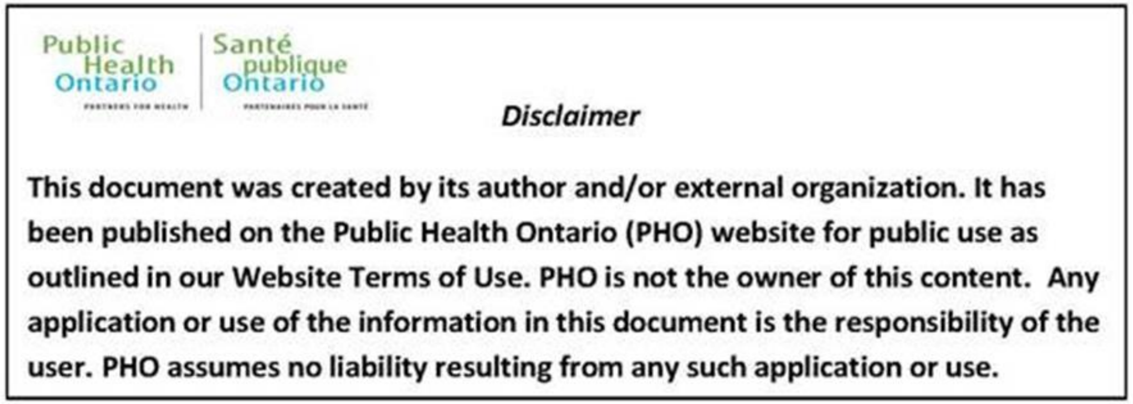
To view an archived recording of this presentation please click the following link: https://youtu.be/FfxrGi-ZL5c

Please scroll down this file to view a copy of the slides from the session.



BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Implementation and Application of Next Generation Sequencing of SARS-CoV-2 in BC

Linda Hoang, MSc, MD, FRCPC, DTM&H
Program Head, Bacteriology & Mycology Laboratory
BCCDC Public Health Microbiology & Reference Laboratory
Associate Medical Director, BCCDC Public Health Laboratory
Clinical Associate Professor, Dept of Pathology and Lab Medicine

Public Health Ontario Rounds
Dec 16, 2021

CONFLICT OF INTEREST

None to Declare

Provincial Health
Services Authority
Province-wide solutions.
Better health.

UBC

BC Centre for Disease Control

Objectives

1. Identify the role of SARS-CoV-2 WGS in the different phases of the COVID-19 pandemic in BC

2. Provide examples of public health applications of SARS-CoV-2 WGS

3. Understand the approach to operationalization of WGS in a Public Health Laboratory

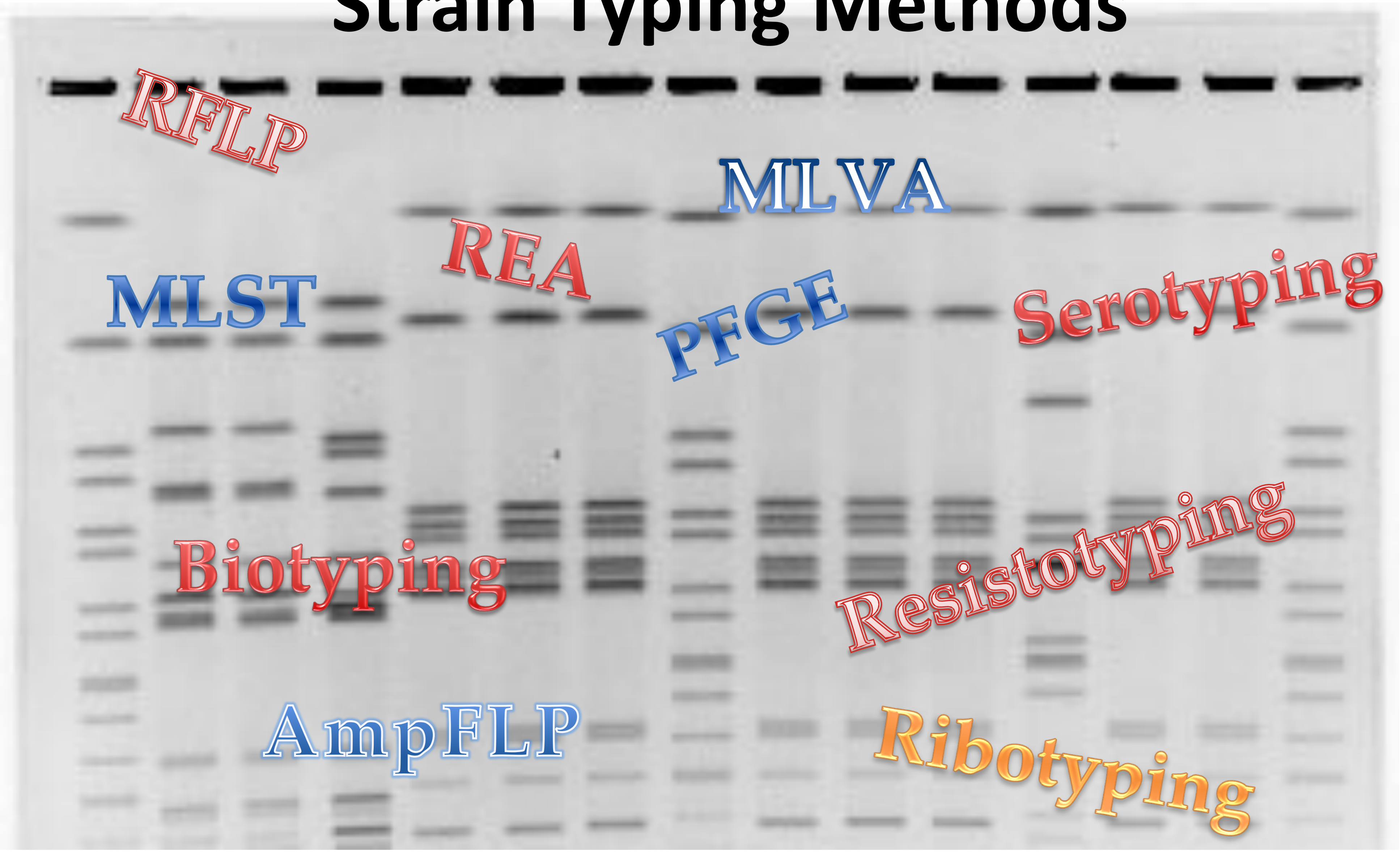
Once Upon a Time

…..before COVID-19

BCCDC Public Health Laboratory (PHL)

WGS limited role and capacity prior to the Pandemic

Strain Typing Methods



RFLP

MLVA

REA

MLST

PFGE

Serotyping

Biotyping

Resistotyping

AmpFLP

Ribotyping

Why "Fingerprint"

Outbreak response: Confirm **epidemiological relationships** in the spread of disease

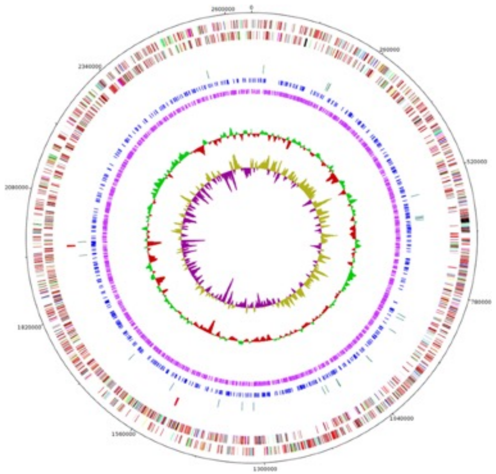
Cluster detection: Provide epidemiological **hypotheses** about epi relationships in the absence of epi data

Surveillance: Describe the **distribution** of pathogen types and identification of potential affecting factors

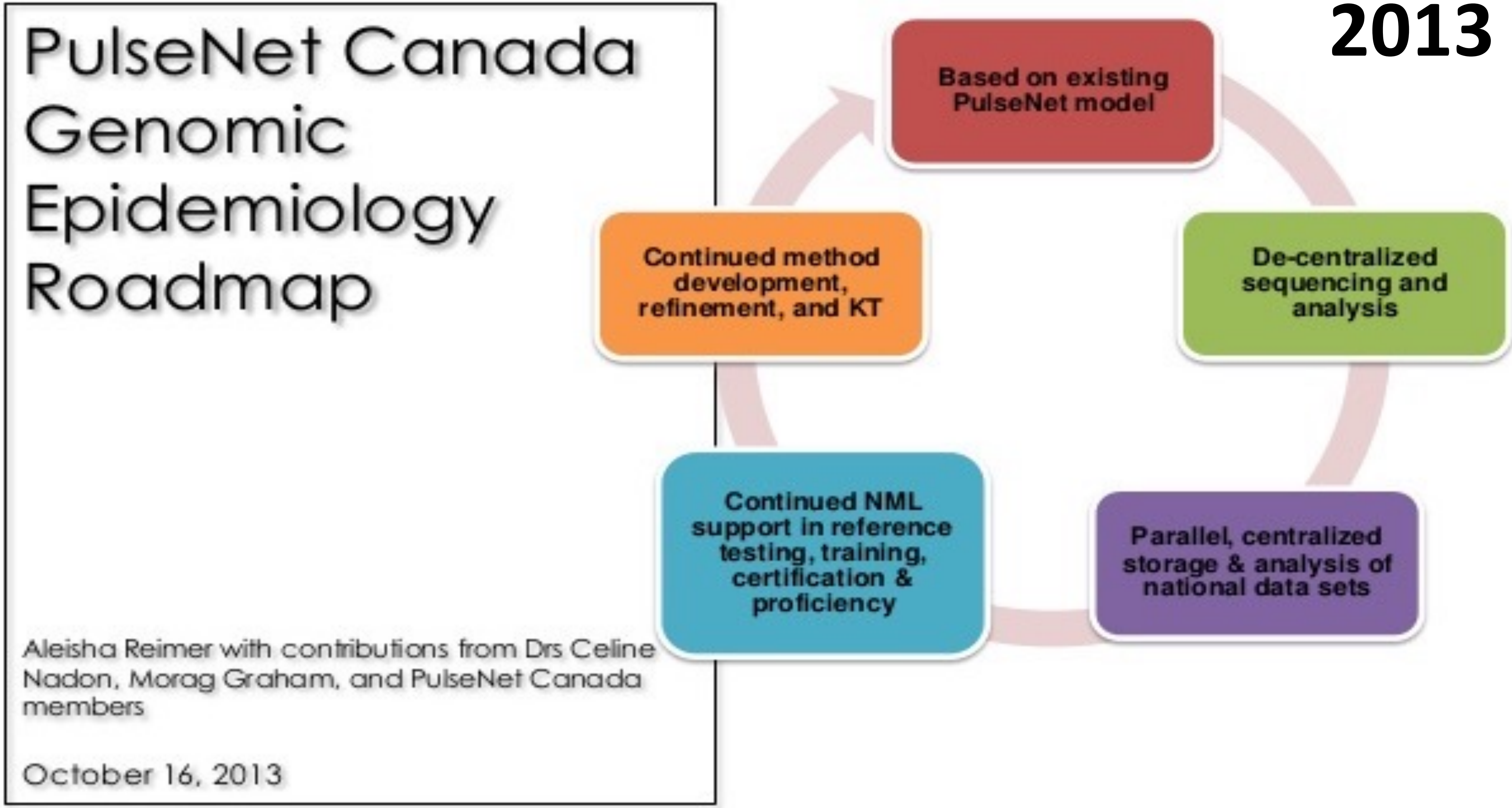
Attributes of typing methods

- Discriminatory power
- Rapid
- Inexpensive
- Easy to perform
- Highly reproducible
- Standardized nomenclature

Next generation sequencing



2013



PulseNet Canada Genomic Epidemiology Roadmap

Aleisha Reimer with contributions from Drs Celine Nadon, Morag Graham, and PulseNet Canada members

October 16, 2013

Based on existing PulseNet model

De-centralized sequencing and analysis

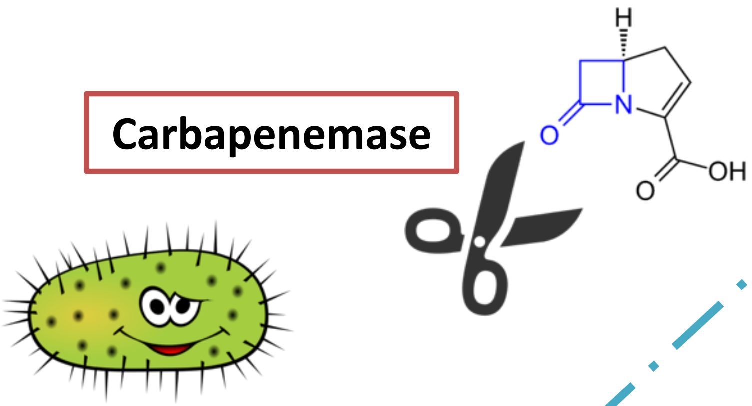
Parallel, centralized storage & analysis of national data sets

Continued NML support in reference testing, training, certification & proficiency

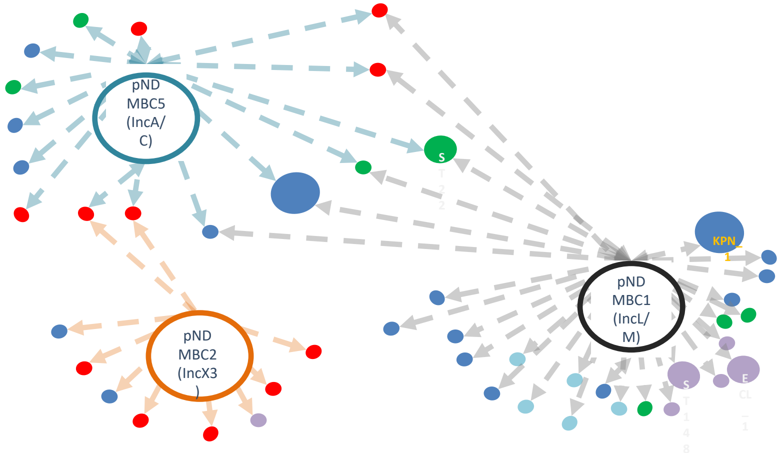
Continued method development, refinement, and KT

Emergence of Carbapenemase-Producing Organisms (CPO) in 2008

Carbapenemase

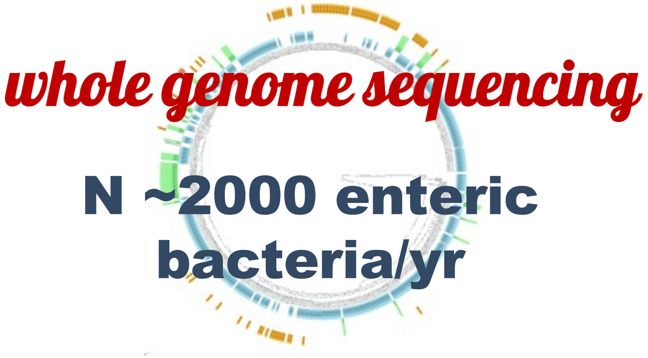


Enterobacterieaceae and other Gram Negative Bacteria



Clonal and plasmid transmissions

PFGE to WGS



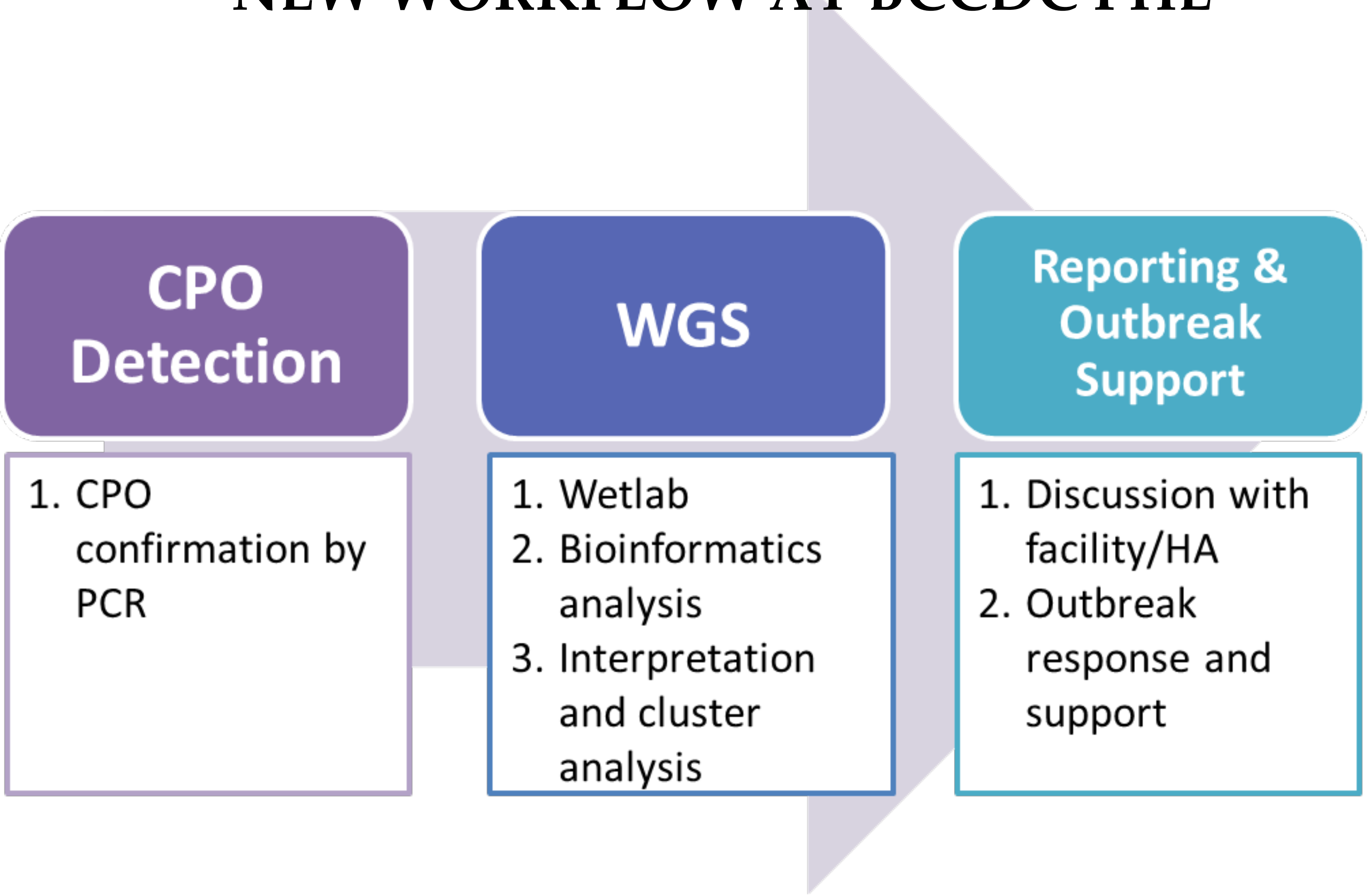
whole genome sequencing

N ~2000 enteric bacteria/yr

- New skillsets
- New technologies and infrastructure
- Higher discriminatory power, greater demand
- Regular connections with Public Health for review and discussions
- Nomenclature standardization

2008 - 2019

NEW WORKFLOW AT BCCDC PHL



CPO Detection

1. CPO confirmation by PCR

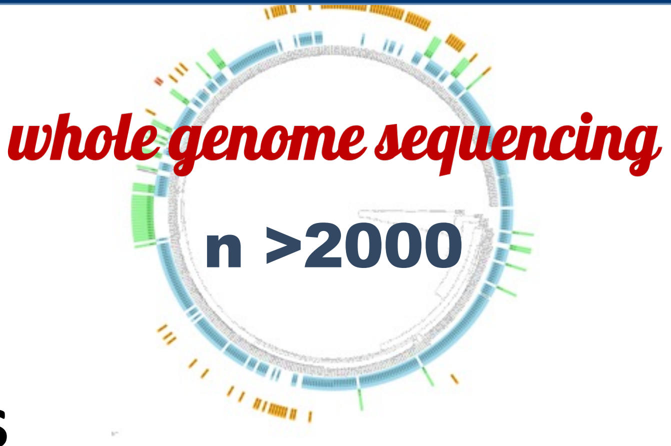
WGS

1. Wetlab
2. Bioinformatics analysis
3. Interpretation and cluster analysis

Reporting & Outbreak Support

1. Discussion with facility/HA
2. Outbreak response and support

CPO WGS



- **Genomics complexity**
- **Development of WGS pipelines**
- **Need for lab information management and integration tools**
- **Nomenclature standardization**
- **Collaboration with HA facilities and public health**
- **Integrated data access from HA level → PLOVER**

Plover

Public health Lab Operations Data Viewer and Reporter

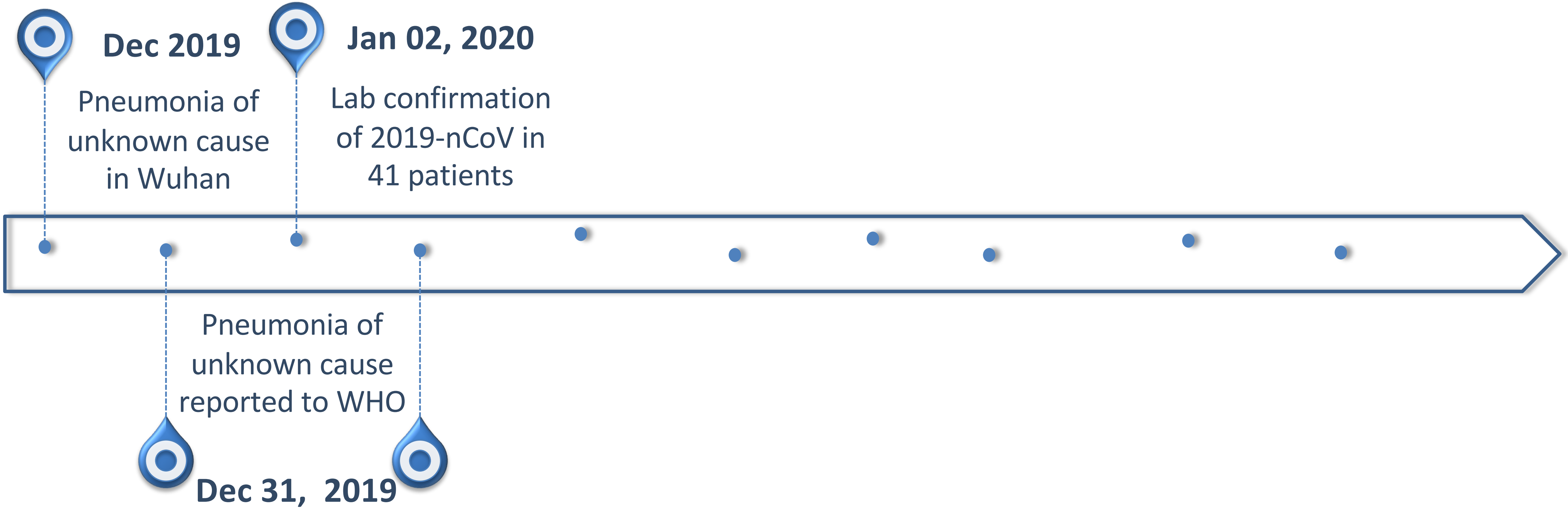
PHL Capacity 2019

- ~24 samples per week
  - PulseNet Enterics samples
  - Carbapenemase Producing Organisms
- Grant funded resources
- Limited operational capacity
- Limited bioinformatics capacity
- Limited data integration capabilities
- Limited data interpretation and reporting capabilities

Ongoing WGS research and collaboration

The Early Days of the Pandemic

Timeline of SARS-CoV-2 Assay Development in BC



Dec 2019

Pneumonia of unknown cause in Wuhan

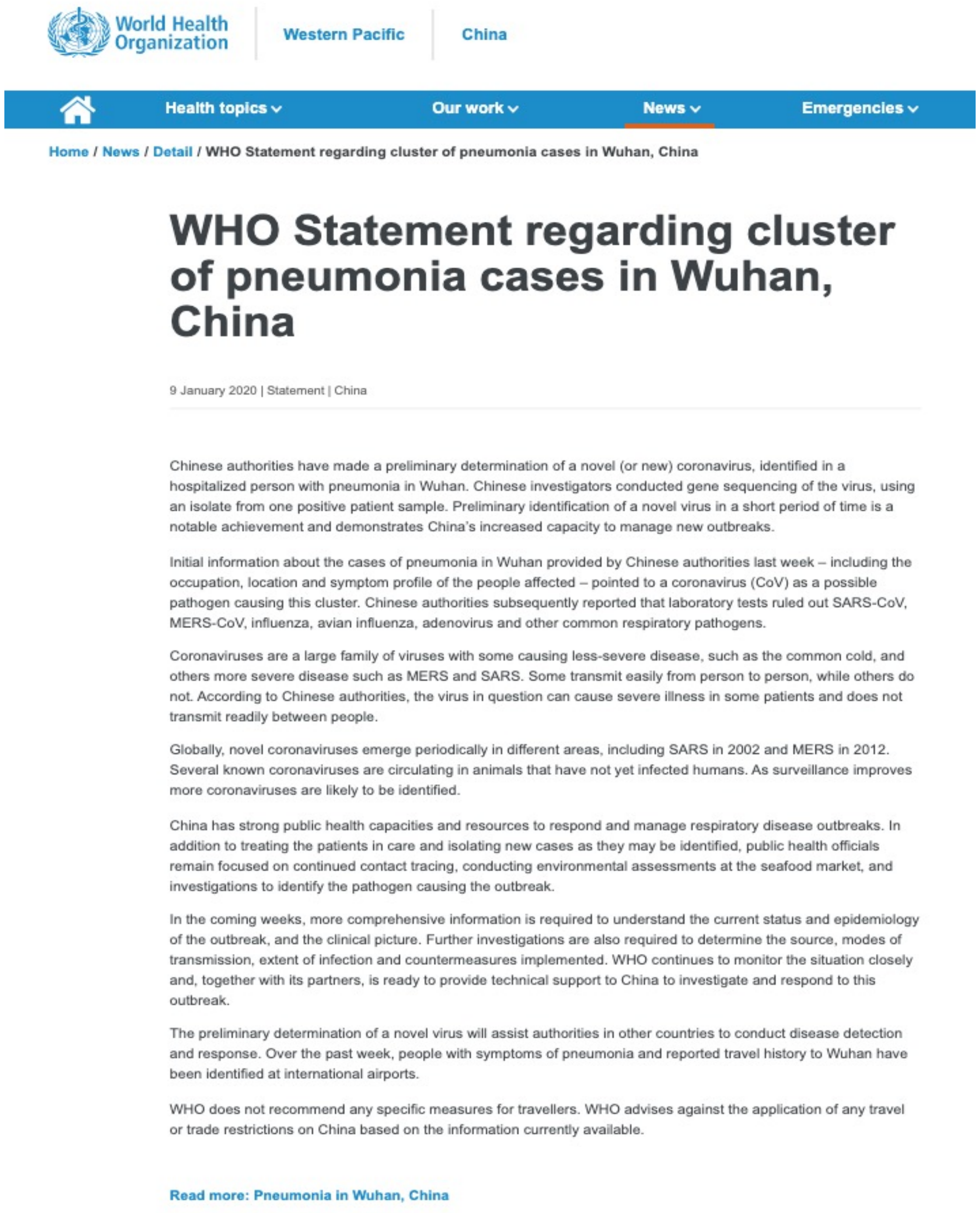
Dec 31, 2019

Pneumonia of unknown cause reported to WHO

Jan 02, 2020

Lab confirmation of 2019-nCoV in 41 patients

January 9, 2020



WHO Statement regarding cluster of pneumonia cases in Wuhan, China

9 January 2020 | Statement | China

Chinese authorities have made a preliminary determination of a novel (or new) coronavirus, identified in a hospitalized person with pneumonia in Wuhan. Chinese investigators conducted gene sequencing of the virus, using an isolate from one positive patient sample. Preliminary identification of a novel virus in a short period of time is a notable achievement and demonstrates China's increased capacity to manage new outbreaks.

Initial information about the cases of pneumonia in Wuhan provided by Chinese authorities last week – including the occupation, location and symptom profile of the people affected – pointed to a coronavirus (CoV) as a possible pathogen causing this cluster. Chinese authorities subsequently reported that laboratory tests ruled out SARS-CoV, MERS-CoV, influenza, avian influenza, adenovirus and other common respiratory pathogens.

Coronaviruses are a large family of viruses with some causing less-severe disease, such as the common cold, and others more severe disease such as MERS and SARS. Some transmit easily from person to person, while others do not. According to Chinese authorities, the virus in question can cause severe illness in some patients and does not transmit readily between people.

Globally, novel coronaviruses emerge periodically in different areas, including SARS in 2002 and MERS in 2012. Several known coronaviruses are circulating in animals that have not yet infected humans. As surveillance improves more coronaviruses are likely to be identified.

China has strong public health capacities and resources to respond and manage respiratory disease outbreaks. In addition to treating the patients in care and isolating new cases as they may be identified, public health officials remain focused on continued contact tracing, conducting environmental assessments at the seafood market, and investigations to identify the pathogen causing the outbreak.

In the coming weeks, more comprehensive information is required to understand the current status and epidemiology of the outbreak, and the clinical picture. Further investigations are also required to determine the source, modes of transmission, extent of infection and countermeasures implemented. WHO continues to monitor the situation closely and, together with its partners, is ready to provide technical support to China to investigate and respond to this outbreak.

The preliminary determination of a novel virus will assist authorities in other countries to conduct disease detection and response. Over the past week, people with symptoms of pneumonia and reported travel history to Wuhan have been identified at international airports.

WHO does not recommend any specific measures for travellers. WHO advises against the application of any travel or trade restrictions on China based on the information currently available.

Read more: Pneumonia in Wuhan, China

January 10, 2020

Novel 2019 coronavirus genome

SARS-CoV-2 coronavirus

edward_holmes 6 Jan '20

> 10th January 2020
> This posting is communicated by Edward C. Holmes, University of Sydney on behalf of the consortium led by Professor Yong-Zhen Zhang, Fudan University, Shanghai

The Shanghai Public Health Clinical Center & School of Public Health, in collaboration with the Central Hospital of Wuhan, Huazhong University of Science and Technology, the Wuhan Center for Disease Control and Prevention, the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control, and the University of Sydney, Sydney, Australia is releasing a coronavirus genome from a case of a respiratory disease from the Wuhan outbreak. The sequence has also been deposited on GenBank (accession MN908947 31.7k) and will be released as soon as possible.

Update: This genome is now available on GenBank and an updated version has been posted 31.7k.

Disclaimer:
Please feel free to download, share, use, and analyze this data. We ask that you communicate with us if you wish to publish results that use these data in a journal. If you have any other questions –then please also contact us directly.

