Shotgun Metagenomics:
A Success Story of the rapid development and implementation of a genomic surveillance method during the 2022 human Monkeypox outbreak

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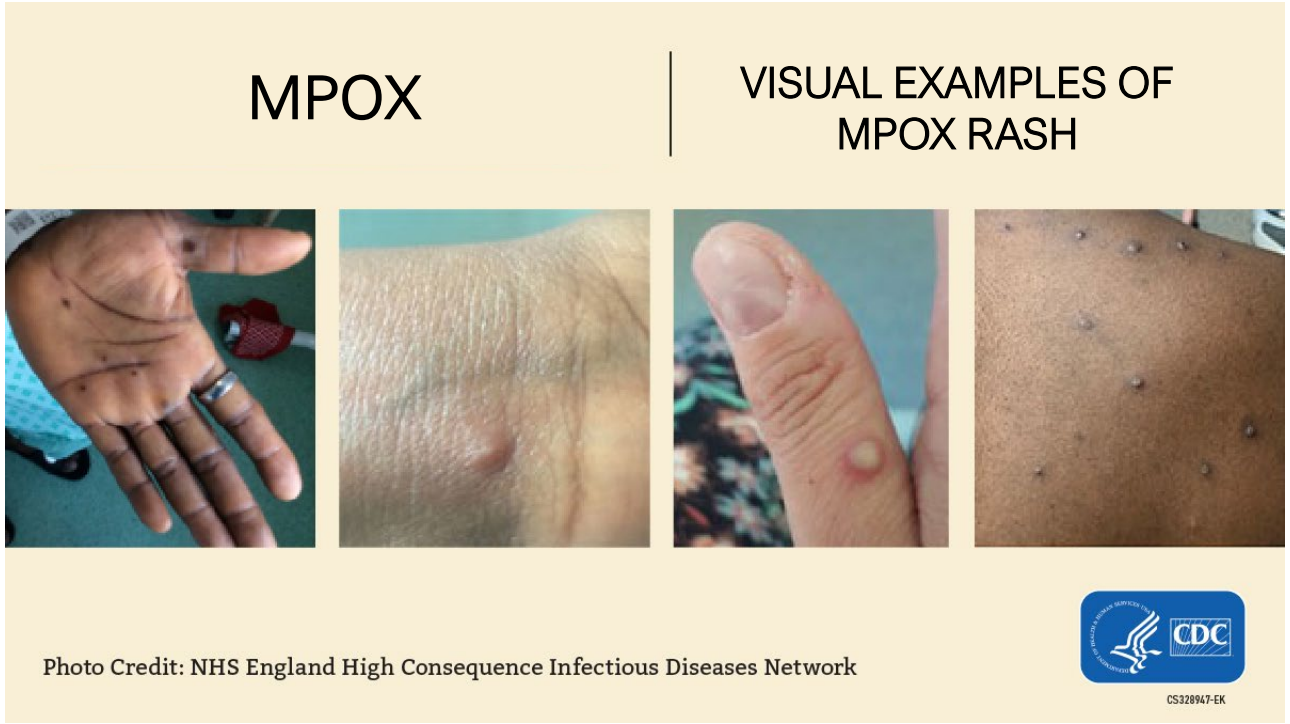
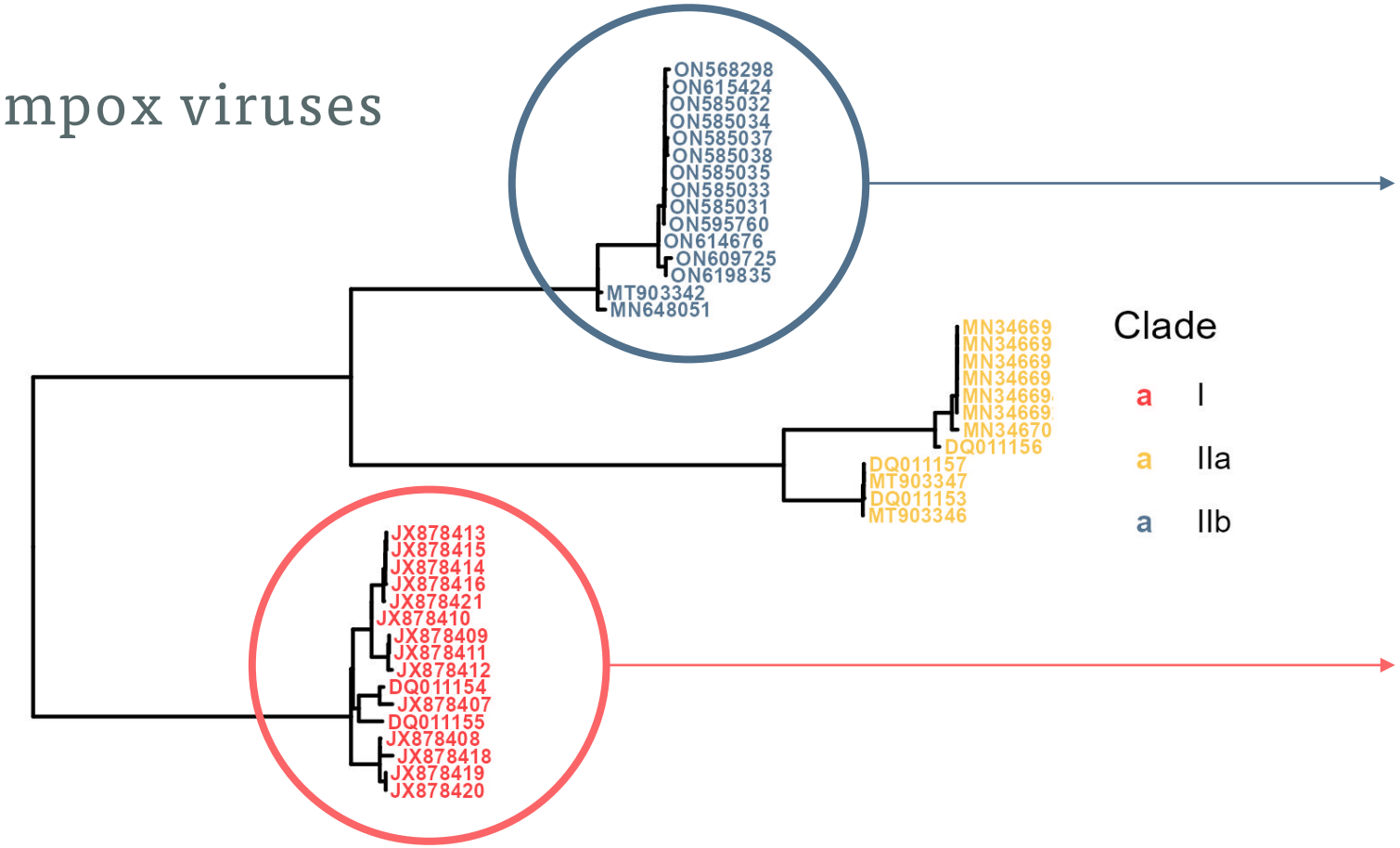
Mpox background

