Monitoring variants of SARS-CoV-2 in wastewater: Overview and UC Berkeley experience

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UC Berkeley
May 18, 2022

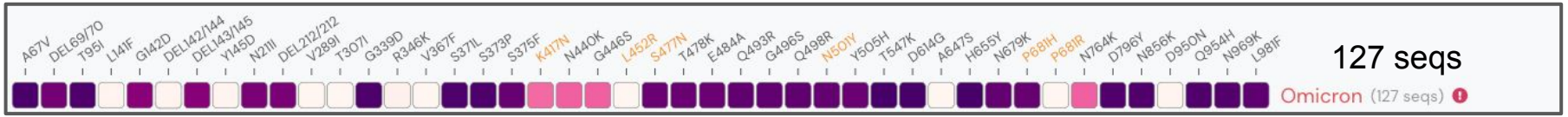
Background: challenges for variant detection in WW

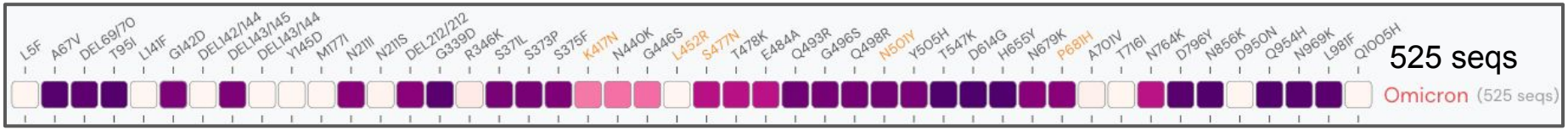
1. SARS-CoV-2 RNA is at relatively low concentrations in wastewater

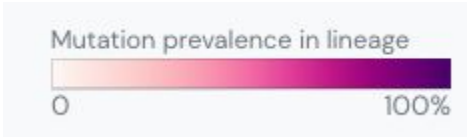
- Replicate/repeat samples to account for heterogeneity & false negatives
- Concentrate virus particles
- Enrich for SARS-CoV-2 RNA (or deplete other WW components)
- Amplify RNA targets

Background: challenges for variant detection in WW

1. SARS-CoV-2 RNA is at relatively low concentrations in wastewater
2. Changing variant definitions and new variants



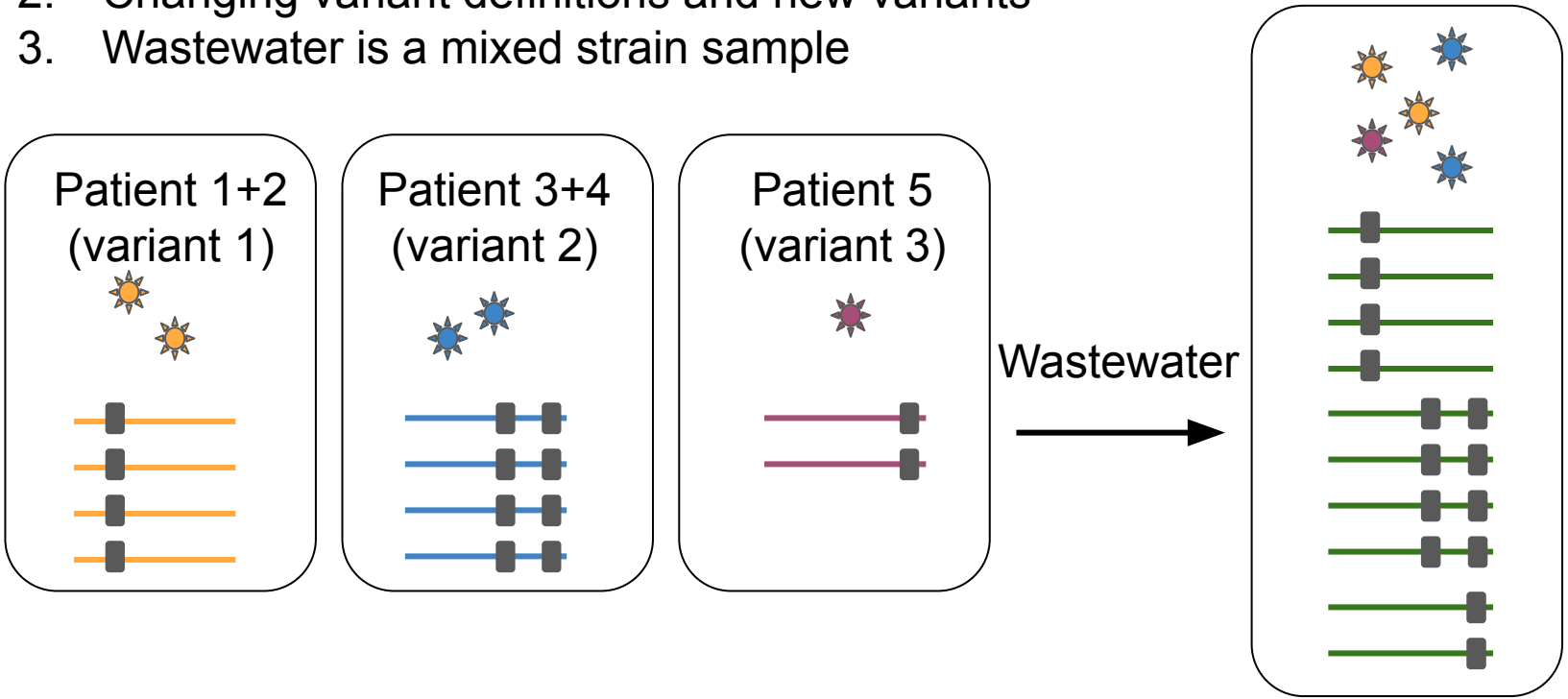




Screenshots from outbreak.info

Background: challenges for variant detection in WW

1. SARS-CoV-2 RNA is at relatively low concentrations in wastewater
2. Changing variant definitions and new variants
3. Wastewater is a mixed strain sample



