SARS-CoV-2 variant surveillance in the COVID public health response in Victoria, Australia

A/Prof Aaron Jex

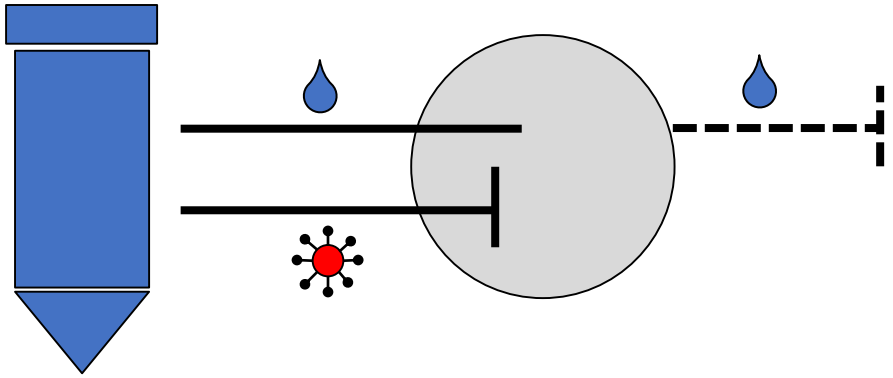
Population Health and Immunity Division

Walter and Eliza Hall Institute of Medical Research

Detecting SARS-CoV-2 in wastewater

Viral concentration

1.

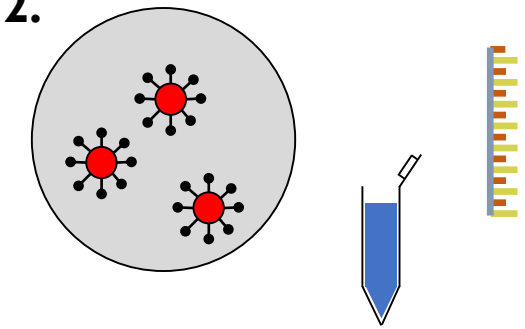


2 x 250mL WWTP Influent

-ve membrane (SW method)

RNA extraction

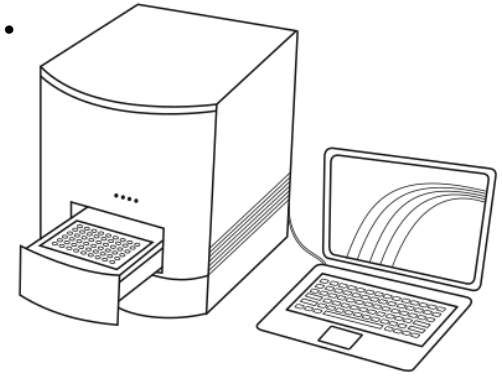
2.



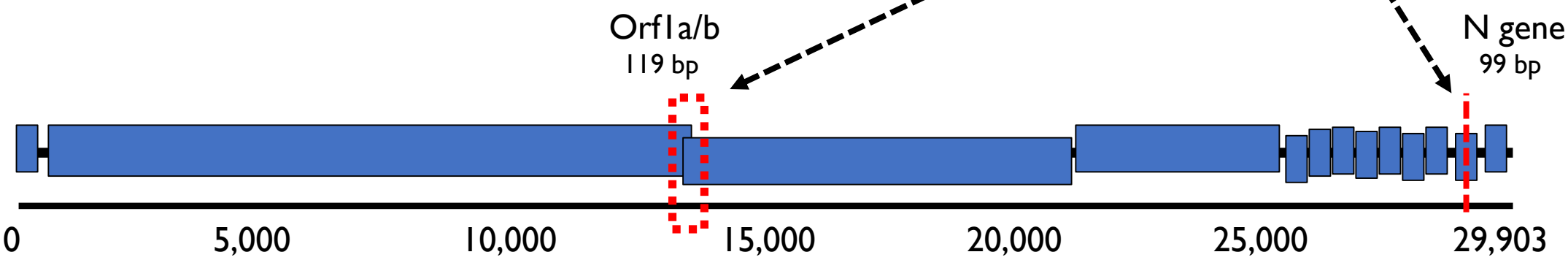
PowerWater Kit (Qiagen) + others

PCR detection

3.

