



WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL



Genomic Surveillance of Influenza and SARS-CoV-2

—
Clyde Dapat, PhD

WHO Collaborating Centre for Reference
and Research on Influenza



**CENTRE FOR
PATHOGEN
GENOMICS**



A joint venture between The University of Melbourne and The Royal Melbourne Hospital





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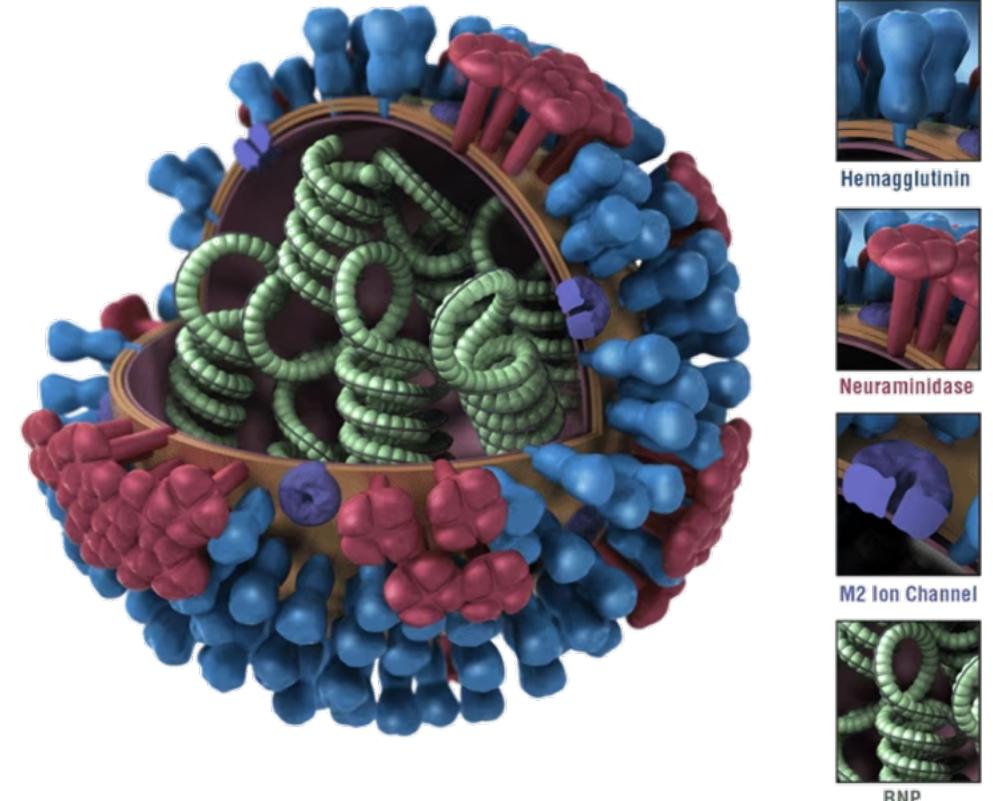
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Objective

- To familiarize with the genomics epidemiology of influenza and SARS-CoV-2

Influenza virus

- Family *Orthomyxoviridae*
- Influenza types A, B, C, D
- Single-stranded, negative-sense segmented RNA
- Genome size of ~13,600 nt
- Major surface proteins
 - Hemagglutinin (HA)
 - Neuraminidase (NA)
- Naming of subtypes is a combination of HA and NA
 - A(H1N1), A(H3N2)



<https://www.cdc.gov/flu/about/viruses/types.htm>

Influenza genome structure

1	PB2 (2,277 nt)	Virus replication
2	PB1 (2,271 nt)	Virus replication, apoptosis
3	PA (2,148 nt)	Virus replication
4	HA (1,698 nt)	Virus attachment, antigenic determinant, subtype specific (H1-H16)
5	NP (1,494 nt)	RNA coating, nuclear targeting, RNA transcription, type specific (A,B,C)
6	NA (1,407 nt)	Virus release from cell, antigenic determinant, subtype specific (N1-N9)
7	M1/M2 (756 nt M1; 291 nt M2)	M1—membrane stability, type specific (A,B,C) M2—virus uncoating, type specific (A,B,C)
8	NS1/NS2 (690 nt NS1; 362 nt NS)	NS1—non-structural protein NS2—mRNA transcription

How influenza viruses evade the host immune response?

Antigenic drift

- gradual change in influenza genome
- due to mutations (lack of proof reading RNA polymerase)
- causes continued annual outbreaks and epidemics

Antigenic shift

- sudden change
- due to reassortment of viruses
- e.g. swine viruses + human viruses = H1N1 2009 pandemic
- can create a novel virus - human population susceptible
- may lead to pandemics

Influenza pandemics



1918: "Spanish Flu"

40 to 50 million deaths

A(H1N1)

CFR 2-3%

1957: "Asian Flu"

1 to 4 million deaths

A(H2N2)

CFR <0.2%

1968: "Hong Kong Flu"

1 to 4 million deaths

A(H3N2)

CFR <0.2%

2009: "Swine Flu"

<0.1 million deaths

A(H1N1)

CFR <0.005%

Victorian Infectious Diseases Reference Laboratory (VIDRL)



Outbreak investigations, surveillance, epidemiology, new technologies, training and applied research



Reference collections, proficiency testing and technical advice



Building laboratory capability in the IndoPacific



WHO Collaborating Centres for influenza, viral hepatitis and *Mycobacterium ulcerans*.

WHO Reference Laboratories for polio, measles, rubella and hepatitis B.