Professor Yong-Zhen Zhang,
Shanghai Public Health Clinical Center & School of Public Health,
Fudan University,
Shanghai, China.

email: zhangyongzhen@shphc.org.cn

Timeline of SARS-CoV-2 Assay Development in BC

Dec 2019
Pneumonia of unknown cause in Wuhan

Dec 31, 201
Pneumonia of unknown cause reported to WHO

Jan 02, 2020
Lab confirmation of 2019-nCoV in 41 patients

Jan 10, 2020
1st 2019-nCoV genome released publicly

Jan 13, 2020
BCCDC designs qPCR assays; order primers

Jan 14, 202
1st qPCR assay released on WHO website

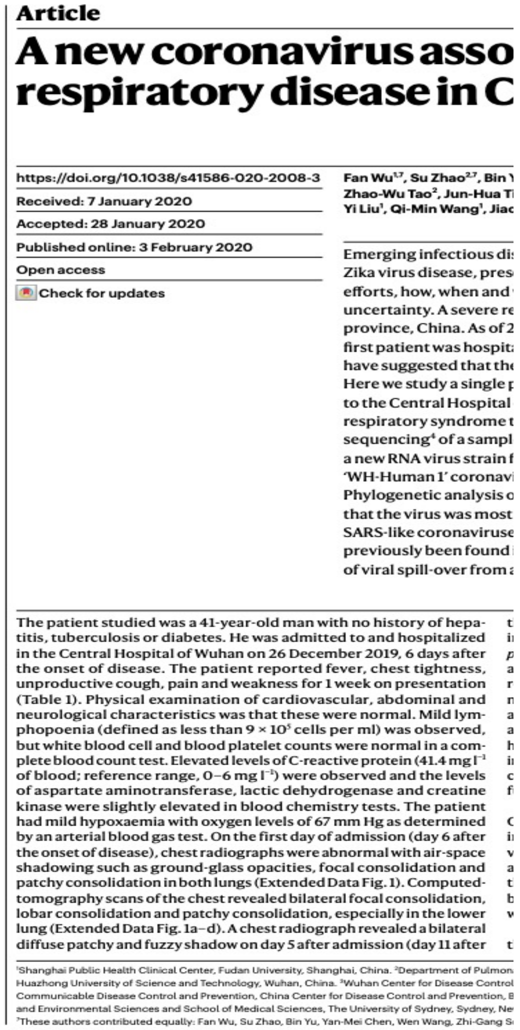
Jan 20, 2020
BCCDC qPCR assay developed; undergoing validation

Jan 23, 2020
1st BC person under investigation (PUI) tested

Jan 27, 2020
1st positive sample from PUI

Feb 2, 2020
151 samples tested,
1 positive

Novel Coronavirus, Novel Illness



Article

A new coronavirus asso
respiratory disease in C

https://doi.org/10.1038/s41586-020-2008-3

Received: 7 January 2020

Accepted: 28 January 2020

Published online: 3 February 2020

Open access

Check for updates

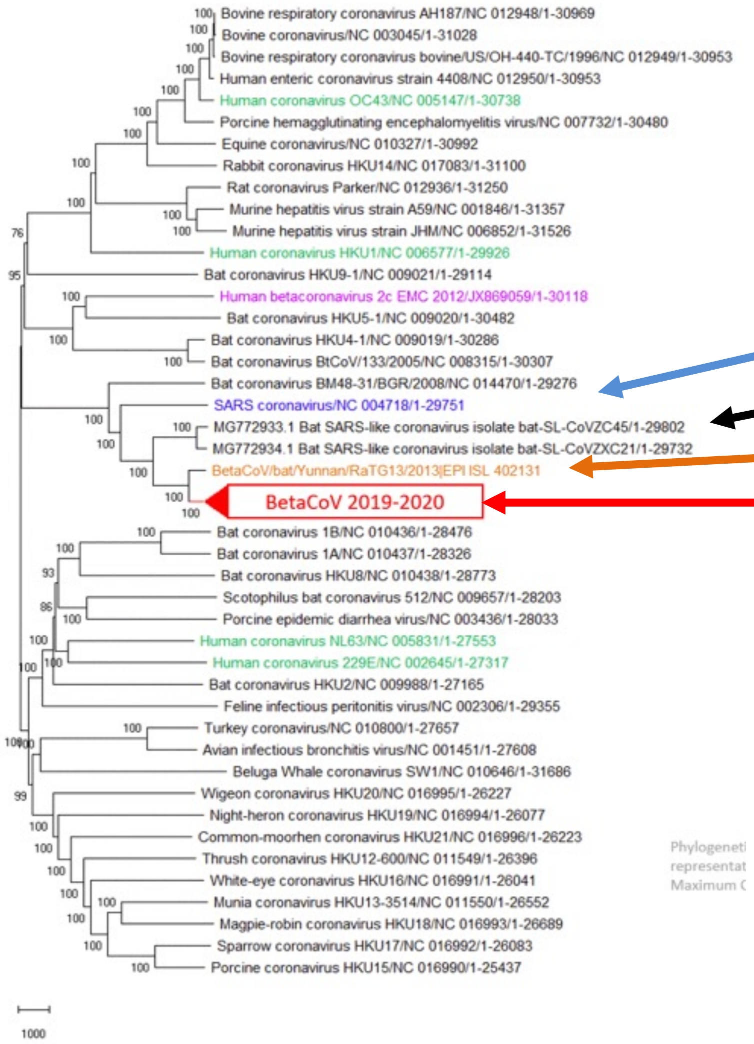
Fan Wu[1,7], Su Zhao[2,7], Bin Y
Zhao-Wu Tao[2], Jun-Hua T
Yi Liu[1], Qi-Min Wang[1], Jiac

Emerging infectious di
Zika virus disease, pres
efforts, how, when and
uncertainty. A severe re
province, China. As of 2
first patient was hospit
have suggested that the
Here we study a single p
to the Central Hospital
respiratory syndrome t
sequencing[4] of a sampl
a new RNA virus strain f
'WH-Human 1' coronavi
Phylogenetic analysis o
that the virus was most
SARS-like coronaviruse
previously been found
of viral spill-over from a

The patient studied was a 41-year-old man with no history of hepatitis, tuberculosis or diabetes. He was admitted to and hospitalized in the Central Hospital of Wuhan on 26 December 2019, 6 days after the onset of disease. The patient reported fever, chest tightness, unproductive cough, pain and weakness for 1 week on presentation (Table 1). Physical examination of cardiovascular, abdominal and neurological characteristics was that these were normal. Mild lymphopoenia (defined as less than 9×10^5 cells per ml) was observed, but white blood cell and blood platelet counts were normal in a complete blood count test. Elevated levels of C-reactive protein (41.4 mg l^{-1} of blood; reference range, 0–6 mg l^{-1}) were observed and the levels of aspartate aminotransferase, lactic dehydrogenase and creatine kinase were slightly elevated in blood chemistry tests. The patient had mild hypoxaemia with oxygen levels of 67 mm Hg as determined by an arterial blood gas test. On the first day of admission (day 6 after the onset of disease), chest radiographs were abnormal with air-space shadowing such as ground-glass opacities, focal consolidation and patchy consolidation in both lungs (Extended Data Fig. 1). Computed-tomography scans of the chest revealed bilateral focal consolidation, lobar consolidation and patchy consolidation, especially in the lower lung (Extended Data Fig. 1a–d). A chest radiograph revealed a bilateral diffuse patchy and fuzzy shadow on day 5 after admission (day 11 after

---

[1]Shanghai Public Health Clinical Center, Fudan University, Shanghai, China. [2]Department of Pulmon
Huazhong University of Science and Technology, Wuhan, China. [3]Wuhan Center for Disease Control
Communicable Disease Control and Prevention, China Center for Disease Control and Prevention, B
and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, Ne
[7]These authors contributed equally: Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang S

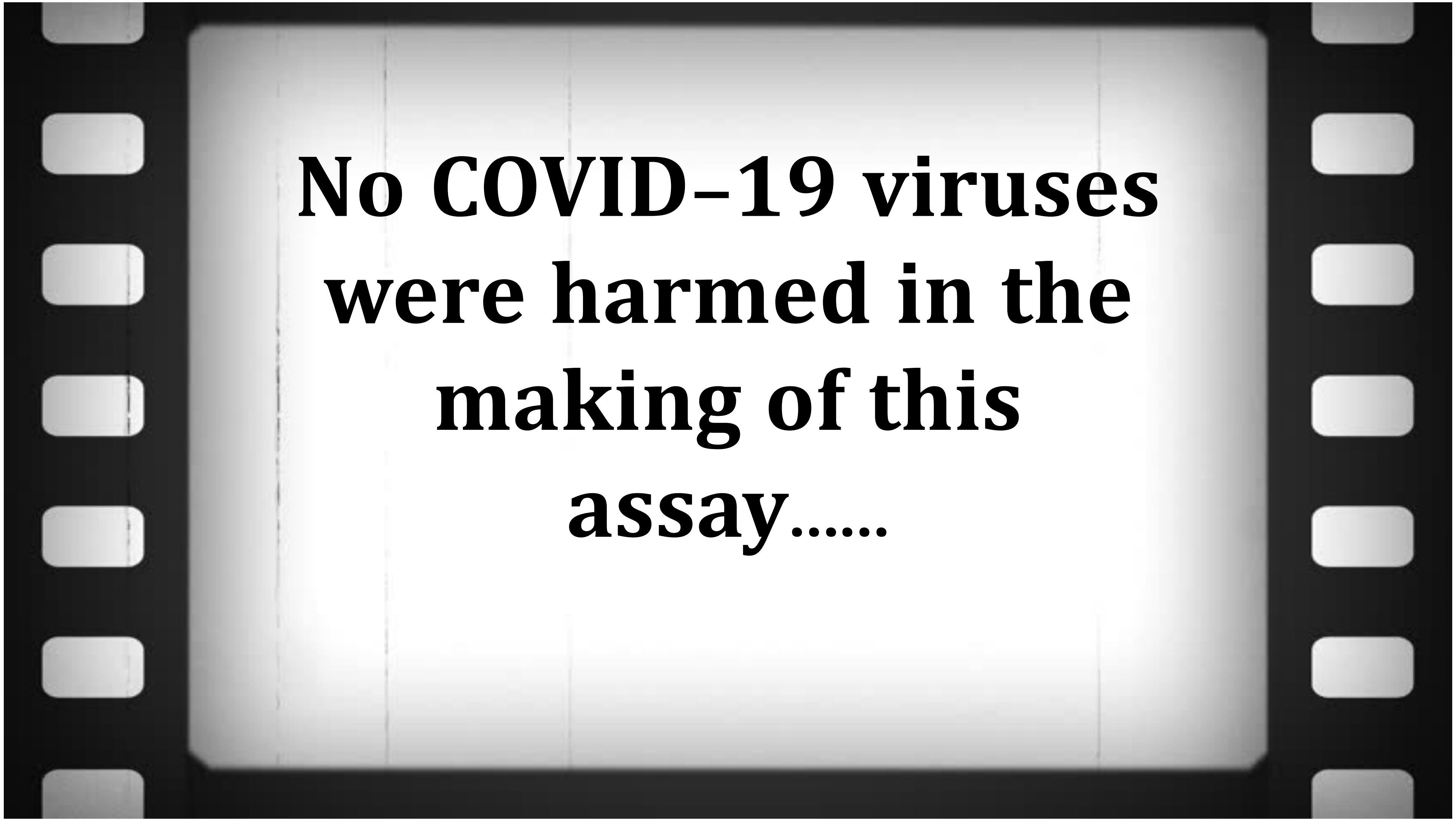


Genome identity to BetaCoV:

- 80% SARS-CoV-1
- 88% ZC45/ZX21 bat precursor
- 96% RaTG13 (nearest bat precursor)

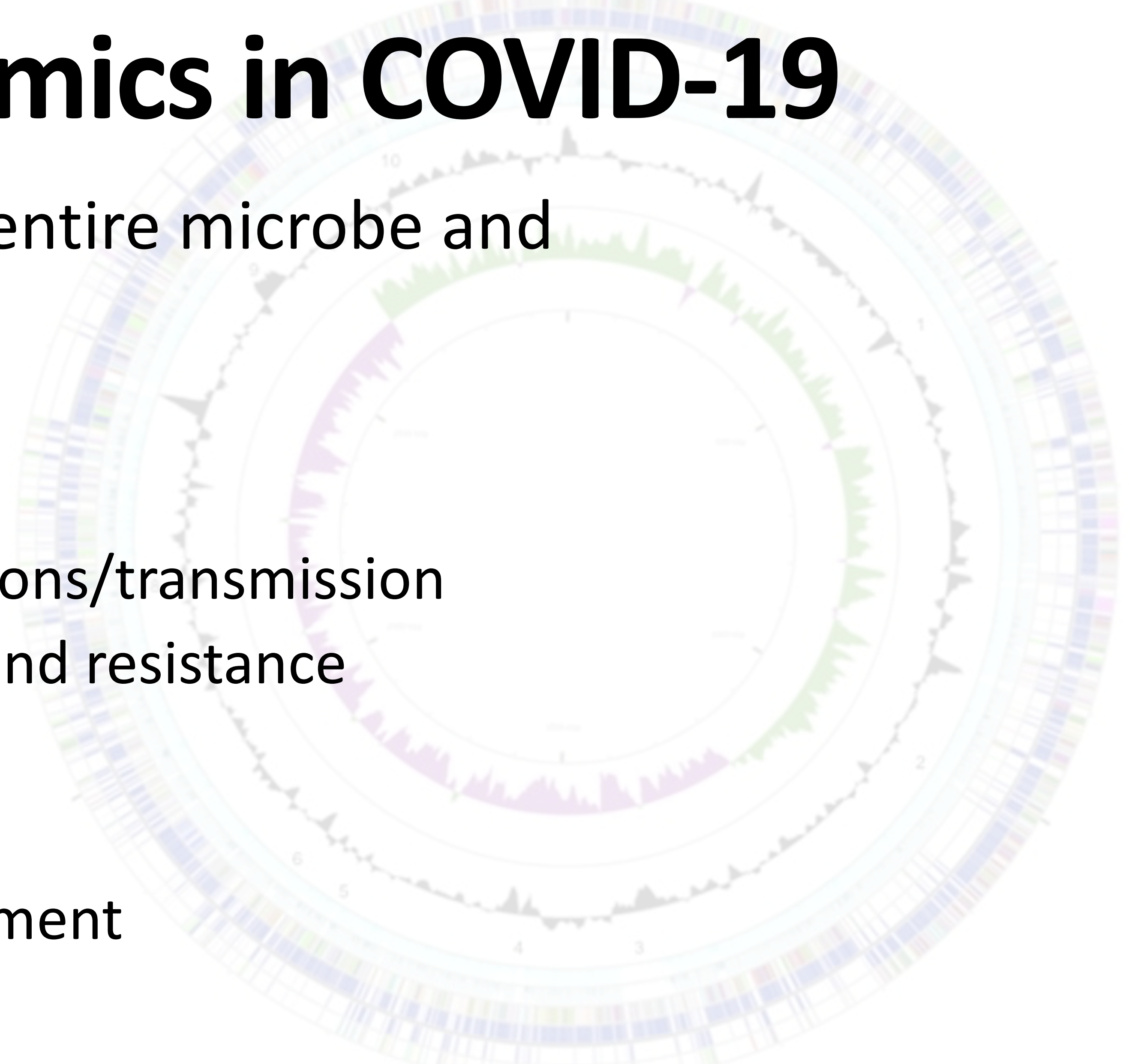
Wu et al, 2020. Nature

by BII, A*STAR Singapore



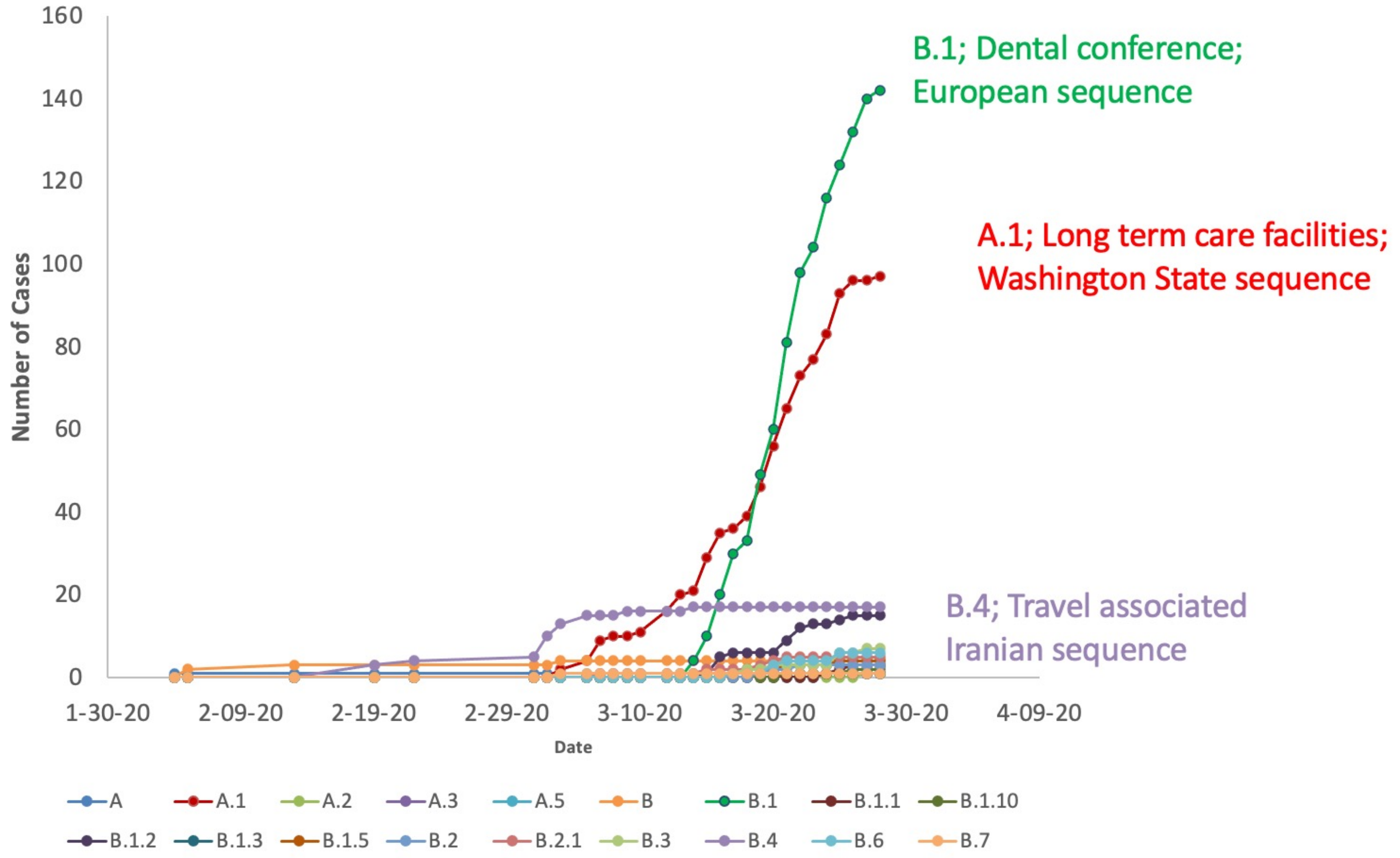
No COVID-19 viruses were harmed in the making of this assay……

Role of genomics in COVID-19



- Genomic Sequence of the entire microbe and molecular fingerprint
- Examples of Applications:
  - Molecular assay design
  - Cluster/Outbreak investigations/transmission
  - Identify drug susceptibility and resistance
  - Virulence factors prediction
  - Detecting new variants
  - Vaccine design and development

COVID-19 Genomics Informed Understanding of Travel-Related Introductions

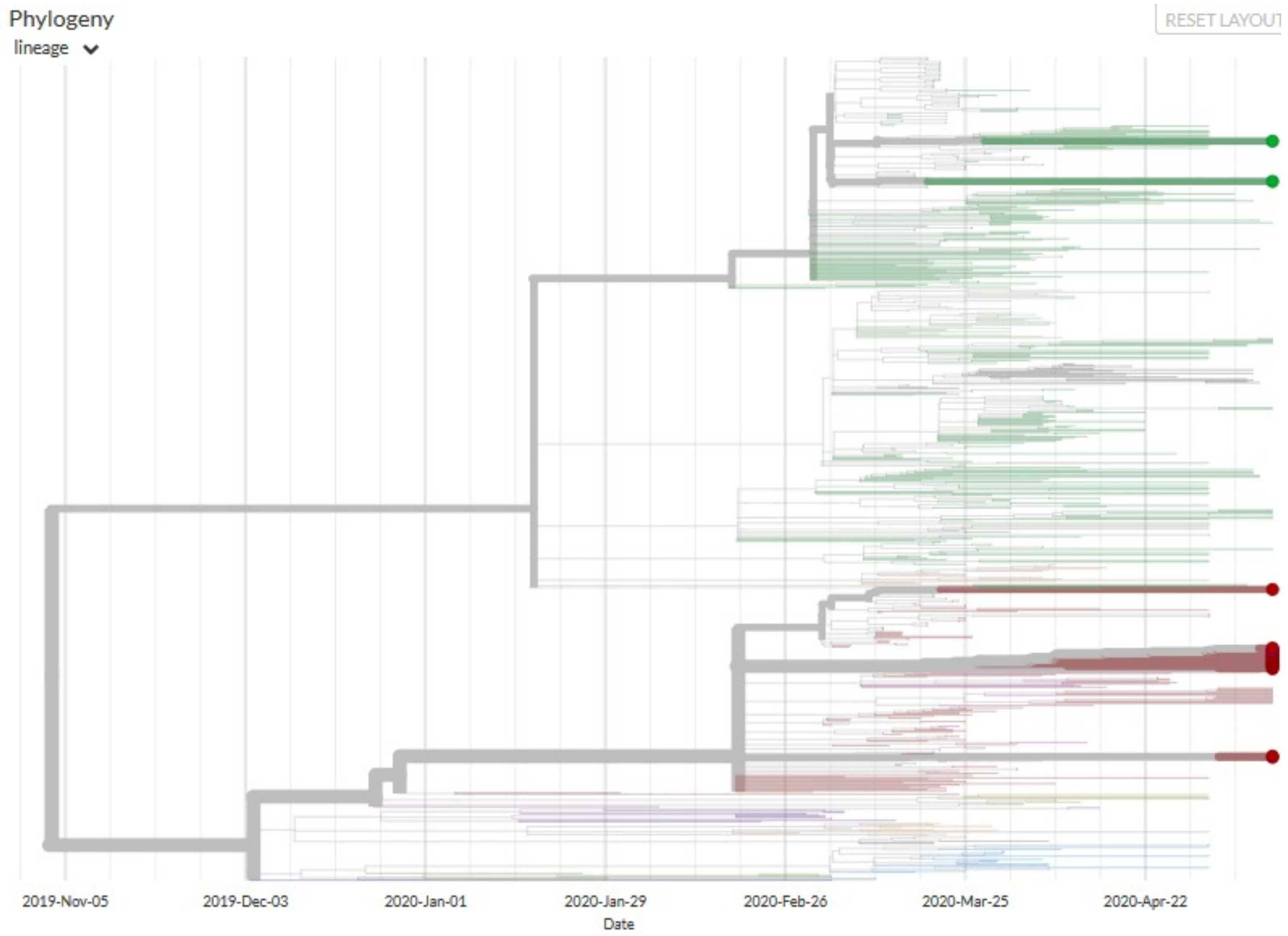


B.1; Dental conference; European sequence

A.1; Long term care facilities; Washington State sequence

B.4; Travel associated Iranian sequence

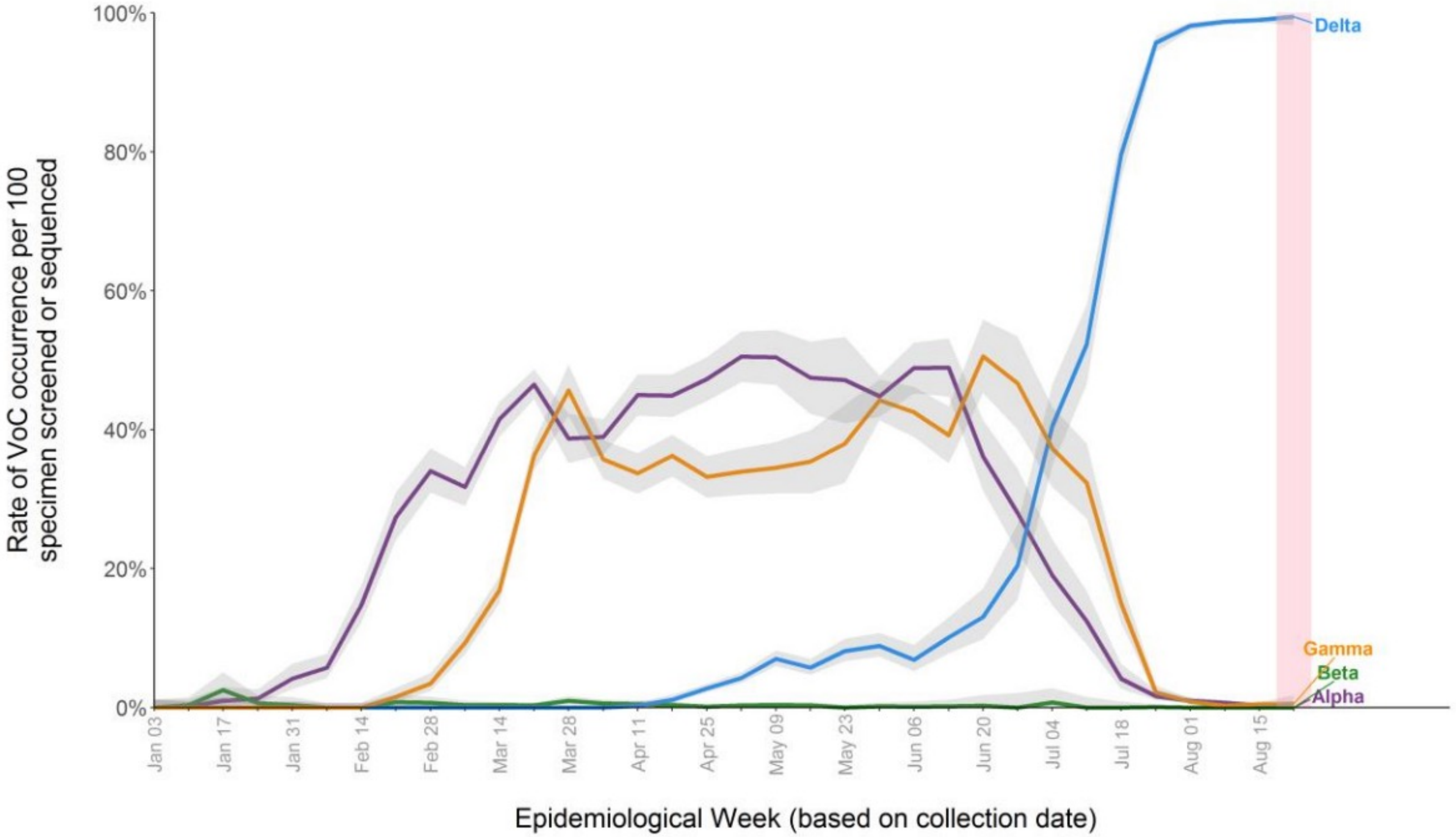
Outbreak Investigations



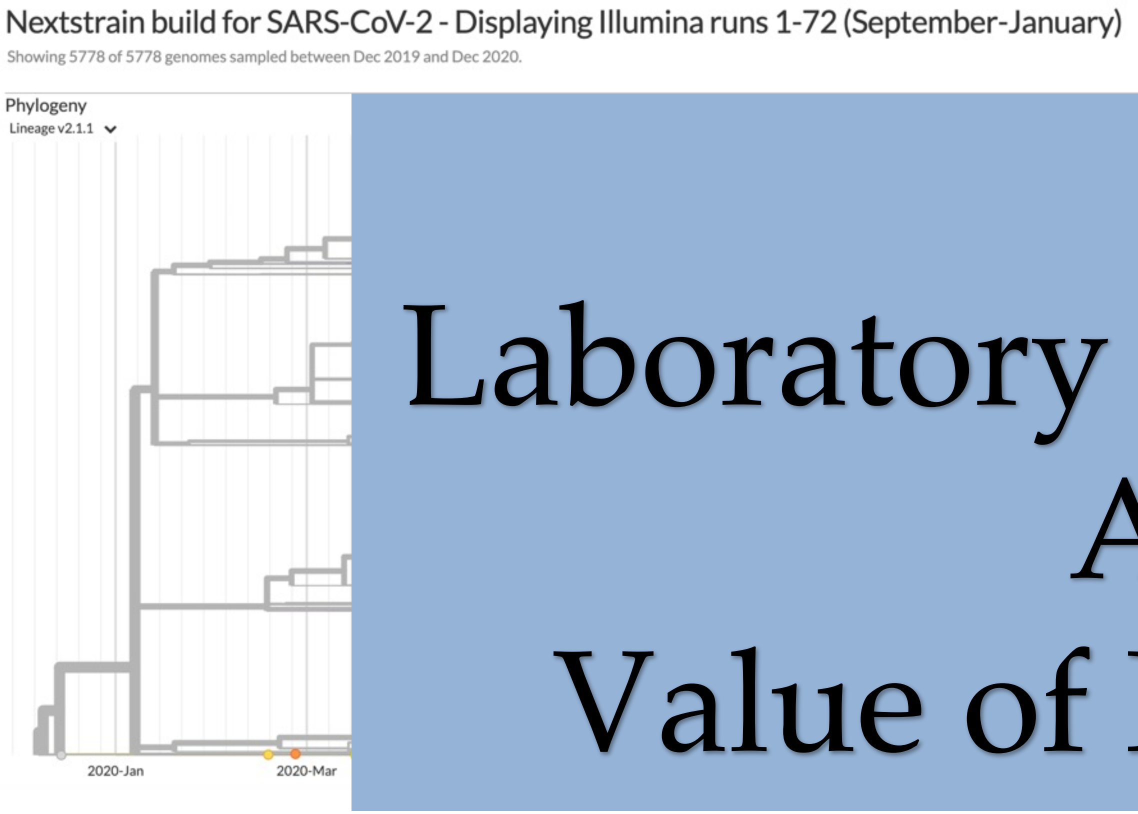
Ward A

Ward B

Wave 3: Variant Detection & Surveillance



Public Health Driven Sequencing Priorities


Nextstrain build for SARS-CoV-2 - Displaying Illumina runs 1-72 (September-January)

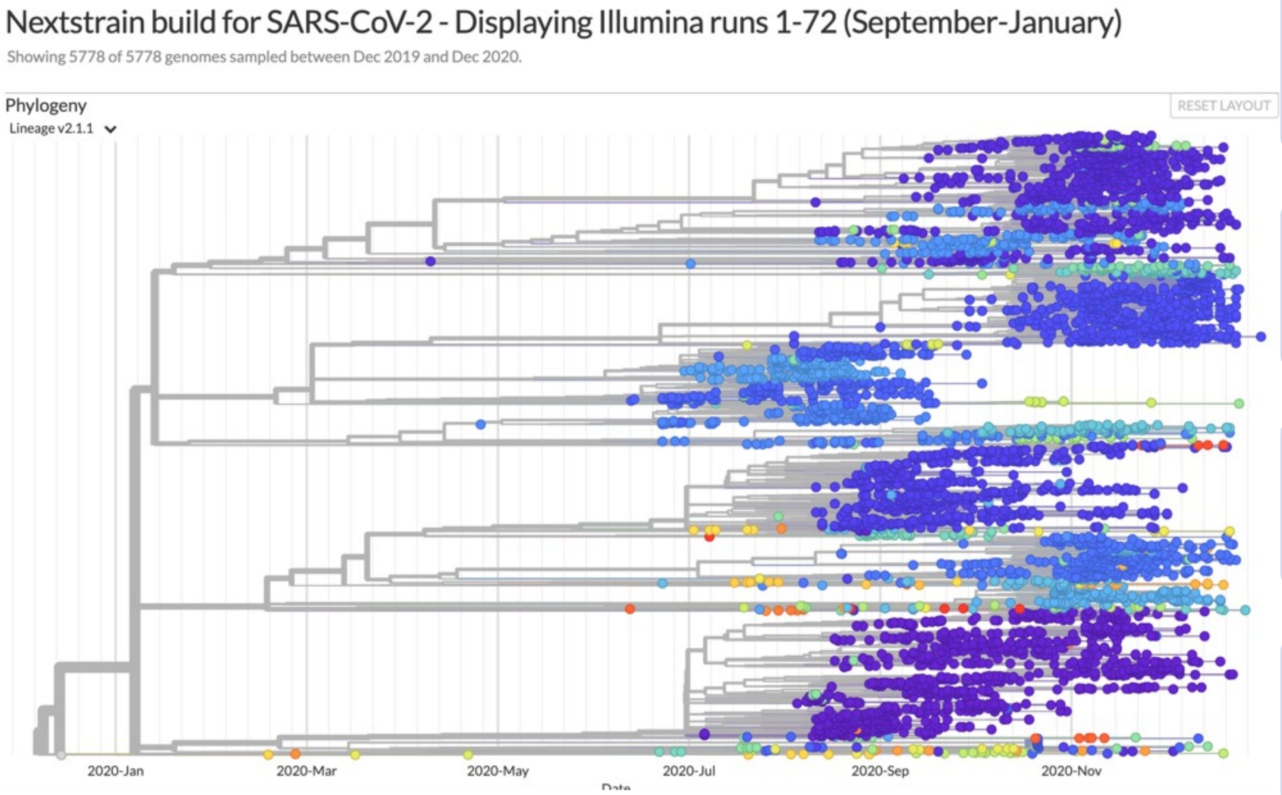
Showing 5778 of 5778 genomes sampled between Dec 2019 and Dec 2020.

