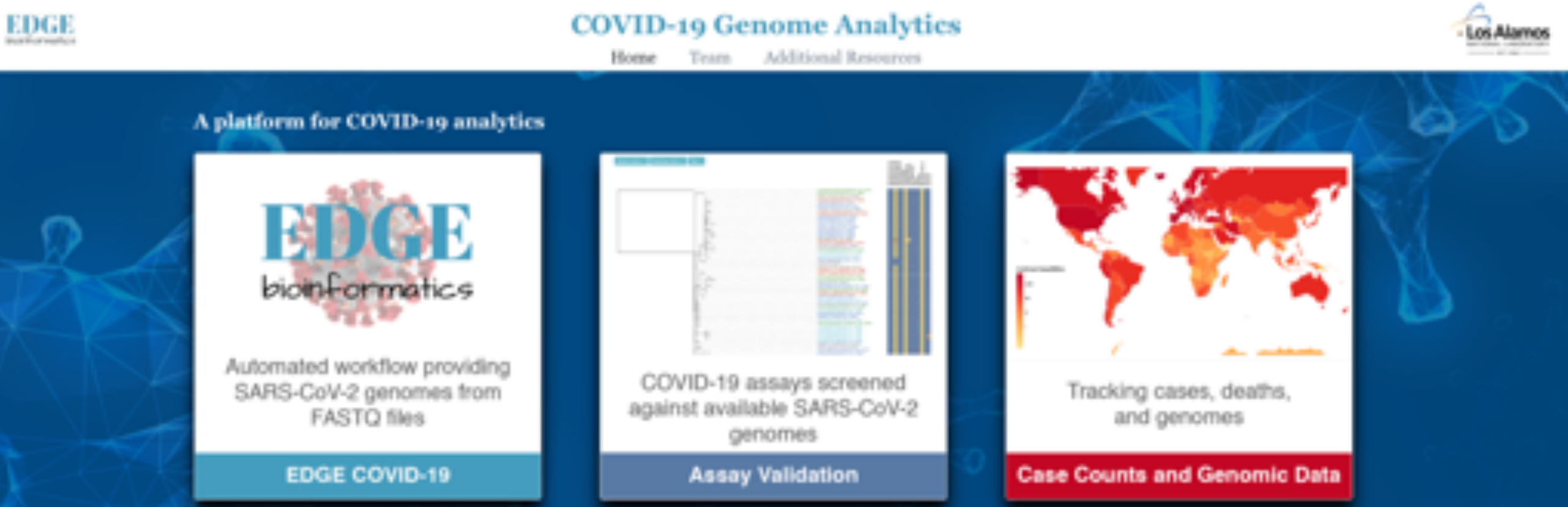


- As genomics is used for biosurveillance of outbreak pathogens, it can help reveal where diagnostic assays may fail – thus we advocate for:
- 1) robust genomic data to be continually generated (even prior to outbreaks) to inform us of pathogen presence and diversity/evolution; 2) continuous tracking of mutations that may affect diagnostic assays and therapeutic targets; 3) automated re-design of assays and suggestion of alternative targets for therapeutic design