- Potentially life-threatening disease caused by the mpox virus (formerly monkeypox virus) that causes rash lesions and flu-like symptoms.



[1]Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox-A potential threat? A systematic review. PLoS Negl Trop Dis.

Classifying mpox viruses



2022 Outbreak
~0.08% mortality rate[1]
Not select agent!

~10.6% mortality rate
Is select agent!

Species

mpox

[1]Scarpa F, Sanna D, Azzena I, Cossu P, Locci C, Angeletti S, et al. Genetic Variability of the Monkeypox Virus Clade IIb B.1. J Clin Med. 2022;11(21).

Mpox surveillance

Real-time PCR:

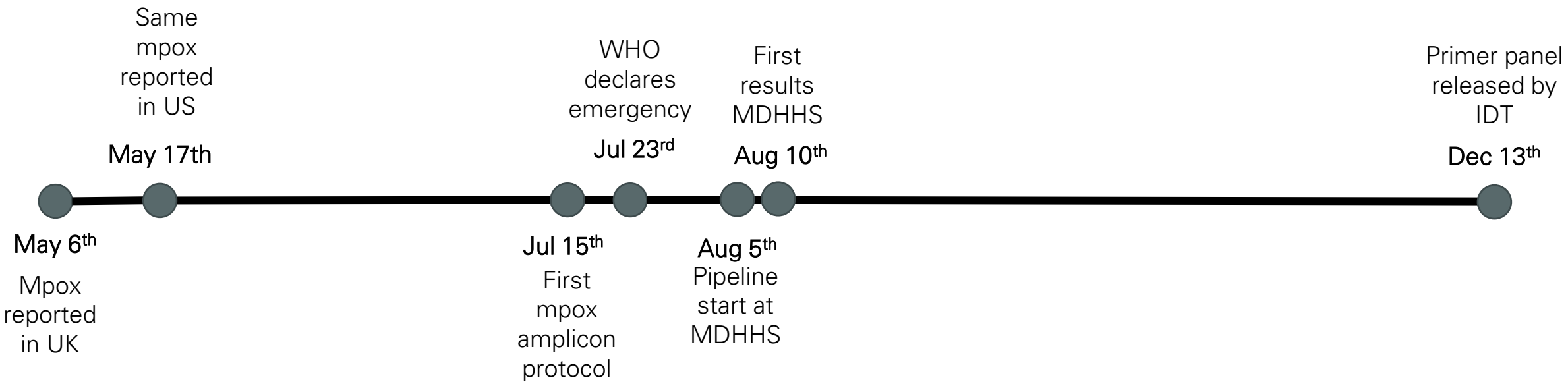
- Non-Variola Orthopoxvirus assay (VAC1)
- MPXV-Specific assay
    - ➢ Primers in terminal regions – prone to mutations

Mpox surveillance

Tiled amplicon sequencing

- High throughput but does not adapt well to evolving viruses.

