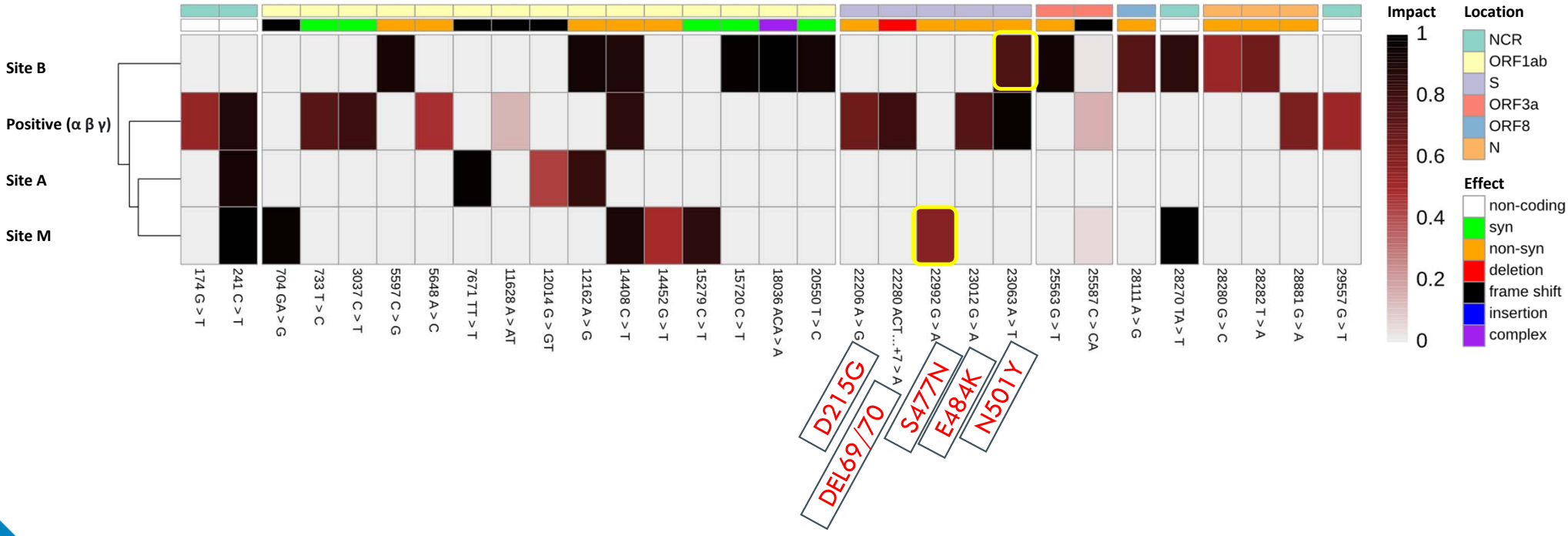
Challenges of mutation identification in wastewater samples

	QC	Clade	Mut.	non-ACGTN	Ns	Gaps	Sequence view
POS1	N M P C	19B	4	0	3339	15	
POS2	N M P C	20A	4	0	3473	9	
POS3	N M P C	19B	3	0	3670	15	
SiteA	N M P C	19A	2	0	20795	1	
SiteB	N M P C	20A	13	0	14632	9	
SiteM	N M P C	20A	8	0	9353	19	



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Lessons Learned

1. Automation in the lab with magnetic beads extraction methods
   → dramatically increase sample throughput.
2. Sample processing spike-in recovery rate
   → Workflow recovery measured by sample spike-in provide necessary information about method efficiency and sample nature.
3. Constantly developing new assays to detect mutations and NGS solutions.
   → RT-ddPCR is a feasible and valid methodology for quantification of mutations.
4. Knowledge sharing helps the public better understand their data and the importance of continuous monitoring effort.
   → Joint efforts from:
   academic research, preprints publications, multi-lab comparison studies, monitoring networks, data sharing platforms, and local community participations.