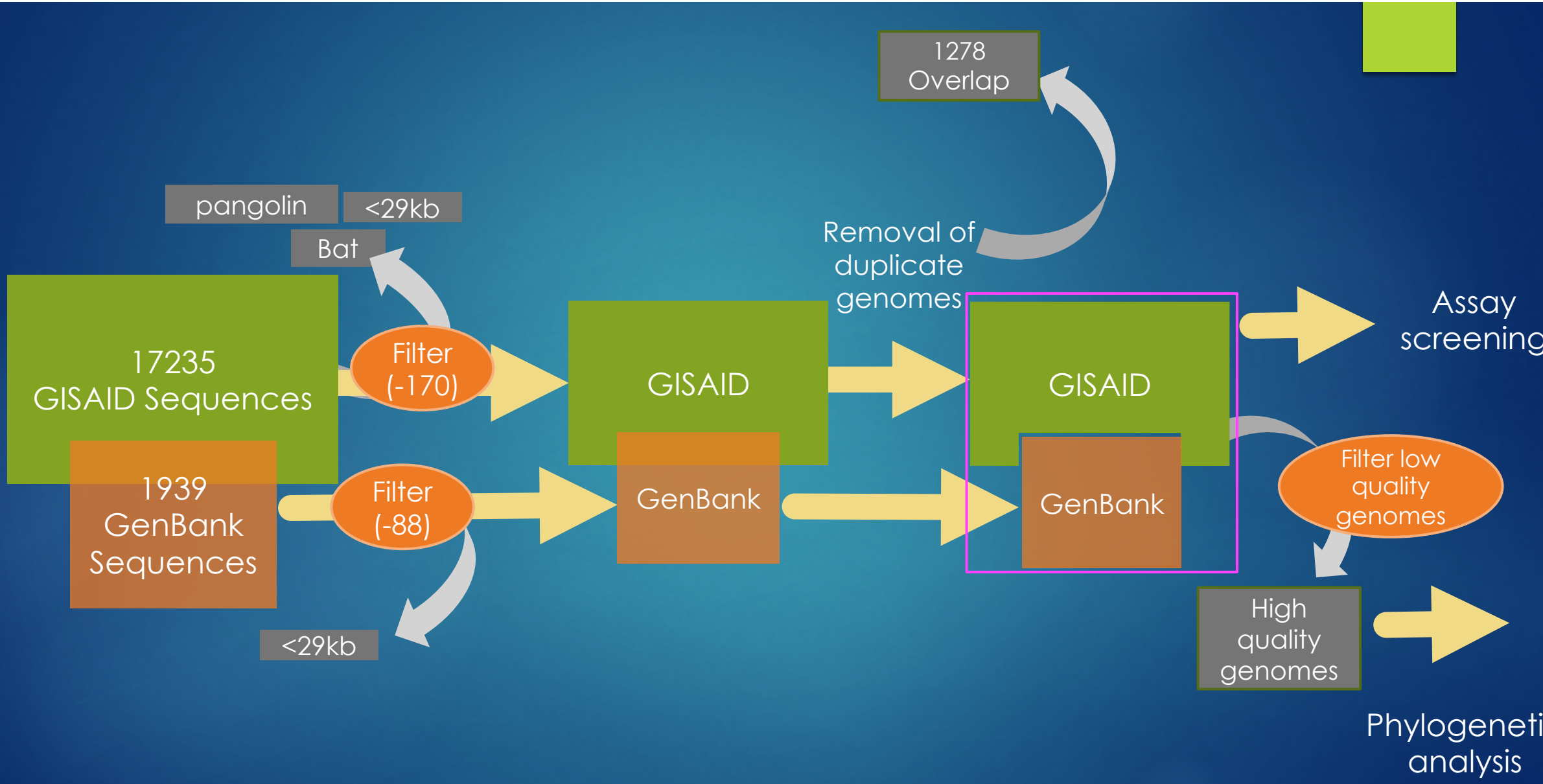
Assay Validation Method

- Our team has developed a web-based application to monitor existing PCR assays that are currently in use around the world
  - Assays from US CDC, China CDC, Charité, HKU, Japan NIID, others
  - SARS-CoV-2 genomes downloaded from GenBank and GISAID daily
- ThermonucleotideBLAST: assesses likelihood of detection success using known thermodynamic parameters (delta G and Tm)
- Visualization
  - Phylogenetic tree created with PhaME and rendered with PhyD3
  - Heatmap of thermodynamic mismatches
  - Table of mismatches and recall
  - Top ranked assays by recall