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High containment PC3 & PC4 laboratories



Genomics platform

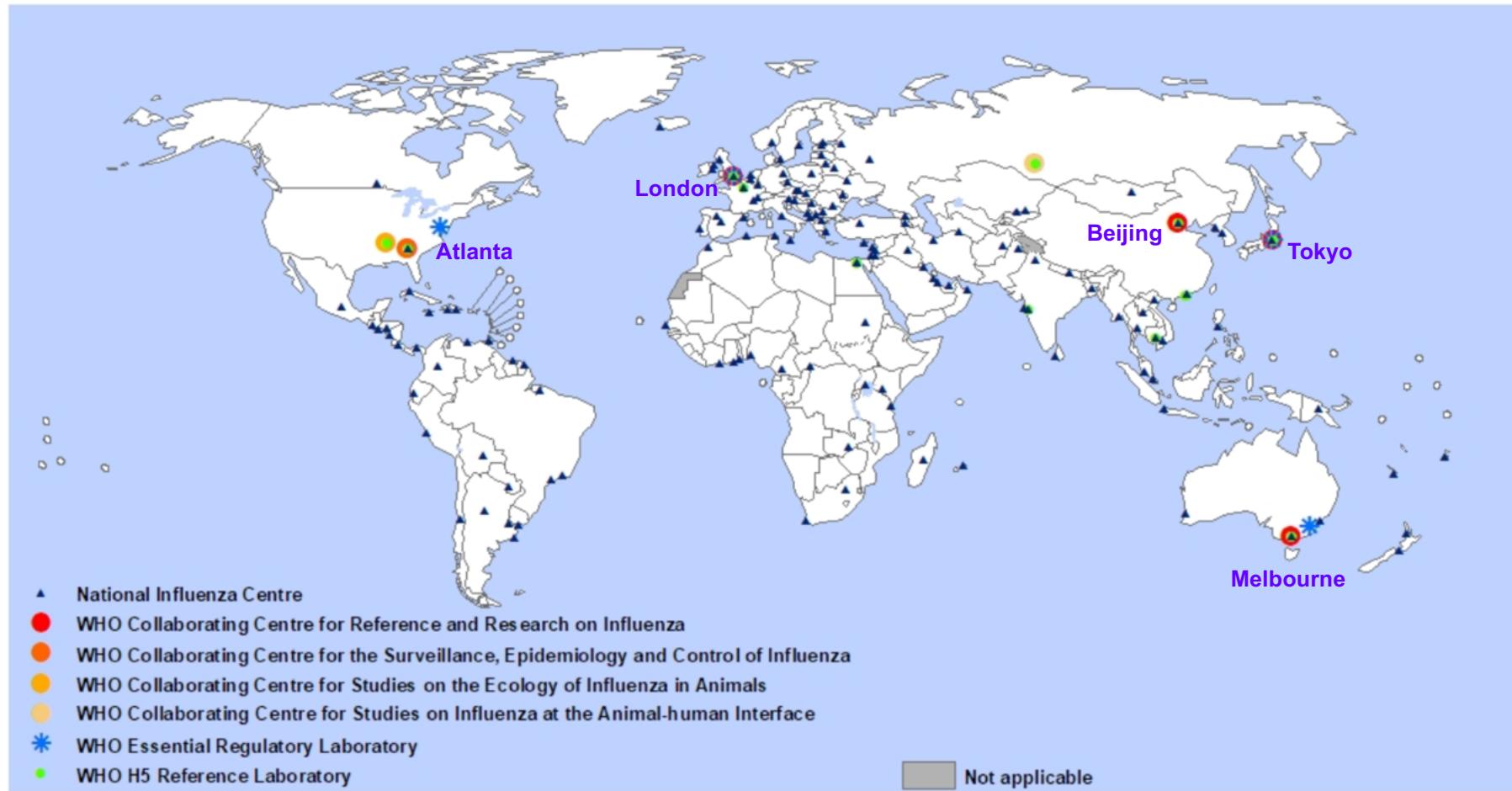


Electron microscopy



High throughput molecular and serological diagnostic assays

WHO Global Influenza Surveillance and Response System



https://www.who.int/images/default-source/departments/global-influenza-programme/gisrs_20210318_1.tmb-1920v.jpg?sfvrsn=517f33f_3



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Major activities at the WHO CCRRI

- Identify & monitor influenza viruses circulating in human population
- Monitor antigenic drift in seasonal viruses
- Monitor emergence of seasonal virus variants resistant to antiviral drugs
- Early detection of unusual influenza viruses in human population
(rapidly identify viruses with pandemic potential)

