

# Development of a rapid, uniform, state-wide surveillance program for COVID variants in New York State wastewater

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## Background

- Sequencing of SARS-CoV-2 positive clinical samples from throughout New York State was initiated at the Wadsworth Center, New York State Department of Health (NYSDOH), in March 2020.
- Throughput was expanded in December 2020 in response to the emergence of variants.
- In mid 2021, a consortium of four academic laboratories was established to perform most of the NYS surveillance sequencing for SARS-CoV-2.
- In 2022, the work of the consortium was further expanded to include the sequencing of SARS-CoV-2 positive wastewater (WW) samples from throughout the state, distributed among the four consortium sites and the Wadsworth Center.
- This expansion was prompted by the successful detection of the virus in wastewater samples, and the decreased availability of community samples for analysis due to increased point of care and home testing.
- Data submission and analysis was centralized to collaborators at Syracuse University, where processed data would be submitted to NCBI and CDC.

## Methods

- To assure rapid, sensitive, and uniform results in this new WW program across the state, ThermoFisher Genexus instruments were installed at all five sites.
  - Data from previous testing demonstrated greater sequence depth and coverage from samples at lower viral loads, with AmpliSeq chemistry on Genexus compared to other methods (ARTIC/Illumina, ClearLabs Dx, and AmpliSeq/S5) with results available within 25 hours of initiation of testing.
- An evaluation panel comprising 16 samples was prepared at the Wadsworth Center and distributed to the five sites, containing total nucleic acid from multiple SARS-CoV-2 isolates, representing multiple circulating variants.
  - Each sample contained varying percentages (from 5-75%) of up to six different lineages.
  - Purity of the isolates was verified by Whole Genome Sequencing (WGS) on Genexus followed by Freyja analysis
  - Absolute quantification was performed with the Qiagen QIAcuity Digital PCR system for each isolate and by real-time RT-PCR using the New York State SARS-CoV-2 assay.
  - Sequencing data from the laboratories was uploaded to a centralized cloud and assessed for performance and consistency.
- Participating sequencing laboratories started receiving SARS-CoV-2 positive wastewater nucleic acid extracts from throughout the state in November, from four contracted detection laboratories.
- Sequencing data was uploaded to a Google Cloud account at Syracuse University, where analysis included deconvolution with Freyja and other software to determine composite lineages. Sequence data flow is shown in Figure 1.