Provincial surveillance

cts (i.e.

Laboratory WGS Capacity
AND
Value of Information

Public Health Driven Sequencing Priorities

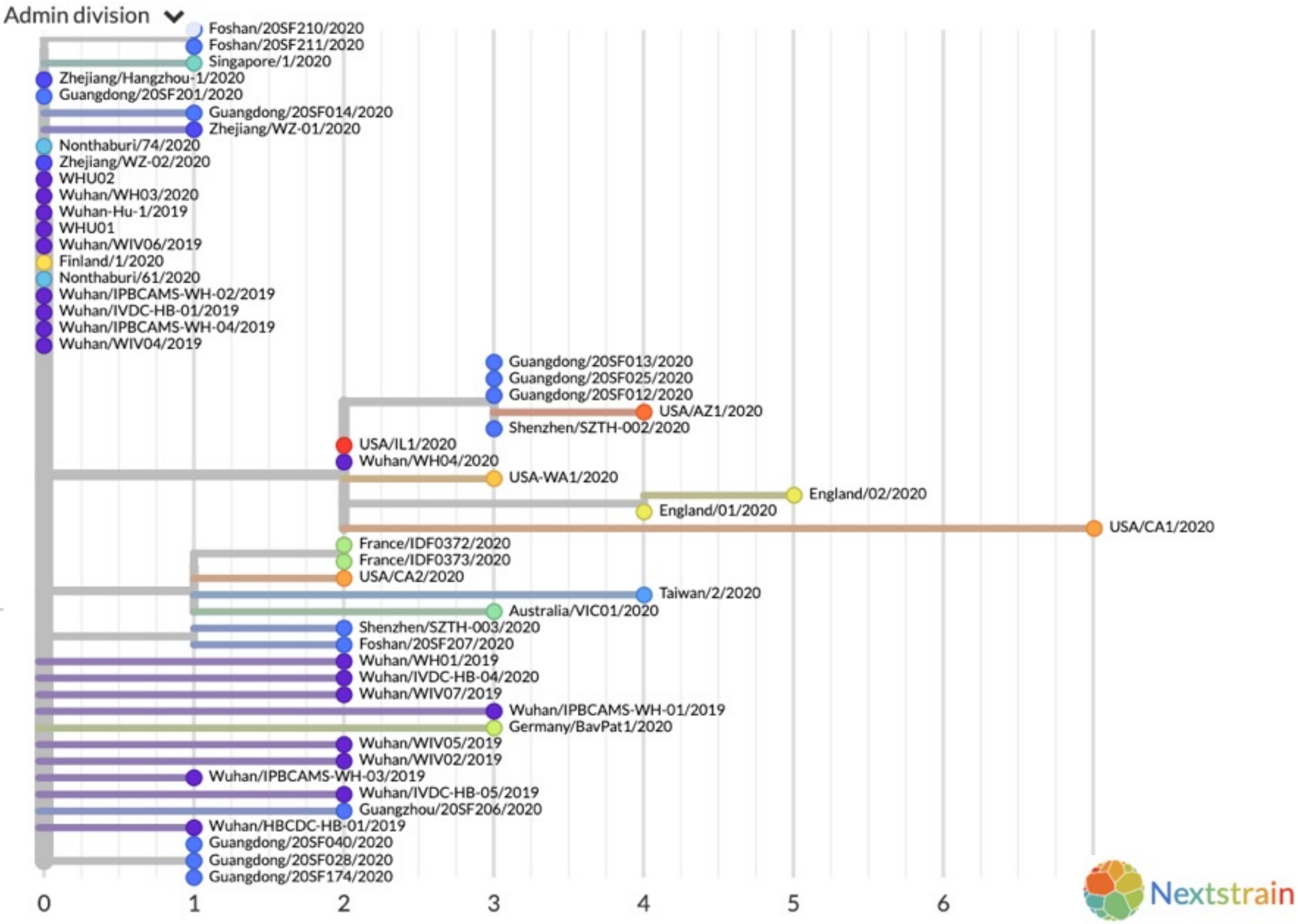


Nextstrain build for SARS-CoV-2 - Displaying Illumina runs 1-72 (September-January)

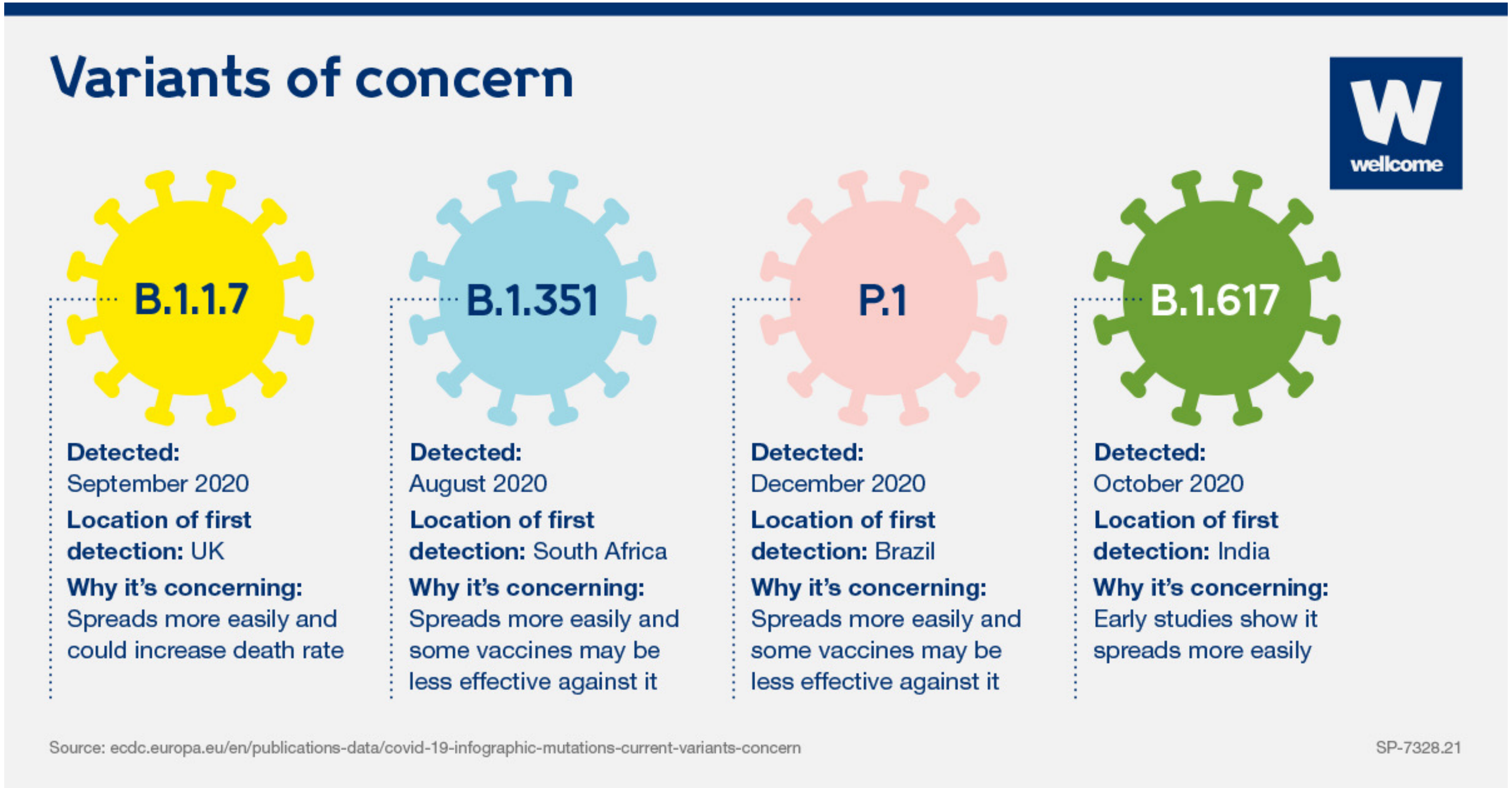
Showing 5778 of 5778 genomes sampled between Dec 2019 and Dec 2020.

- Provincial surveillance
- Outbreak investigations
- Targeted surveillance projects (i.e. pediatric cases)
- Reinfection cases

Late Jan, 2020



Emergence of Variants of Concern Resulted in Greater Pressure to Generate Sequences



Variants of concern

B.1.1.7

Detected: September 2020

Location of first detection: UK

Why it's concerning: Spreads more easily and could increase death rate

B.1.351

Detected: August 2020

Location of first detection: South Africa

Why it's concerning: Spreads more easily and some vaccines may be less effective against it

P.1

Detected: December 2020

Location of first detection: Brazil

Why it's concerning: Spreads more easily and some vaccines may be less effective against it

B.1.617

Detected: October 2020

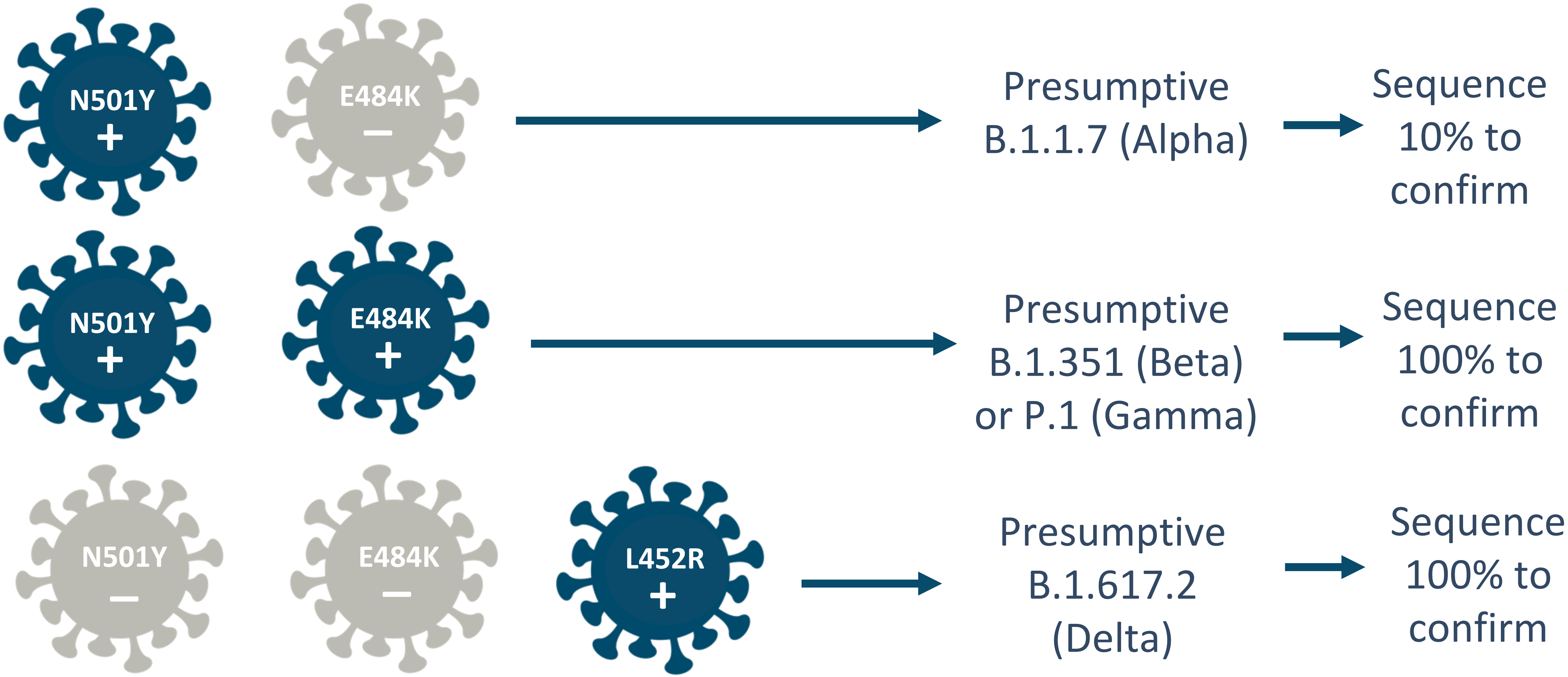
Location of first detection: India

Why it's concerning: Early studies show it spreads more easily

Source: ecdc.europa.eu/en/publications-data/covid-19-infographic-mutations-current-variants-concern

SP-7328.21

qPCR for VOC Detection; Role of Sequencing

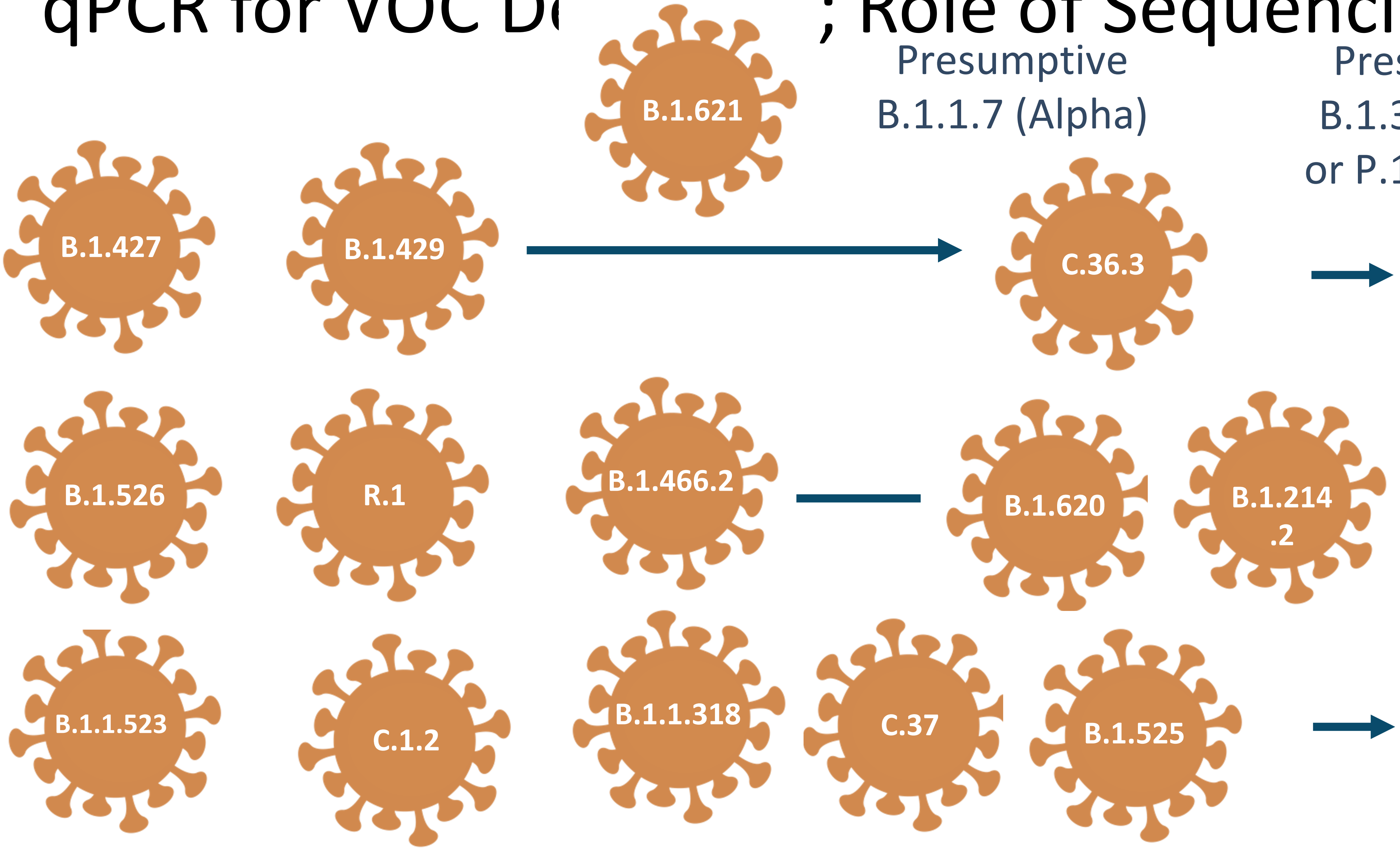


Presumptive
B.1.1.7 (Alpha)
→ Sequence
10% to
confirm

Presumptive
B.1.351 (Beta)
or P.1 (Gamma)
→ Sequence
100% to
confirm

Presumptive
B.1.617.2
(Delta)
→ Sequence
100% to
confirm

qPCR for VOC De ; Role of Sequencing



Presumptive
B.1.1.7 (Alpha)

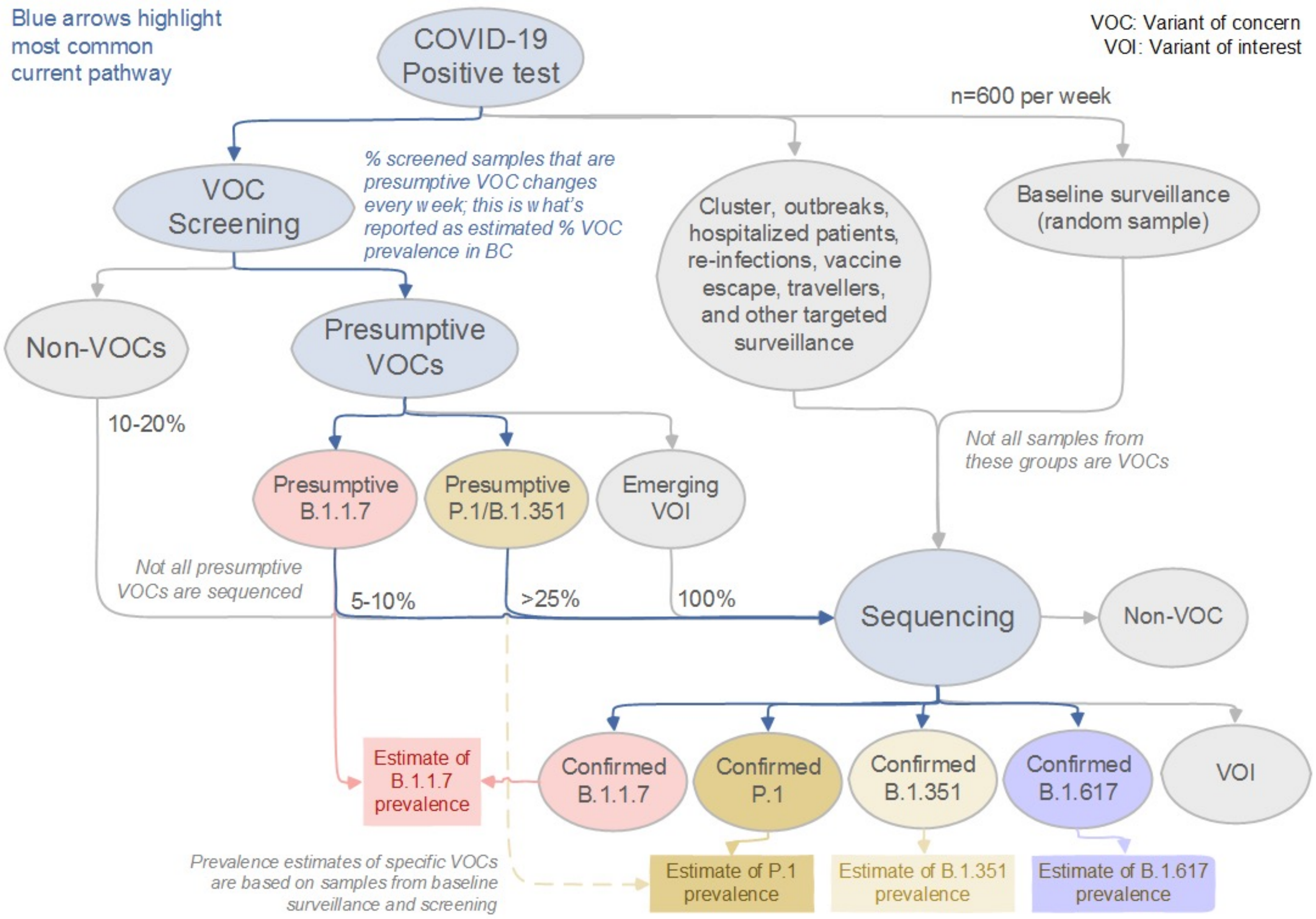
Presumptive
B.1.351 (Beta)
or P.1 (Gamma)

Sequence
10% to
confirm

Sequence
100% to
confirm

Sequence
100% to
confirm

BCCDC Sequencing Strategy (early)



~5000 BC COVID genomes sequenced since Feb 2020 (2400 since August 24th)

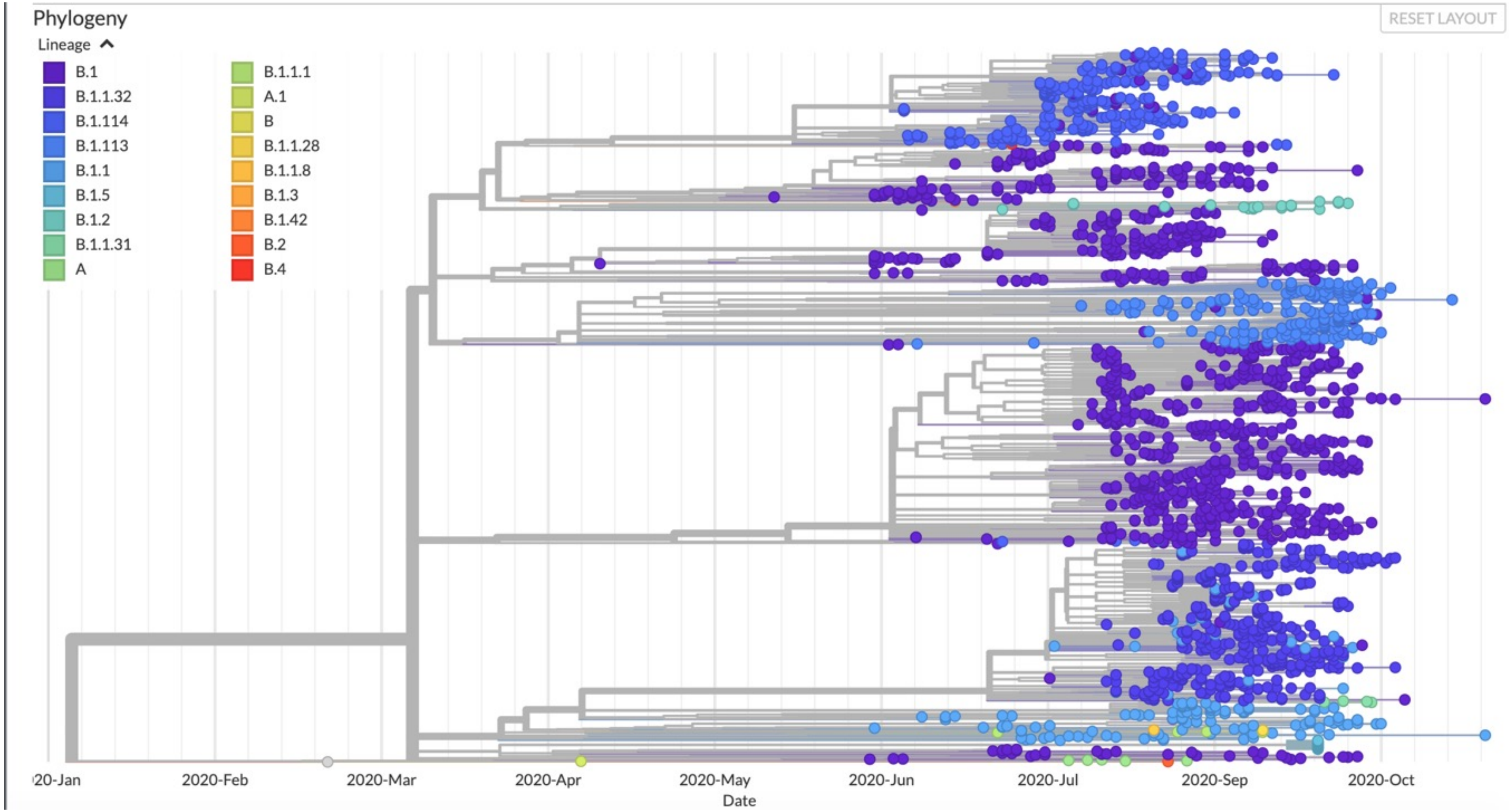
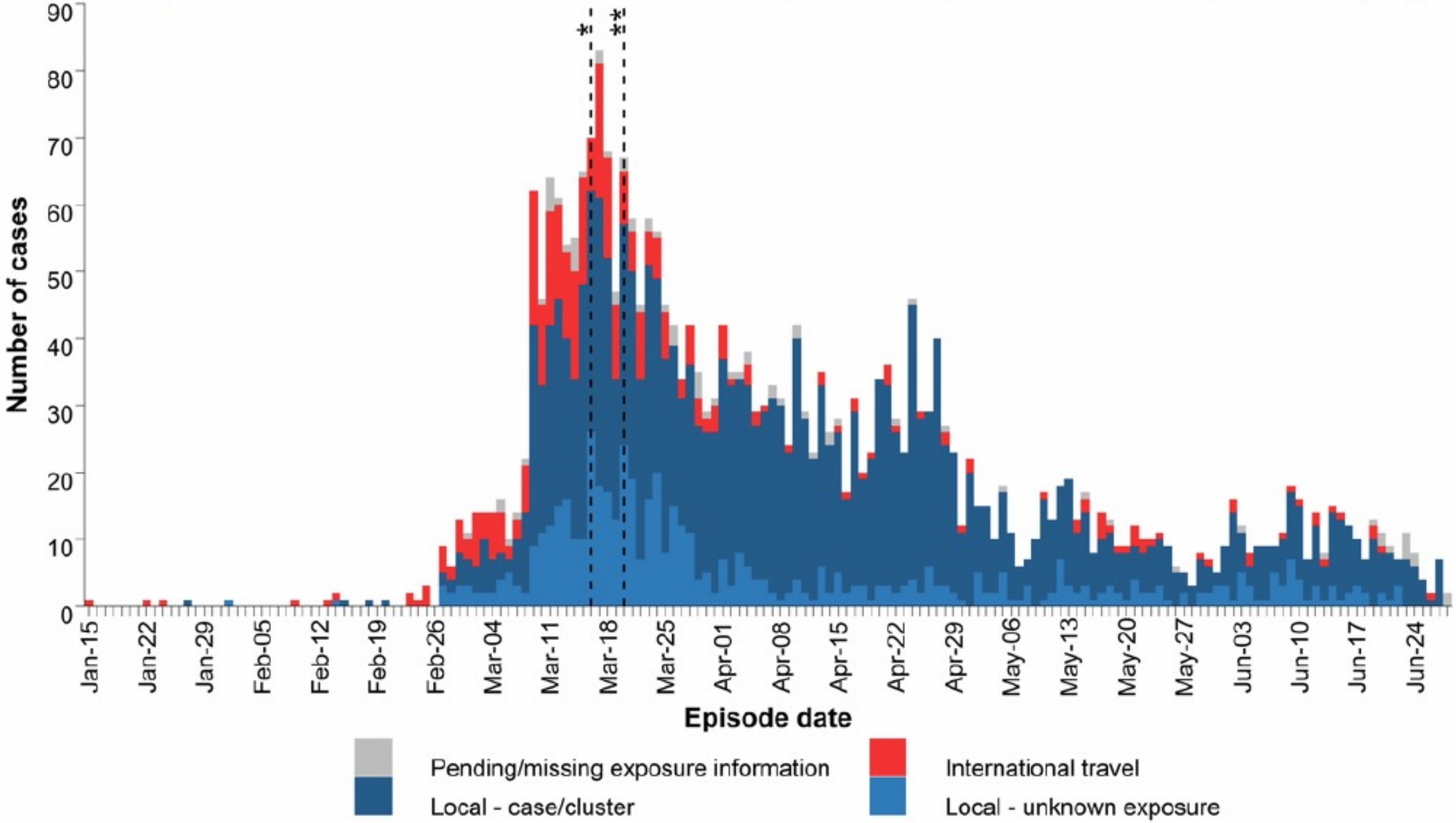


Figure 4: Likely source of infection for COVID-19 cases in BC by episode date§, January 15 – June 28, 2020 **(N=2,904)**

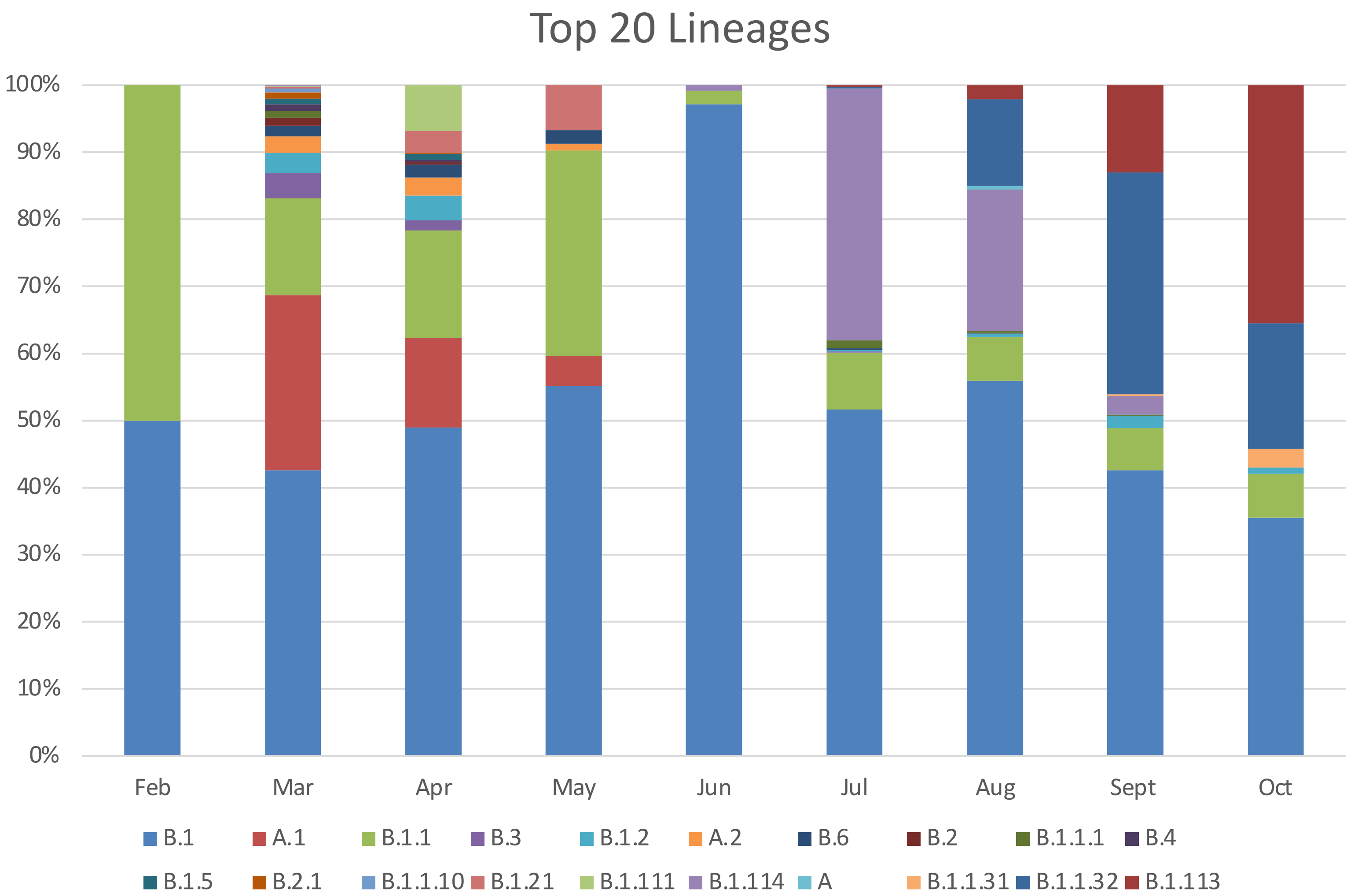


§ Episode date is based on symptom onset date (n=2,712), if not available then date COVID-19 was reported to health authority (n=192).