Viral sequencing

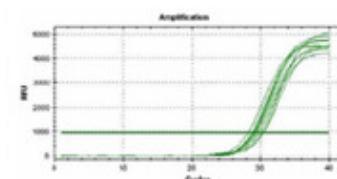


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1. Clinical sample

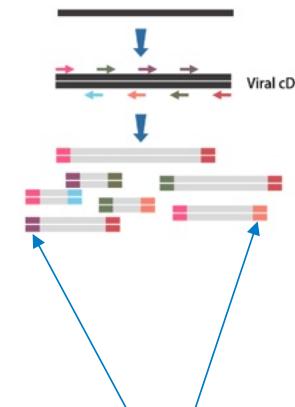


2. Diagnostic PCR



3. Amplify viral RNA

Tiled amplicon PCR



PCR
primers

4. Sequencing

Short-read Illumina sequencing



OR

Long-read Nanopore sequencing

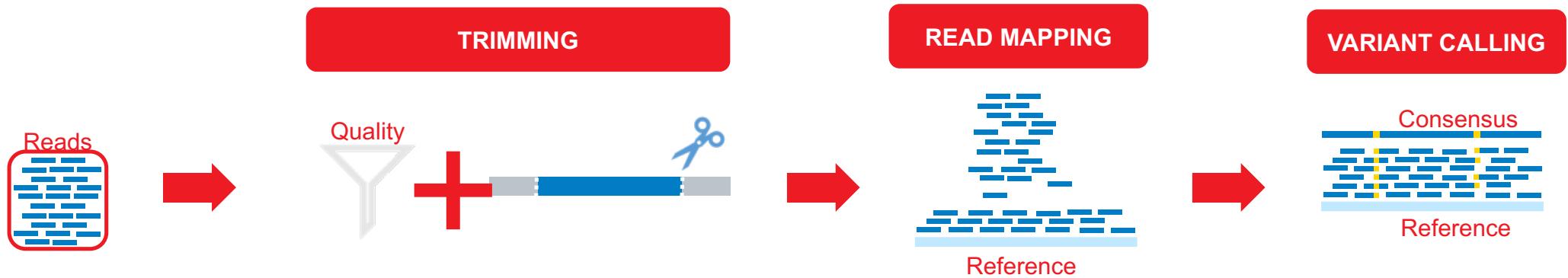


Bioinformatics analysis



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IRMA pipeline of CDC



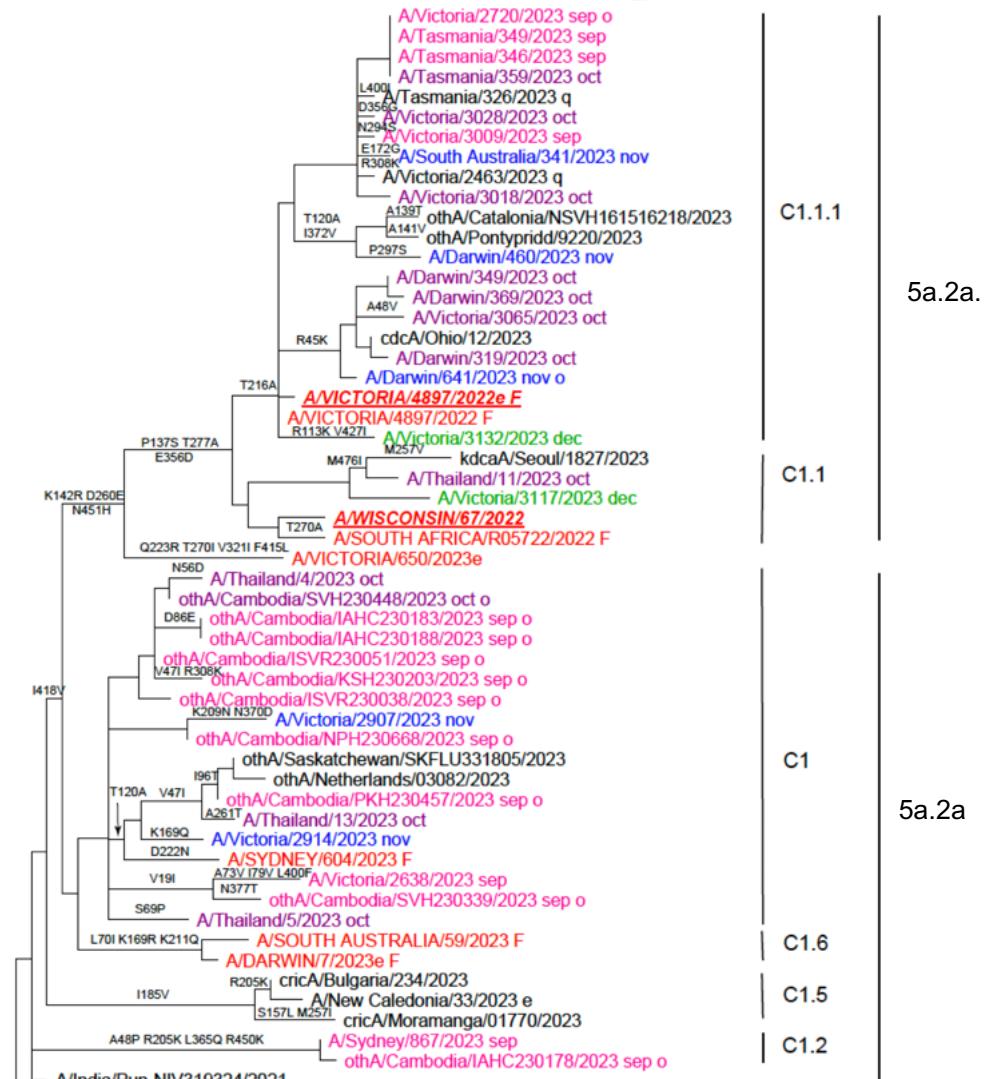
A(H1N1pdm09) HA

Current vaccine strains
for SH and NH

e = Egg isolate
 o = Original Specimen
 q = QMC
 F= Ferret antisera
 LR = Low reactor