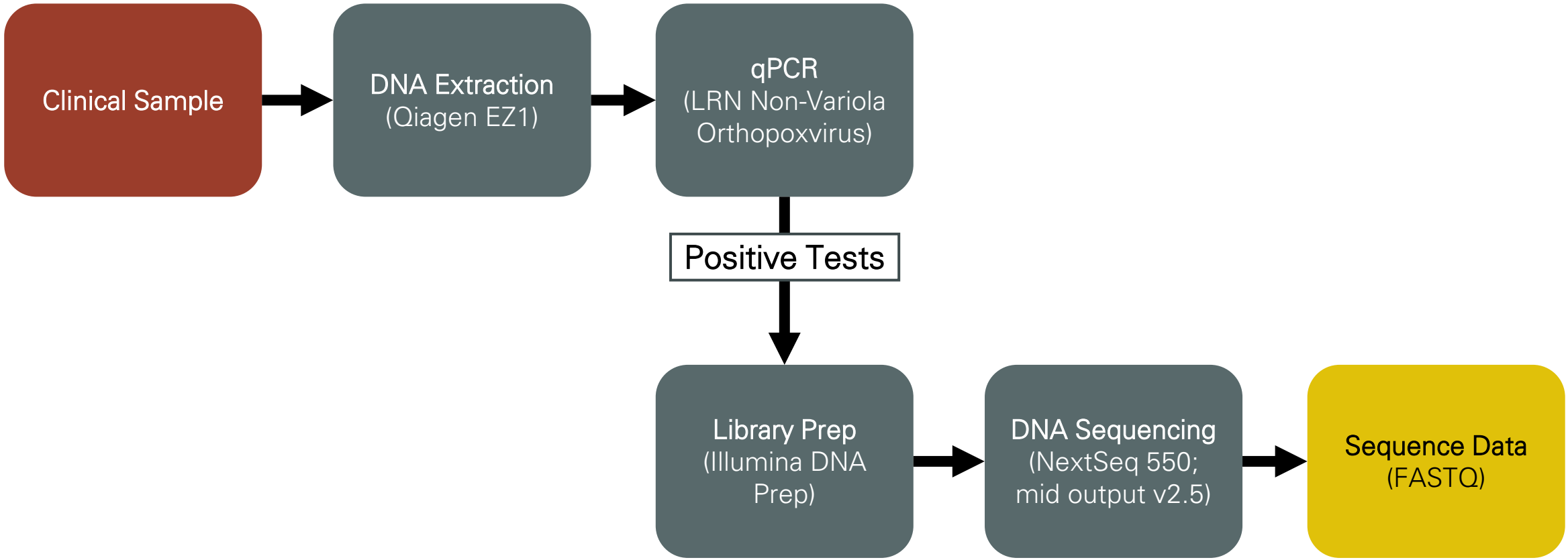
Outbreak and pipeline development timeline



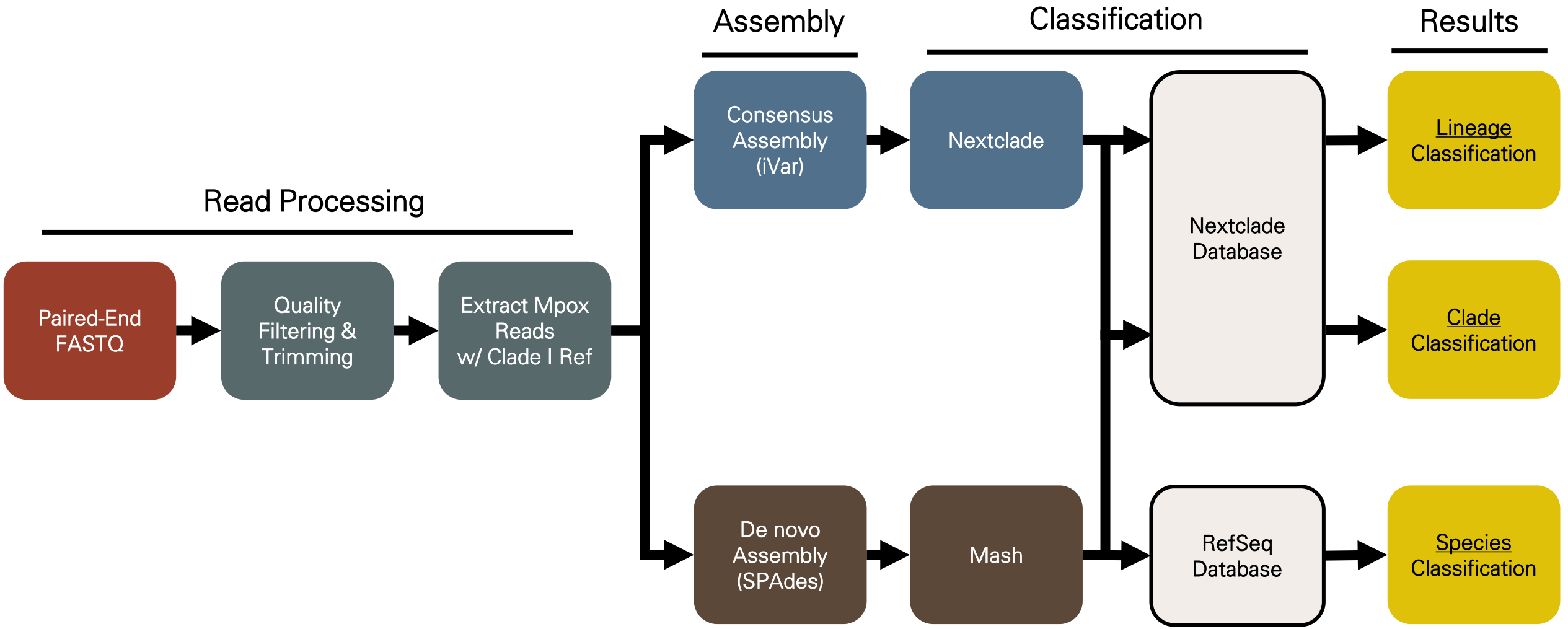
Reasons for selecting shotgun approach:

1. Wanted to explore metagenomic approach for SARS-CoV-2
2. Have the appropriate sequencers
3. Anticipated large workload adopting tiled approach on wet-bench side

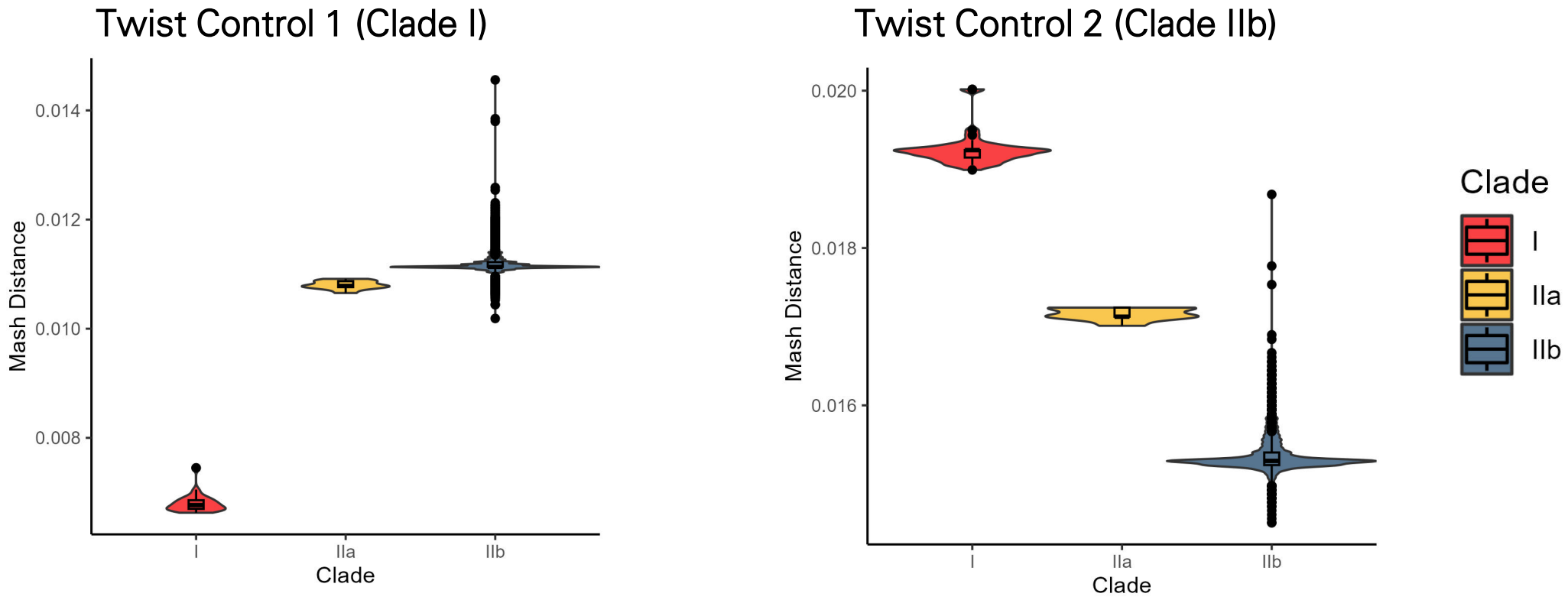
Overview of sequencing process



Overview of bioinformatic pipeline

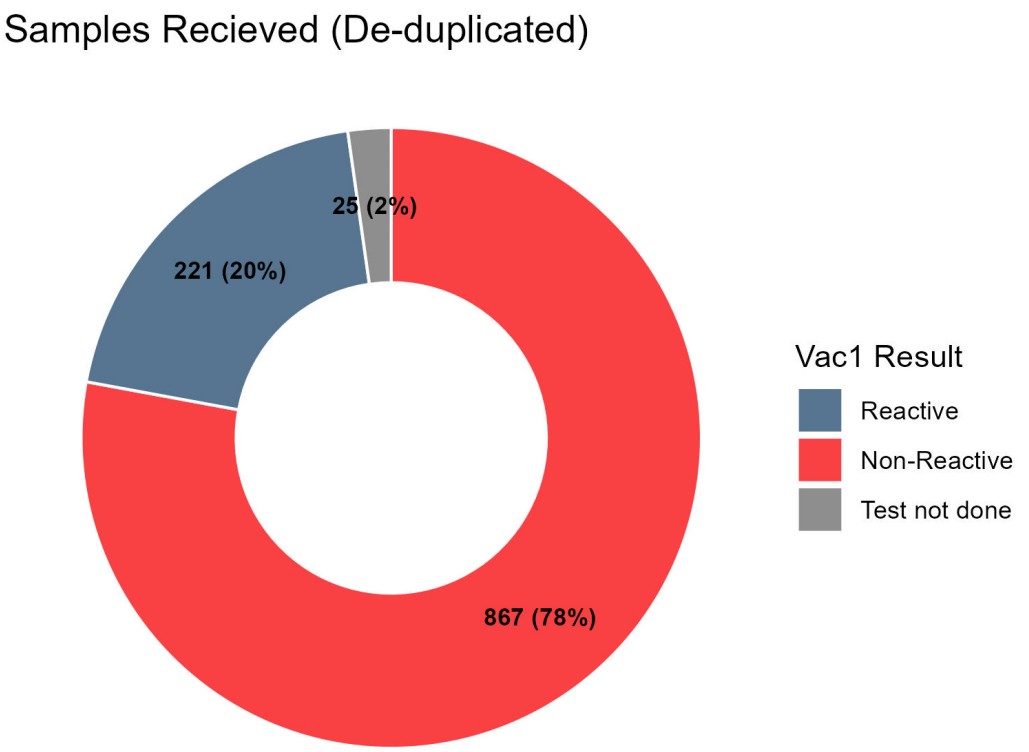