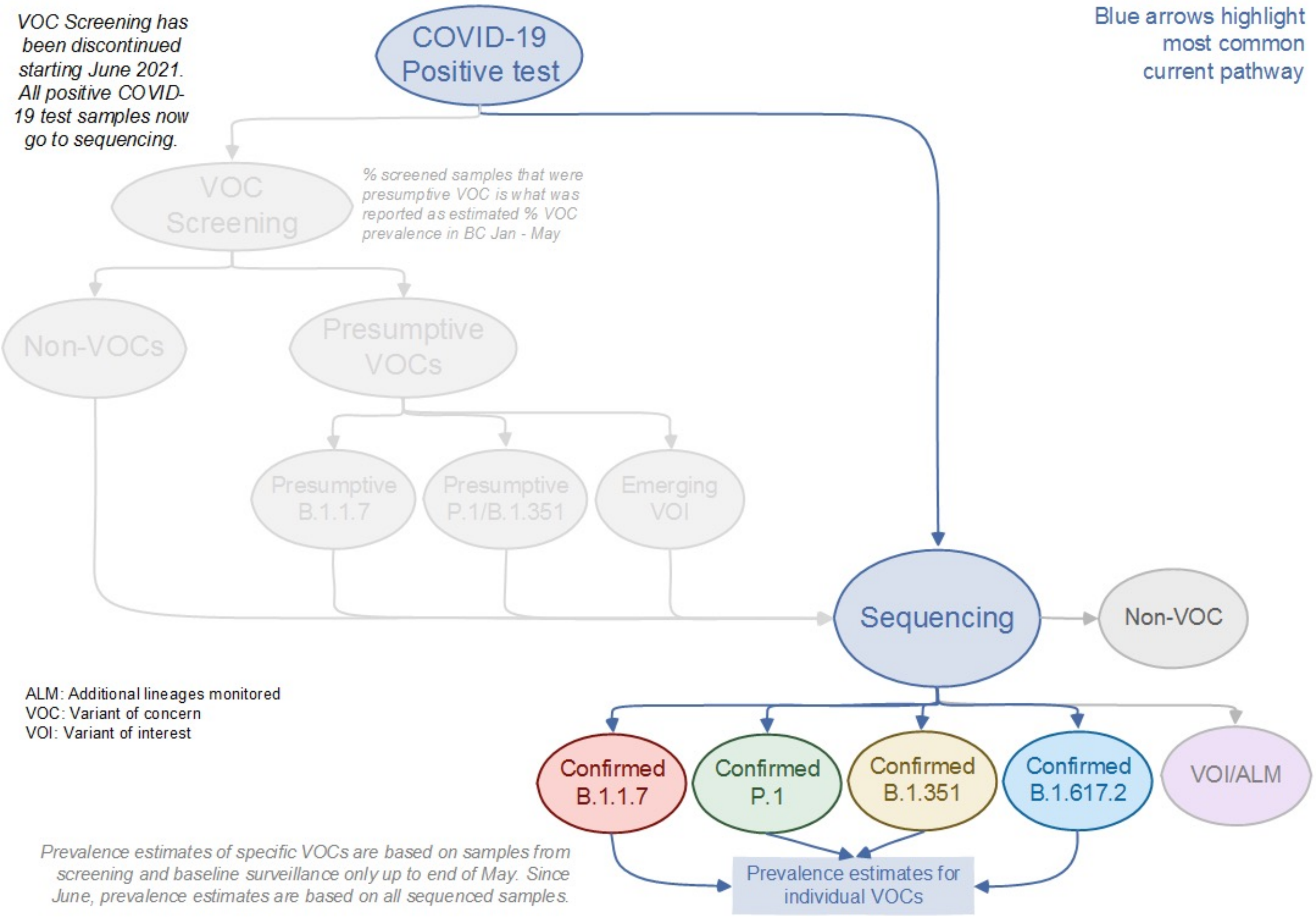
* March 16: Entry of foreign nationals banned; symptomatic individuals banned from flights to Canada; international flights restricted to four national airports.

** March 20: US/Canada border closed to non-essential travel.

Predominant Lineages in BC Changing Over Time



Discontinued VOC Screening



VOC Screening has been discontinued starting June 2021. All positive COVID-19 test samples now go to sequencing.

Blue arrows highlight most common current pathway

COVID-19 Positive test

VOC Screening

% screened samples that were presumptive VOC is what was reported as estimated % VOC prevalence in BC Jan - May

Non-VOCs

Presumptive VOCs

Presumptive B.1.1.7

Presumptive P.1/B.1.351

Emerging VOI

Sequencing

Non-VOC

Confirmed B.1.1.7

Confirmed P.1

Confirmed B.1.351

Confirmed B.1.617.2

VOI/ALM

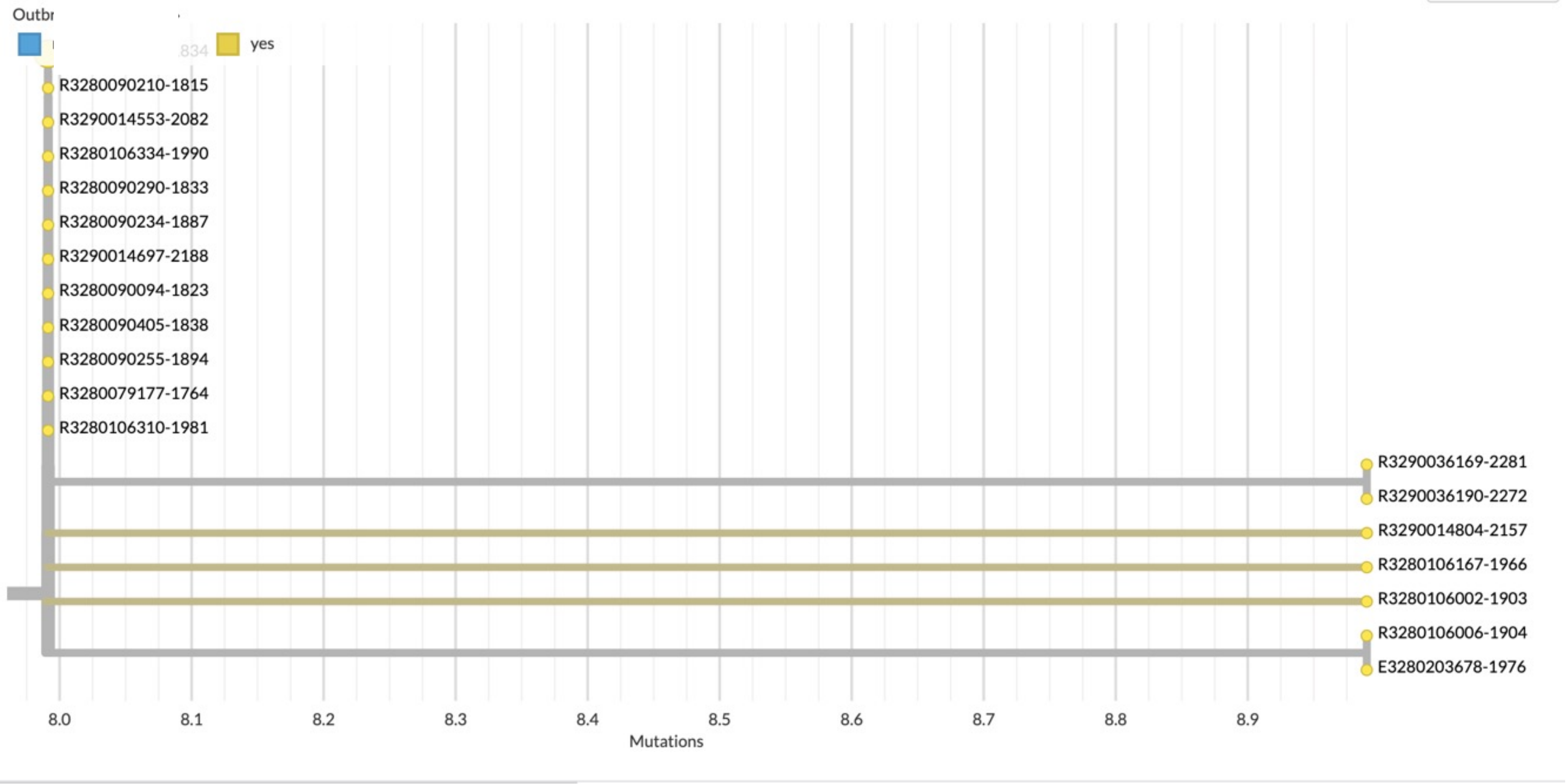
Prevalence estimates for individual VOCs

ALM: Additional lineages monitored
VOC: Variant of concern
VOI: Variant of interest

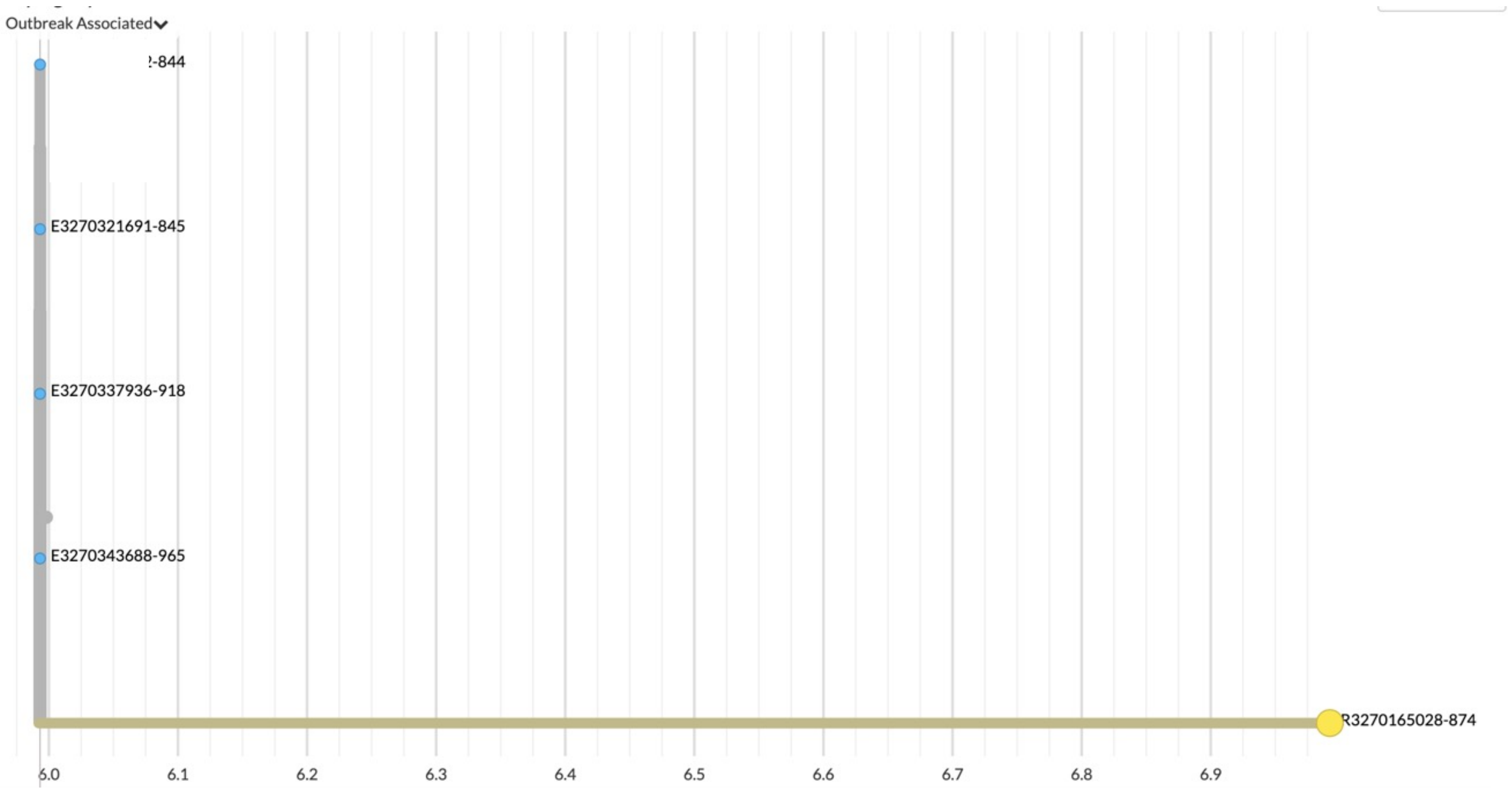
Prevalence estimates of specific VOCs are based on samples from screening and baseline surveillance only up to end of May. Since June, prevalence estimates are based on all sequenced samples.

WGS Cluster Detection

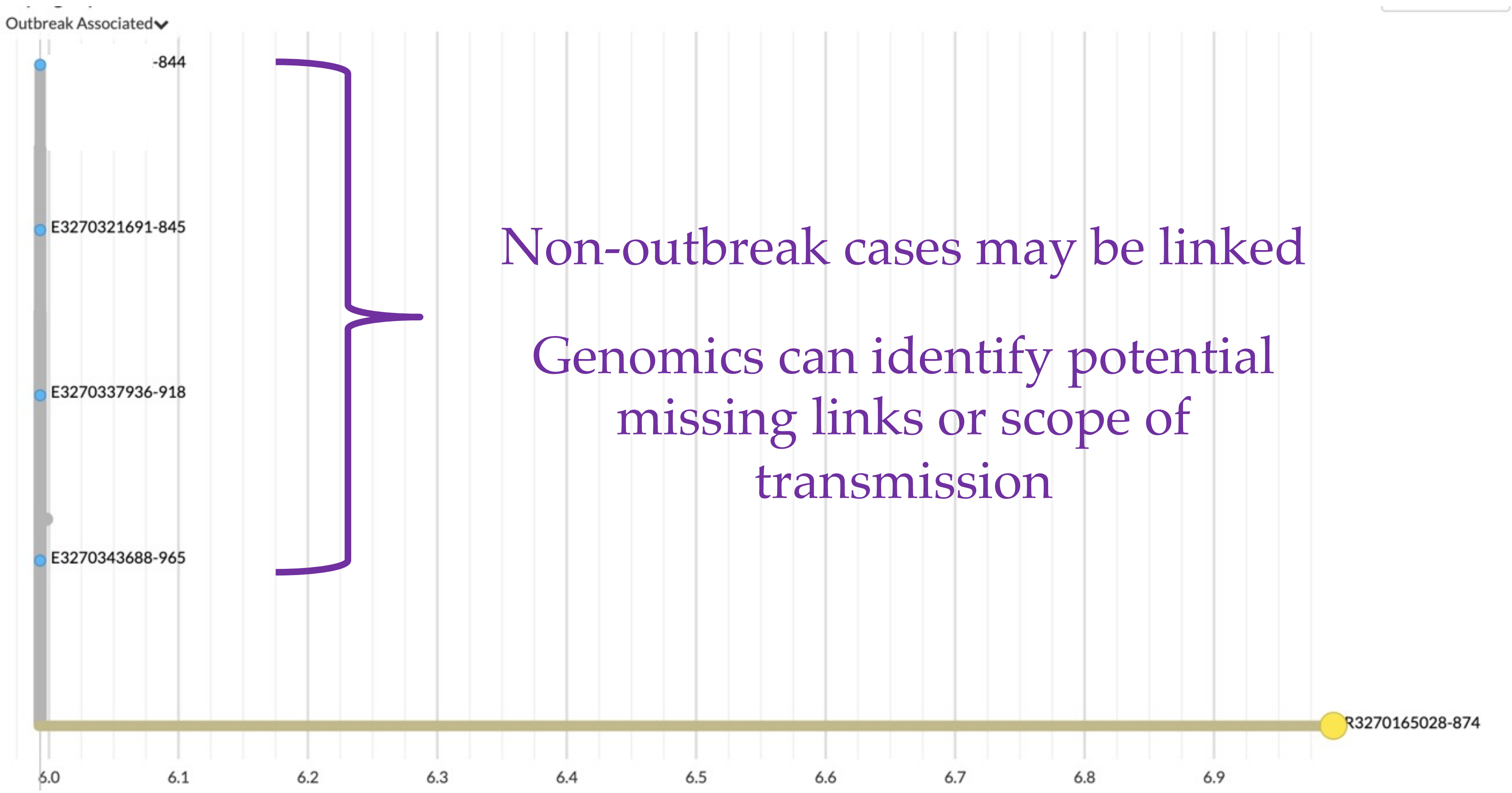
Outbreak cases are linked to each other



“outbreak’ case clusters with non-outbreak cases



“Outbreak’ case clusters with non-outbreak cases



Non-outbreak cases may be linked

Genomics can identify potential missing links or scope of transmission

Feedback from health authorities

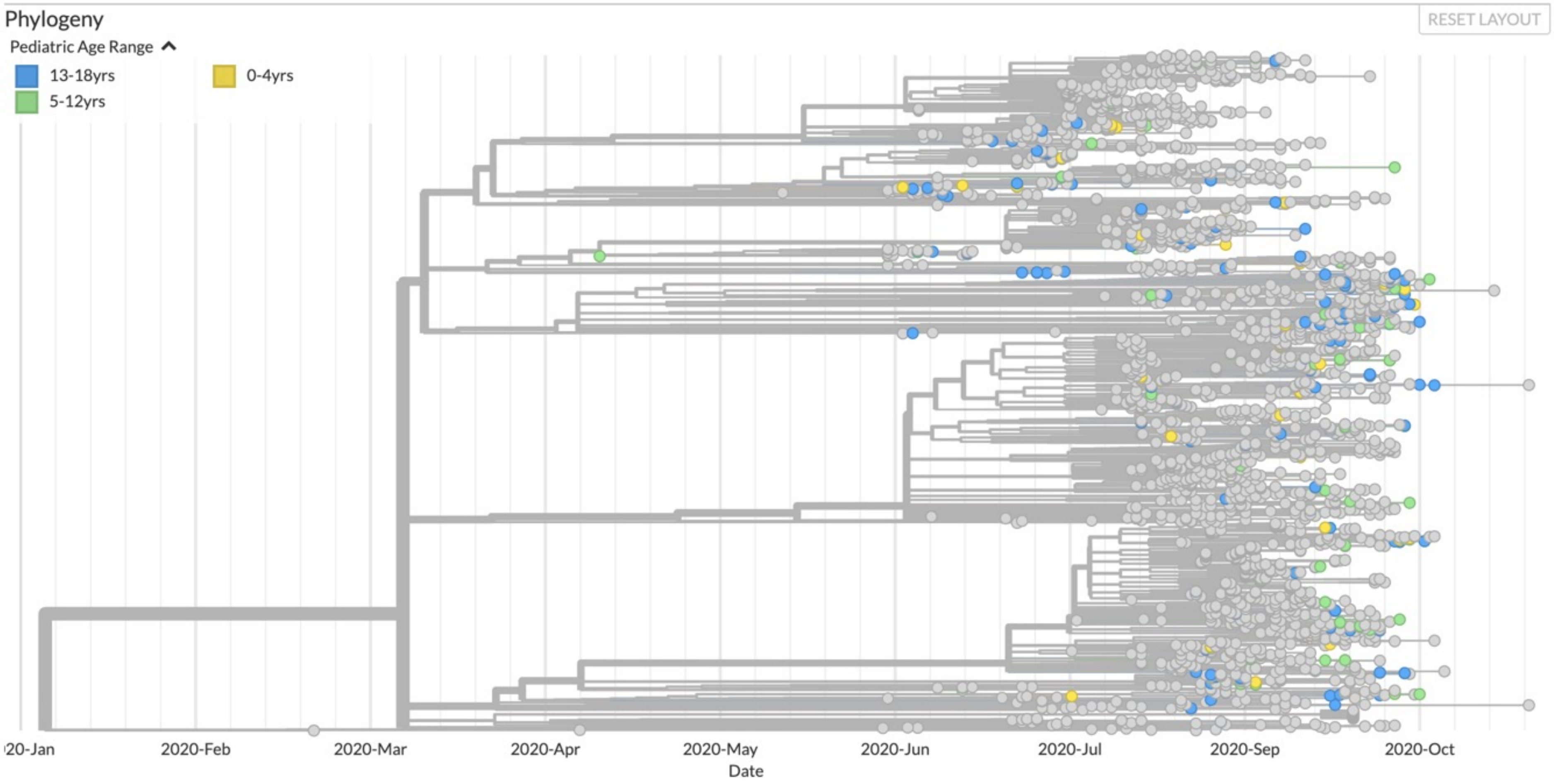
1. **School cluster investigation:** When variants first emerged, a few schools with clusters had broader testing performed. **WGS helped in confirming that most of the cases were community-acquired** rather than transmissions that were occurring in school. This helped inform public health measures and helped confirm that schools weren't high transmission environments when mitigating measures are in place.

2. **Poultry Factory:** This was a large workplace outbreak where the employers argued that new introductions of COVID-19 were **occurring from the community rather than being workplace-associat**ed. WGS confirmed that cases were highly related and helped confirm that workplace transmission was the primary mechanism of spread.

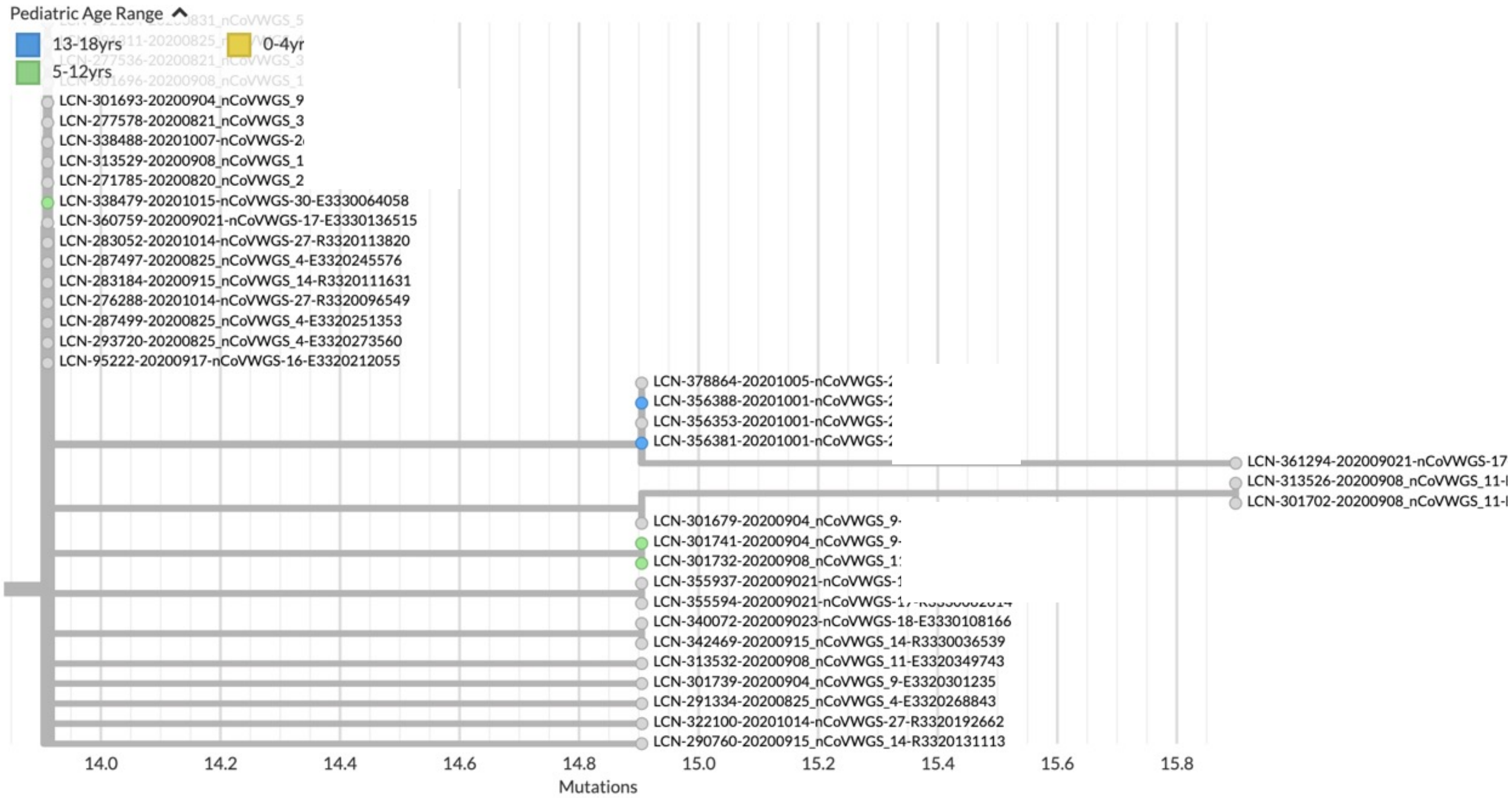
3. **LTC outbreak:** WGS helped with understanding the **impact of cohorting decisions** in the site where movement of residents unfortunately resulted in seeding of cases in an unaffected unit and contributed to a secondary surge of cases in the outbreak. This has **helped Community IPC refine cohorting guidance to providers moving forward.**

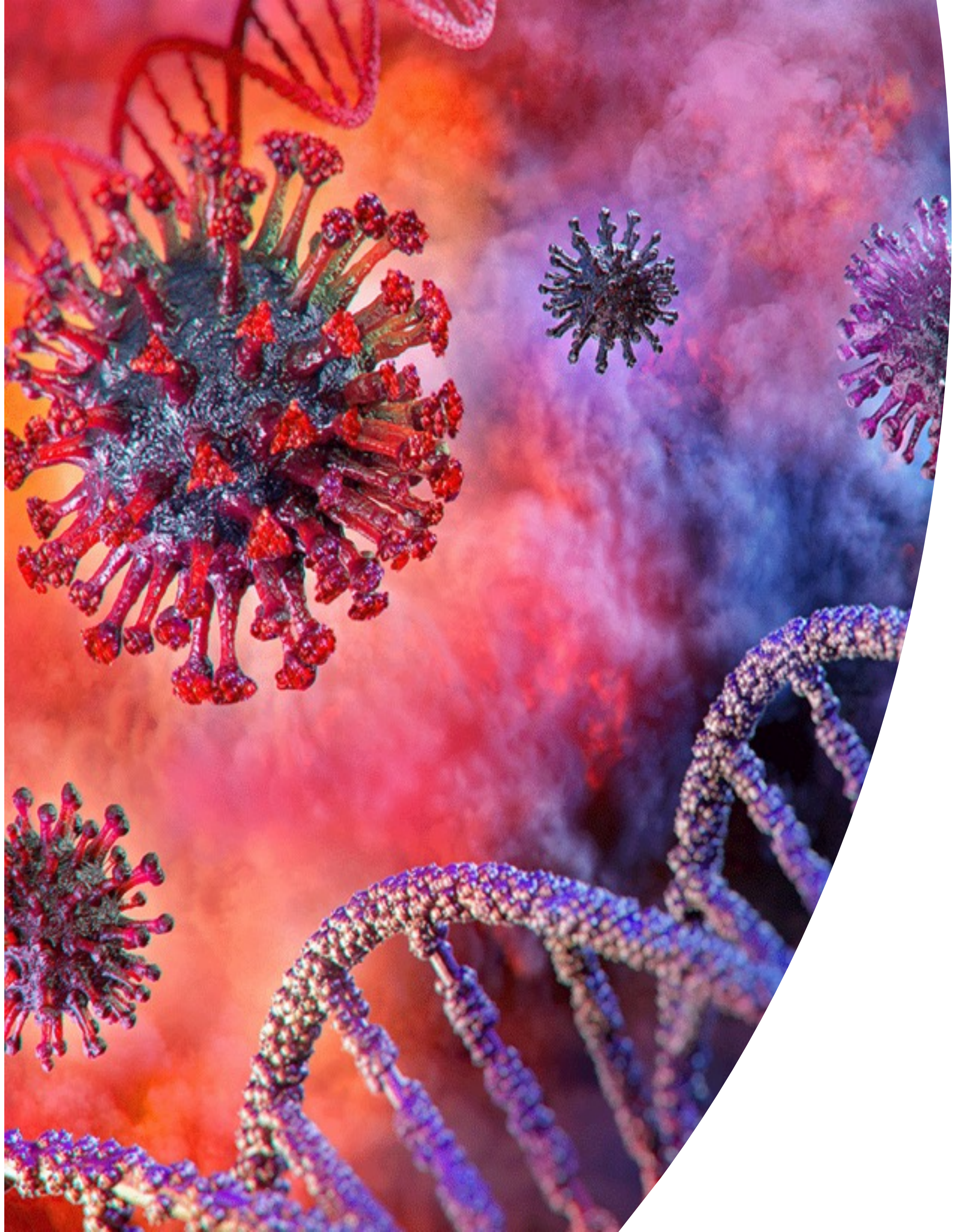
Dr Aamir Bharmal, MHO

Genomic Sequencing of Pediatric Cases (n=223)