Reference Antigen

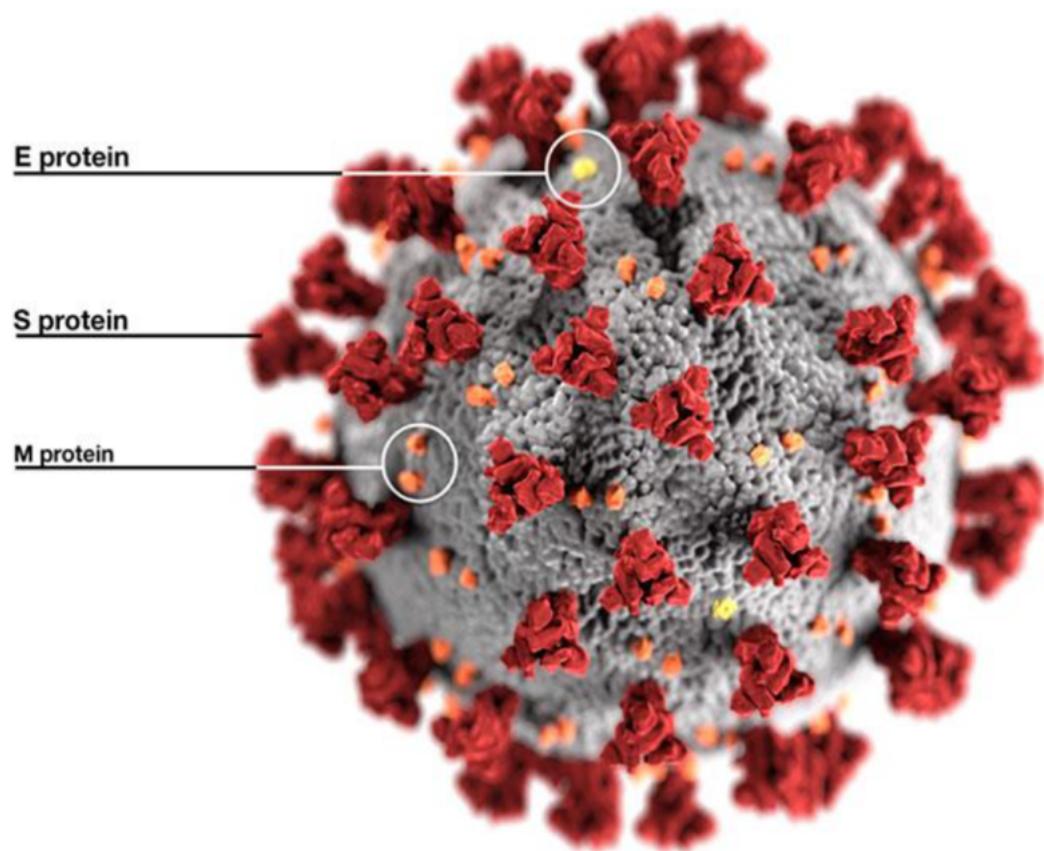
Sep 2023
 Oct 2023
 Nov 2023
 Dec 2023



Influenza vaccines

- Trivalent vaccines
 - A(H1N1)pmd09, A(H3N2), B/Victoria
- Quadrivalent vaccines
 - A(H1N1)pmd09, A(H3N2), B/Victoria, B/Yamagata
- Northern hemisphere vaccine and Southern hemisphere vaccine
 - 2023 WHO Southern Hemisphere recommendations:
 - an A/Sydney/5/2021 (H1N1)pdm09-like virus
 - an A/Darwin/9/2021 (H3N2)-like virus
 - an B/Austria/1359417/2021 (B/Victoria lineage)-like virus; and
 - an B/Phuket/3073/2013 (B/Yamagata lineage)-like virus
 - 2023-2024 WHO Northern Hemisphere recommendations
 - an A/Victoria/4897/2022 (H1N1)pdm09-like virus
 - an A/Darwin/9/2021 (H3N2)-like virus
 - an B/Austria/1359417/2021 (B/Victoria lineage)-like virus; and
 - an B/Phuket/3073/2013 (B/Yamagata lineage)-like virus

SARS-CoV-2

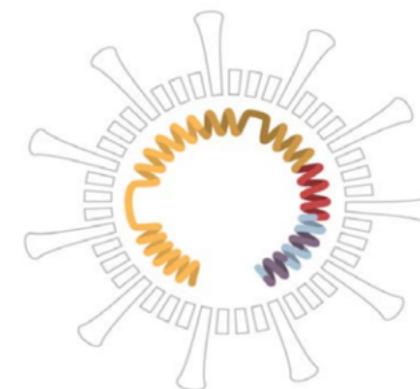


- Family *Coronaviridae*
- Single-stranded, positive sense RNA
- Genome size of 30,000 nt
- 11 coding regions (genes)