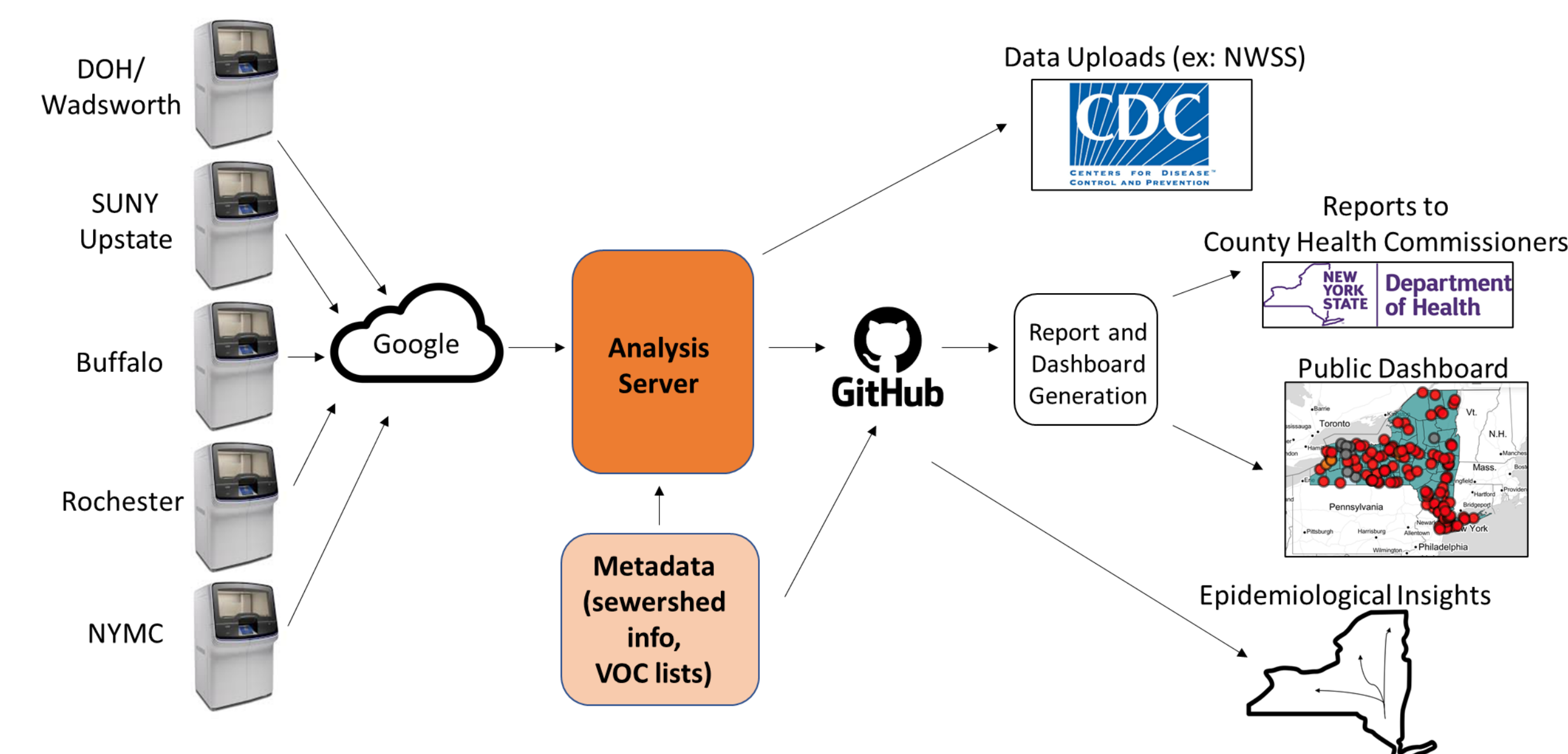


Figure 1: Sequence data flow, analysis and posting of results

## Results

A sampling network of more than 200 sewer sheds was established in NYS during 2021-23 for the detection of SARS-CoV-2 in wastewater (see Figure 1)

The NYS sequencing consortium, established in 2021 for SARS-CoV-2 sequencing of positive clinical samples, was expanded for sequencing of positive wastewater samples in 2022. Comparison of the sequencing data across the five consortium sites was tested with a proficiency testing panel and demonstrated a high consistency in the detection and percentage composition of lineages detected (Figure 2).

After the initiation of SARS-CoV-2 sequencing from wastewater across the state, data processing and visualizations demonstrated the interesting variation in lineage distribution across the counties, regions and state over time (figures 3, 4 and 5) and the ability to visualize individual lineages by county and sewer shed source (figure 6).