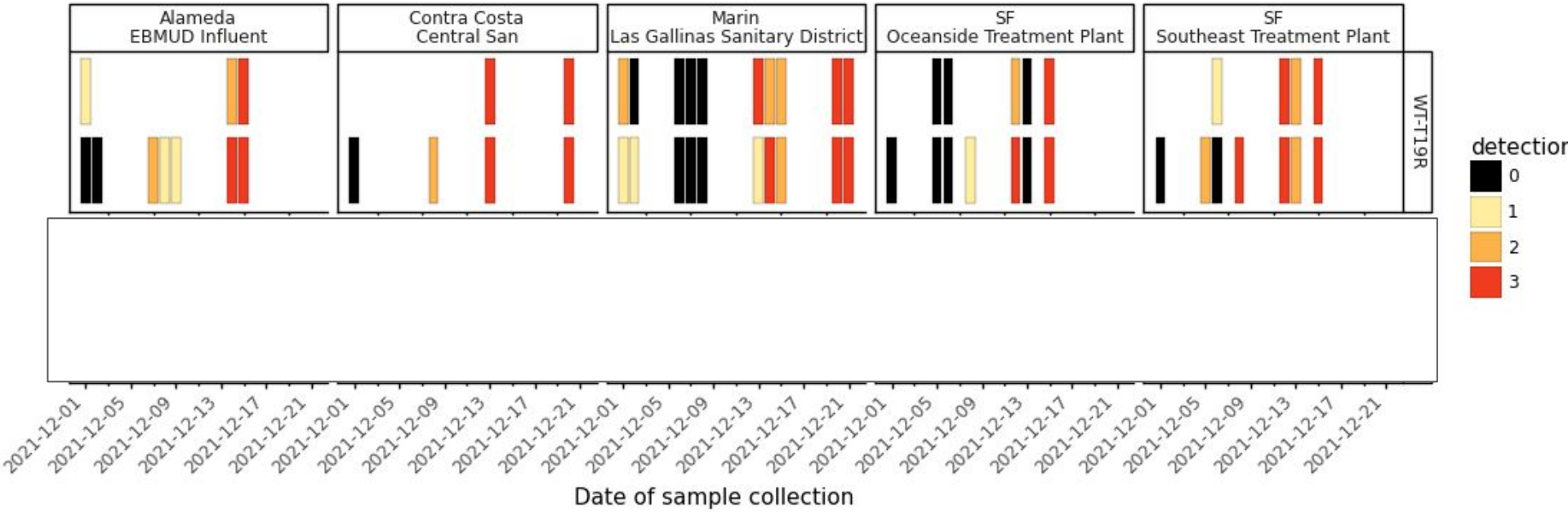
Methods choice and interpretation considerations

Consideration	**PCR-based**	**Sequencing-based**
Sensitivity		
Novel vs. known/targeted		
Quantification		
Time-to-results		
Development and reagent lead time w/ new variant		
Cost per sample		

Methods choice and interpretation considerations

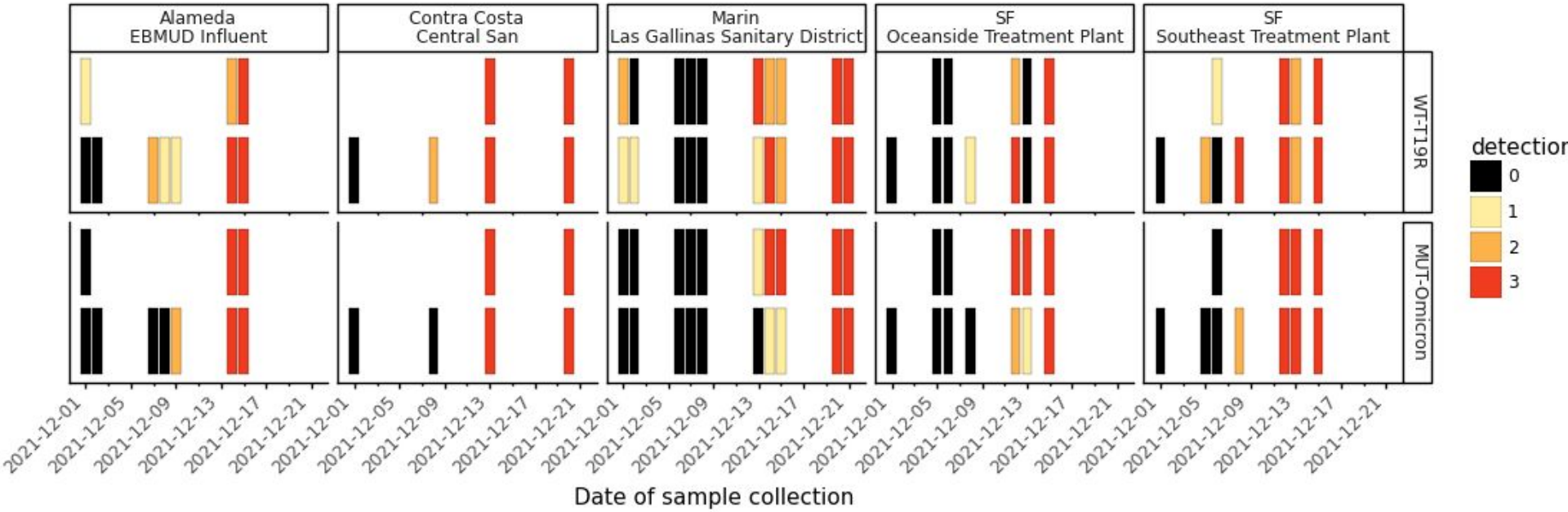
Consideration	**PCR-based**	**Sequencing-based**
Sensitivity	Higher	Lower
Novel vs. known/targeted	Known (targeted) only	Novel and known
Quantification	Absolute	Relative
Time-to-results	Faster	Slower
Development and reagent lead time w/ new variant	Longer	Shorter
Cost per sample	Lower	Higher

Results from RT-qPCR: early Omicron detection



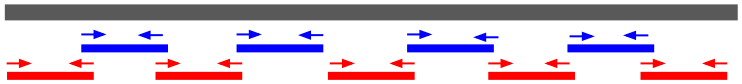
WT-T19 is a non-Delta assay

Results from RT-qPCR: early Omicron detection



Methods: Tiled Amplicon Whole Genome Sequencing

Primers designed to tile across the SARS-CoV-2 genome



Primer sets

- ARTIC
- Swift
- “Midnight”

Sequencing platforms:

- Illumina
- Nanopore

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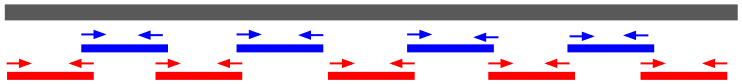