SARS-CoV-2 genome

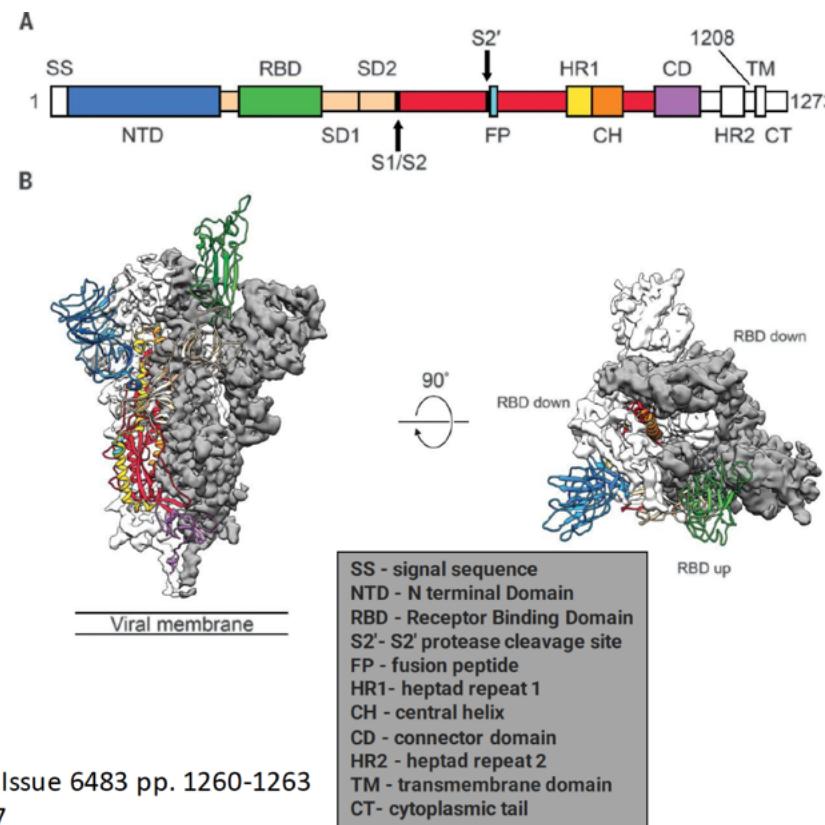


Structural Protein	Function of Protein
Nucleocapsid Protein (N)	<ul style="list-style-type: none"> Bound to RNA genome to make up nucleocapsid
Spike Protein (S)	<ul style="list-style-type: none"> Critical for binding of host cell receptors to facilitate entry of host cell
Envelope Protein (E)	<ul style="list-style-type: none"> Interacts with M to form viral envelope
Membrane Protein (M)	<ul style="list-style-type: none"> Central organiser of CoV assembly Determines shape of viral envelope



SARS-CoV-2 Spike

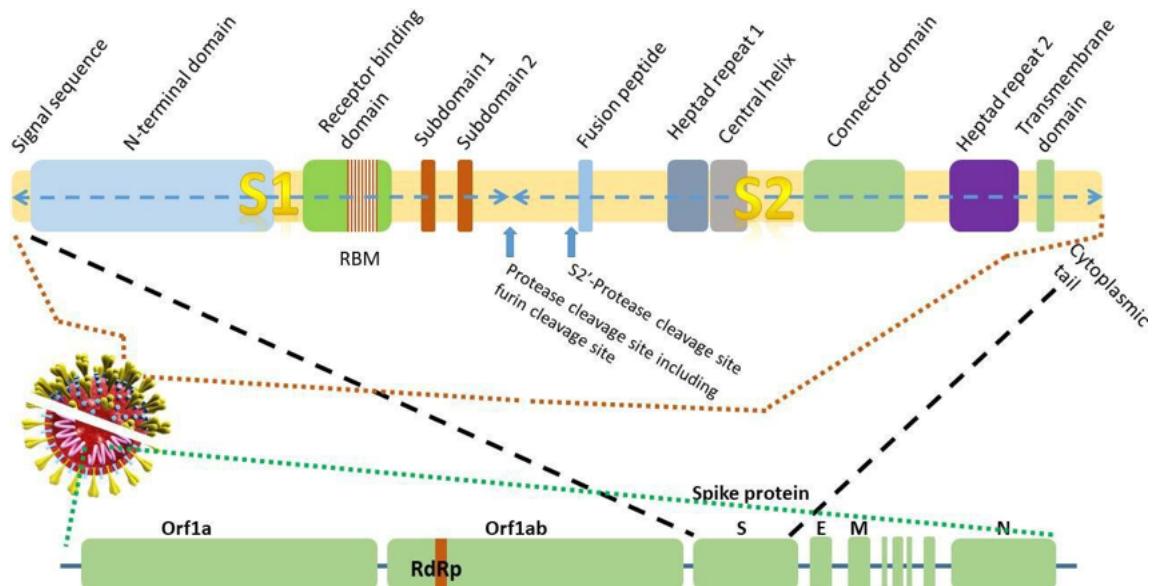
- 3.6KB gene
- Target of mRNA vaccines
- **Main antigenic determinant of the SARS-CoV-2 virus**
- Target antibody therapies
- Accumulation of many mutations during the pandemic



SCIENCE 19 Feb 2020 Vol 367, Issue 6483 pp. 1260-1263
DOI: 10.1126/science.abb2507

• Why Sequence SARS-CoV-2?

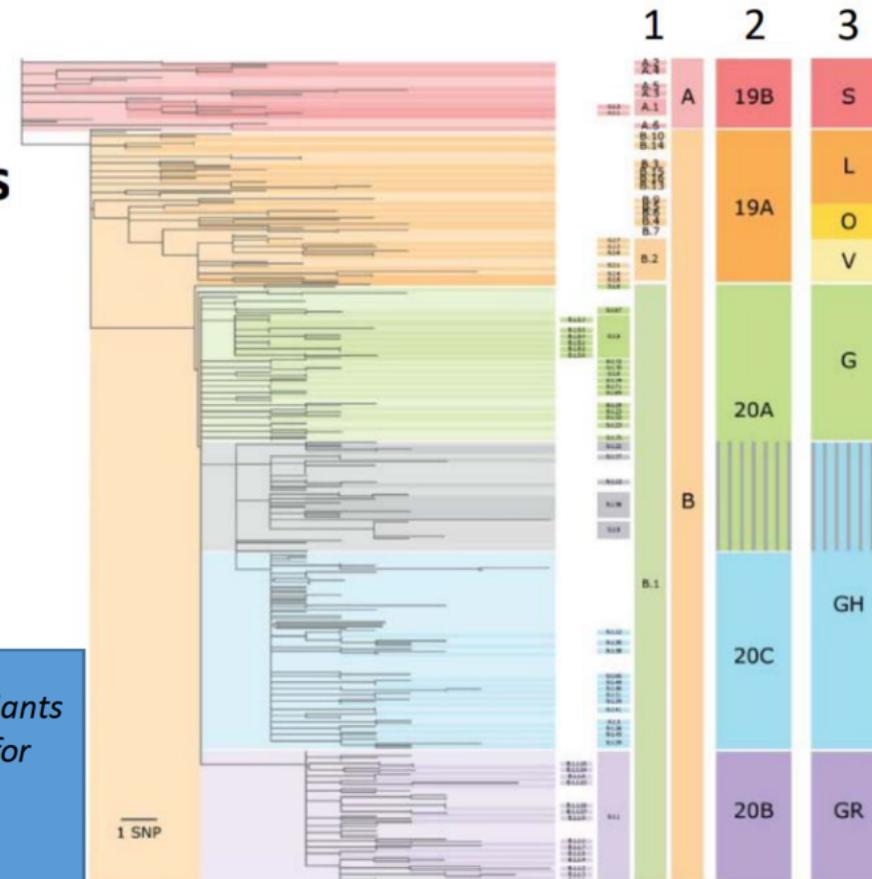
- Identification
- Characterisation
- Genetic diversity
- Complete strain profiling
- Investigate spread
 - Contact tracing
 - Trace the emergence
- Estimate prevalence in population
- Evolution
- Develop new diagnostic tests
- Develop new therapeutics
- Vaccine development