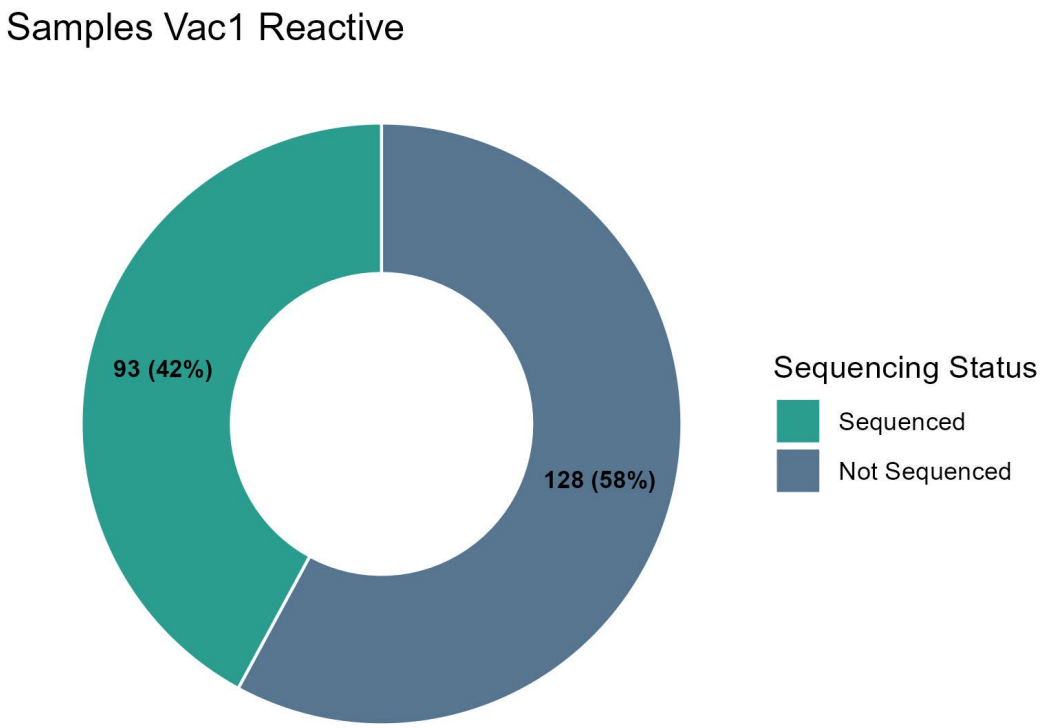
Clade classification using Mash



Mash was fast and effective method for classifying mpox clades!

Sample summary





- Approximately 42% of Vac1 reactive samples were sequenced.
- Those not sequenced typically had high Ct values or pre-dated the pipeline creation.