Workflow

- Genome sequences downloaded daily from GenBank and GISAID
  - GenBank: 1624, GISAID: 15498 (as of 2020-04-30 08:36)
  - GenBank: 1939, GISAID: 17235 (as of May 7th 3PM)
- Filter out bat, pangolin, and sequences shorter than 29 kb
  - GenBank: 86 (+2) removed. GISAID: 170 removed
- Remove overlapping sequences
  - Overlap: 1227 (+51). Final database total: 15639 (+1999)
- Each assay validated against every sequence, producing thermodynamic mismatches and recall value (True positive rate, sensitivity)
  - $Recall = \frac{True\ Positives}{True\ Positives + False\ Negatives}$
- Additional low quality genome removal for phylogenetic analysis
- Render visualizations

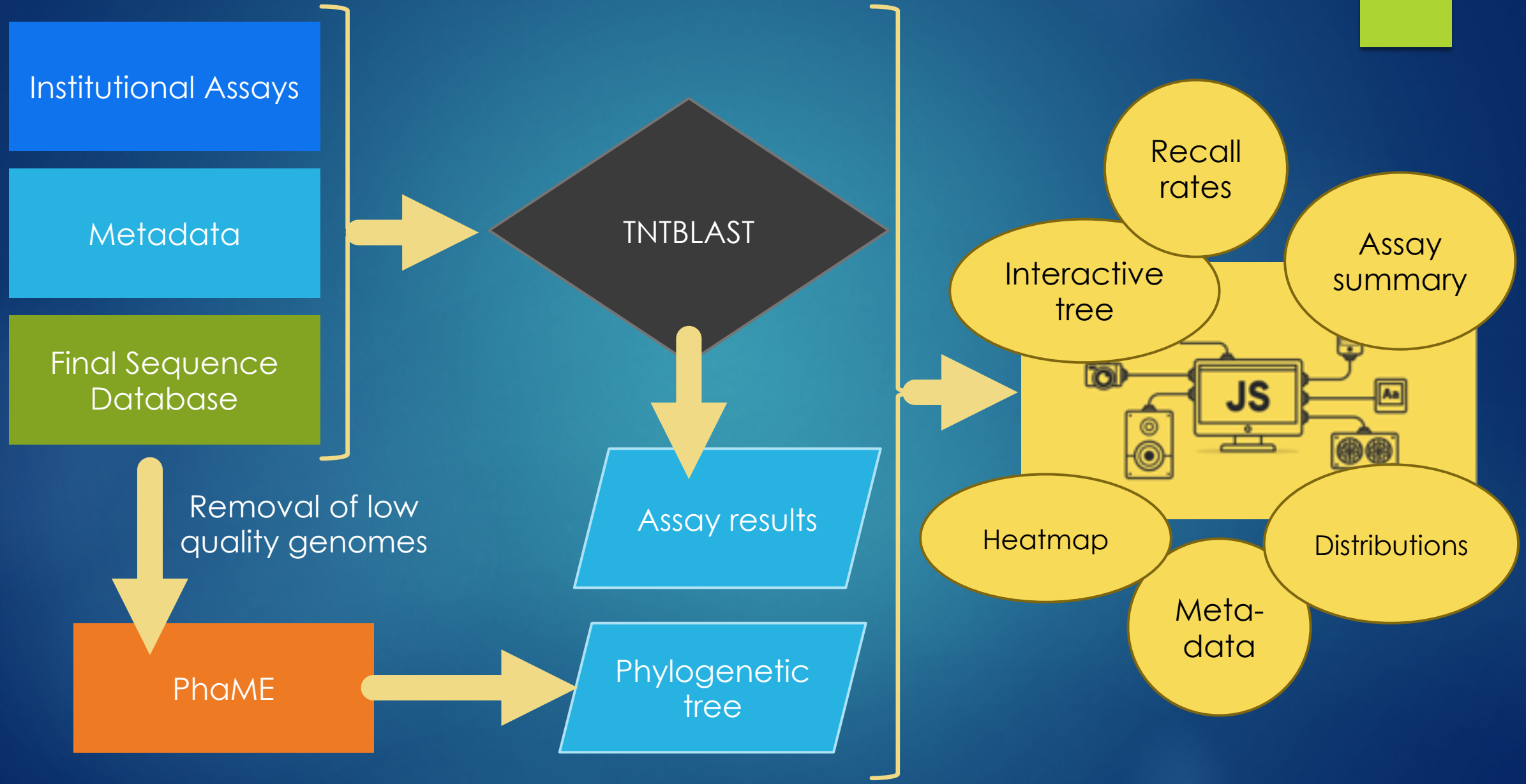