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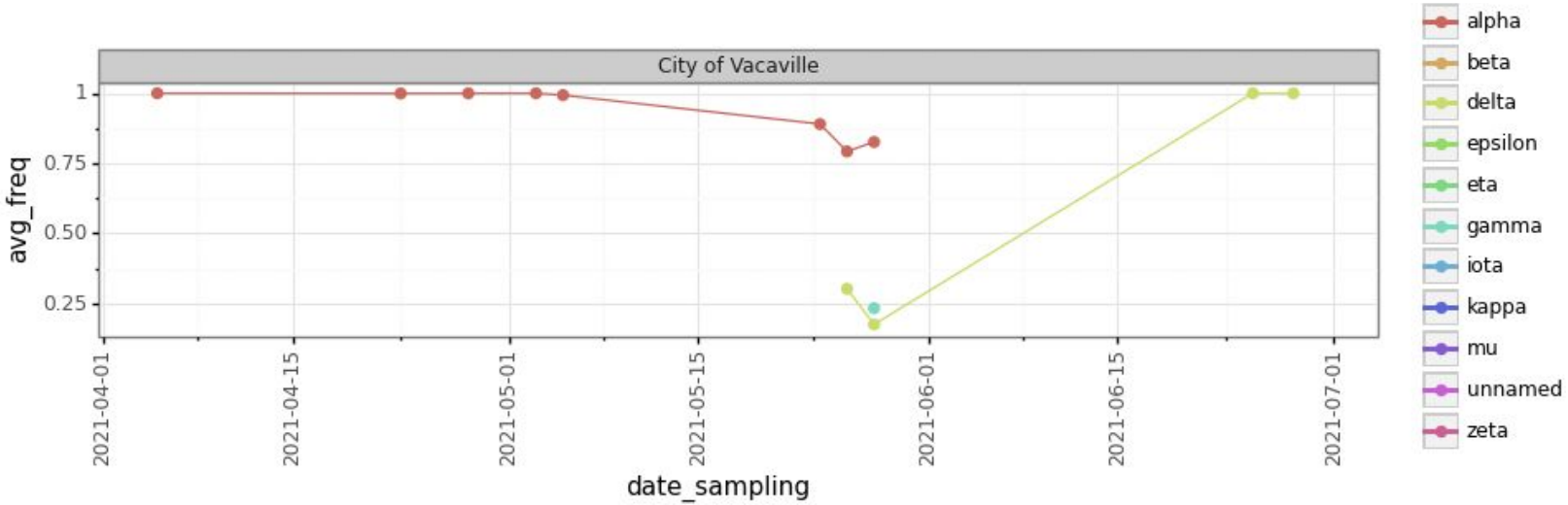
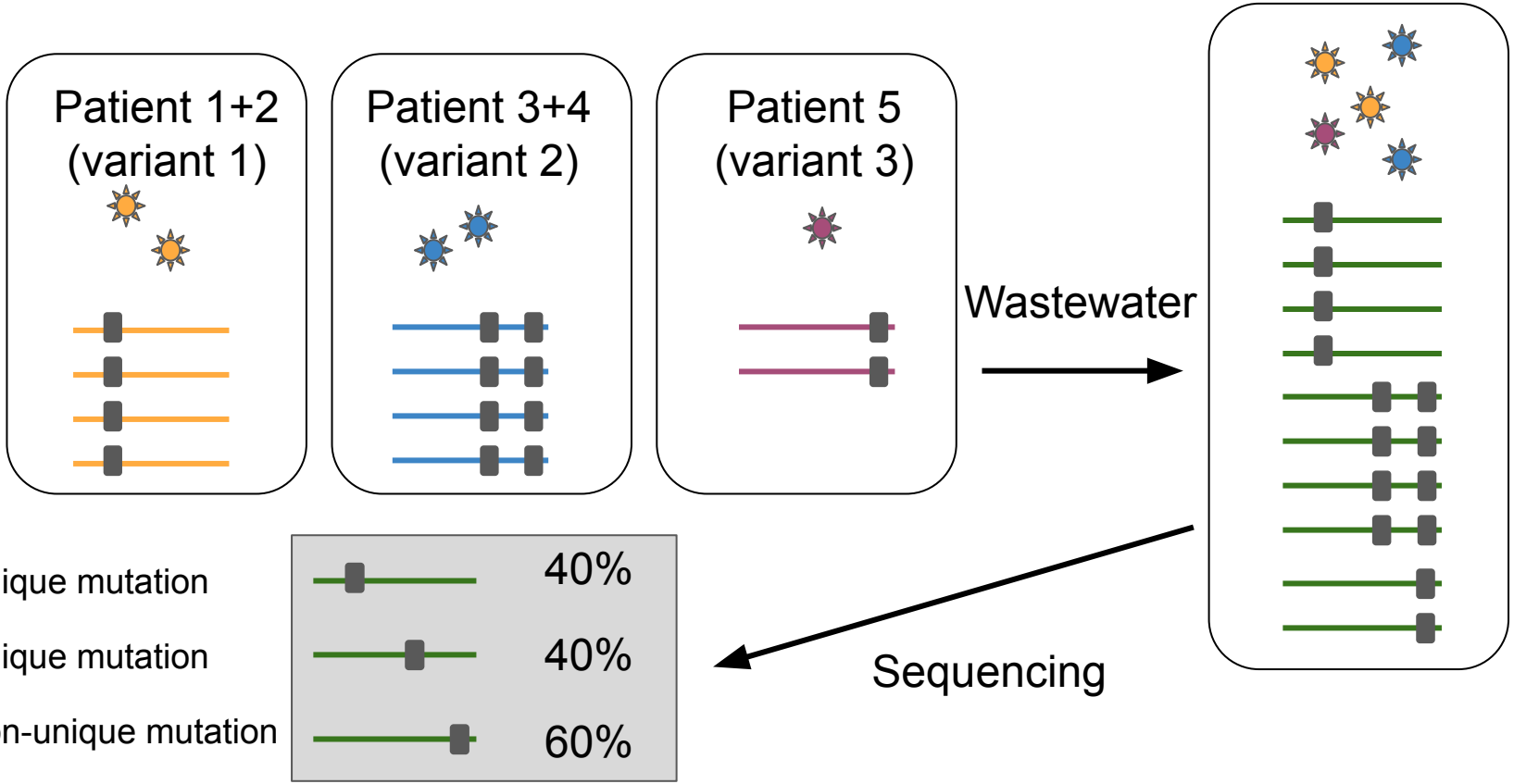
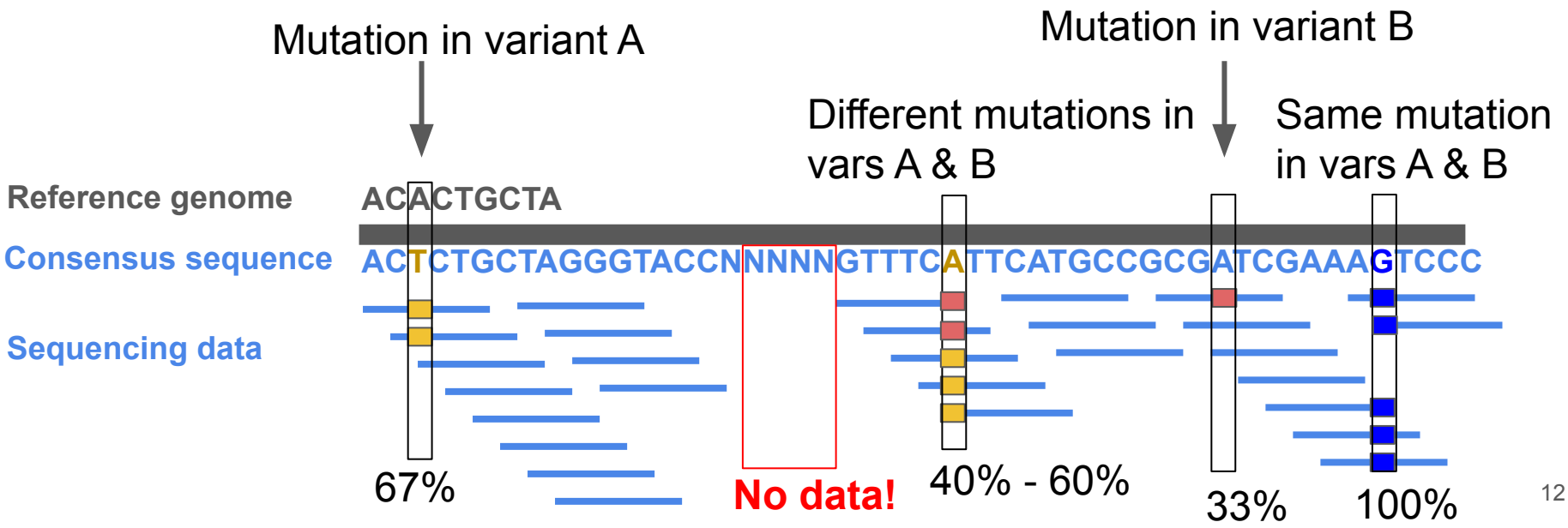