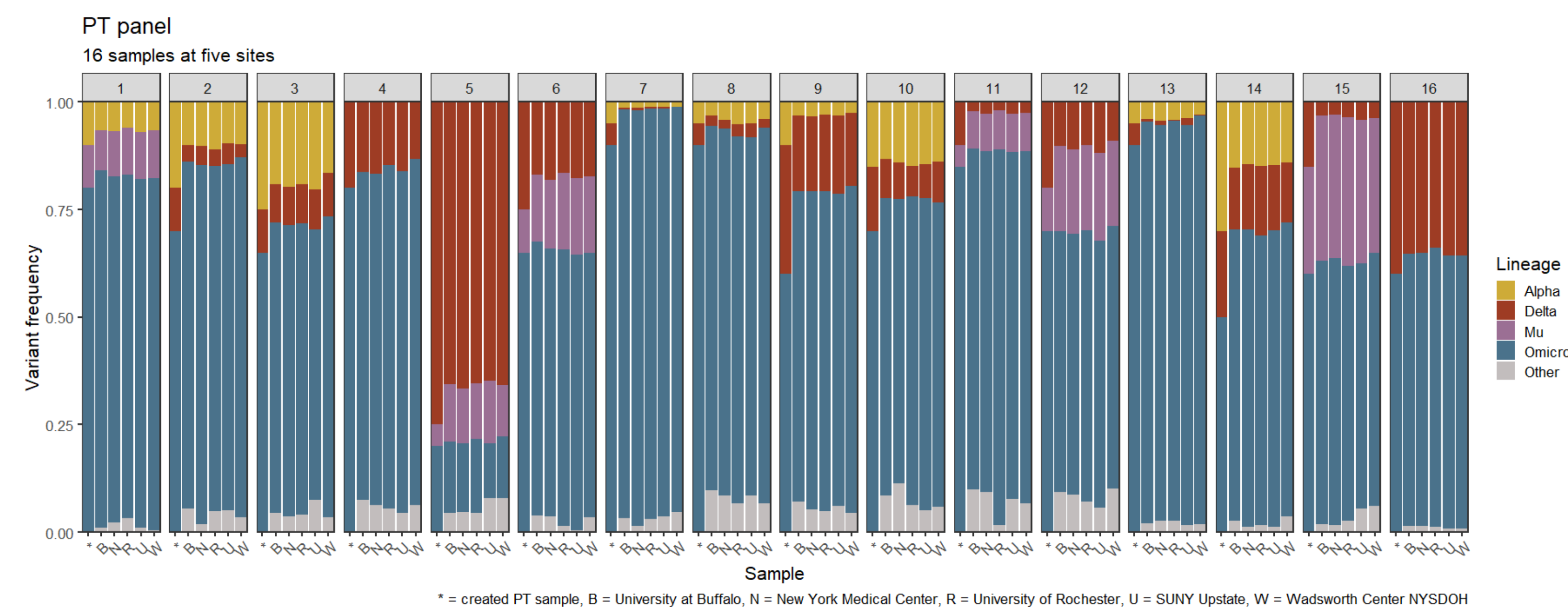


Figure 2: Results of wastewater SARS-CoV-2 sequencing proficiency testing across the five sequencing laboratories.