COVID-19 Genome Analytics



EDGE bioinformatics

COVID-19 Genome Analytics

Los Alamos NATIONAL LABORATORY EST. 1943

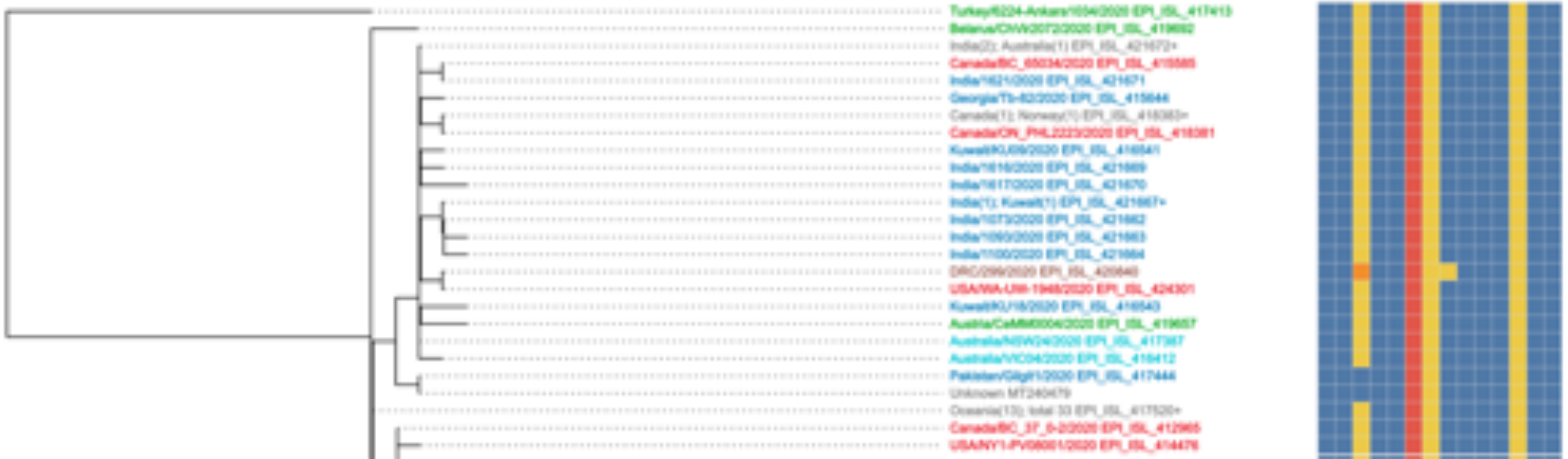


Assay Validation Dashboard

- Top ranked assays by recall
  - Institut Pasteur: Two assays against RdRP (nCoV_IP2, nCoV_IP4)
  - Charité, Berlin: Two RdRP, one Sarbeco screening assay against Envelope
  - Hong Kong University: Assay targeting ORF1b

Tree/heatmap view of assay results

- Heatmap with tree can show evolutionary patterns of mismatches
  - Charité: probe with two mms (P1), reverse primer with one mm (P2)
    - P2 designed originally for SARS and bat-SARS coronaviruses
  - USA CDC: Mismatch in forward primer of N3