Targeted amplicon sequencing reveals linked mutations



Caveats of whole genome sequencing

1. **Mutations are unlinked in analysis and wastewater is a mixed sample**
2. **Genomes are not always complete**



Methods: Targeted Amplicon Sequencing

Targeted S-gene regions:

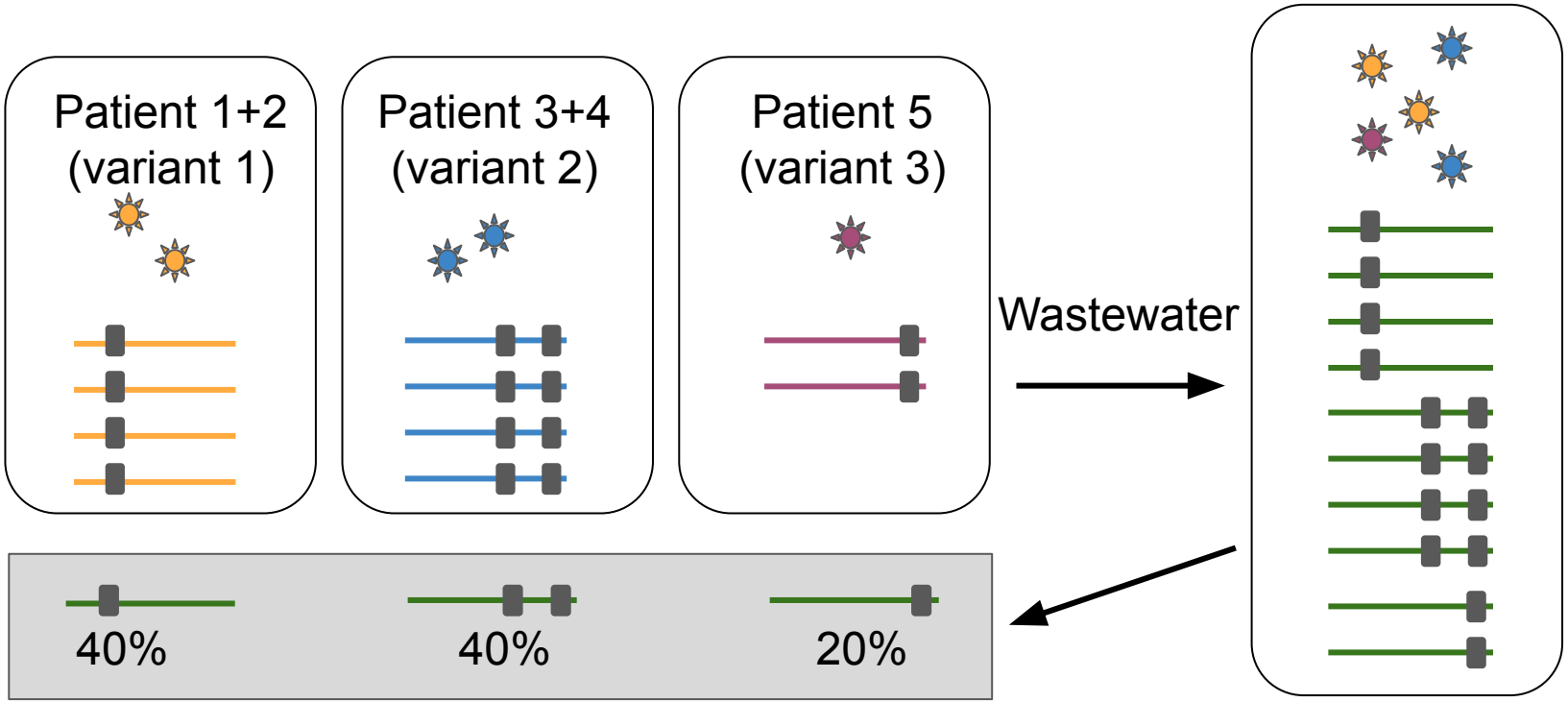
- Receptor binding domain
- N-terminal domain
- S1S2 domain