Pediatric Cases Typically Cluster in Larger Clusters with Many Adults





Operationalizing SARS-CoV-2 Sequencing in BC

Basic WGS infrastructure scaled up to support COVID-19

Transition from Research to Clinical

Scale Up For High Volume

- PLOVER for sample management
- Improved workflow (LEAN processes)
- Liquid handling to automate library generation
- Acquisition of 2 NextSeq instruments
- Training and recruitment of staff
- Automated analysis pipelines and data visualization
- Integration with epidemiology

Pre-analytic

- Ct values acceptable for WGS
- Data entry
- Indications for testing

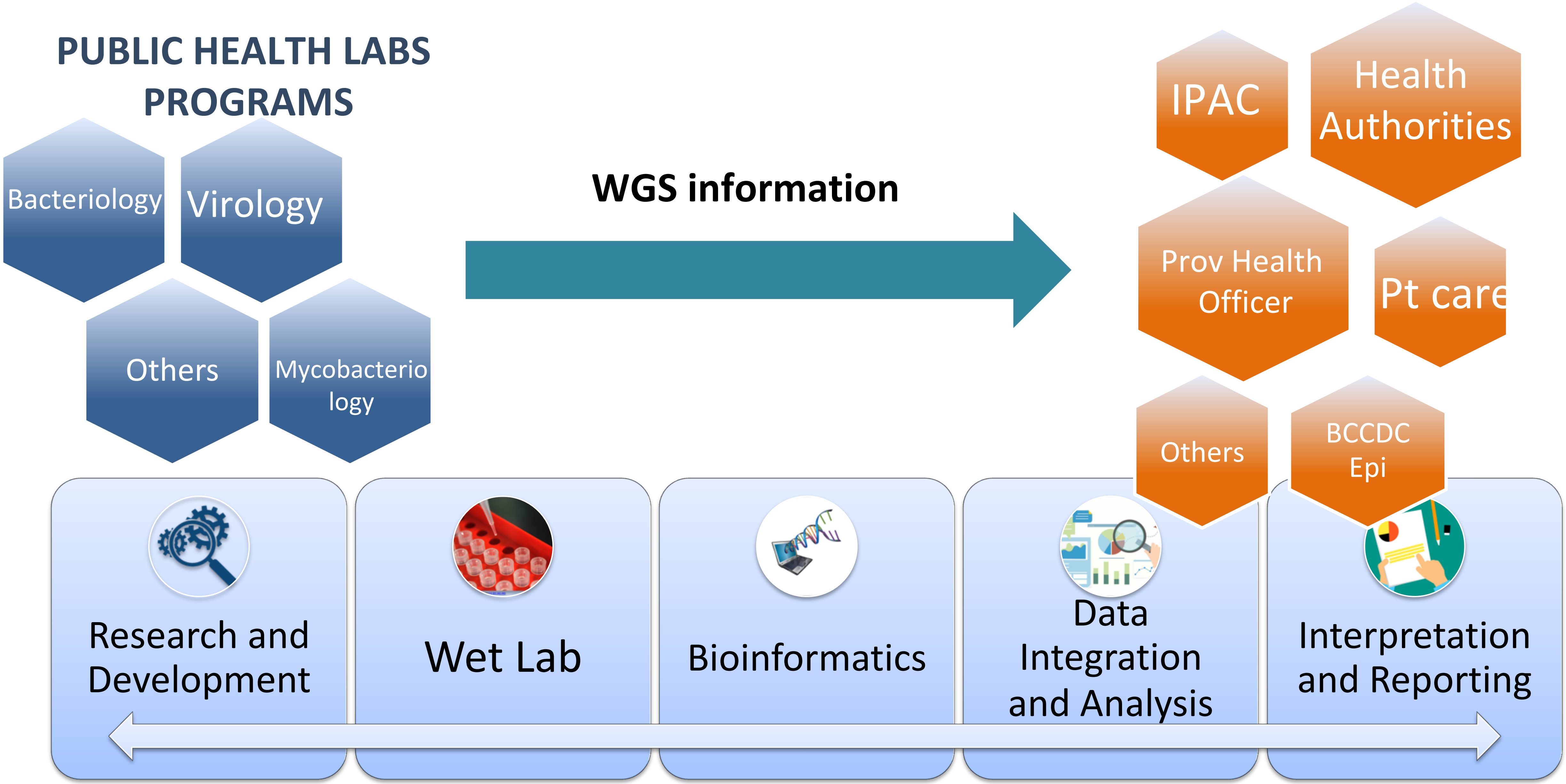
Analytic

- Wet lab assay
- Bioinformatics pipelines
- LIMS???

Post-analytic

- Analysis
- Interpretation
- Reports

Operationalized over a Quality Framework (QA/QC)



END USERS

PUBLIC HEALTH LABS PROGRAMS

Bacteriology

Virology

Others

Mycobacteriology

WGS information

IPAC

Health Authorities

Prov Health Officer

Pt care

Others

BCCDC Epi

Research and Development

Wet Lab

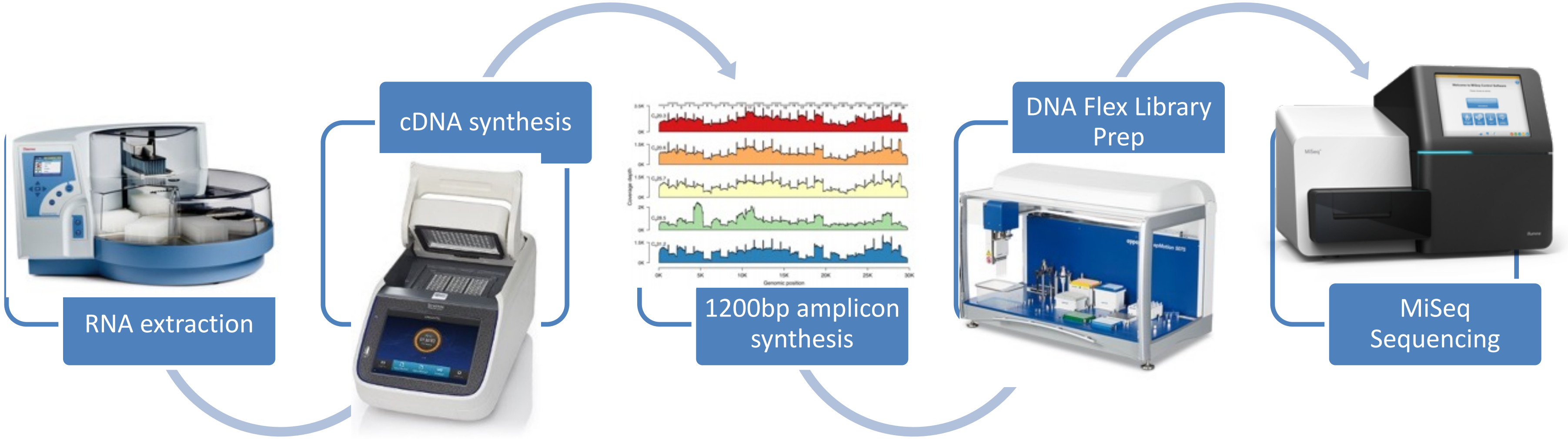
Bioinformatics

Data Integration and Analysis

Interpretation and Reporting

Wet Lab Method Validation & Operationalization

- Extensive validation of sequencing protocol → robust and reproducible



RNA extraction

cDNA synthesis

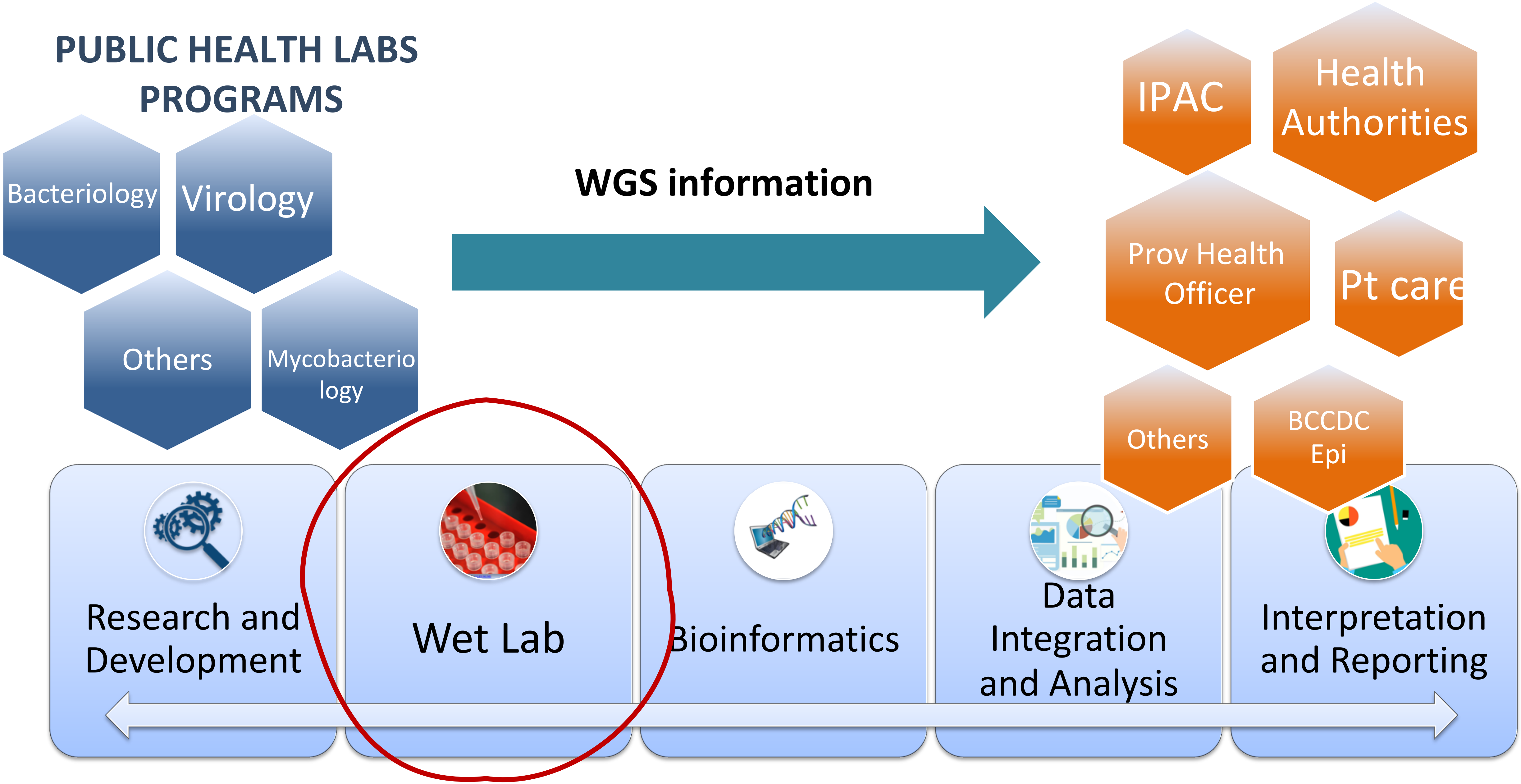
1200bp amplicon synthesis

DNA Flex Library Prep

MiSeq Sequencing

Assay validation

- With introduction of each equipment, method modifications, or kit modifications
  - Requires extensive validations
  - Cannot be performed within daily work environment
  - Important to have a parallel group of technologists to support method improvement work in a separate environment



END USERS

PUBLIC HEALTH LABS PROGRAMS

Bacteriology

Virology

Others

Mycobacteriology

WGS information

IPAC

Health Authorities

Prov Health Officer

Pt care

Others

BCCDC Epi

Research and Development

Wet Lab

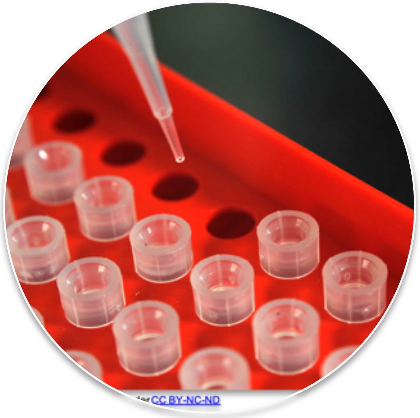
Bioinformatics

Data Integration and Analysis

Interpretation and Reporting

Wetlab

- Standardization of methods
- Training, SOP’s etc
- “LIMS”
- Efficiency and feasibility
- Modular workflows
  - Not linear



Capital


20:18
illumina
illumina


Haier
Salvum

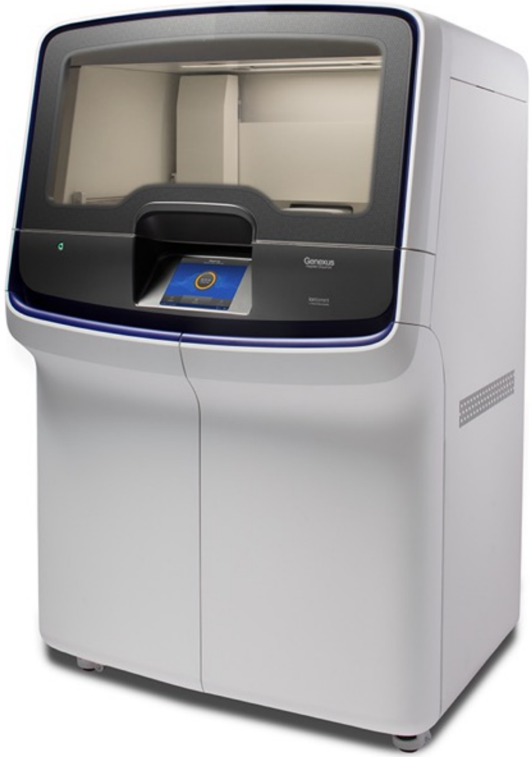

MICROLAB STAR
HAMILTON

Sequencing Platforms: The Options

MinION, GridION (ONT)

NextSeq, MiSeq, MiniSeq (Illumina)

Others

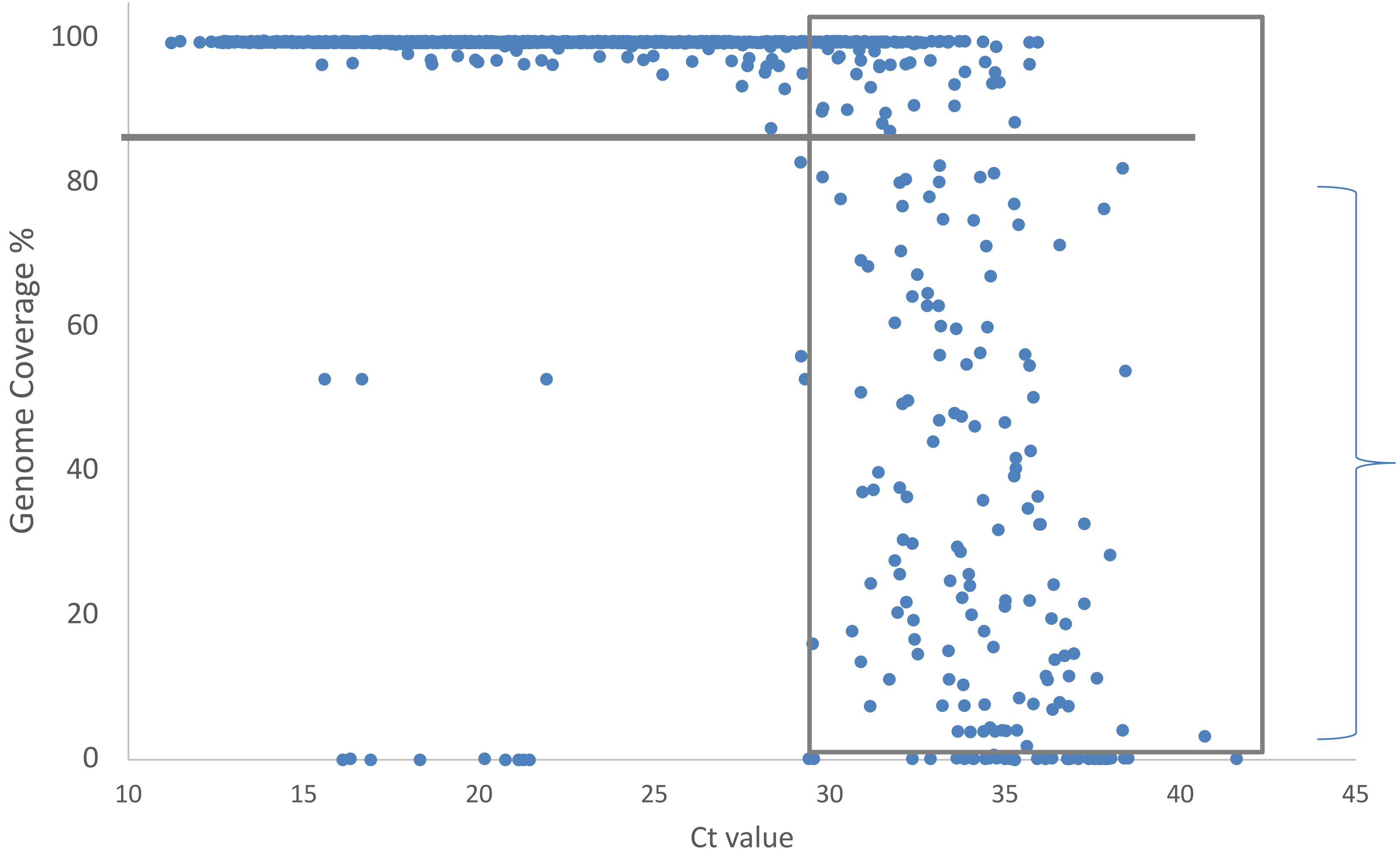


Automation of Library Preparation

- Equivalent library prep times vs manual
- Ergonomic gains
- Overall increase in in library quality
- More consistent performance

Eppendorf EpMotion 7075

Genome Coverage Decreases for Samples Beyond Ct 30, But Still Some Success



QC Threshold 85%

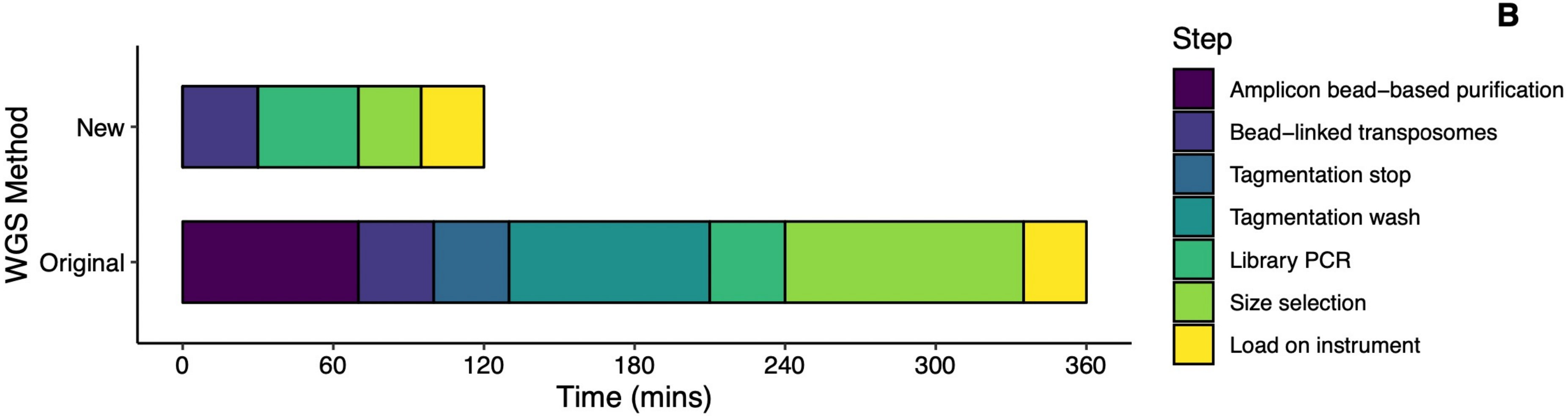
50% sequencing success at higher Cts

Table 1. Libraries genome completeness for each method

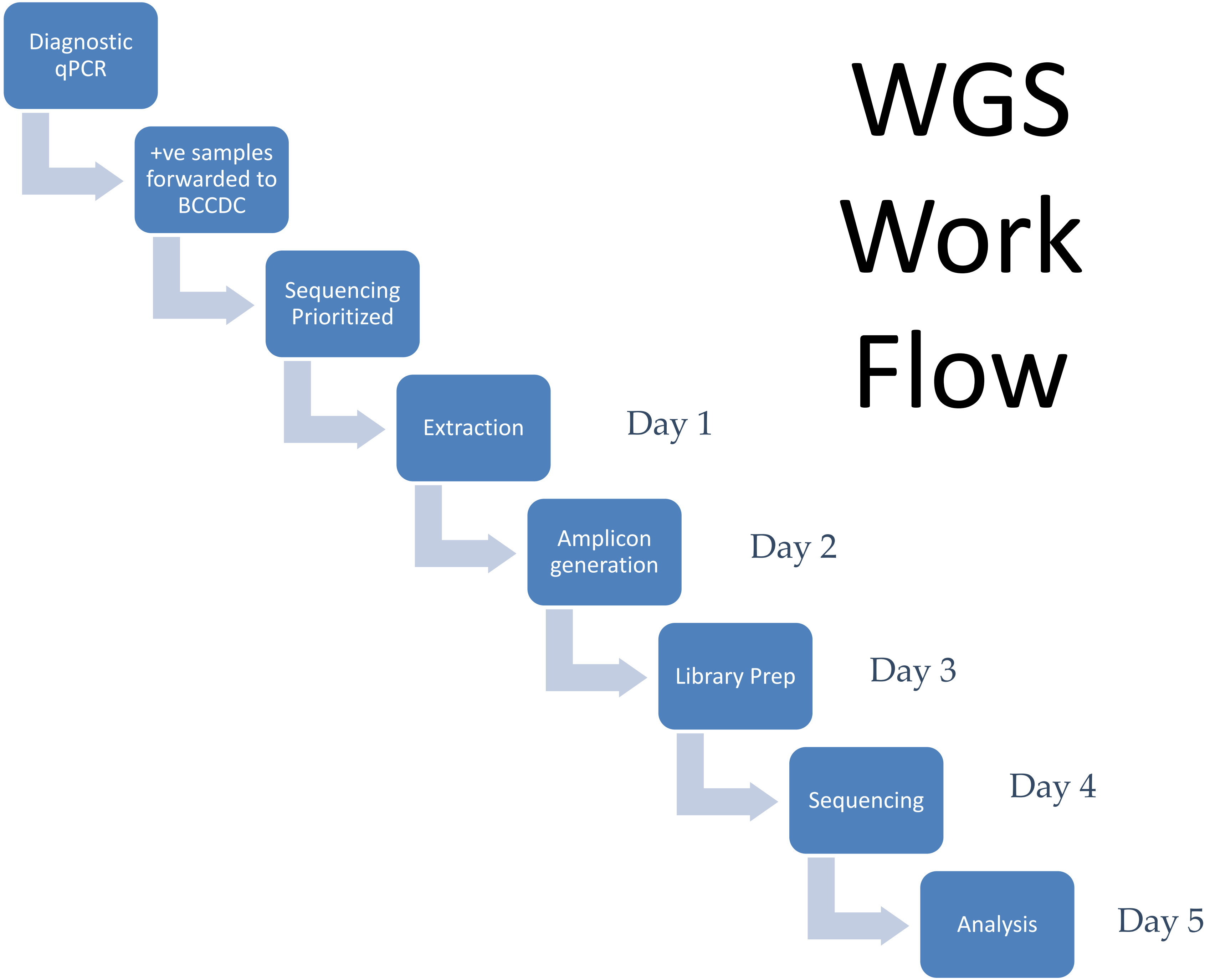
	Original method	New method
Number of samples	450	450
% Passed quality metric (>85%)	86.0 (387/450)	86.4 (389/450)
% Complete genome (>99%)	80.7 (363/450)	81.3 (366/450)
% Lineages called (>70%)	86.8 (391/450)	87.1 (392/450)

Table 2. Time, cost, and consumable savings

	Original method	New method
Hands-on time (manual)	4 hours, 30 minutes	1 hour
Total time to completion (manual)	6 hours	2 hours
Time on instrument (epMotion)	6 hours	45 minutes
Total time to completion (epMotion)	6 hours, 30 minutes	1 hour, thirty minutes
Cost of library prep (CAD list price)	$68	$31
Number of tip boxes	34	4



WGS Work Flow



Diagnostic qPCR

+ve samples forwarded to BCCDC

Sequencing Prioritized

Extraction — Day 1

Amplicon generation — Day 2

Library Prep — Day 3

Sequencing — Day 4

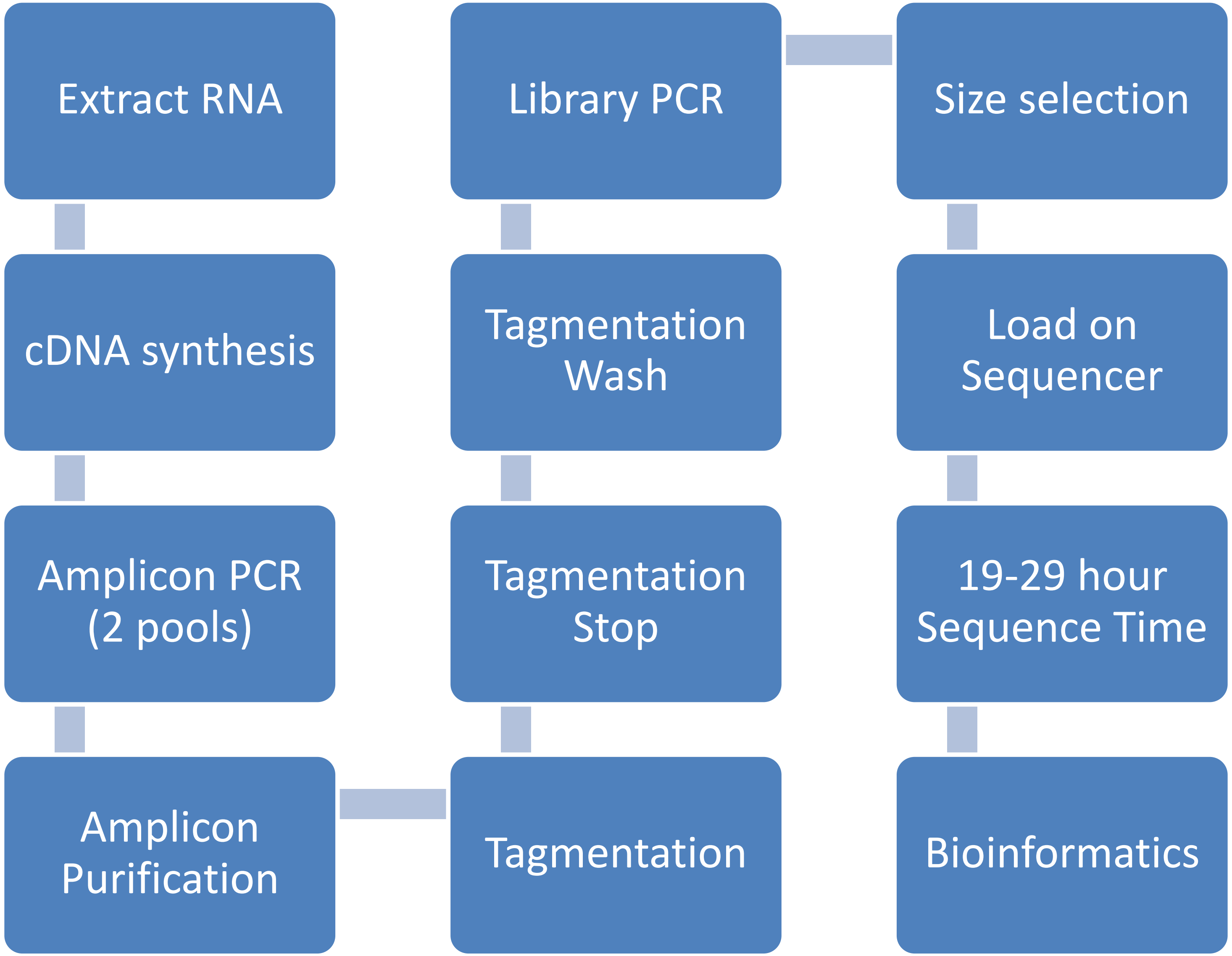
Analysis — Day 5

Increasing Pressure to Sequence More – How?

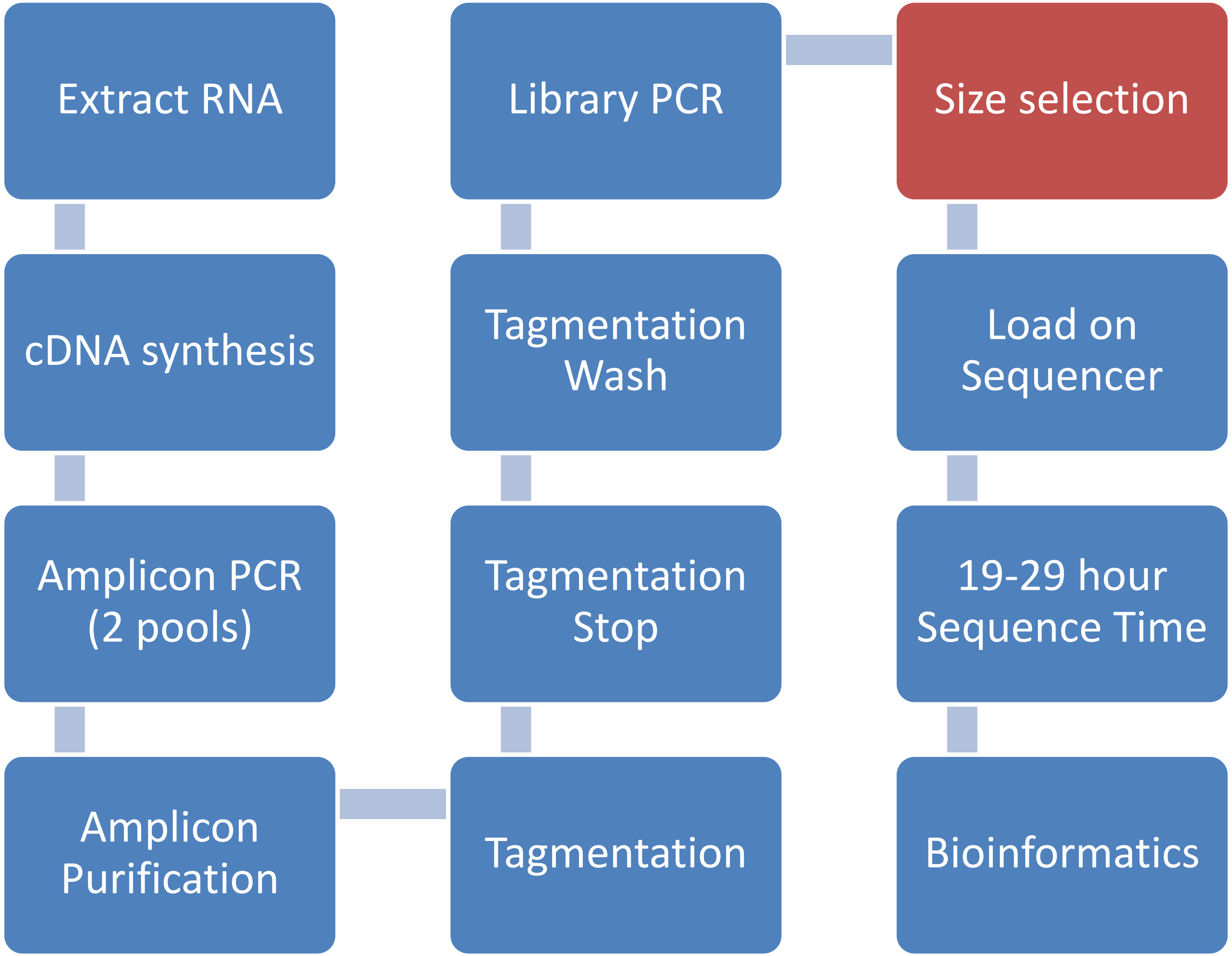
1200bp amplicon scheme

Illumina DNA Prep

MiSeq or NextSeq 2000


Extract RNA
cDNA synthesis
Amplicon PCR (2 pools)
Amplicon Purification
Tagmentation
Tagmentation Stop
Tagmentation Wash
Library PCR
Size selection
Load on Sequencer
19-29 hour Sequence Time
Bioinformatics

Increasing Pressure to Sequence More – How?