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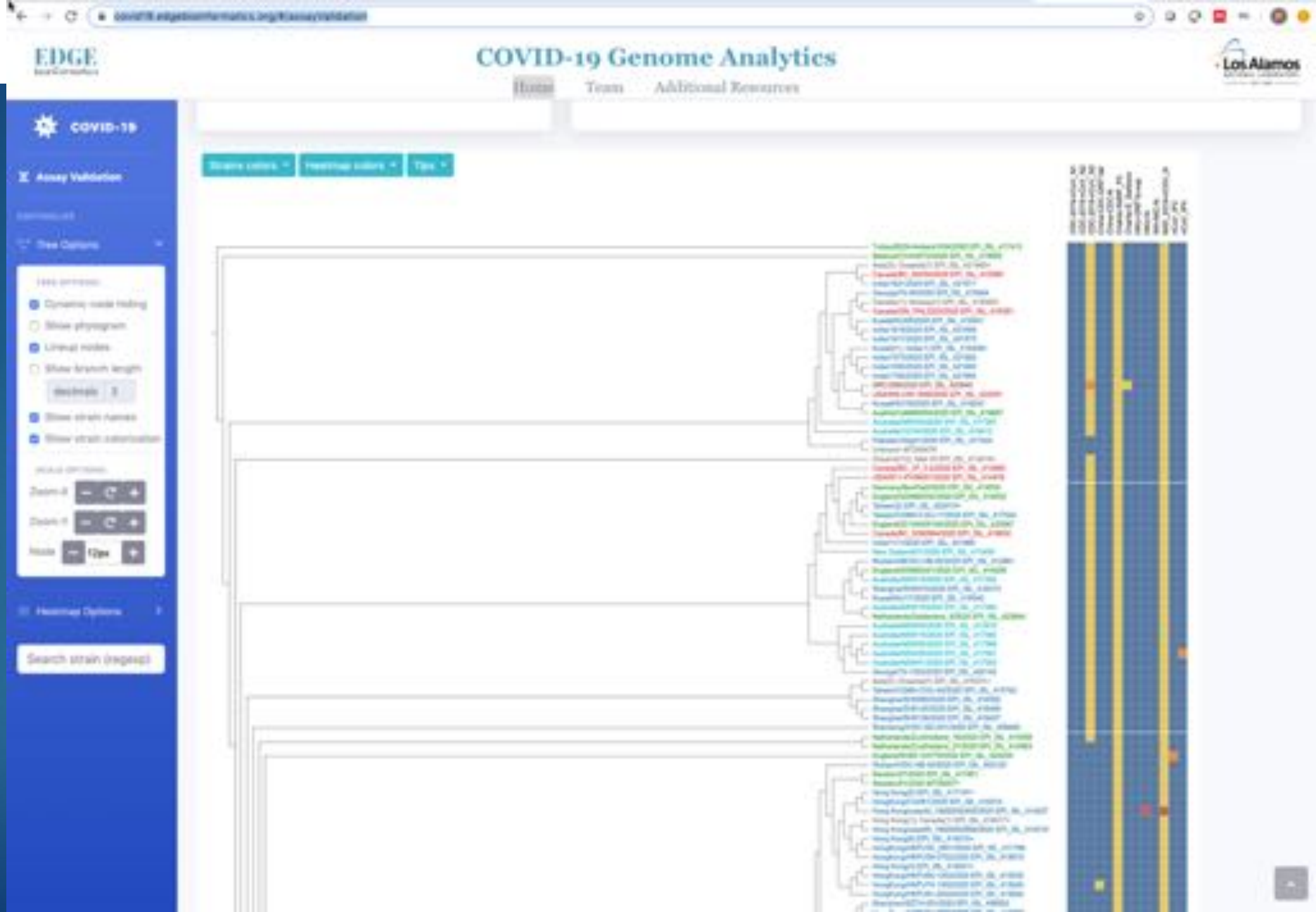
Known issues with CDC N3 assay