Sequencing platforms:

- Illumina

Gregory et al. 2021

Targeted amplicon sequencing reveals linked mutations



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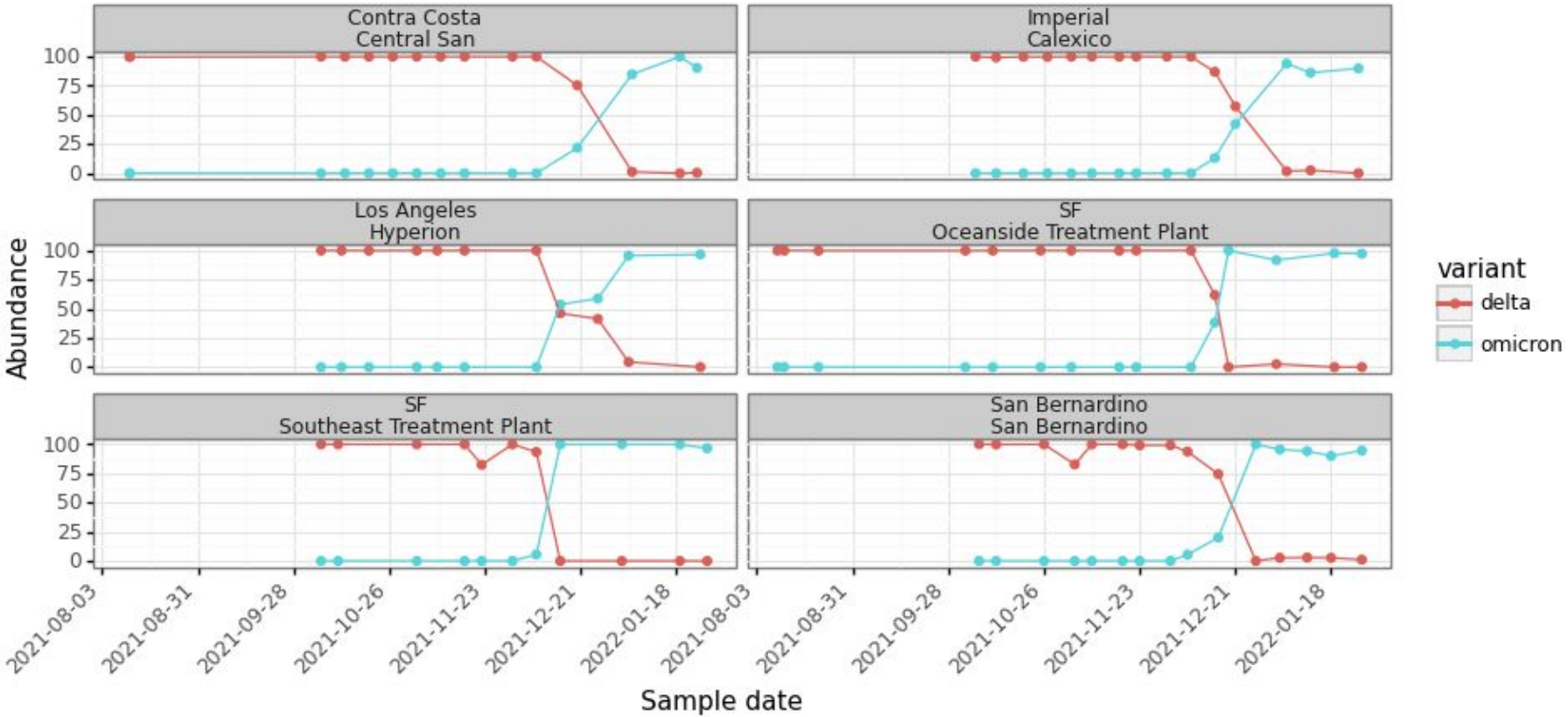

Cryptic lineages found in targeted sequencing data

In New York City Sewage, a Mysterious Coronavirus Signal

For the past year, scientists have been looking for the source of strange coronavirus sequences that have appeared in the city's wastewater.