Sequencing and pipeline metrics

Samples Per Run

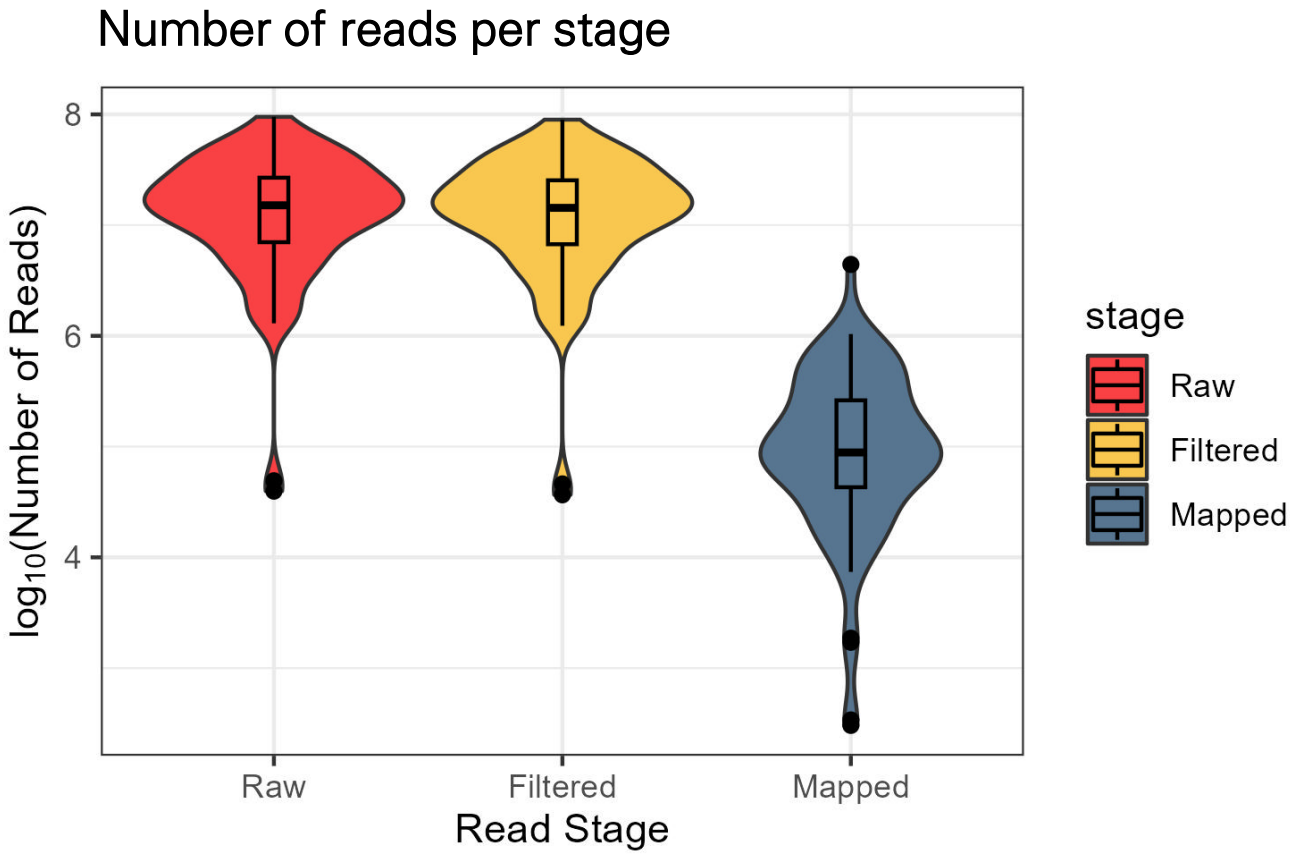
- ~20 samples

Average Read Metrics

- ~19 million high quality reads per sample
- ~1.4 % mapped to the Clade I reference genome