Several clades show potential issues



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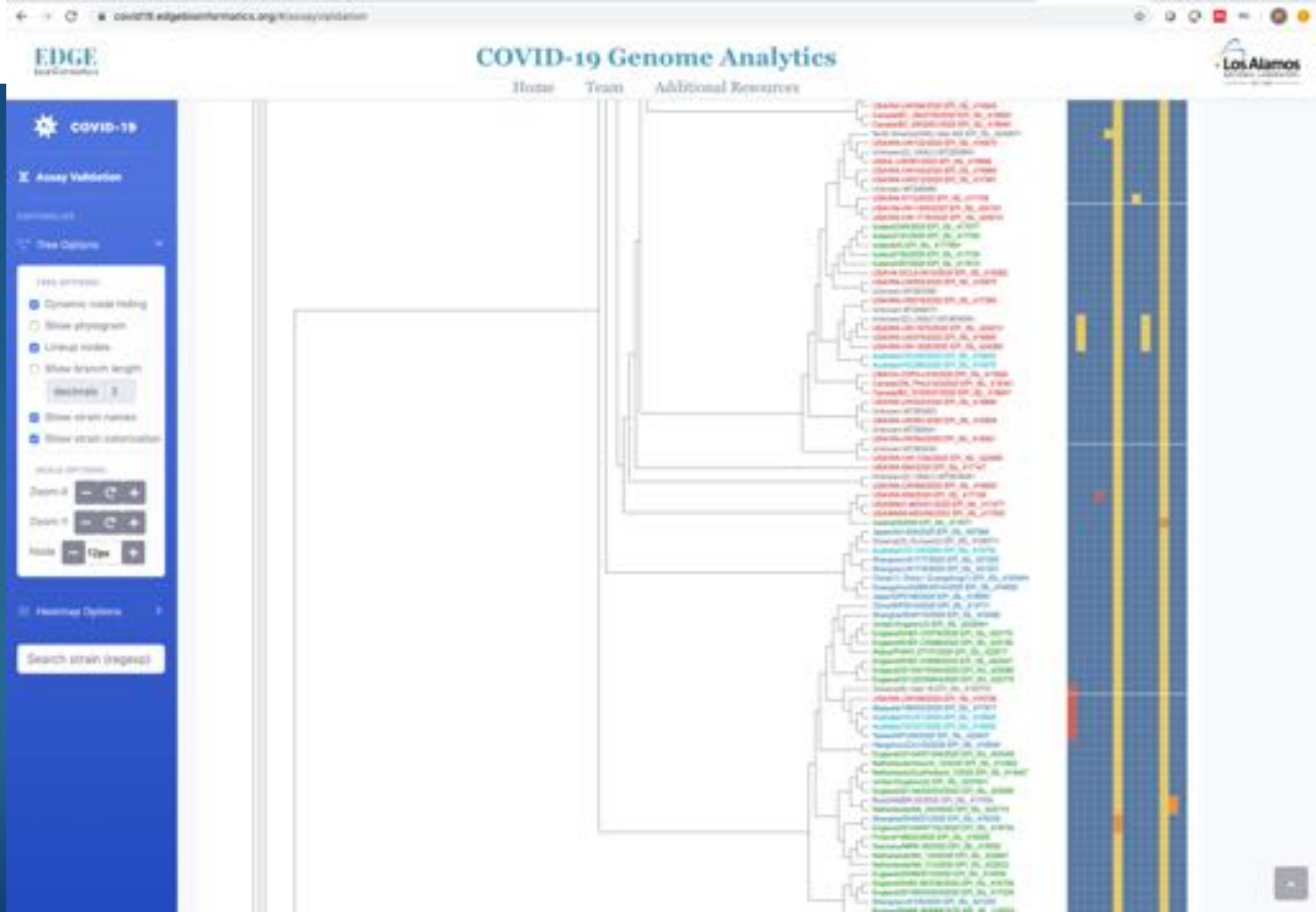
Potential issues for some newer clades with CDC N2 and N1 assays