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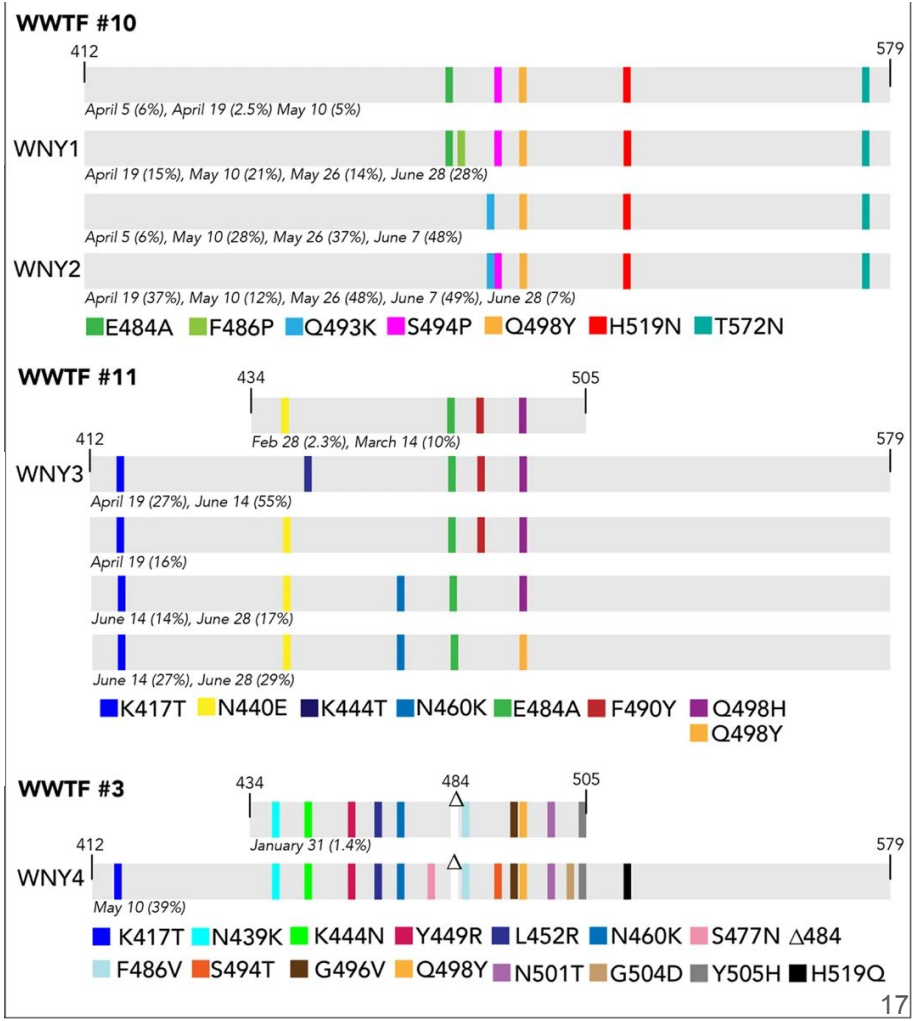
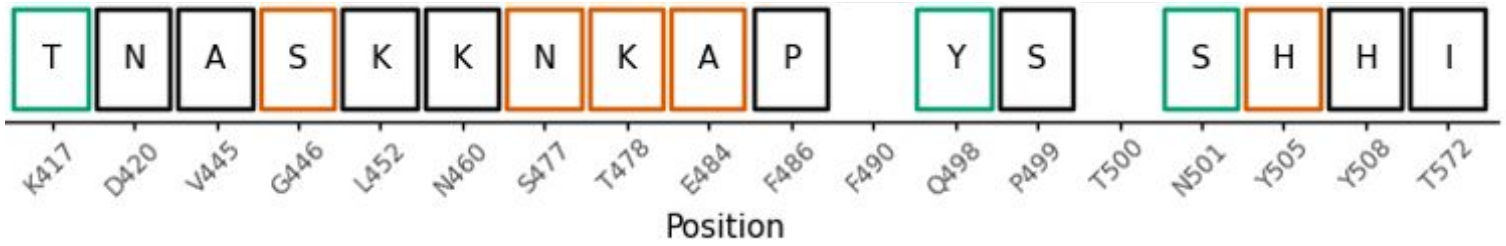
Smyth et al. Nature Comms, 2022