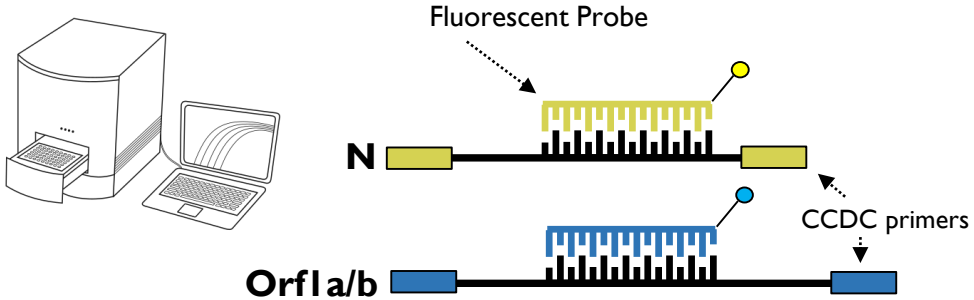


Perkin Elmer SARS-CoV-2 kit

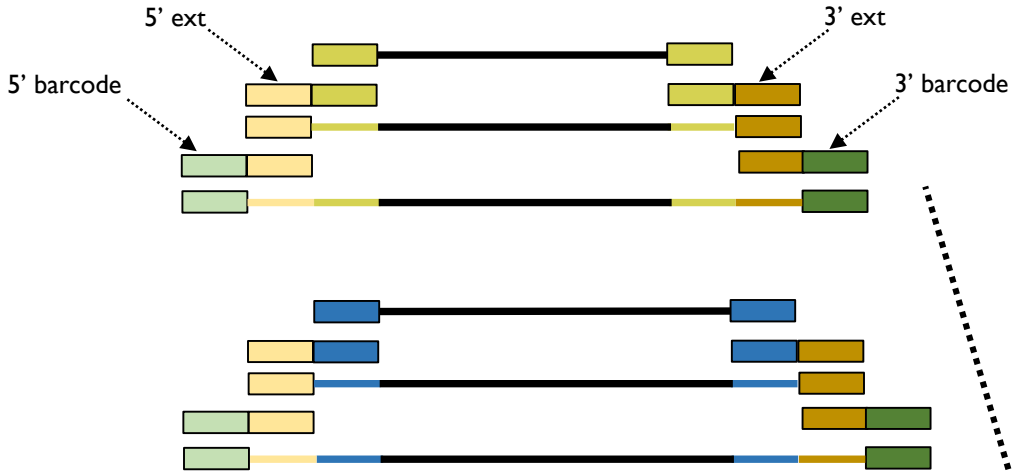


Verifying positive test results

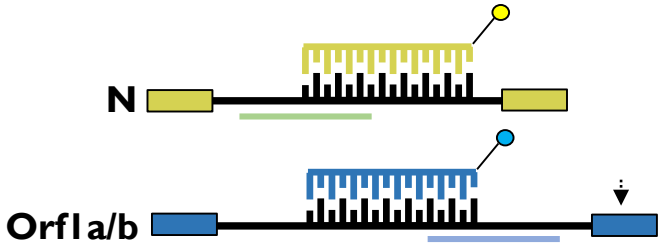
1. RT-qPCR probe

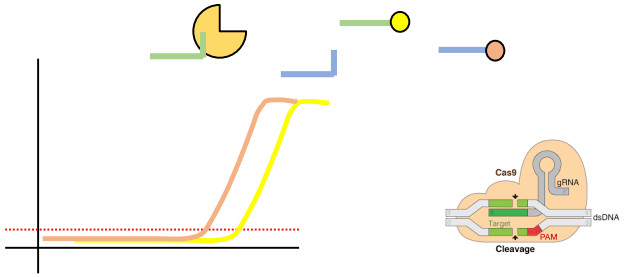


2. Amplicon sequencing (Illumina MiSeq)



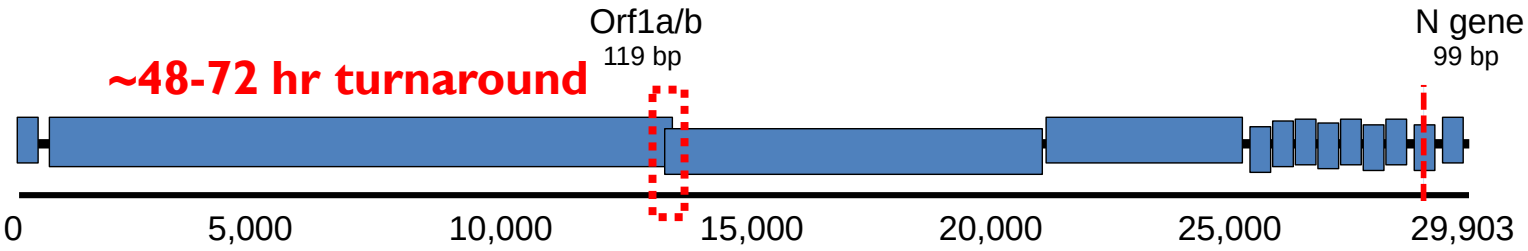
3. CRISPR DETECTR





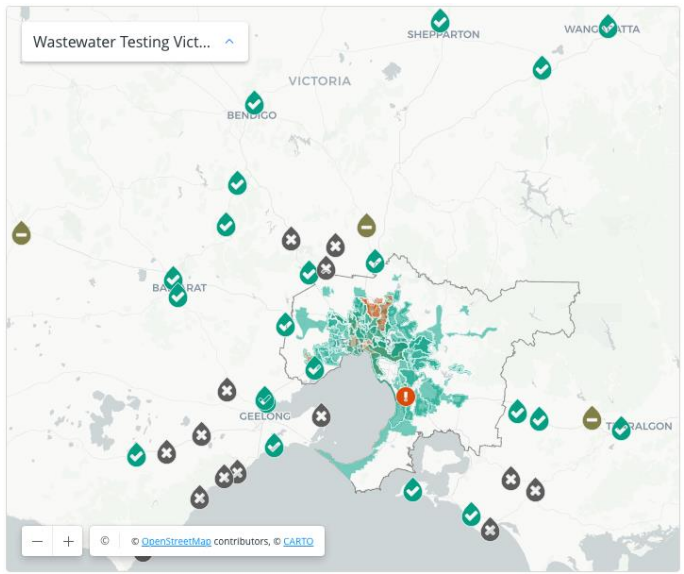