Including some US isolates



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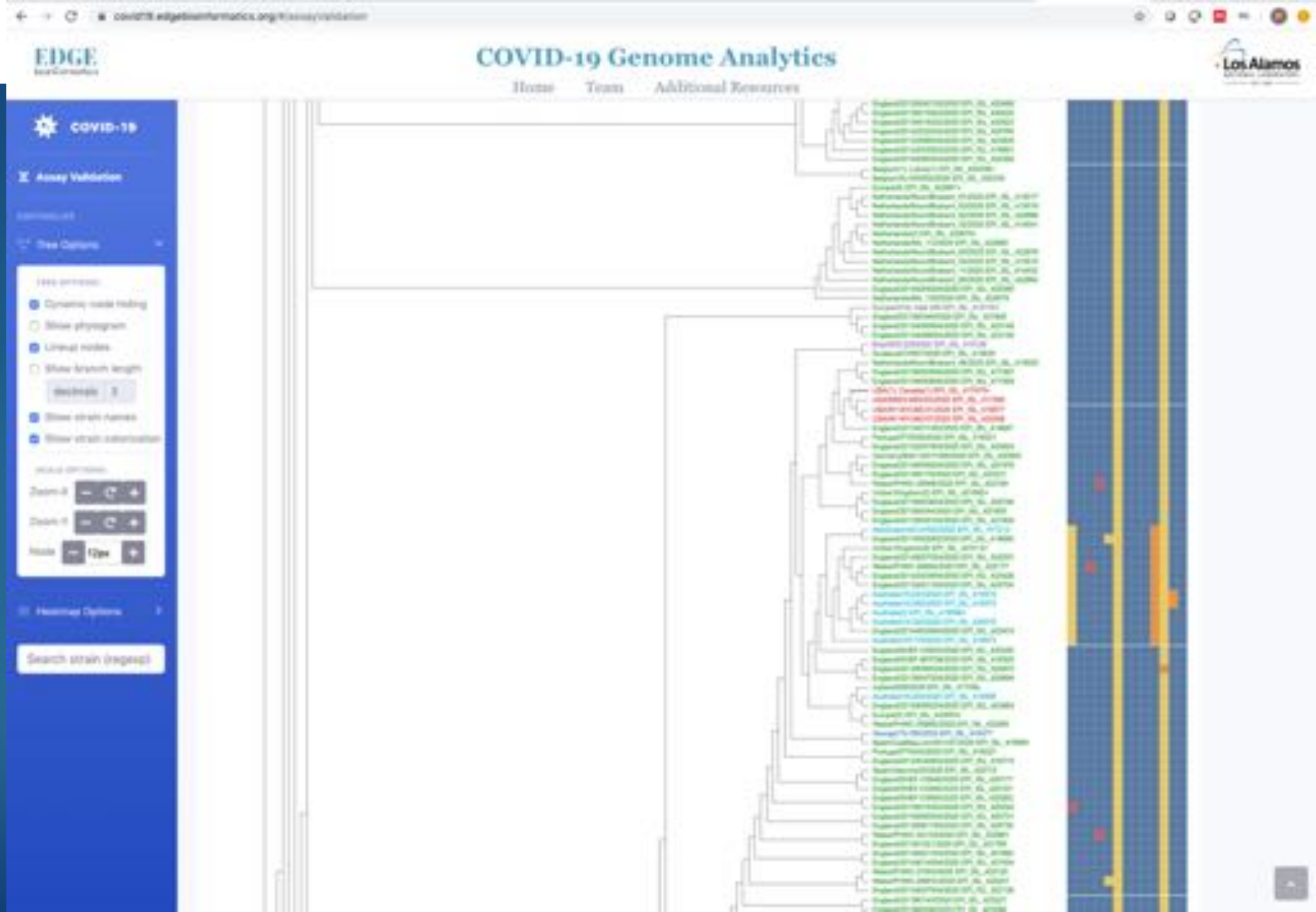
An additional clade shows potential issues with CDC N1

Includes strains from Australia/ England/ New Zealand



Remaining issues

- Display/interaction/presentation
- Genome quality assessment
  - *identified several strains already with errors