Cryptic lineages found in targeted sequencing data, CA

Nov 4, 2021

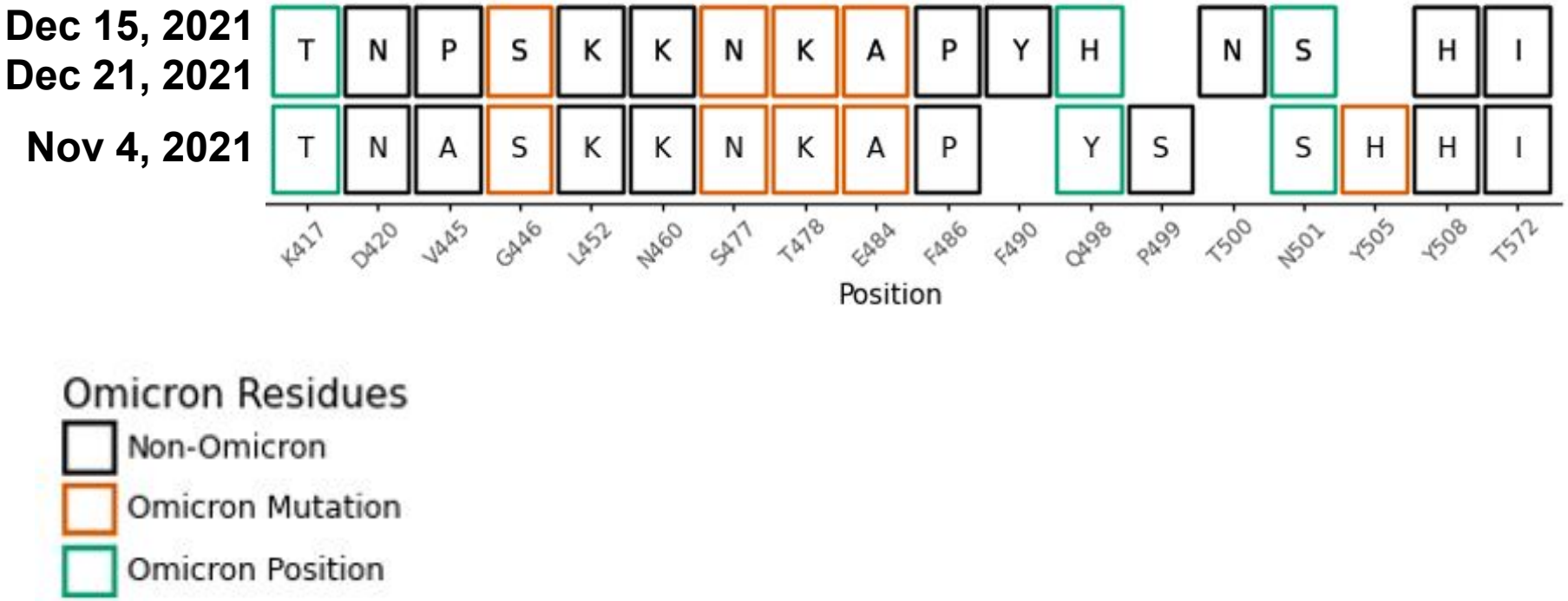


Omicron Residues

- Non-Omicron
- Omicron Mutation
- Omicron Position

Gregory, Johnson, Kantor, Dennehy et al. *in prep*

Cryptic lineages found in targeted sequencing data, CA



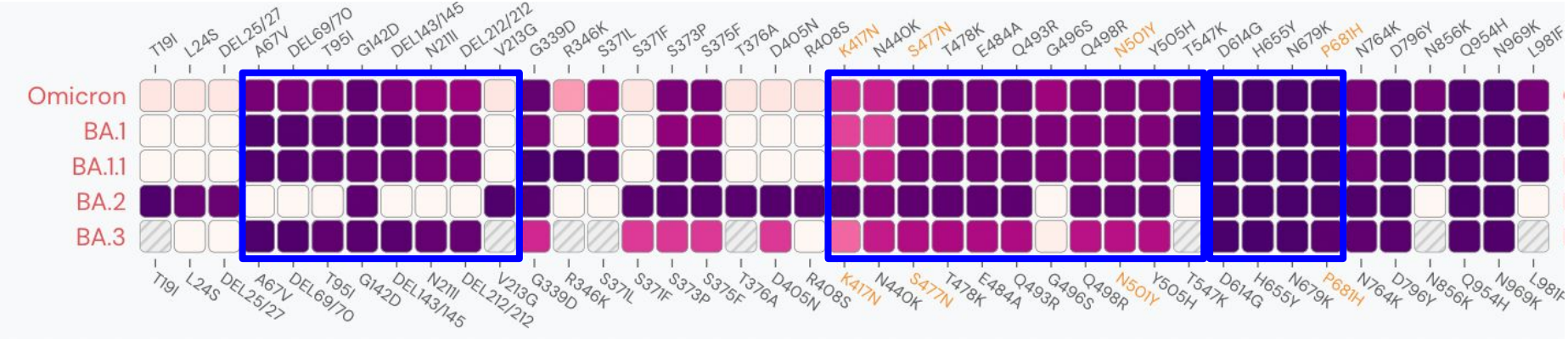
Gregory, Johnson, Kantor, Dennehy et al. *in prep*

Caveats of targeted amplicon sequencing: Sometimes cannot distinguish sublineages

NTD amplicon

RBD amplicon

S1S2 amplicon



- BA.1 + BA.1.1 + BA.3
- BA.2

- BA.1 + BA.1.1
- BA.2 + BA.3

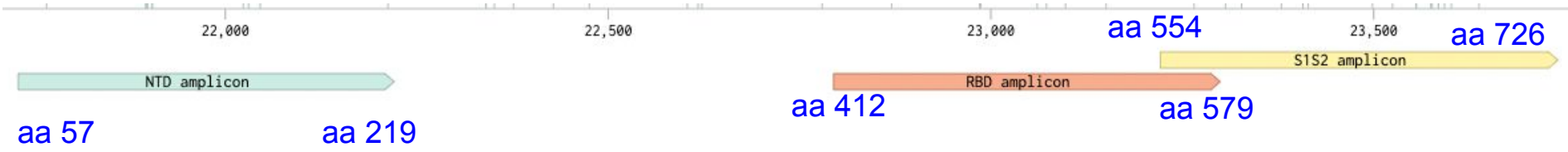
- All Omicron

Variant monitoring: barriers and directions

- Low concentrations in wastewater: sequencing may fail between surges
- Sequence data interpretation as paired with clinical data?
  - Where are there discrepancies?
  - Novel variants or sublineages?
  - Monitor variant sweeps
- Consolidated state-level dashboard?
- Fit-for-purpose monitoring effort:
  - Define the goal (e.g. detect novel variants OR track known variants discovered in clinical data)
  - Choose appropriate method
  - Analyze all data with consistent definitions of variant lineages
  - Visualize, communicate within PH → action? (e.g. decide use of monoclonal antibody treatments)

Extra slides

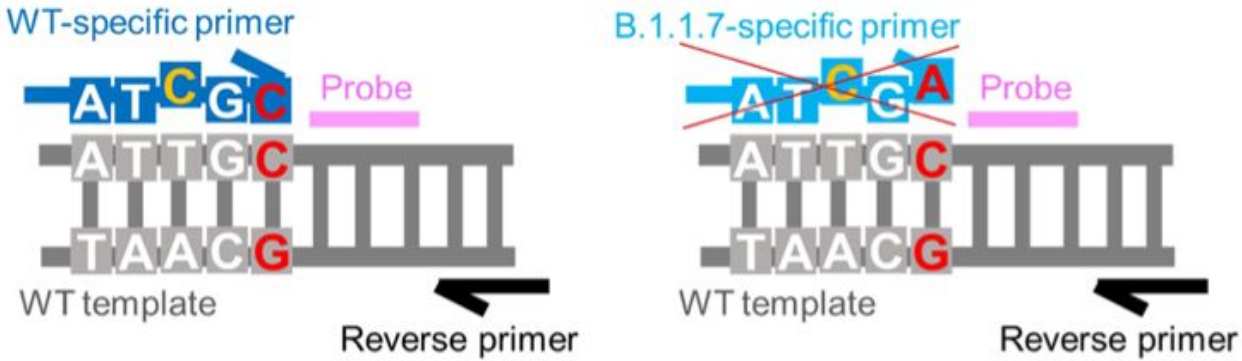
High-throughput sequencing of 3 s-gene regions yields exact amplicon sequences within these regions