Clade naming conventions

1. Pangolin Lineages
 - cov-lineages.org
2. Clades by Nextstrain ****
 - nextstrain.org
3. Clades by GISAID
 - gisaid.org

*The WHO, to assist with public discussion of variants
the uses Geek letters to differentiate variants for
nonscientific audiences
(ex. B.1.1.529= Omicron)*



Adapted from [Alm et al. 2020](#)

Microbiological Diagnostic Unit Public Health Laboratory

State diagnostic
microbiology
laboratories
(Public & private)



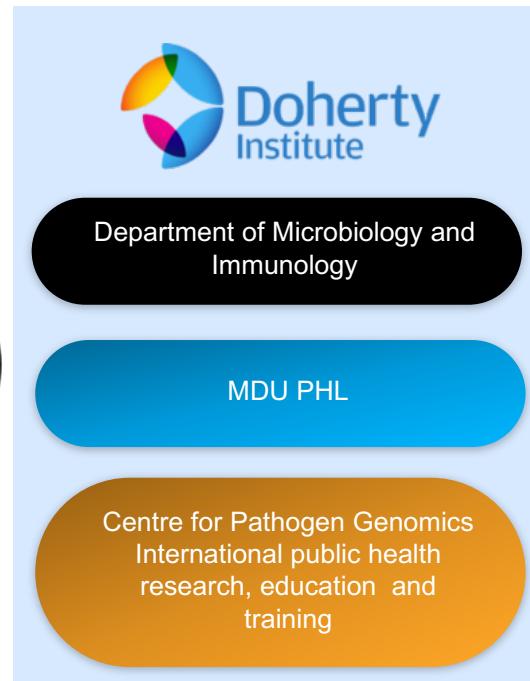
Other state public
health laboratories



Referral laboratories in
the Asia Pacific region



International genomics, WHO CC for AMR
and diagnostic laboratory programs



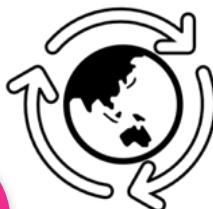
State Department of Health

Integration with detailed
epidemiologic data for local use



**National Department of Health
and other national bodies**

Coordination of national data in public
health genomic data sharing platform
Reporting to national authorities



International networks and partnership

Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL)



- MDU PHL is a leading Australian public health laboratory providing microbiology services to the Victorian Department of Health, delivering excellence in emerging technologies including pathogen genomics for surveillance, outbreak response, global health, capacity building and training.
- Key focus areas for MDU PHL include:



Invasive and vaccine preventable diseases



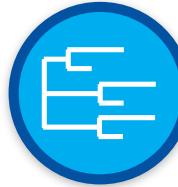
Biothreat agents



Food and waterborne diseases



Pathogen genomics



Outbreak response



Surveillance and infection control



Bioinformatics and genomic epidemiology



Antimicrobial resistance



Sexually transmitted infections

SARS CoV-2 Sequencing Workflow



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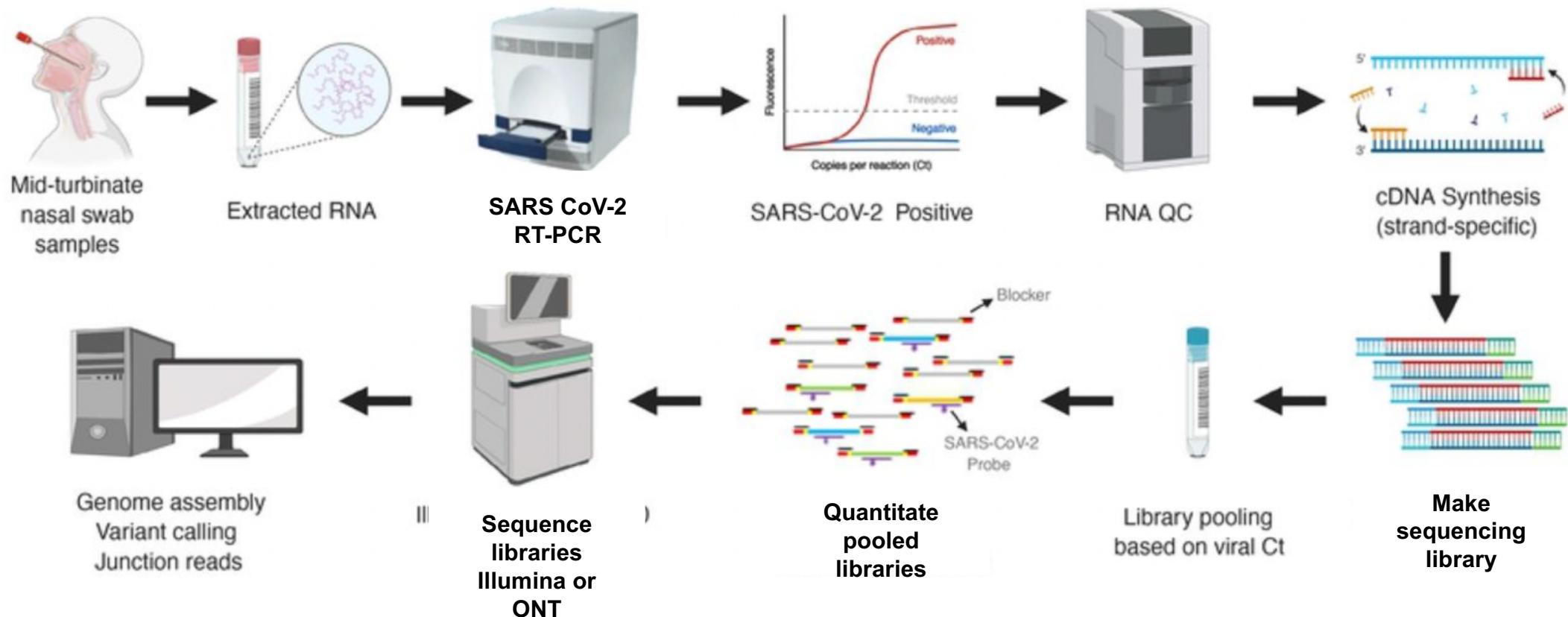
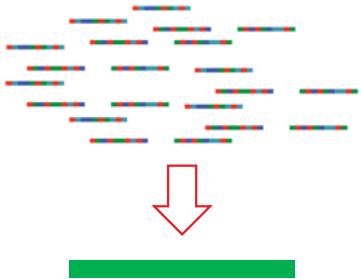