Extract RNA

cDNA synthesis

Amplicon PCR (2 pools)

Amplicon Purification

Tagmentation

Tagmentation Stop

Tagmentation Wash

Library PCR

Size selection

Load on Sequencer

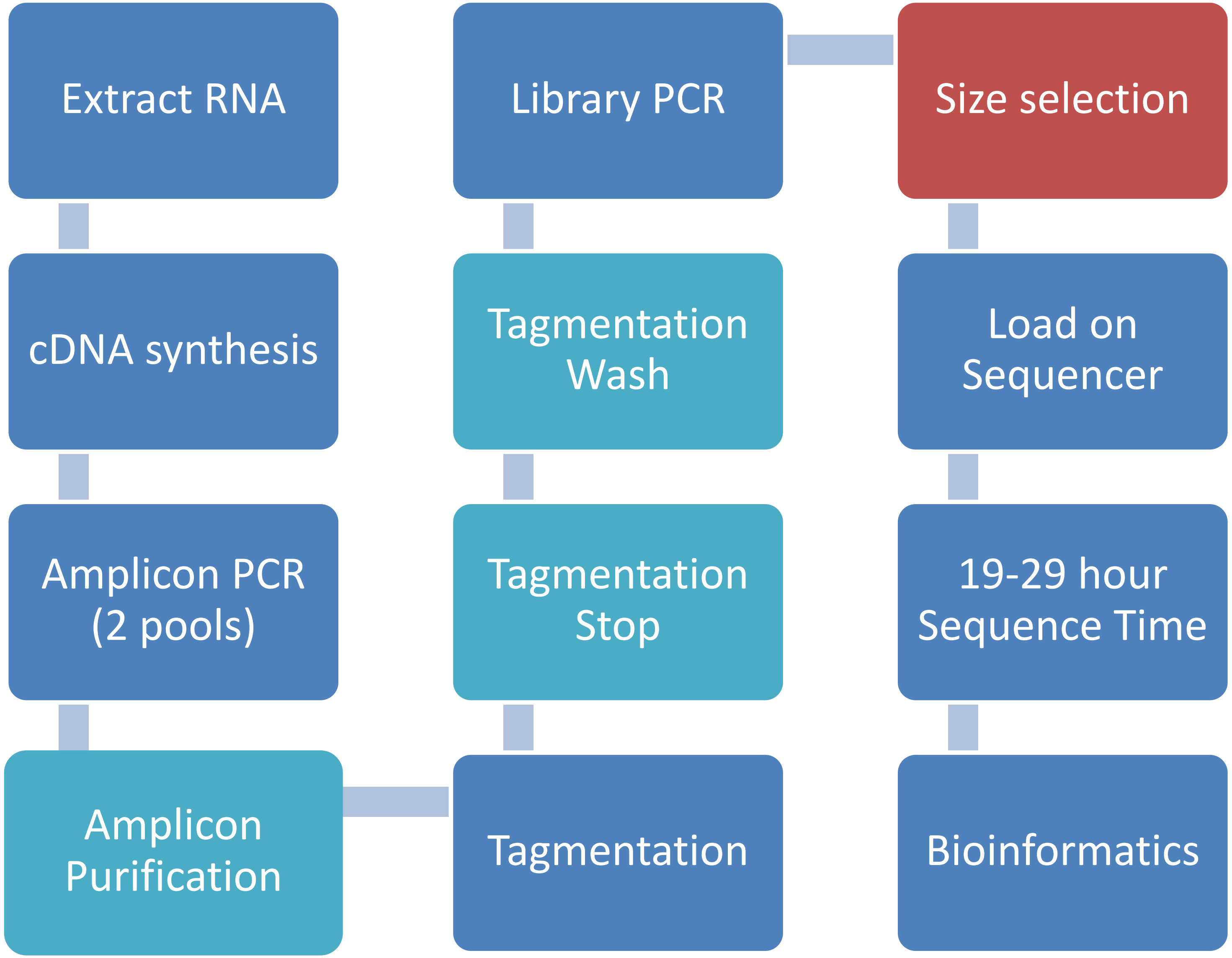
19-29 hour Sequence Time

Bioinformatics

Pooled all samples, then performed size selection manually
↓
made liquid handler available for another library

Increasing Pressure to Sequence More – How?

Removed timely steps without impacting library prep.
↓
Reduced time from 360 min to 120 min.



Extract RNA

cDNA synthesis

Amplicon PCR (2 pools)

Amplicon Purification

Tagmentation

Tagmentation Stop

Tagmentation Wash

Library PCR

Size selection

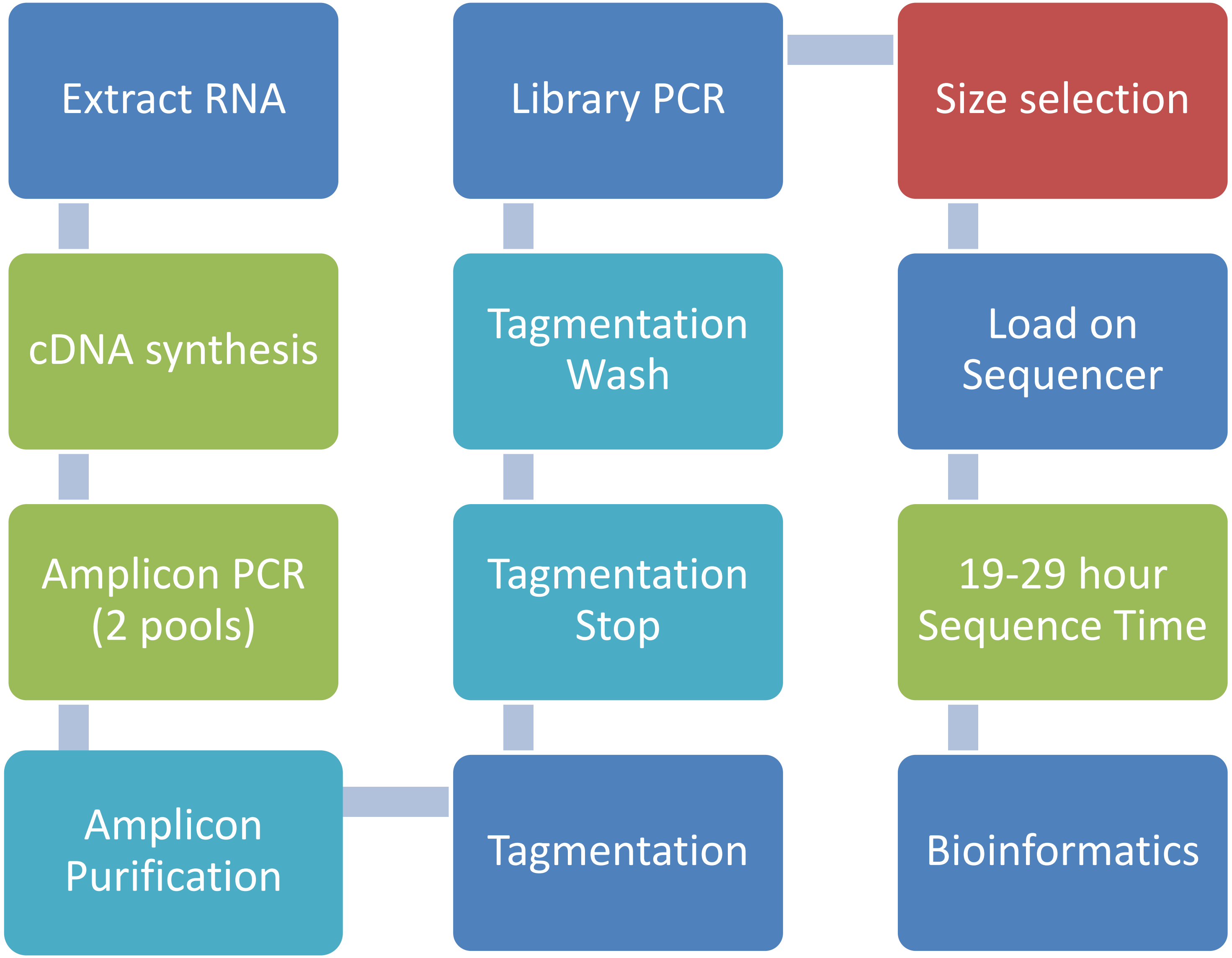
Load on Sequencer

19-29 hour Sequence Time

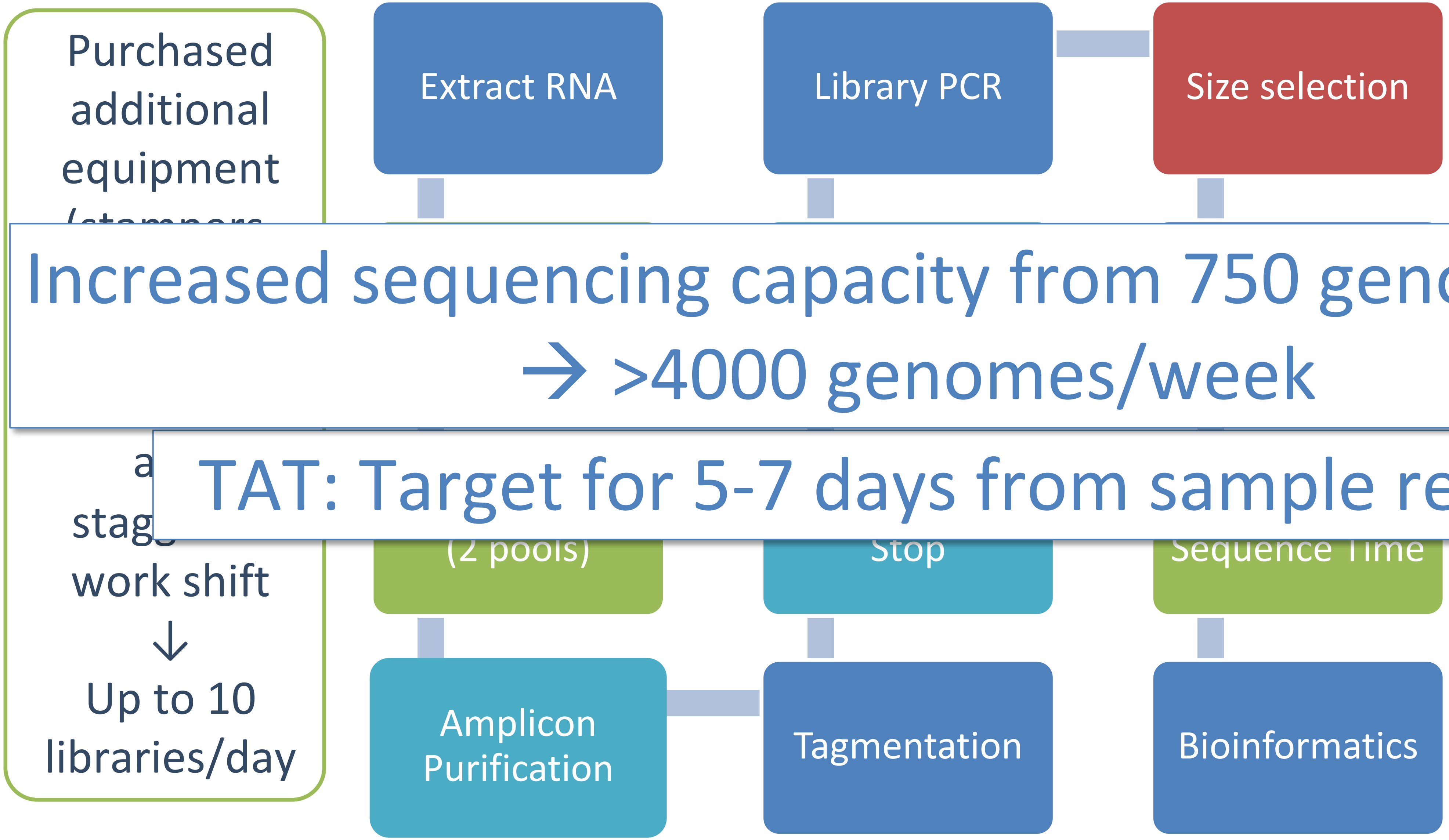
Bioinformatics

Increasing Pressure to Sequence More – How?

Purchased additional equipment (stampers, liquid handlers, sequencers) and staggered work shift
↓
Up to 10 libraries/day


Extract RNA
cDNA synthesis
Amplicon PCR (2 pools)
Amplicon Purification
Tagmentation
Tagmentation Stop
Tagmentation Wash
Library PCR
Size selection
Load on Sequencer
19-29 hour Sequence Time
Bioinformatics

Increasing Pressure to Sequence More – How?



Purchased
additional
equipment
(stampers

a
stag
work shift
↓
Up to 10
libraries/day

Extract RNA

Library PCR

Size selection

(2 pools)

Stop

Sequence Time

Amplicon Purification

Tagmentation

Bioinformatics

Increased sequencing capacity from 750 genomes/week → >4000 genomes/week

TAT: Target for 5-7 days from sample receipt

COVID-19 WGS: Reasons for testing

- Surveillance
  - VOCs, emerging strains, at risk populations, etc
  - Monitor travel related cases (importation of new strains)
  - Monitor vaccinated vs unvaccinated cases
  - Etc

- Support public health and IPAC's cluster and outbreak responses
- Monitor severe cases
- Monitor breakthrough and re-infection cases
- Meet federal surveillance requirements

Sequencing Capacity

- Before the pandemic, capacity was 24 samples/wk
- Currently, throughput is 4000 samples/wk
  - Weekly cases currently >5000 samples
- Federal recommendations ~10-15% of positive cases.
  - BC is testing ~90-100%
  - BC is the only province testing at this capacity

- Current state is not sustainable

Challenges

- Major challenge is sample storage and handling
  - – Freezer capacity limited
- ➢ Going forward
  - – Send ALL positives for first Epi Week each month
  - – Send priority samples and 10% “convenient” background samples
  - – Archive all other positives for E.g. 4 weeks
- ➢ Retrospective
  - – Send un-sequenced positive samples to BCCDC PHL or
  - – Follow BCCDC PHL’s retention strategy

Recommendation

- Combine a monthly point prevalence testing with targeted testing by Epi weeks:

Week 1
- All positive cases by collection date

Week 2
- 10% convenient sampling
- Priority List

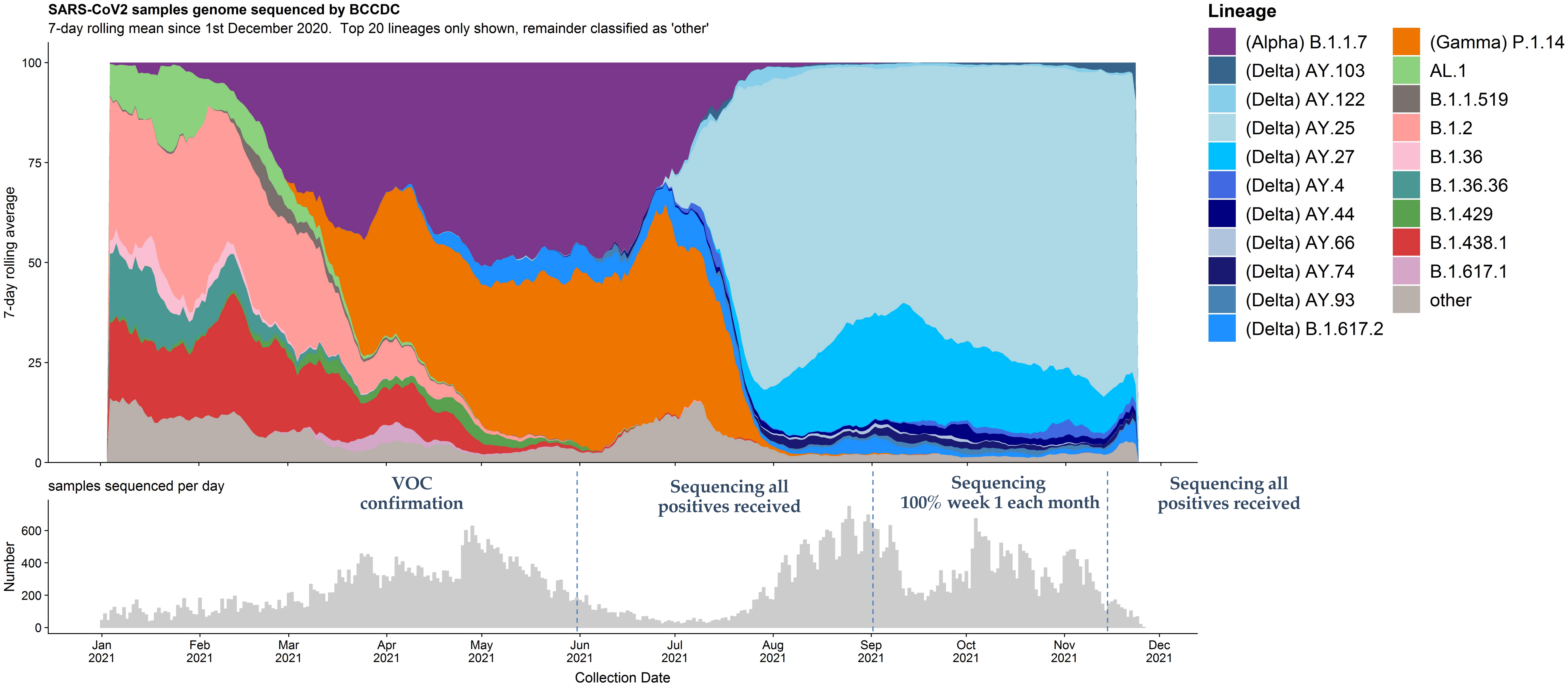
Week 3
- 10% convenient sampling
  Priority List

Week 4
- 10% convenient sampling
- Priority List

Priority List:
- Clusters & outbreaks
- Travel
- Hospitalized/LTCF
- Re-infections
- Vaccine breakthroughs
- PHAC surveillance
- Other MHO requests

-This strategy will be modified as weekly volumes increase or decrease

Sequencing at BCCDC PHL shows temporal evolution of pandemic


SARS-CoV2 samples genome sequenced by BCCDC

7-day rolling mean since 1st December 2020. Top 20 lineages only shown, remainder classified as 'other'

samples sequenced per day

VOC confirmation

Sequencing all positives received

Sequencing 100% week 1 each month

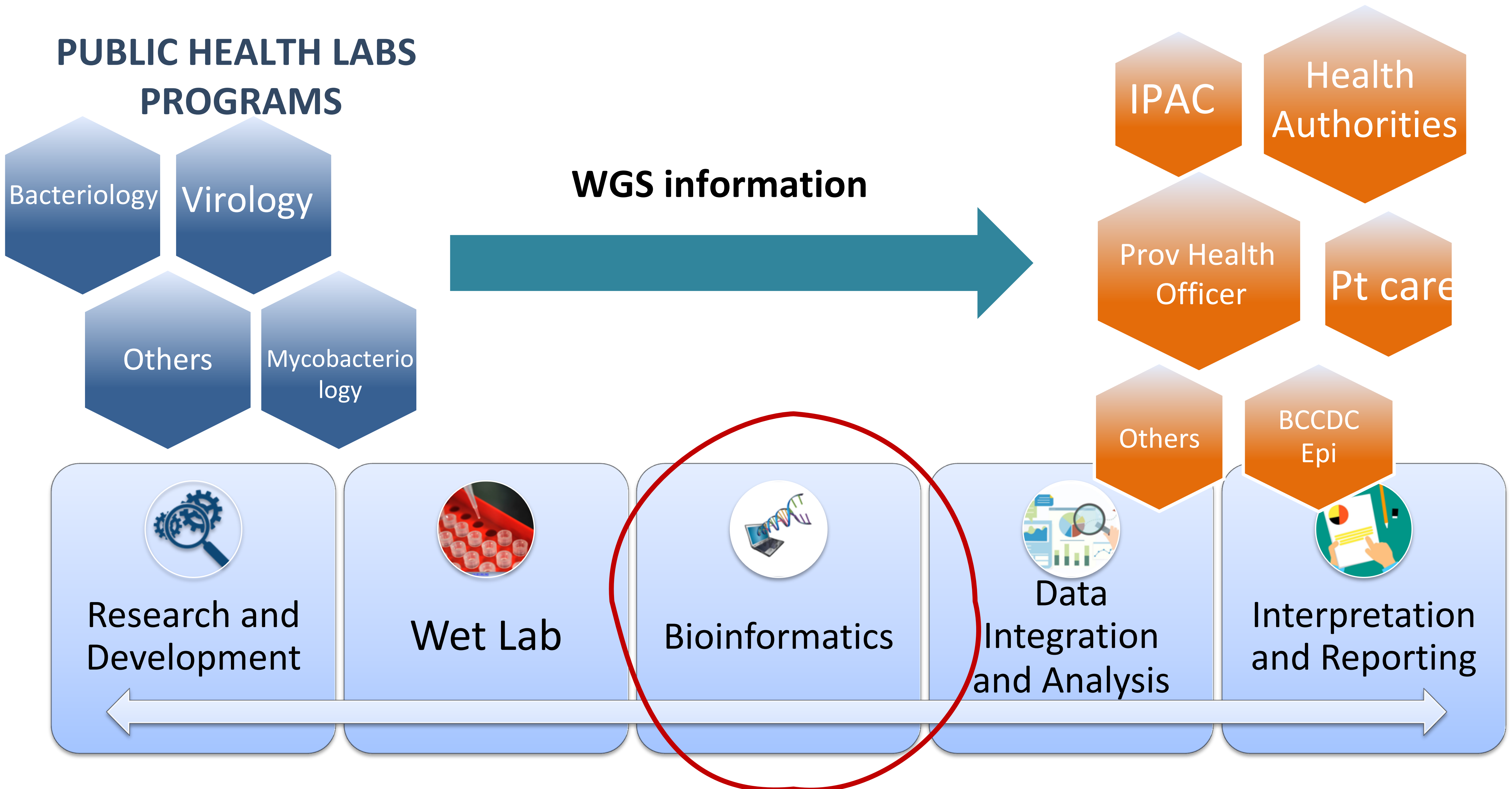
Sequencing all positives received

Pangolin version: 3.1.16, PangoLEARN: 2021-11-18, Total sequences: 85423

Key messages

WGS by BC CDC PHL shows:

- **March → July 2021:** co-dominance of Alpha and Gamma.
- **April → July 2021:** Delta introduction remaining at low levels for nearly 3 months between Gamma and Alpha.
- **July 2021 → present:** Delta established dominance quickly at a time of low case counts.
- **July 2021 → present:** the current predominant lineage of Delta is AY.25, with AY.27 being the other main lineage we are seeing.
- **By HA in the Delta era:** trends similar, but some differences (e.g. AY.4 in VIHA).
  - Note, AY.4 is parent lineage of AY.4.2 flagged by PHE as having enhanced growth.



PUBLIC HEALTH LABS PROGRAMS

- Bacteriology
- Virology
- Others
- Mycobacteriology

WGS information

END USERS

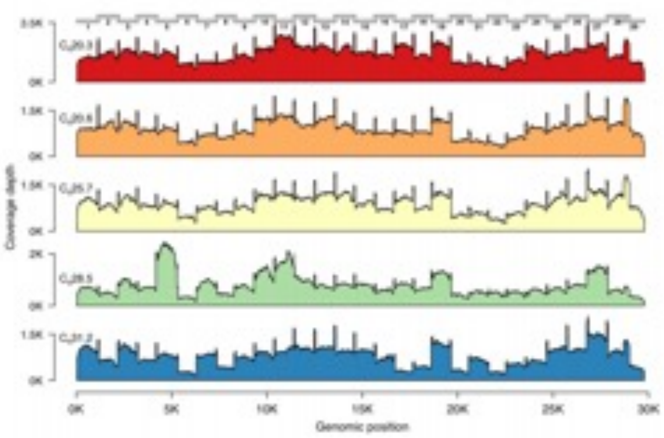
- IPAC
- Health Authorities
- Prov Health Officer
- Pt care
- Others
- BCCDC Epi

- Research and Development
- Wet Lab
- Bioinformatics
- Data Integration and Analysis
- Interpretation and Reporting

Bioinformatics

- People, people, people
- Automated wgs quality checks
  - Technologists with bioinformatics support
- Standardized pipelines for data integration

Analysis



OICR Fork of Connor Pipeline

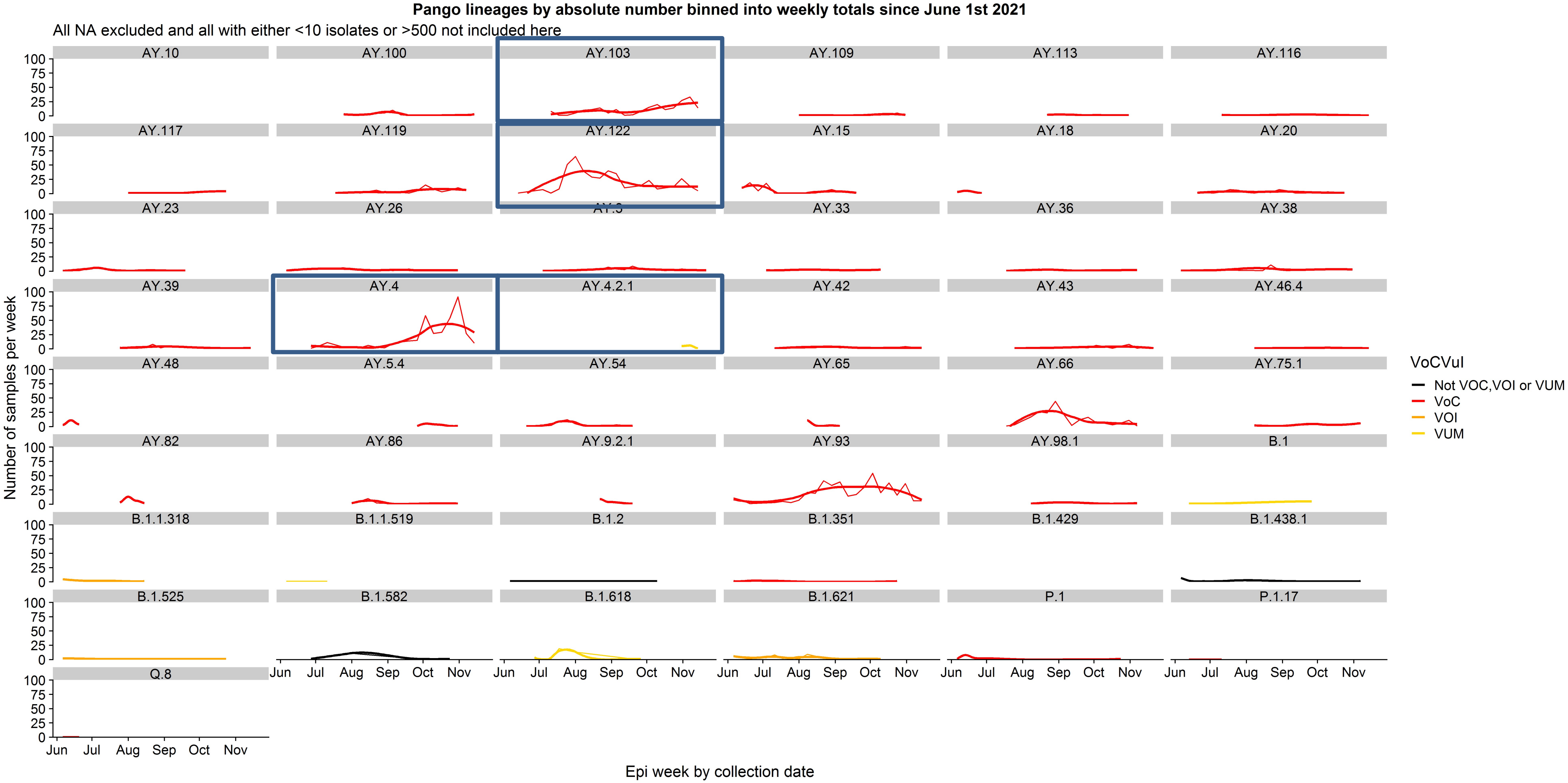
1. Quality filtering & adaptor removal
2. Mapping against human
3. Mapping against reference (Wuhan-Hu-1)
4. Primer trimming, generate consensus sequence (ivar)

Analysis

```
AAAGAAACTATAGCTGAGAGCGGCG
ATCGTACGATGCATGCTAGCTAGCG
AGAGCGGCGATCGTACGATGCATGC
TAGCTAGCGAGAGCGGCGATCGTAC
GATGCATGCTAGCTAGCGAGAGCGG
CGATCGTACGATGCATGCTAGCTAG
CGAGAGCGGCGATCGTACGATGCAT
```

1. ncov-tools (OICR)
2. Pangolearn
3. Mutation watchlist
4. Phylogeny w/ epi on NextStrain