Average Assembly Metrics

- ~86.3% genome fraction
- ~140-165X depth of coverage



Establishing Ct thresholds

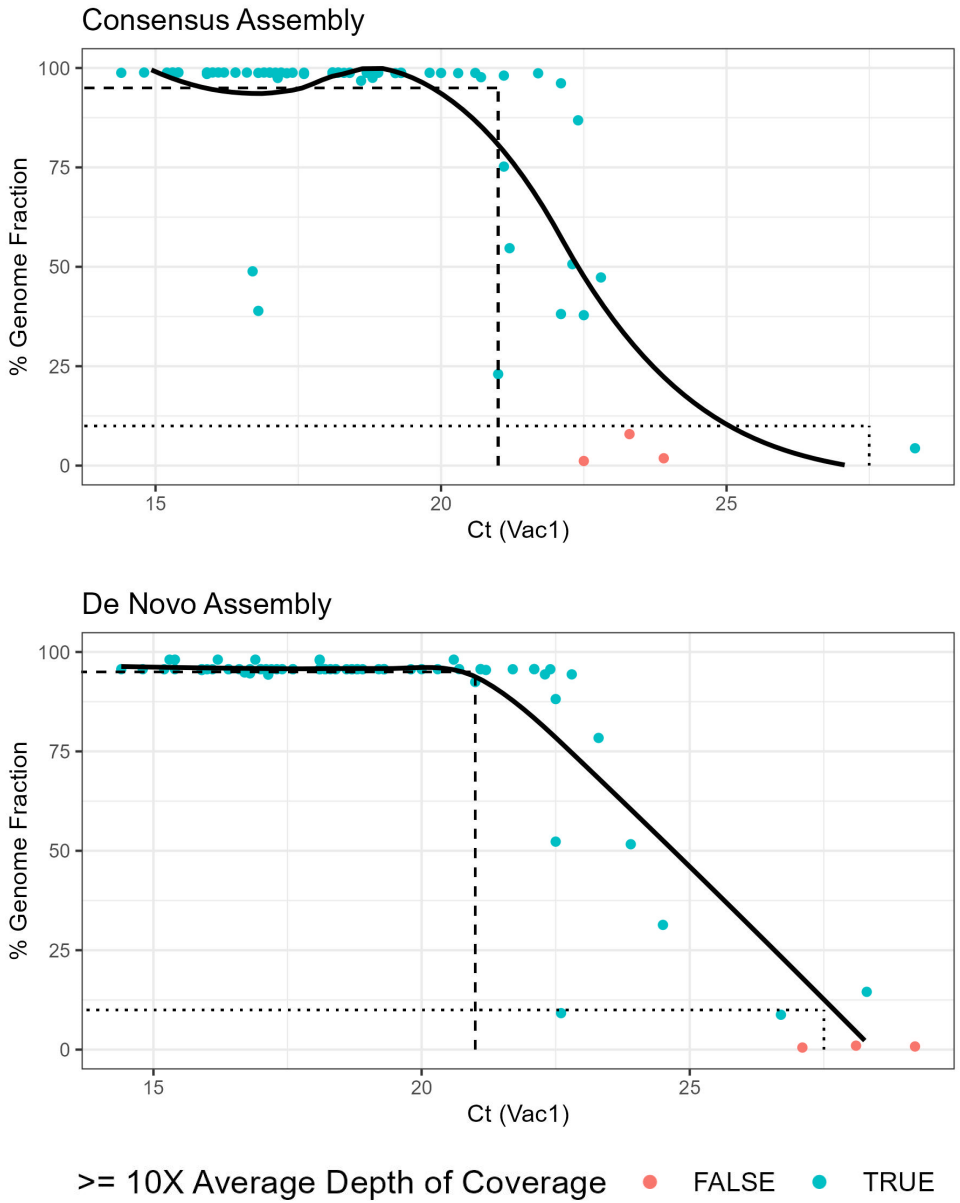
De novo assembly generally resulted in higher genome recovery rates.

Lineage-level:

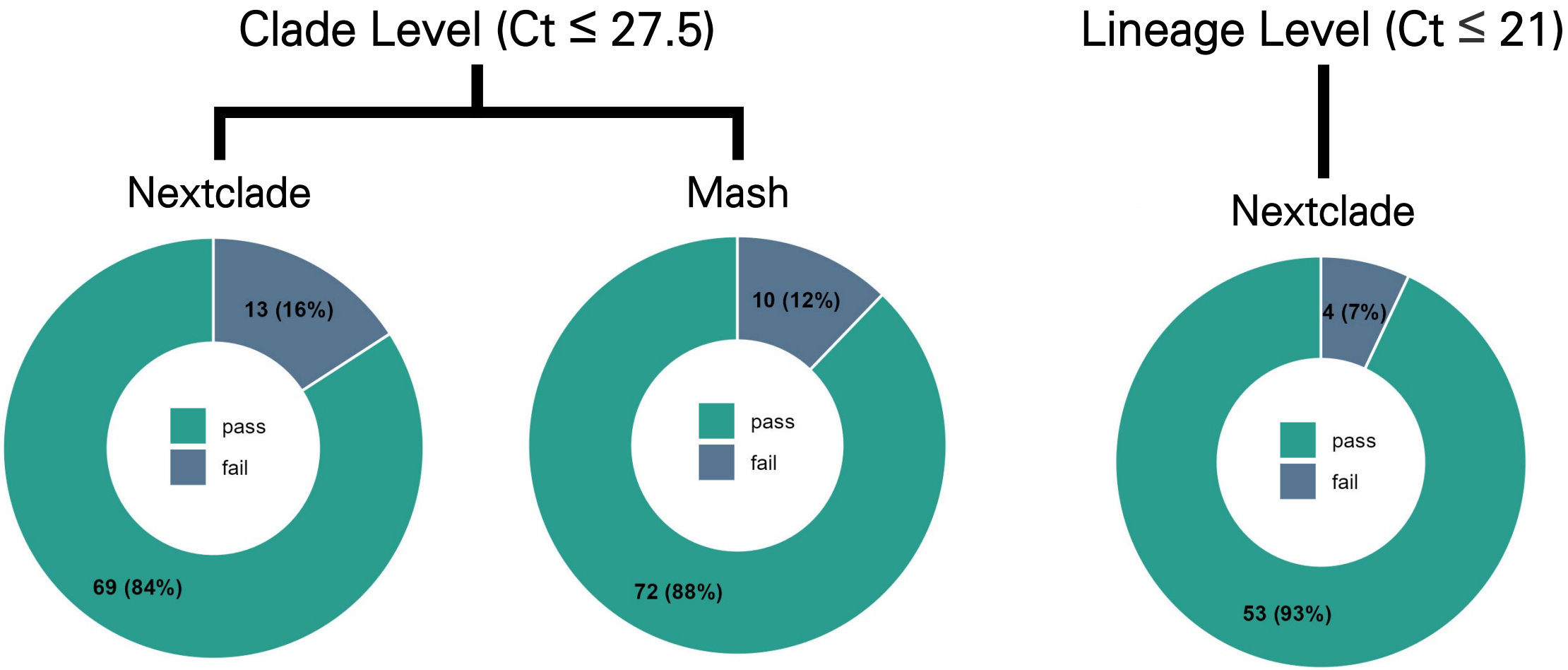
- Target: 95% genome recovery
- Threshold: Ct ≤ 21