Image Source Modified From: Doddapaneni H, Cregeen SJ, Sucgang R, Meng Q, Qin X, Avadhanula V, et al. (2021) Oligonucleotide capture sequencing of the SARS-CoV-2 genome and subgenomic fragments from COVID-19 individuals. PLoS ONE 16(8): e0244468. <https://doi.org/10.1371/journal.pone.0244468>

SARS-CoV-2 sequencing



Reconstruct genome



Generate consensus genome

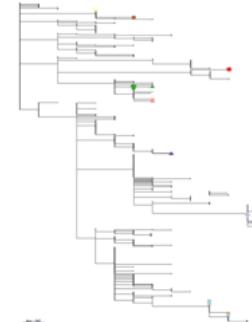
Align to reference

```
AGTCTGATTAGCTTAGCTTGAGCGCTATATTAT  
AGTCTGATTAGCTTAGATTGTAGC-CTATATTAT  
AGTCTGATTAGCTTAGATTGTAGC-CTATATTAT  
AGTCTGATTAGCTTAGATTGTAGC-CTATATTAT  
  
AGTCTTATTGCTGAGCTTGTAGC-CTAT--AT  
AGTCGGATTACCCCTAGCTTGAGCGCTTATTAT  
AGTCTGATTAGCTTAGCTTGAGCGCTATATTAT  
AGTCTGATTAGCTTAGCTTGAGCGCTATATTAT  
AGGCTGTTAGCTTAGCCGTAGCGCTATATTAT
```

A block of DNA sequence data showing two sets of aligned sequences. The first set is in black text, and the second set is in blue text. The blue text sequences are identical to the black ones but include additional characters at the beginning and end, likely representing gaps or different reference points.