Lineages by HA over time



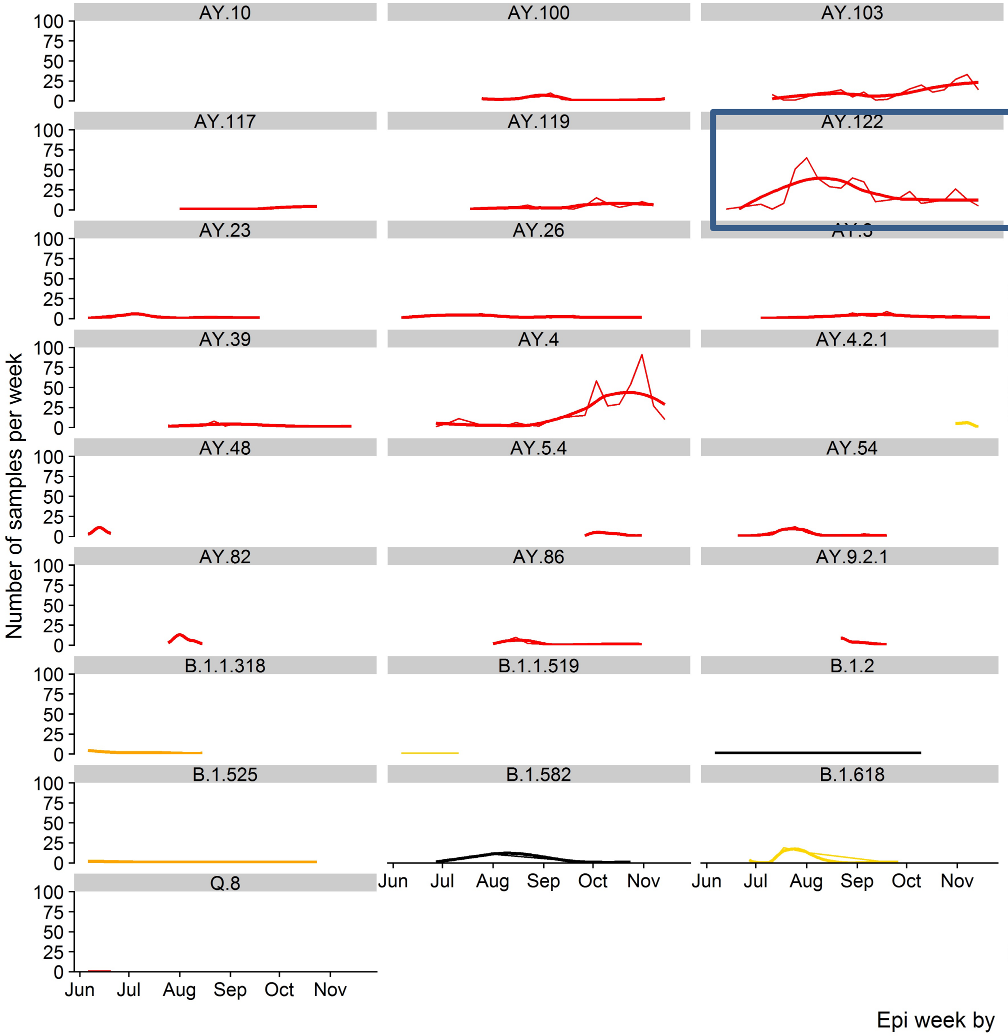
Pango lineages by absolute number binned into weekly totals since June 1st 2021

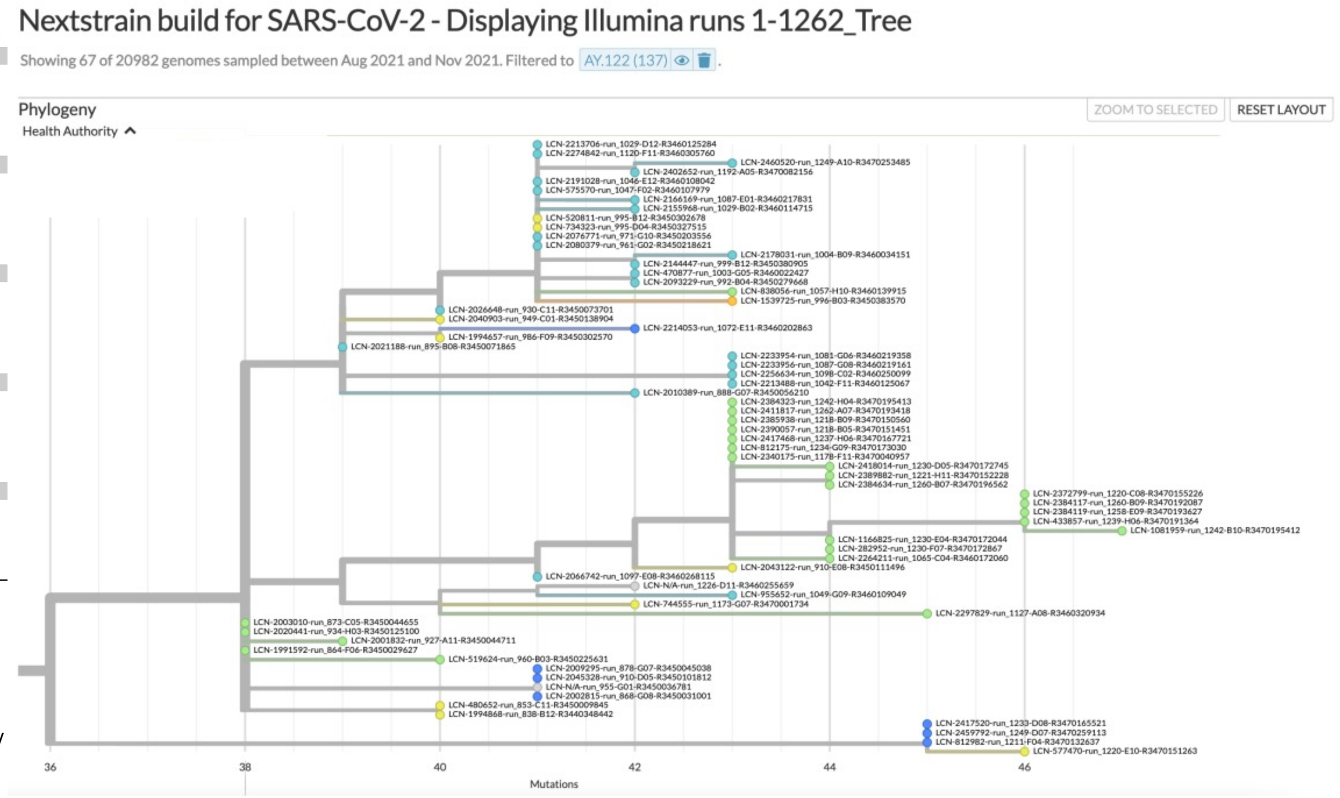
All NA excluded and all with either <10 isolates or >500 not included here

Lineages by HA and phylogeny

Pango lineages by absolute number binned into weekly totals since June 1st 2021

All NA excluded and all with either <10 isolates or >500 not included here

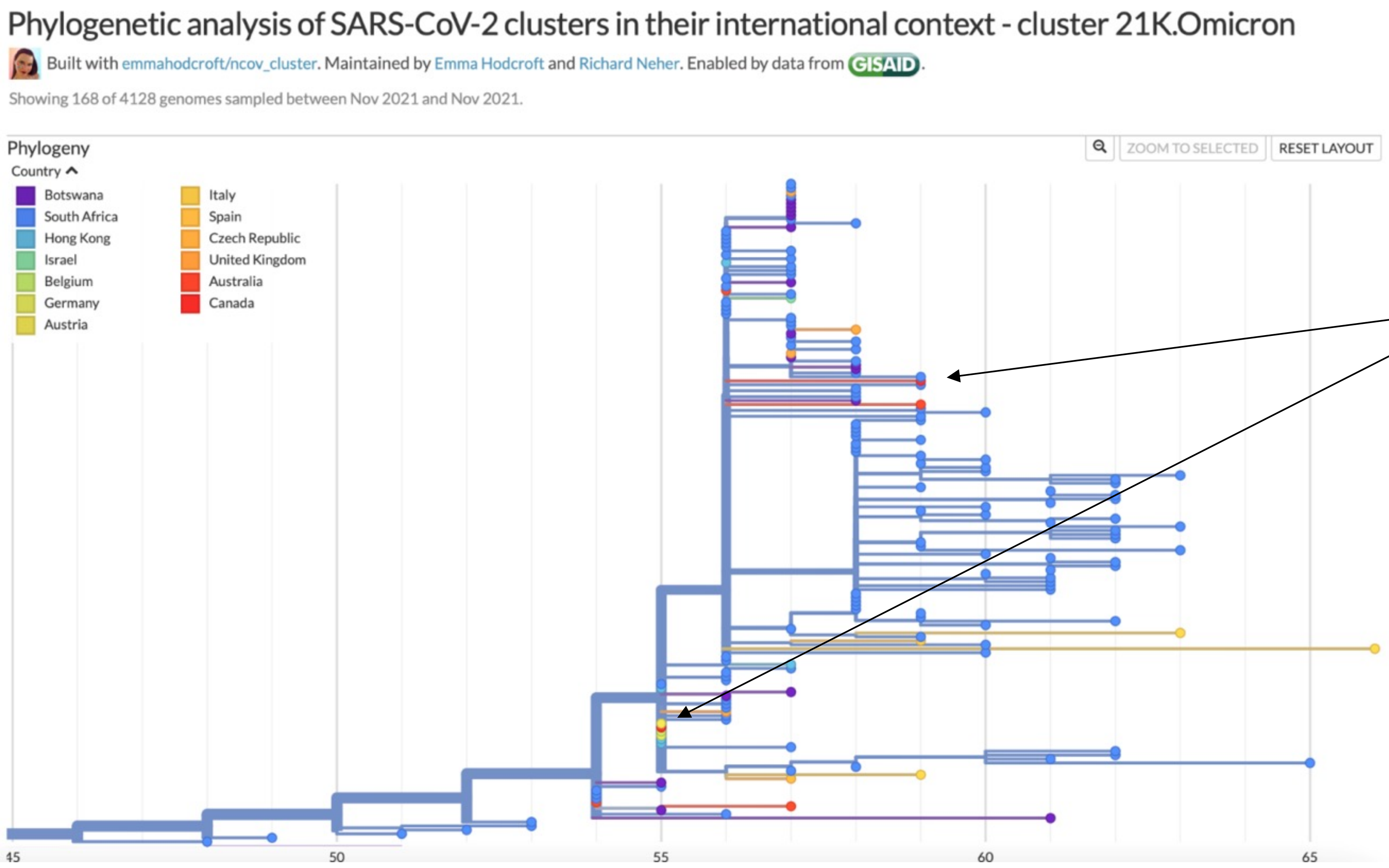




Nextstrain build for SARS-CoV-2 - Displaying Illumina runs 1-1262_Tree

Showing 67 of 20982 genomes sampled between Aug 2021 and Nov 2021. Filtered to AY.122 (137)

December 2nd Omicron



Phylogenetic analysis of SARS-CoV-2 clusters in their international context - cluster 21K.Omicron

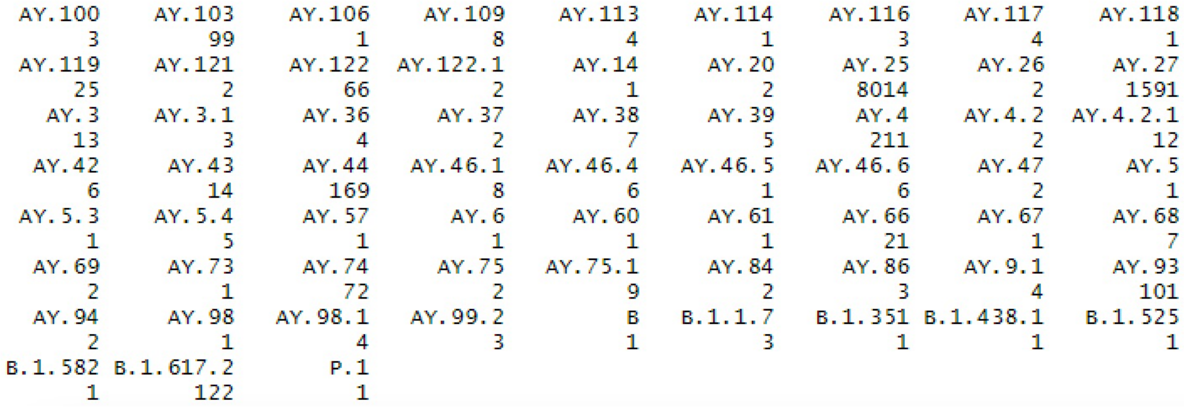
Built with emmahodcroft/ncov_cluster. Maintained by Emma Hodcroft and Richard Neher. Enabled by data from GISAID.

Showing 168 of 4128 genomes sampled between Nov 2021 and Nov 2021.

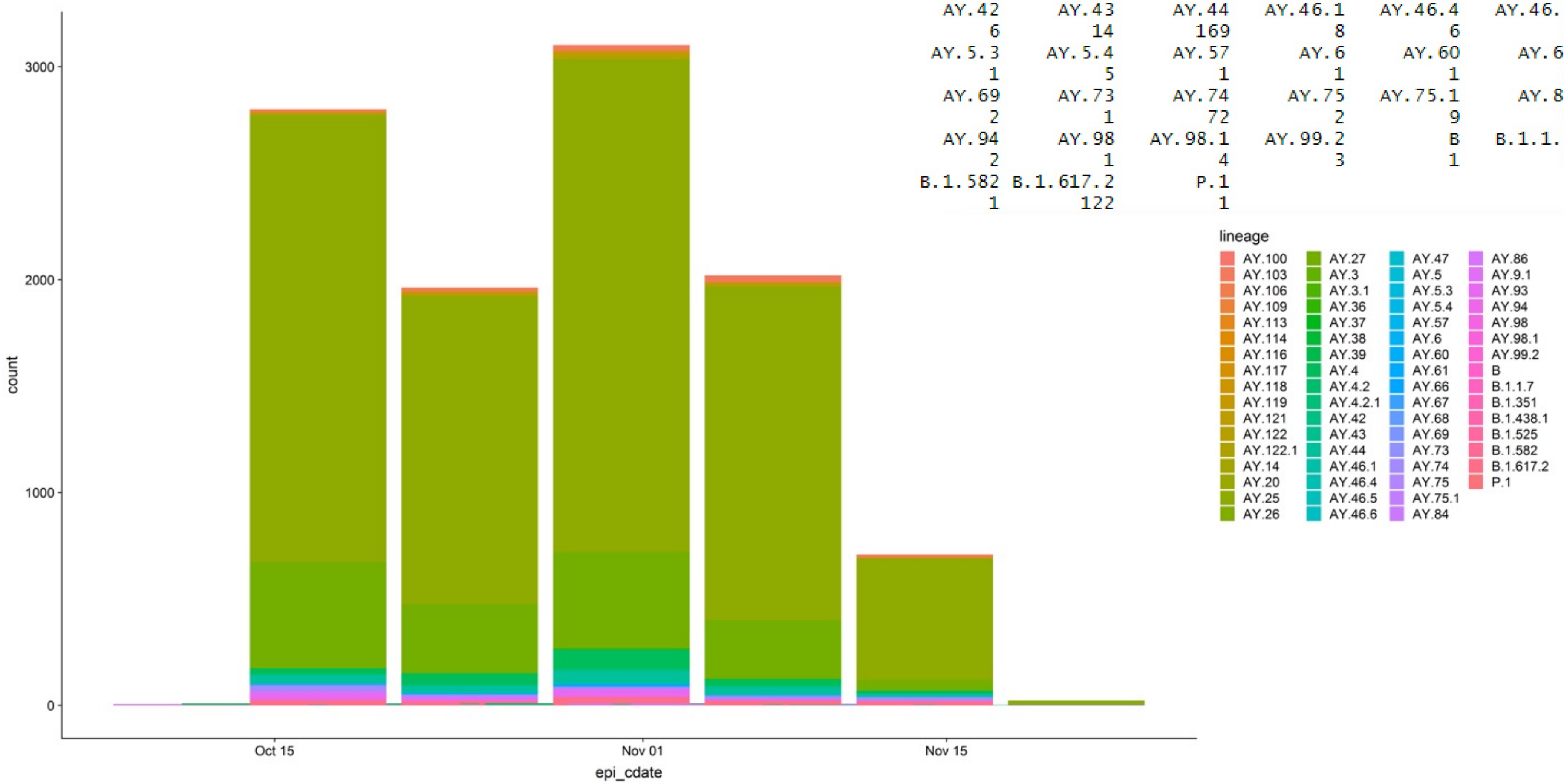
Canada (Ontario)

Already some diversity (+ 2 intros in Ontario?)

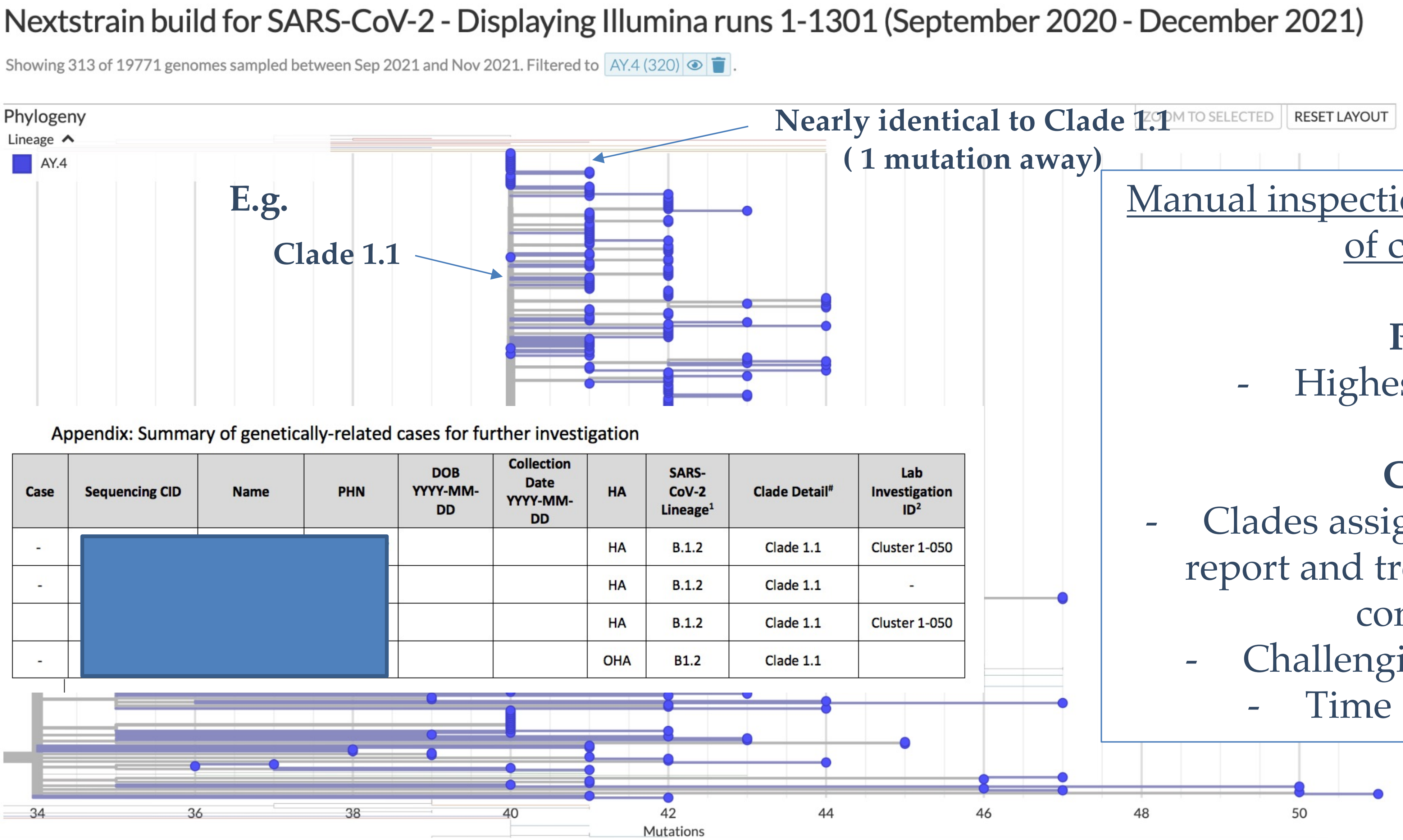
Lineages over time



```
   AY.100    AY.103    AY.106    AY.109    AY.113    AY.114    AY.116    AY.117    AY.118
        3        99         1         8         4         1         3         4         1
   AY.119    AY.121    AY.122  AY.122.1     AY.14     AY.20     AY.25     AY.26     AY.27
       25         2        66         2         1         2      8014         2      1591
     AY.3    AY.3.1     AY.36     AY.37     AY.38     AY.39      AY.4    AY.4.2  AY.4.2.1
       13         3         4         2         7         5       211         2        12
    AY.42     AY.43     AY.44   AY.46.1   AY.46.4   AY.46.5   AY.46.6     AY.47      AY.5
        6        14       169         8         6         1         6         2         1
   AY.5.3    AY.5.4     AY.57      AY.6     AY.60     AY.61     AY.66     AY.67     AY.68
        1         5         1         1         1         1        21         1         7
    AY.69     AY.73     AY.74     AY.75   AY.75.1     AY.84     AY.86    AY.9.1     AY.93
        2         1        72         2         9         2         3         4       101
    AY.94     AY.98   AY.98.1   AY.99.2         B   B.1.1.7   B.1.351 B.1.438.1   B.1.525
        2         1         4         3         1         3         1         1         1
  B.1.582 B.1.617.2       P.1
        1       122         1
```



Current practice: Manual clade calling



Nextstrain build for SARS-CoV-2 - Displaying Illumina runs 1-1301 (September 2020 - December 2021)

Showing 313 of 19771 genomes sampled between Sep 2021 and Nov 2021. Filtered to AY.4 (320).

Nearly identical to Clade 1.1
(1 mutation away)

E.g.

Clade 1.1

Appendix: Summary of genetically-related cases for further investigation

Case	Sequencing CID	Name	PHN	DOB YYYY-MM-DD	Collection Date YYYY-MM-DD	HA	SARS-CoV-2 Lineage[1]	Clade Detail[#]	Lab Investigation ID[2]
-	[redacted]	[redacted]	[redacted]			HA	B.1.2	Clade 1.1	Cluster 1-050
-	[redacted]	[redacted]	[redacted]			HA	B.1.2	Clade 1.1	-
	[redacted]	[redacted]	[redacted]			HA	B.1.2	Clade 1.1	Cluster 1-050
-	[redacted]	[redacted]	[redacted]			OHA	B1.2	Clade 1.1	

<u>Manual inspection and assignment of clades:</u>

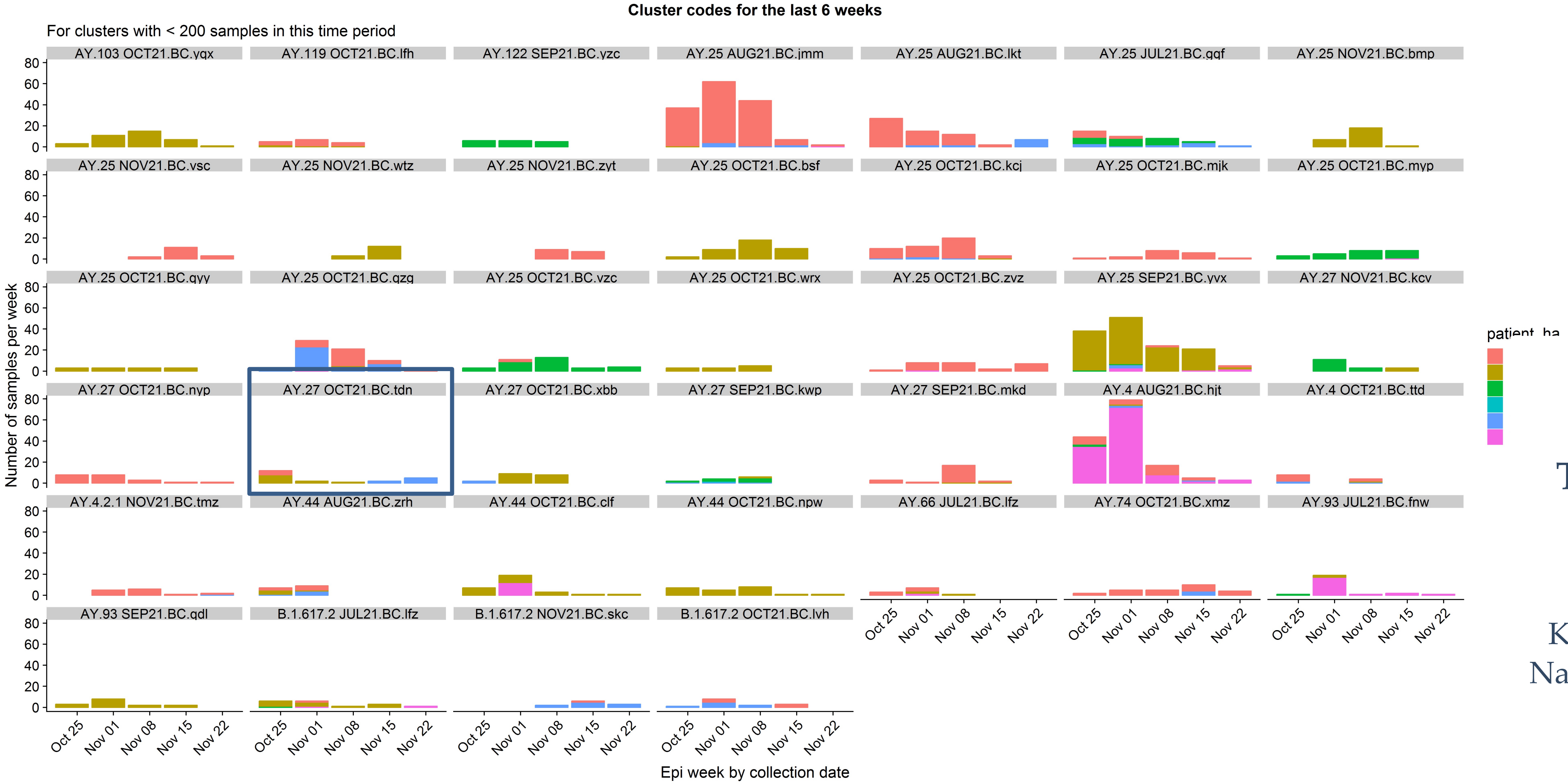
Pro:

- Highest resolution

Con:

- Clades assignment specific to report and tree (cannot be cross compared).
- Challenging to interpret.
- Time consuming.

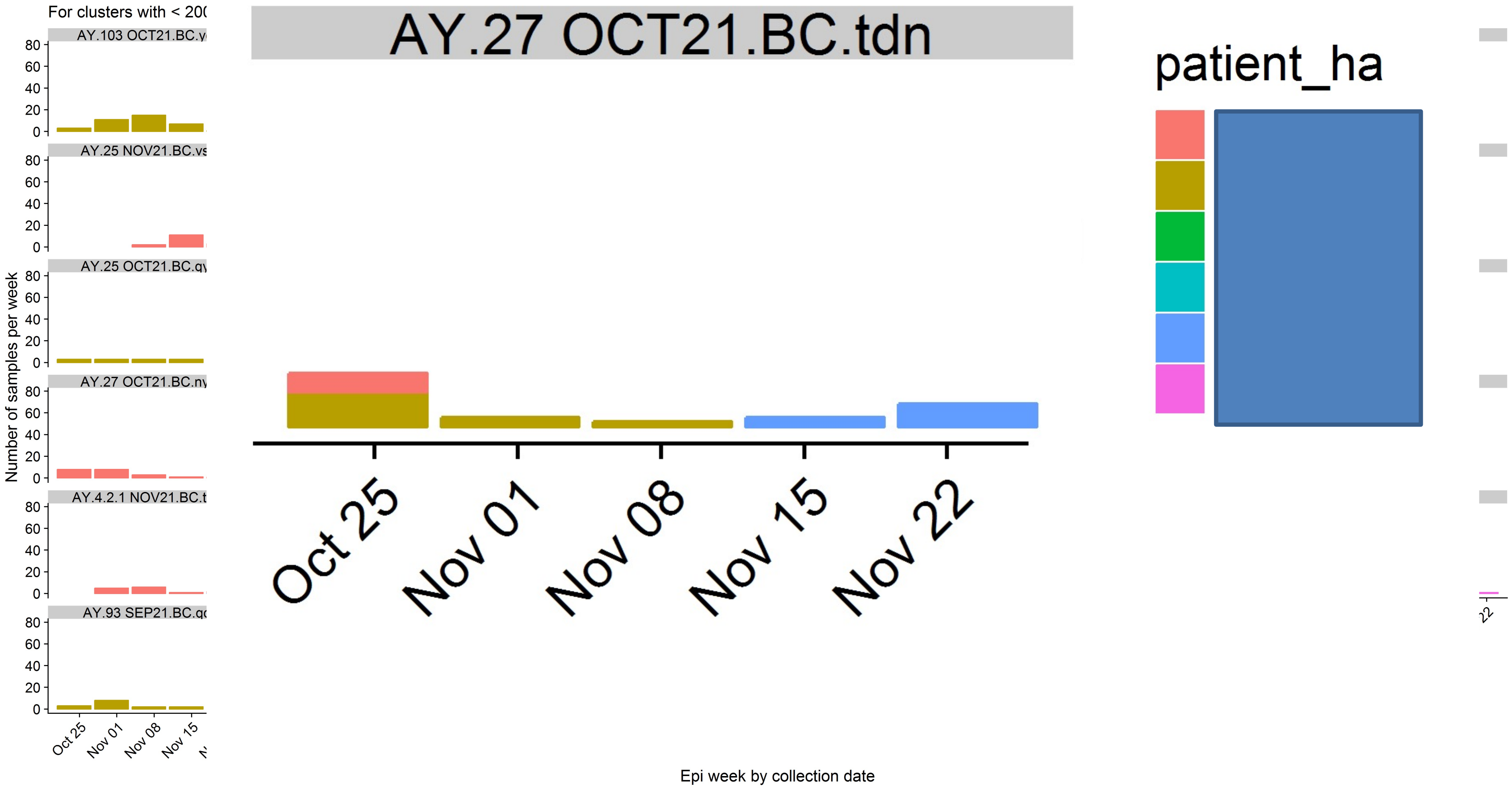
Cluster codes allow surveillance



Tool developed by:
Caroline Colijn (SFU)
Ben Sobkowiak (SFU)

Kimia Kamelian (BCCDC)
Natalie Prystajecky (BCCDC)
Linda Hoang (BCCDC)