Clade-level:

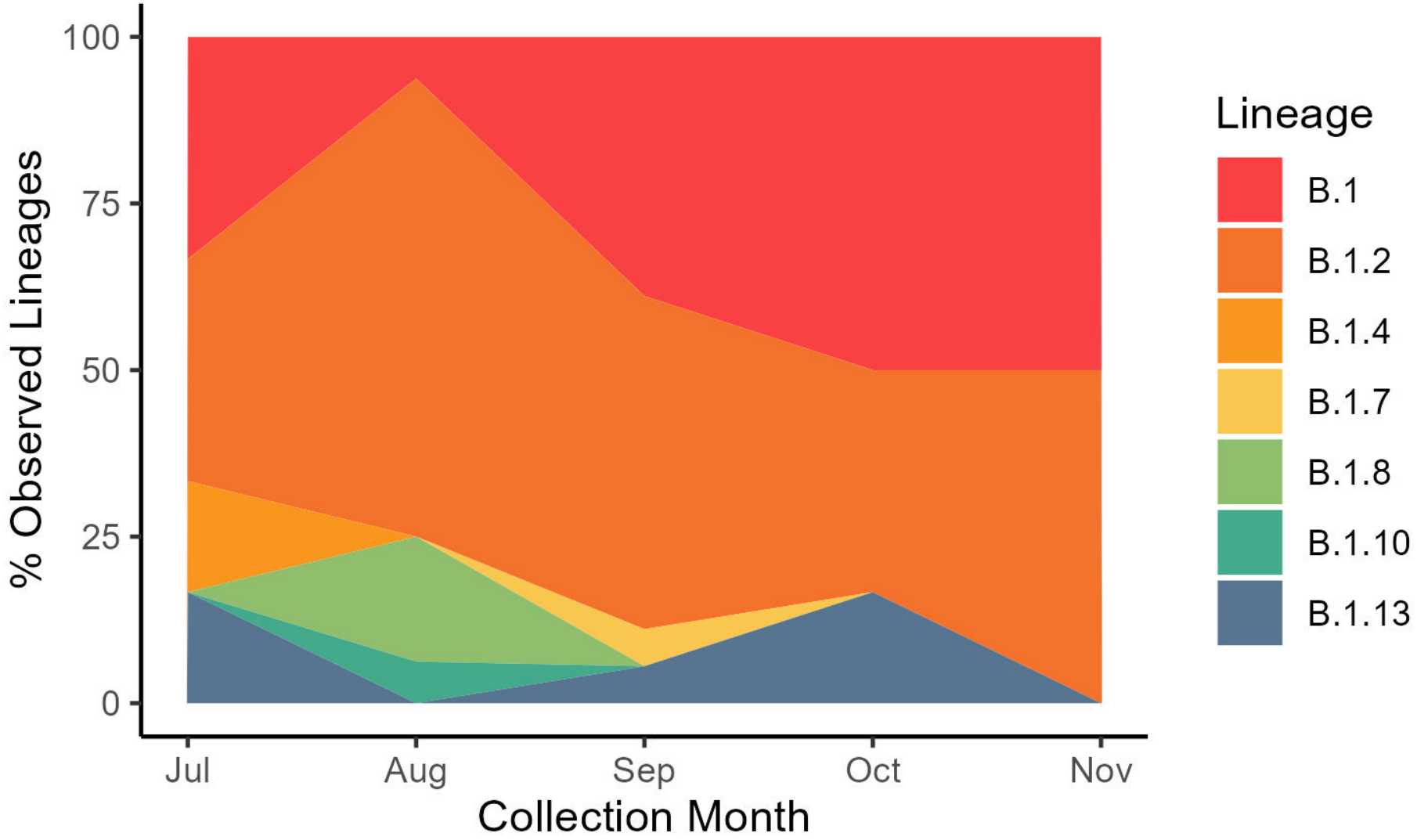
- Target: 10% genome recovery
- Threshold: Ct ≤ 27.5



Classification performance



Further use of lineage data



Summary

- Metagenomic approach allowed for rapid development of a surveillance method during an emerging threat.
- This was only possible because we had the appropriate sequencers to handle the low depth of metagenomic samples.
- Mash is a versatile tool that may be useful for classifying organisms during the early stages of an outbreak.

Next Steps

- Continue to refine metagenomic approach.
- Apply what we learned from mpox to our SARS-CoV-2 pipeline.
- Explore the use of Twist Bioscience controls for validation purposes.

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