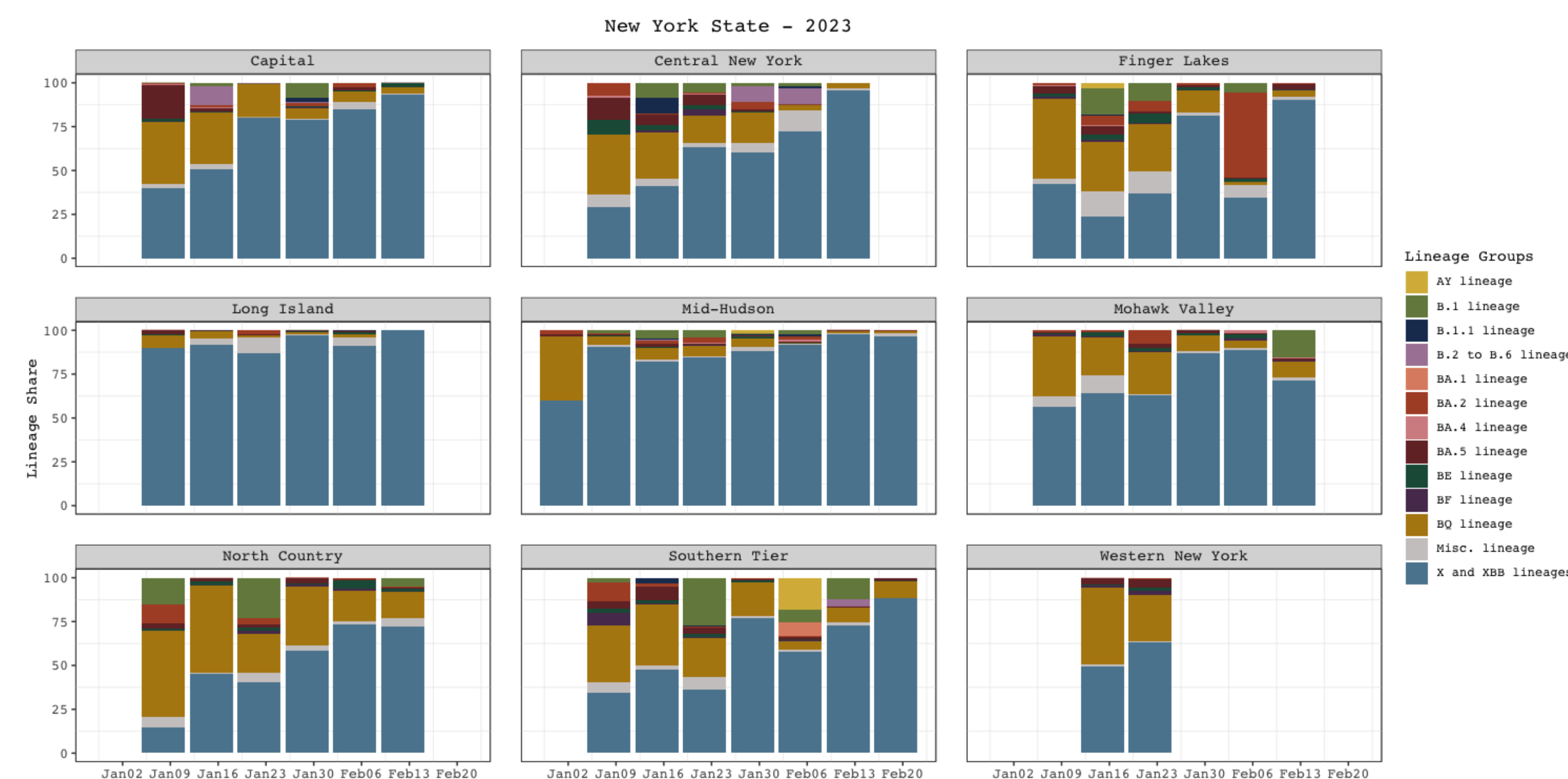


Figure 3: Results of weekly NYS wastewater SARS-CoV-2 lineage analysis, aggregated by economic region, Jan-Feb 2023.

## Results

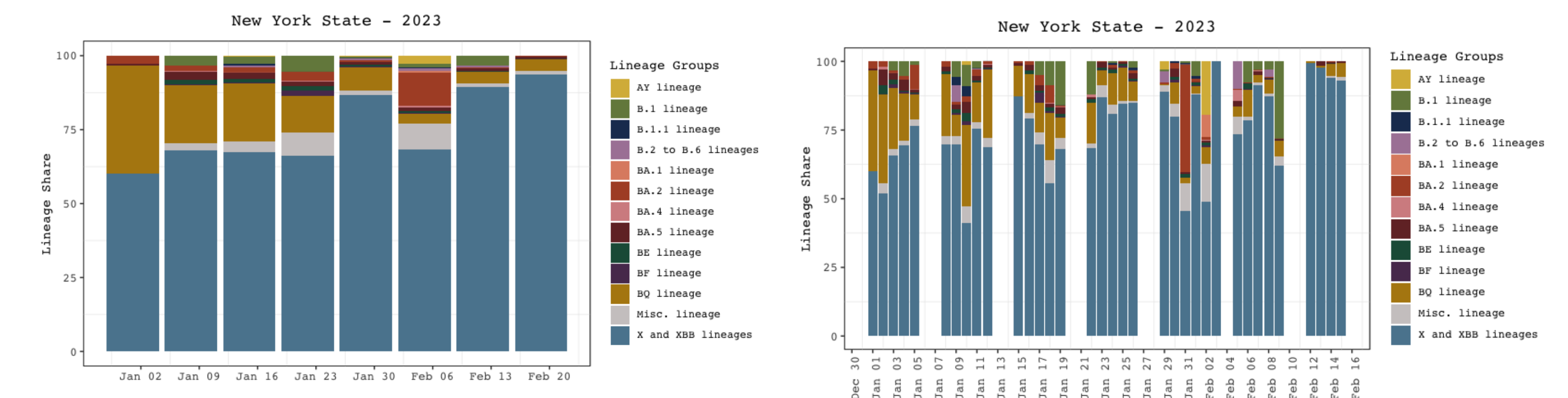


Figure 4: Weekly NYS wastewater SARS-CoV-2 lineage analysis, aggregated across state, Jan-Feb 2023

Figure 5: Weekly wastewater NYS aggregate data, with daily measures indicated within weekly groupings. Jan-Feb 2023