Align all the sequences to reference genome to compare

Phylogenetic tree

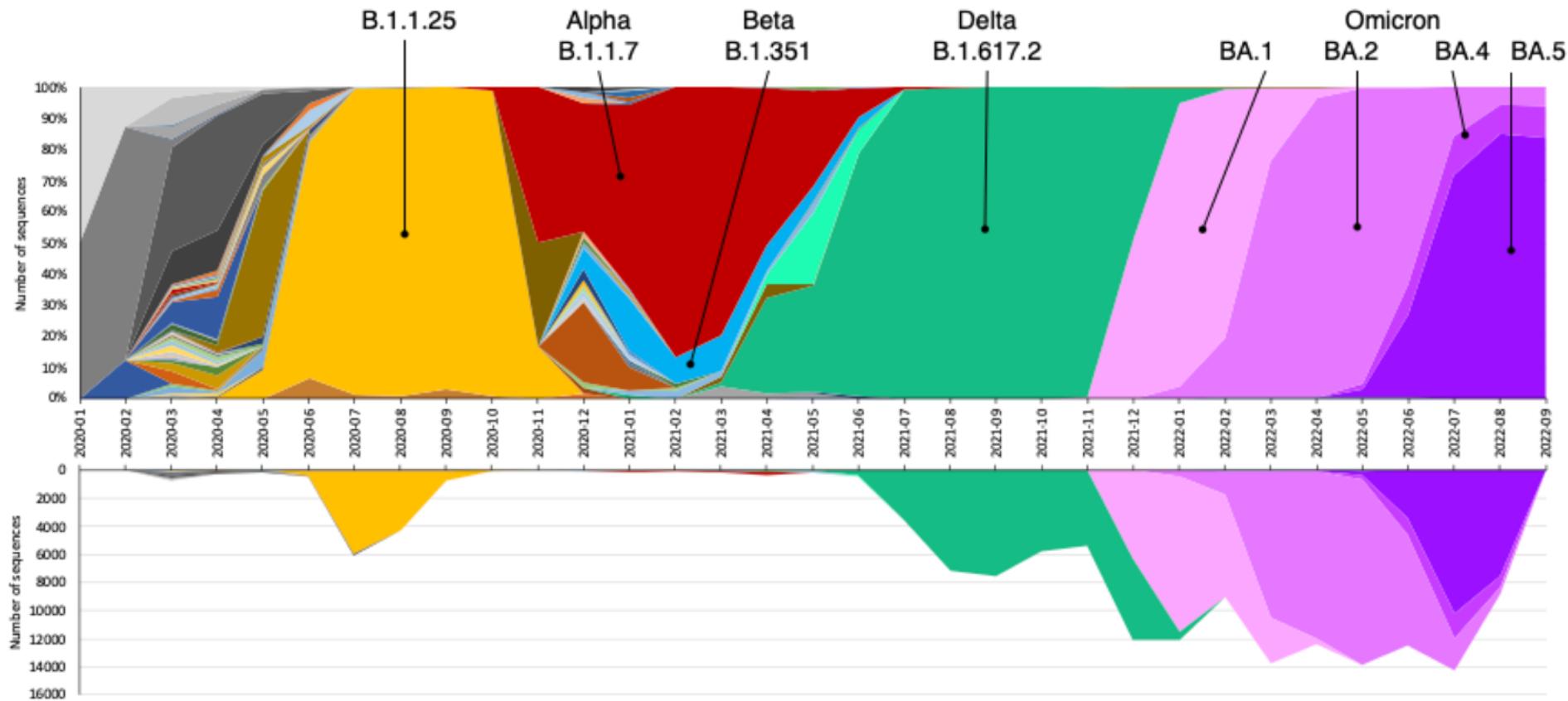


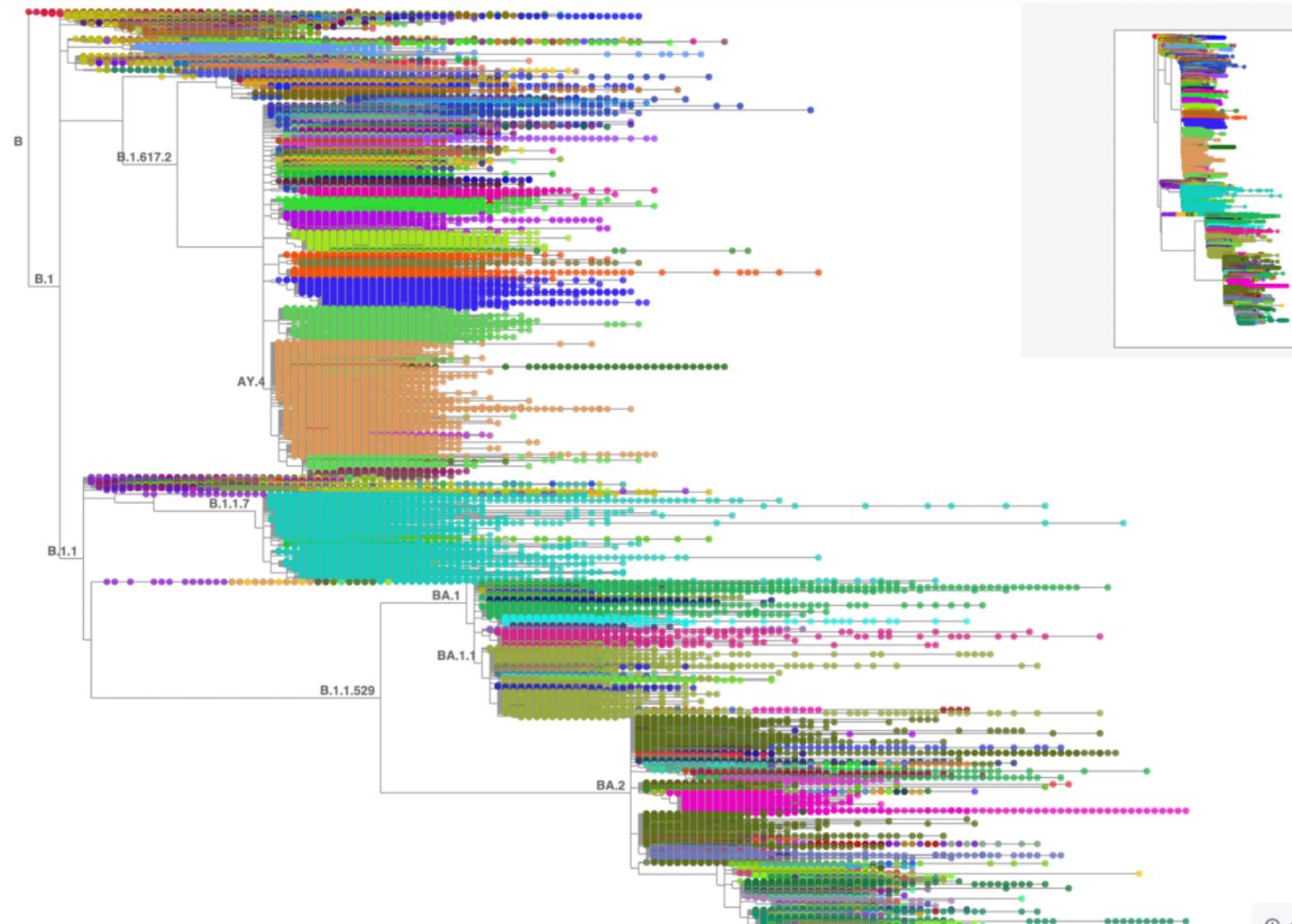
Visualize relationships between sequences

Integrate epidemiology & report



Australian sequencing effort over time





Summary

- Influenza and SARS-CoV-2 remain public health threats
- NGS is a powerful tool for the discovery of novel variants and ongoing surveillance in a timely manner
- Use of genomic data in the development of vaccine, antiviral drug, diagnostic test



World Health
Organization
Philippines



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THANK YOU

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KDCA

Korea Disease Control and
Prevention Agency



Doherty
Institute

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THE UNIVERSITY OF
MELBOURNE



The Royal
Melbourne
Hospital