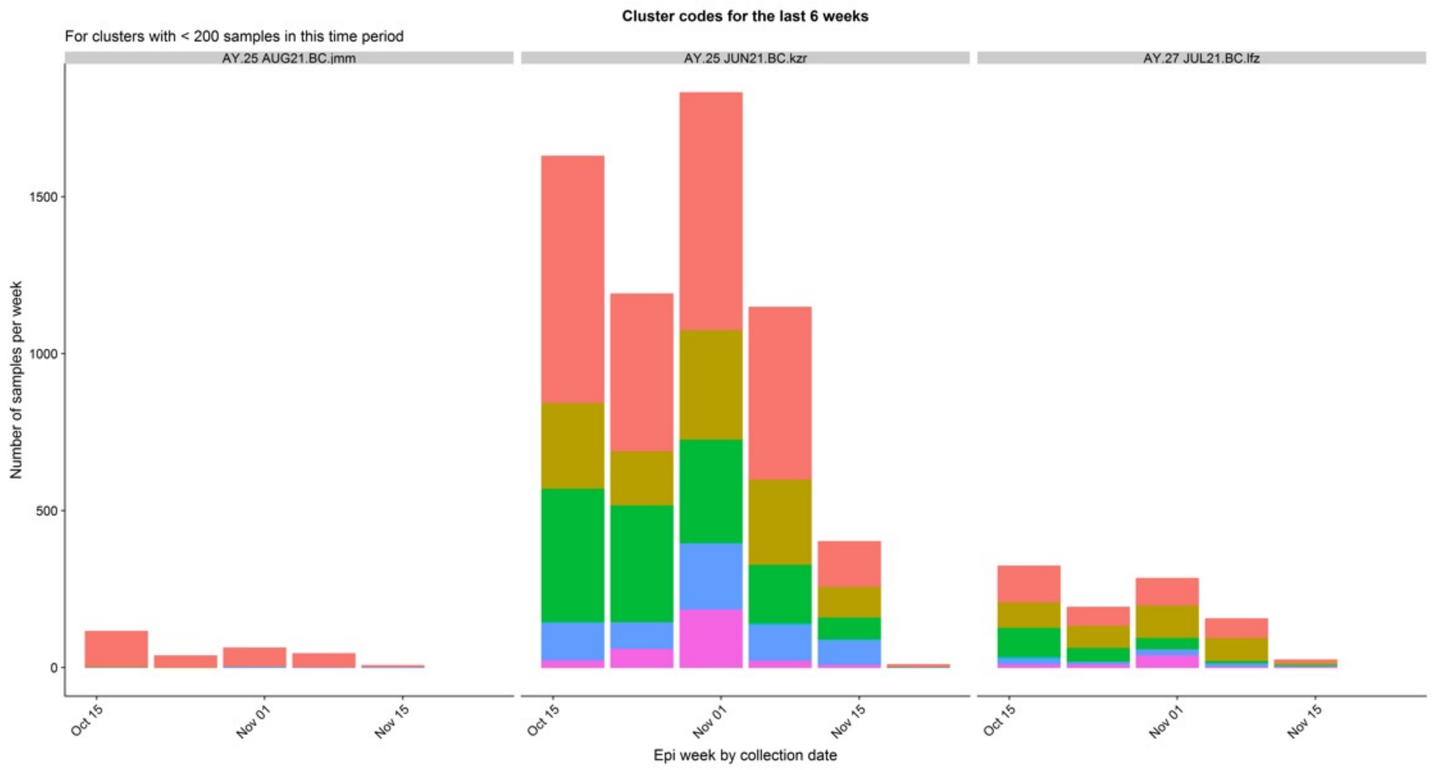
Cluster codes allow surveillance



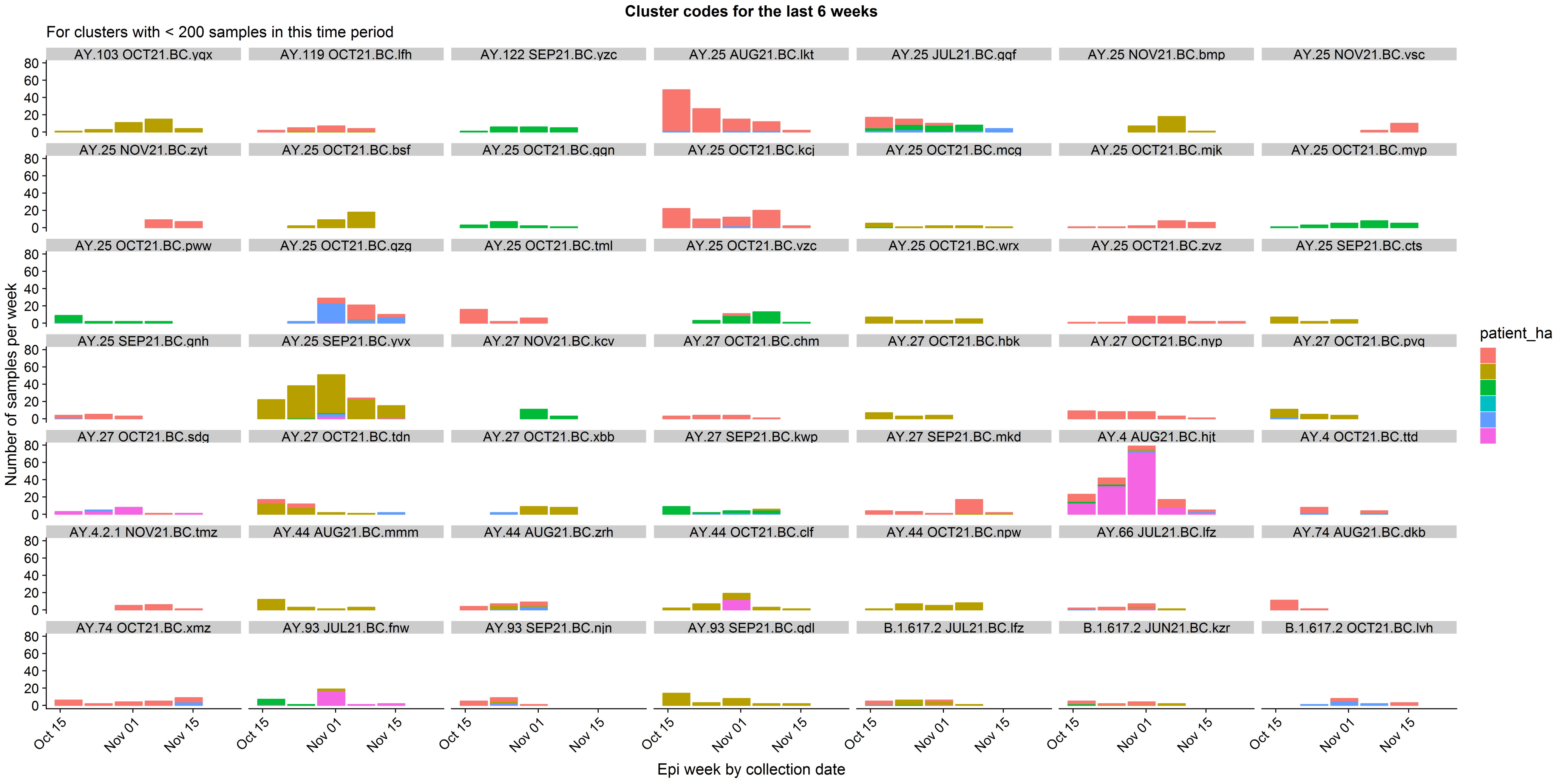
Tool developed by:
Caroline Colijn (SFU)
Ben Sobkowiak (SFU)

Kimia Kamelian (BCCDC)
Natalie Prystajecky (BCCDC)
Linda Hoang (BCCDC)

Cluster codes – major clusters



Cluster codes – major clusters and Health Authorities



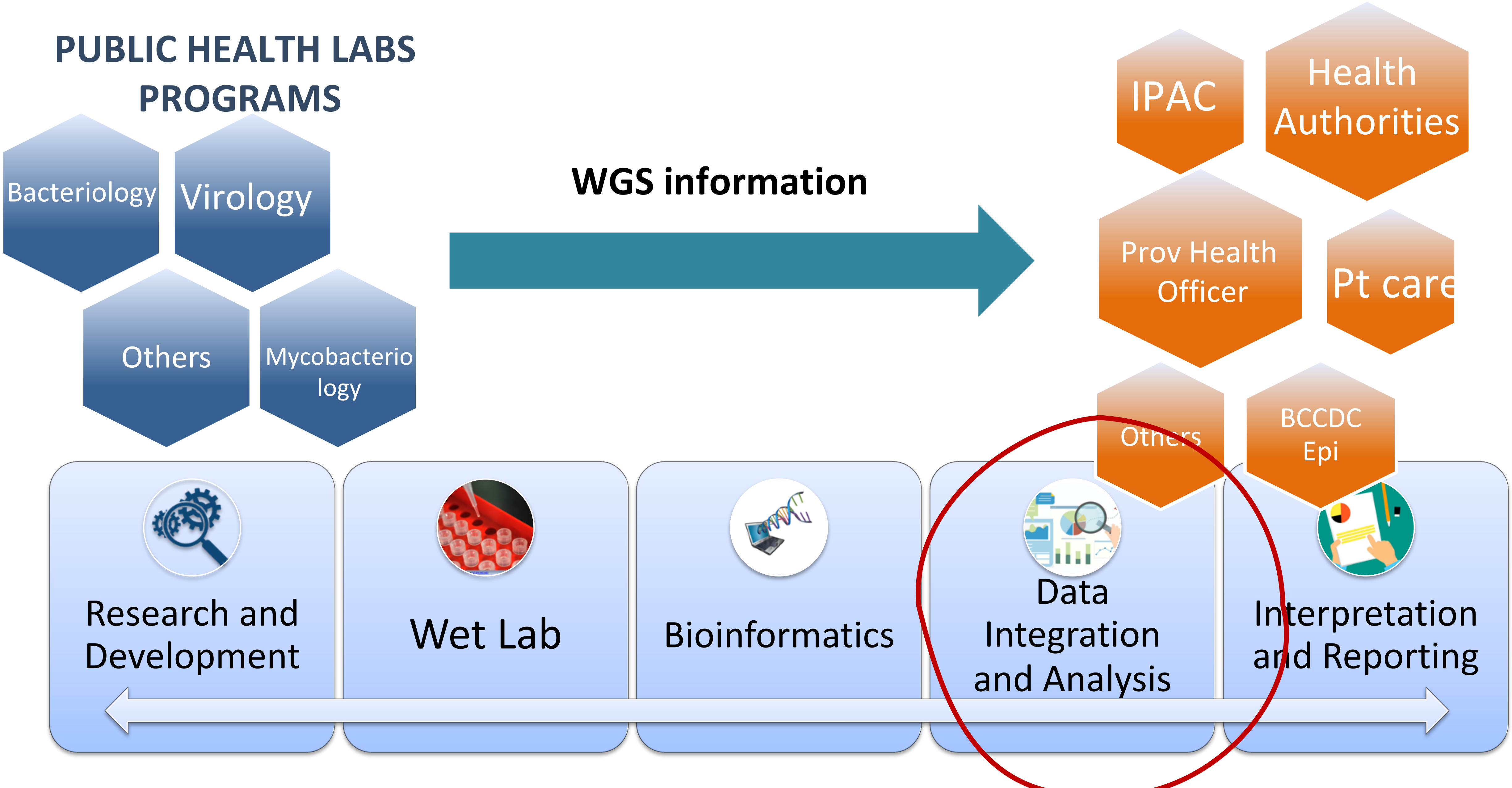
Key messages

WGS by BC CDC PHL:

- Assigns lineages to samples.
- Allow tracking of the pandemic across time.
- Phylogenetic tree analysis of WGS allows even higher resolution.
- To date, manual inspection of trees has allowed reporting of outbreak clusters in response to requests from health authorities.
- Cluster tool developed through collaboration with SFU.
  - Allows automatic assignment of sequences to cluster codes.
  - Cluster codes will be consistent between trees

END USERS

PUBLIC HEALTH LABS PROGRAMS



Bacteriology

Virology

Others

Mycobacteriology

WGS information

IPAC

Health Authorities

Prov Health Officer

Pt care

Others

BCCDC Epi

Research and Development

Wet Lab

Bioinformatics

Data Integration and Analysis

Interpretation and Reporting

December 2019

- Servers in place
- Support in alpha for AMR bacteria WGS
- FluWatch in prod

January 2020

- *Wuhan virus warning*
- PHO Bonnie Henry orders PHL to receive test records and make available
- January 28, initial site goes live for PHO, MHOs, BCCDC

February 2020

- MHOs request support for contact tracers etc
- New types of users added

2021

- VOC tests
- Point of Care (Rapid) tests
- Support for BCCDC hotline
- Automatic data transfers (file and email)
- Private lab data (discontinued)

December 2019
- Servers in place
- WGS in alpha for AMR bacteria
- Watch in prod

February 2020
- MHOs est supp contact tra
- N of users

2021

VOC tests
of Care (Rapid) tests
upport for BCCDC hotline
tomatic data transfers (file and email)
data (discontinued)

Current numbers:
- 130 active users (600 total)
- 4.1 million COVID-19 tests
- 94,400 VOC (PCR) tests
- 25.6 million Sunquest records
- 21,300 emails sent (batch case notifications to HAs)
- 92,900 WGS results

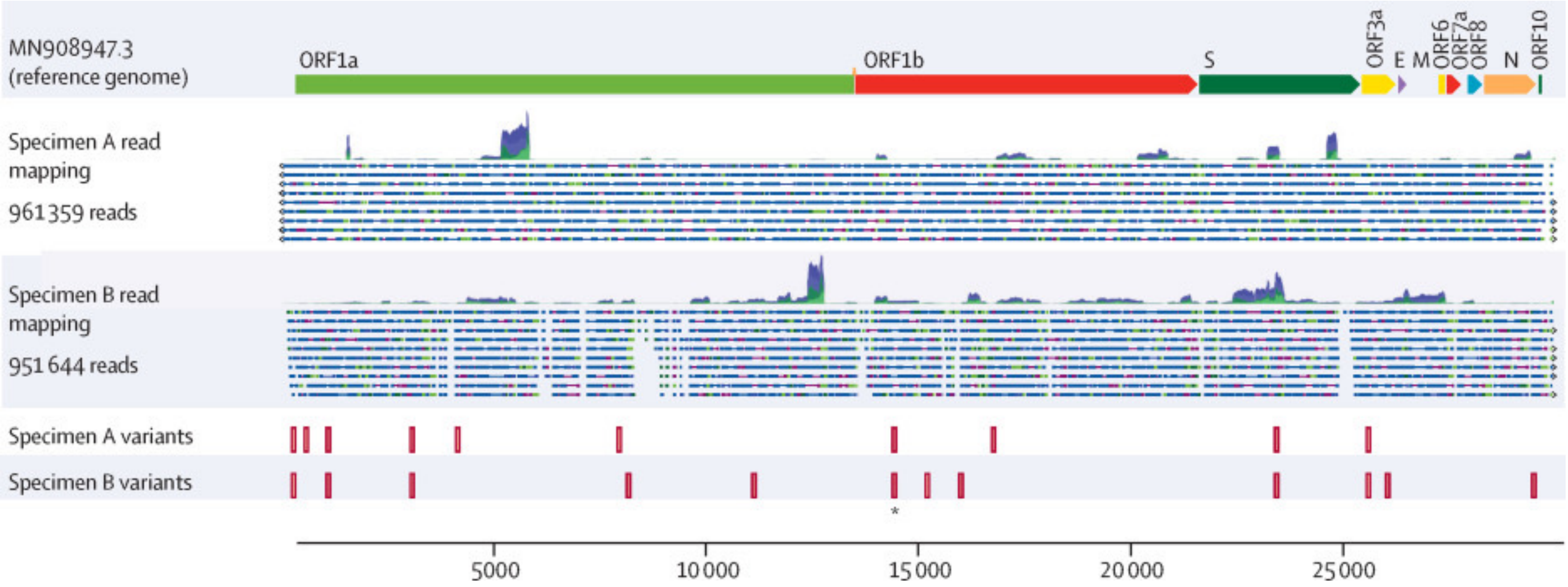
Data linkage

- Realtime
- Automated
- Quality assured
- Laboratory as source for data linkage
  - Lab metadata (e.g. age, gender, HA, etc)
  - Reason for testing - flagged at sample collection and data entry)
  - Lab results
  - Significant Epi meta data (e.g. vaccination, hospitalization, travel)
- Data accessible by both labs and Epi
- Tight working relationship, avoid duplication

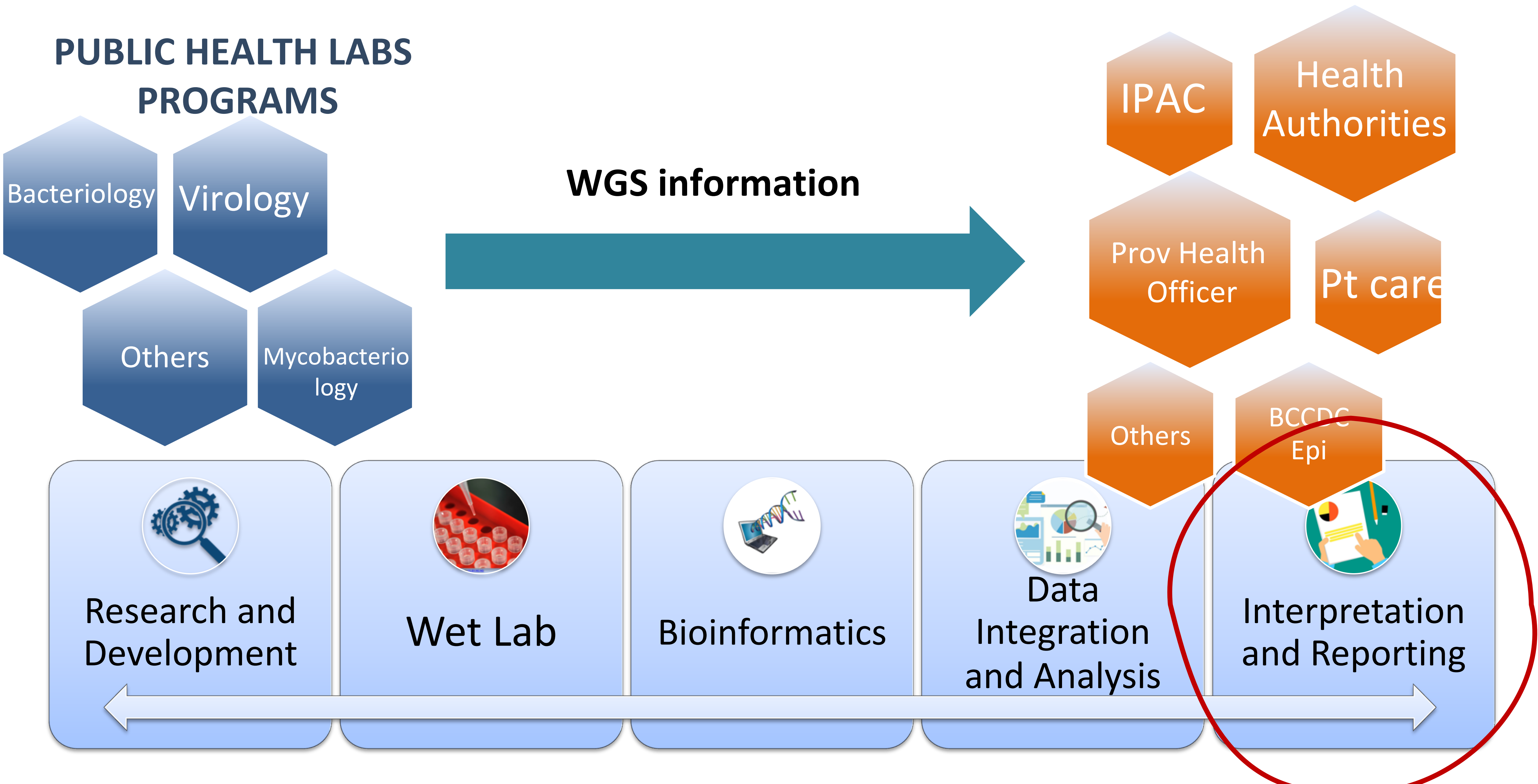
COVID-19 health outcomes by vaccination status and age, BC, Nov 2021

Data include cases from Nov 03-Nov 30, hospitalizations, critical care admissions and deaths from Oct 31-Nov 27, and vaccination statistics on Nov 16, 2021

Reinfections



Tillet et al, 2021. Lancet Infectious Diseases



END USERS

PUBLIC HEALTH LABS PROGRAMS

Bacteriology

Virology

Others

Mycobacteriology

WGS information

IPAC

Health Authorities

Prov Health Officer

Pt care

Others

BCCDC Epi

Research and Development

Wet Lab

Bioinformatics

Data Integration and Analysis

Interpretation and Reporting

WGS Request Form -standardized request format for end users (e.g. Health authorities, IPAC)

BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Public Health Laboratory
655 West 12th Avenue, Vancouver, BC V5Z 4R4
www.bccdc.ca/publichealthlab

LM LABS

CLUSTER INVESTIGATION
Genomic Subtyping Request for SARS-CoV-2

Date	____________	**Contact Person**	____________
Organism (Genus, species)	SARS-CoV-2	**Telephone Number**	____________
CD Unit/Health Authority	____________	**Fax Number**	____________
Acute Care Facility	____________	**Send Report To**	____________

Cluster Details: ______________________________

Cases are epidemiologically linked? ☐ Yes ☐ No If Yes, indicate how:

☐ Health care exposure ☐ Contact of case(s) ☐ Other risk factors:
☐ Common event ☐ Travel ______________________________

Line List: (Please attach spreadsheet/line list if more cases/specimens are in your cluster)

Patient Name:	**PHN:**	**DOB** (DD/MM/YY)**:**	**CT Values from COVID NAT (if available):**	
			RdRP Gene**:**	E Gene**:**

If these specimens were tested for COVID-19 at a lab **other** than the BCCDC, please ensure the samples have been sent to BCCDC for WGS analysis.

Comments:

INTERNAL USE ONLY

Approved by: ☐ Yes ☐ No

☐ Dr. L. Hoang, Program Head, Bacteriology & Mycology Laboratory, BCCDC Public Health Laboratory
OR
☐ Dr. N. Prystajecky, Program Head, Environmental Microbiology, BCCDC Public Health Laboratory
OR
☐ Medical Microbiologist on call, (Name): ______________________

Please email completed form to **_BCCDC_NCOV.PUI@bccdc.ca**

Form PHBM_COVIDWGS Version 1.0 04/2020

Whole genome sequencing of SARS-Co-V2 at BC CDC

Samples received

Whole genome Sequencing

Lineage assignment

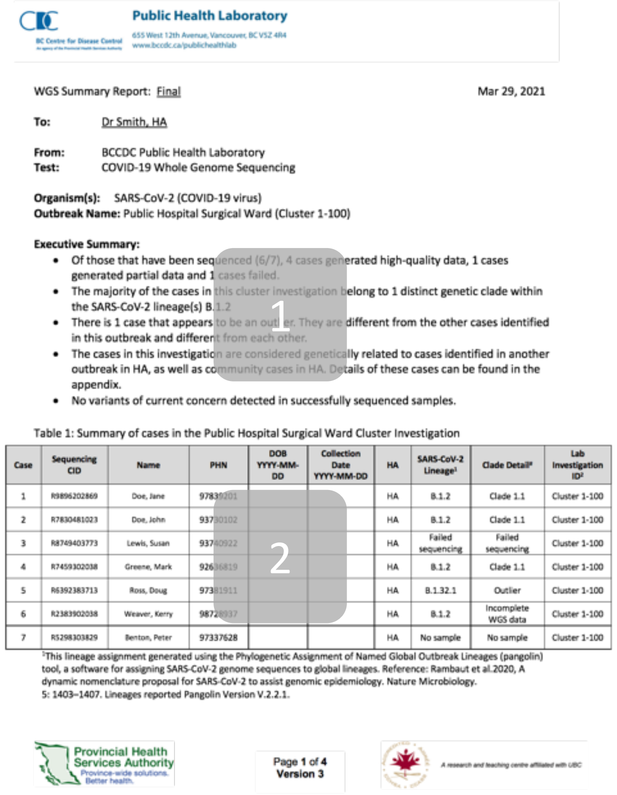
Incorporation into phylogenetic tree & Clade assignment

Report

Fully operationalised workflow!!!

All stages performed by technical staff with scientific and clinical oversight!

The report back to end users…



Public Health Laboratory
655 West 12th Avenue, Vancouver, BC V5Z 4R4
www.bccdc.ca/publichealthlab

WGS Summary Report: Final Mar 29, 2021

To: Dr Smith, HA

From: BCCDC Public Health Laboratory
Test: COVID-19 Whole Genome Sequencing

Organism(s): SARS-CoV-2 (COVID-19 virus)
Outbreak Name: Public Hospital Surgical Ward (Cluster 1-100)

Executive Summary:

- Of those that have been sequenced (6/7), 4 cases generated high-quality data, 1 cases generated partial data and 1 cases failed.
- The majority of the cases in this cluster investigation belong to 1 distinct genetic clade within the SARS-CoV-2 lineage(s) B.1.2
- There is 1 case that appears to be an outlier. They are different from the other cases identified in this outbreak and different from each other.
- The cases in this investigation are considered genetically related to cases identified in another outbreak in HA, as well as community cases in HA. Details of these cases can be found in the appendix.
- No variants of current concern detected in successfully sequenced samples.

Table 1: Summary of cases in the Public Hospital Surgical Ward Cluster Investigation

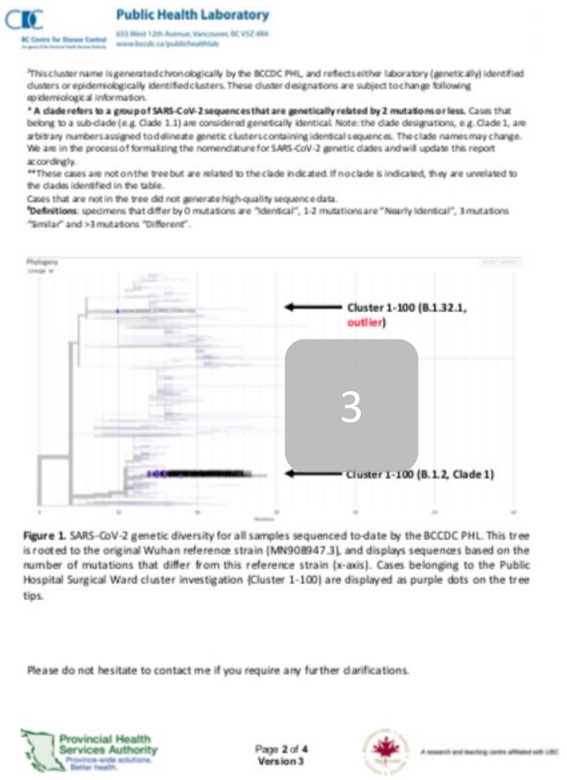
Case	Sequencing CID	Name	PHN	DOB YYYY-MM-DD	Collection Date YYYY-MM-DD	HA	SARS-CoV-2 Lineage[1]	Clade Detail*	Lab Investigation ID[2]
1	R9896202869	Doe, Jane	97839201			HA	B.1.2	Clade 1.1	Cluster 1-100
2	R7830481023	Doe, John	93730102			HA	B.1.2	Clade 1.1	Cluster 1-100
3	R8749403773	Lewis, Susan	93740922			HA	Failed sequencing	Failed sequencing	Cluster 1-100
4	R7459302038	Greene, Mark	92636819			HA	B.1.2	Clade 1.1	Cluster 1-100
5	R6392383713	Ross, Doug	97381911			HA	B.1.32.1	Outlier	Cluster 1-100
6	R2383902038	Weaver, Kerry	98728937			HA	B.1.2	Incomplete WGS data	Cluster 1-100
7	R5298303829	Benton, Peter	97337628			HA	No sample	No sample	Cluster 1-100

[1] This lineage assignment generated using the Phylogenetic Assignment of Named Global Outbreak Lineages (pangolin) tool, a software for assigning SARS-CoV-2 genome sequences to global lineages. Reference: Rambaut et al.2020, A dynamic nomenclature proposal for SARS-CoV-2 to assist genomic epidemiology. Nature Microbiology. 5: 1403–1407. Lineages reported Pangolin Version V.2.2.1.

Provincial Health Services Authority
Province-wide solutions. Better health.

Page 1 of 4
Version 3

A research and teaching centre affiliated with UBC



Public Health Laboratory
655 West 12th Avenue, Vancouver, BC V5Z 4R4
www.bccdc.ca/publichealthlab

[2] This cluster name is generated chronologically by the BCCDC PHL, and reflects either laboratory (genetically) identified clusters or epidemiologically identified clusters. These cluster designations are subject to change following epidemiological information.

* **A clade refers to a group of SARS-CoV-2 sequences that are genetically related by 2 mutations or less.** Cases that belong to a sub-clade (e.g. Clade 1.1) are considered genetically identical. Note: the clade designations, e.g. Clade 1, are arbitrary numbers assigned to delineate genetic clusters containing identical sequences. The clade names may change. We are in the process of formalizing the nomenclature for SARS-CoV-2 genetic clades and will update this report accordingly.

**These cases are not on the tree but are related to the clade indicated. If no clade is indicated, they are unrelated to the clades identified in the table.

Cases that are not in the tree did not generate high-quality sequence data.

Definitions: specimens that differ by 0 mutations are "Identical", 1-2 mutations are "Nearly Identical", 3 mutations "Similar" and >3 mutations "Different".

Figure 1. SARS-CoV-2 genetic diversity for all samples sequenced to-date by the BCCDC PHL. This tree is rooted to the original Wuhan reference strain (MN908947.3), and displays sequences based on the number of mutations that differ from this reference strain (x-axis). Cases belonging to the Public Hospital Surgical Ward cluster investigation (Cluster 1-100) are displayed as purple dots on the tree tips.

Please do not hesitate to contact me if you require any further clarifications.

Provincial Health Services Authority
Province-wide solutions. Better health.

Page 2 of 4
Version 3

A research and teaching centre affiliated with UBC

4 components:

1. **Executive summary** of the findings specific to the outbreak cluster
2. **Line list** of the cases you asked to be sequenced.
3. **Tree:** A Figure showing the position of your cases on the tree relative to other BC cases for which we have WGS data.
4. **Appendix:** If applicable, an appendix containing cases that the tree suggests are similar to your cases

Role of Genomics in Current Phases of Pandemic

- Transition from detecting variants → surveillance of variants
  - What is circulating?
  - Is anything emerging?
- Targeted surveillance of specific populations
  - Hospitalized
  - Reinfections
  - Breakthrough cases
  - Outbreaks
- Surveillance critical as we transition from pandemic to endemic stage

People

- Technologists
- Scientists
- Bioinformaticians!!!!!!!!
- Medical Microbiologists

Today’s Research is tomorrow’s Healthcare!!!

Acknowledgments

BCCDC PHL

- Natalie Prystajecky
- John Tyson
- James Zlosnick
- Hind Sbihi
- Mel Krajden
- Shannon Russell
- Tracy Lee
- Rebecca Hickman
- Jason Nguyen
- Dan Fornika
- Kimia Kamelian
- Kim Macdonald
- Chris Fjell
- Diane Eisler
- Ana Paccagnella
- Corrinne Ng
- Rob Azana
- Loretta Janz
- Karen Mooder

BCCDC

- Eleni Galanis
- Marsha Taylor
- Kyle Noftall
- Danuta Skowronski
- Naveed Janjua

Elsewhere

- Front-line Laboratories
- MHOs and Health Authorities
- Anders Goncalves da Silva
- Caroline Colijn
- National Microbiology Laboratory

Funders

20YEARS GenomeBritishColumbia
LEADING ▸ INVESTING ▸ CONNECTING

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

MICHAEL SMITH FOUNDATION FOR HEALTH RESEARCH
BC's health research funding agency

CanCOGeN

Provincial Health Services Authority
Province-wide solutions. Better health.

Thank you!