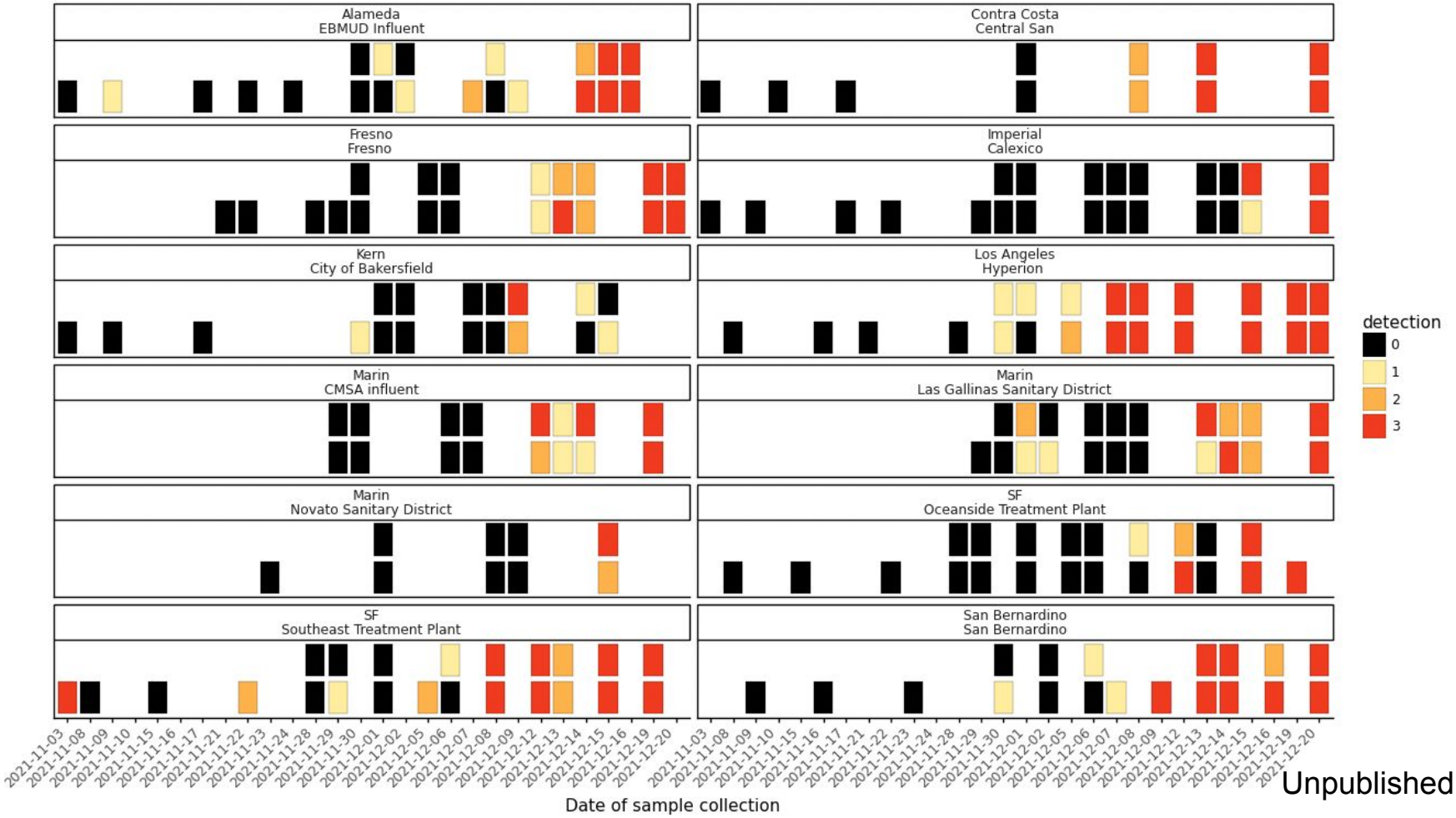
Primers and approach from Gregory et al. 2021

Methods: PCR-based assays

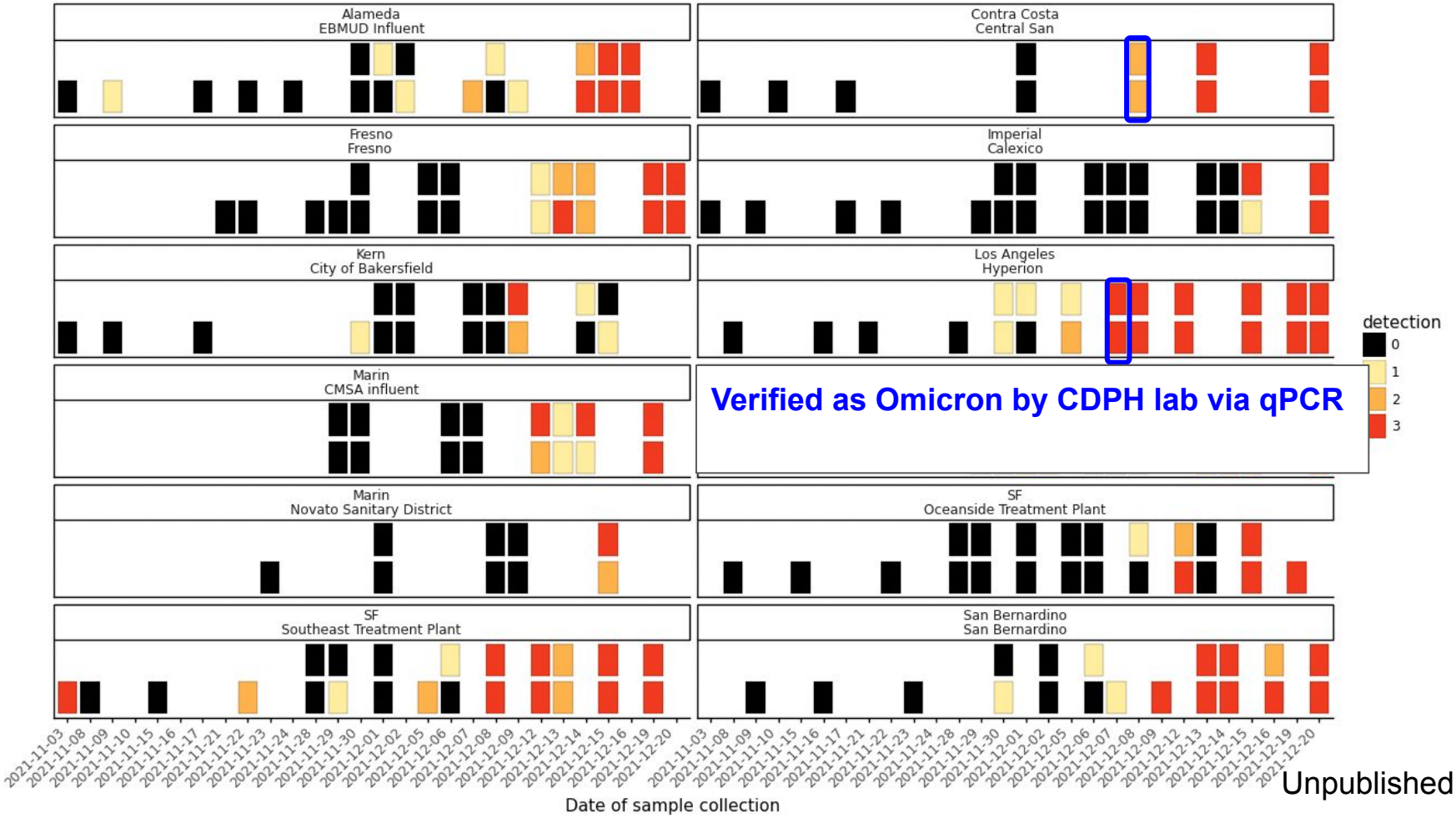
Allele-specific qPCR



Lee et al. 2021 medRxiv



Unpublished data



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