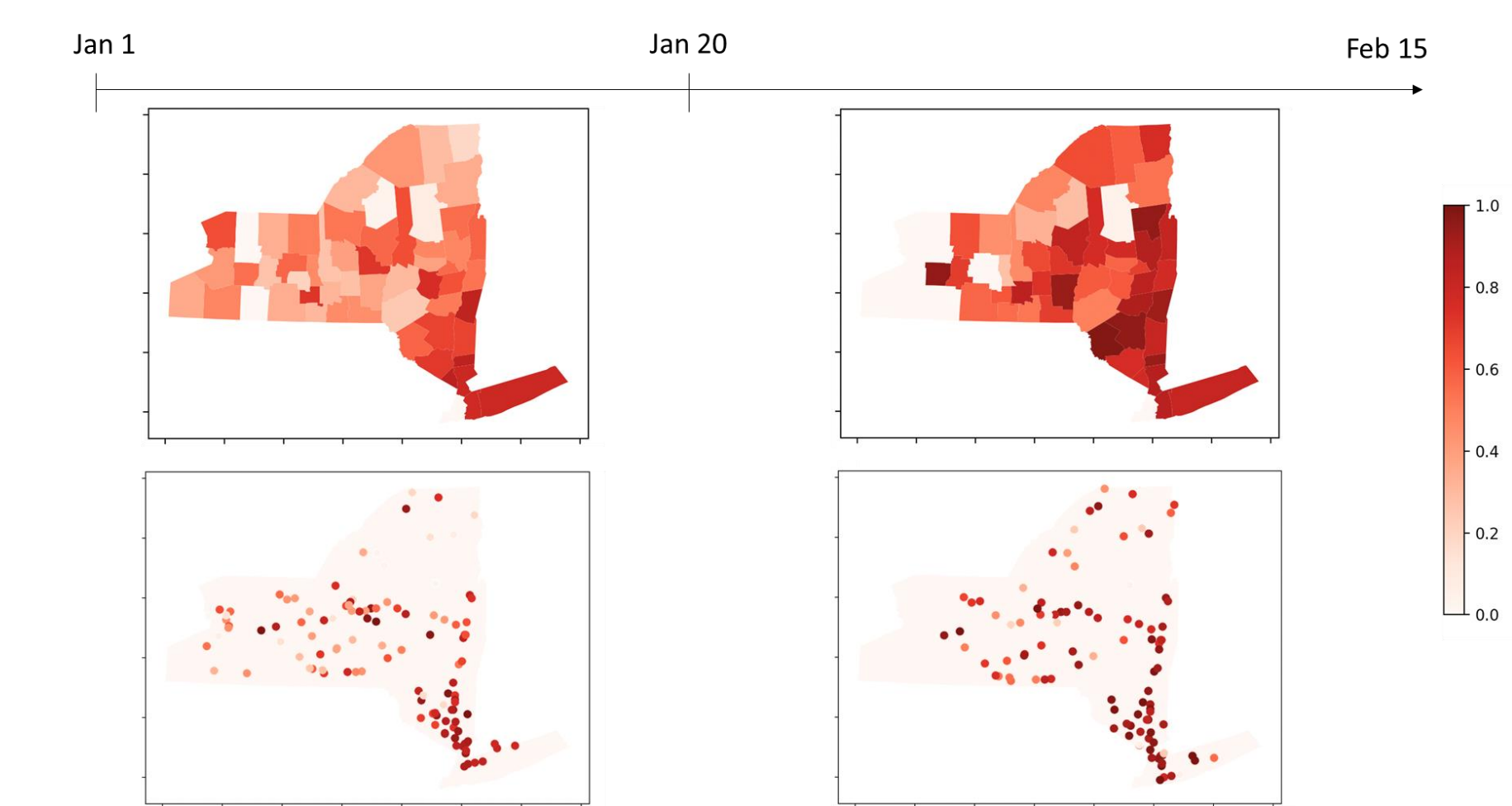


Figure 6: Aggregate data for January and February 2023, of the relative abundance of SARS-CoV-2 lineage XBB.1.5 indicated by county (top maps) and by sewer shed source (bottom maps).

## Conclusions

- A network was established for the rapid monitoring of SARS-CoV-2 lineages in NYS wastewater
- A proficiency testing panel clearly demonstrated a high degree of consistency in the detection and percentage lineage composition from the five participating sequencing laboratories
- With multiple laboratories participating, continual testing and data flow is assured, since testing can be diverted to alternative sites in the event of an issue at any one site
- An efficient data network was established for the centralized analysis of wastewater sequencing data from throughout the state, with uploads of analyzed data to the state dashboard, CDC and NCBI
- Multiple visualizations were developed to demonstrate detected lineages across the state, within regions, counties, and from individual sewer sheds

## Acknowledgements

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