~2-3 hr turnaround

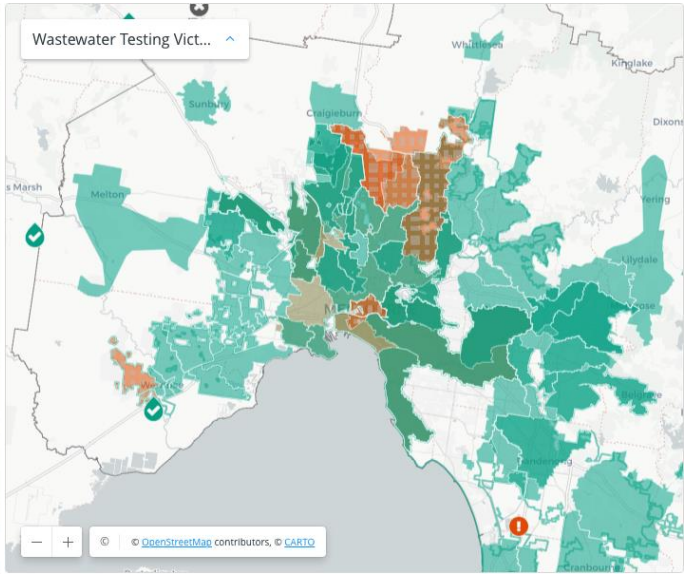
~48-72 hr turnaround



Public reporting in real-time

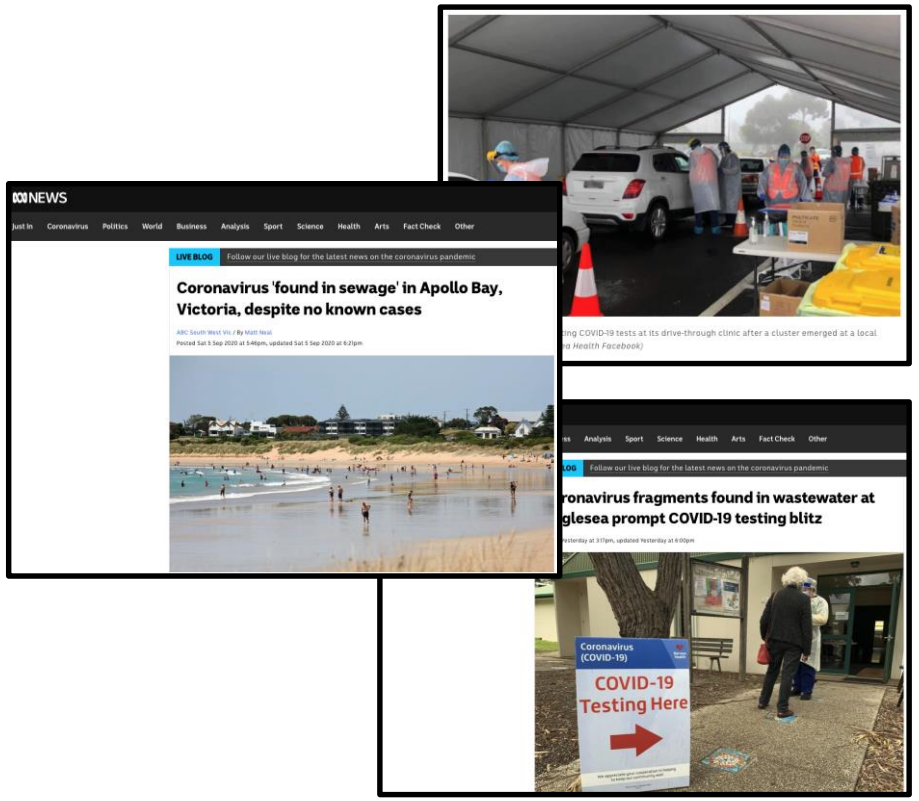
https://www.dhhs.vic.gov.au/wastewater-testing-covid-19





Status	Wastewater treatment plant	Network area
Unexpected detection		
Anticipated detection		
No detection		
Test result pending		
Inconclusive		
No tests performed in this period		
Testing site suspended		Not applicable

Site/Last 28 day results	Localities include...
Altona	Altona, Altona Meadows, Seaholme, Sanctuary Lakes, and parts of Laverton, Point Cook, and Williams Landing
Altona Outfall - Laverton North	Ravenhall, Deer Park, Altona, Laverton North, Derrimut, Truganina
Aurora	Epping



Wastewater surveillance has provided:

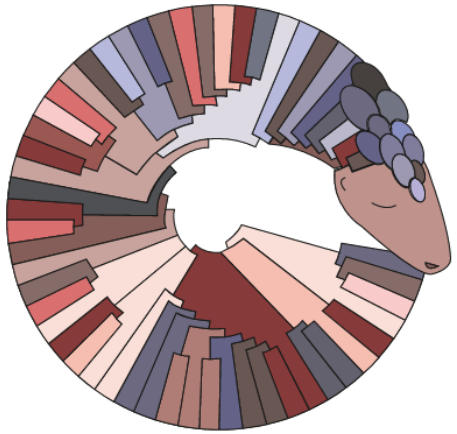
- Early case detection in all Victoria Health districts
- Warning of undetected community transmission, e.g
  - Lakes Entrance – Dec 2020
  - Maidstone (Delta variant) – May, 2021
  - Shepparton (Delta variant) – Oct, 2021

WGS in SARS-CoV-2 wastewater surveillance

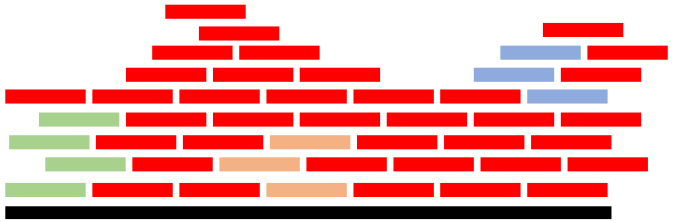
Dominant variant ID

pangolin

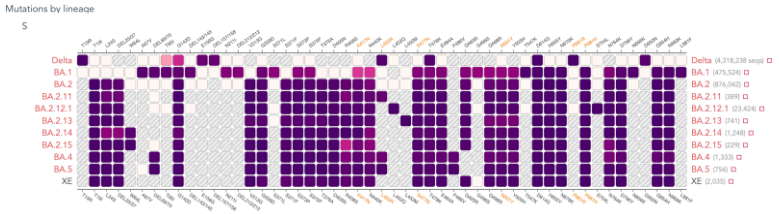
Phylogenetic Assignment of Named Global Outbreak Lineages



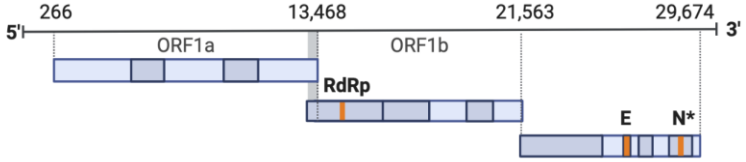
Priority SNP tracking



Lineage-specific SNPs

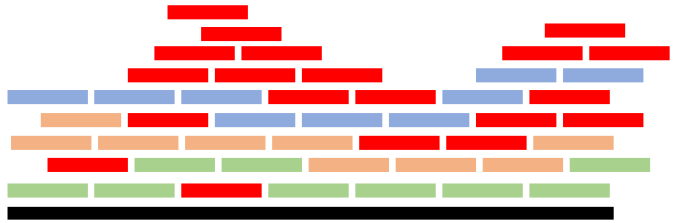


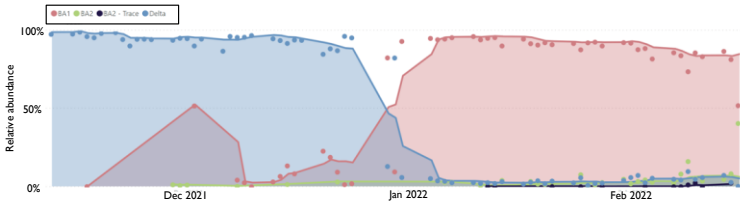
Phenotypically-relevant SNPs



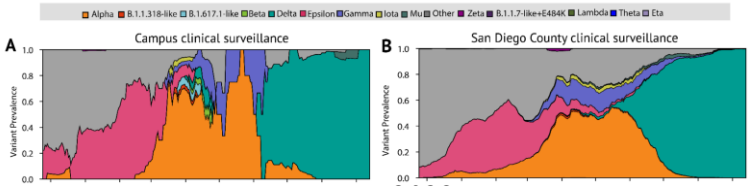
- nsp6 SNPs – Paxlovid
- N SNPs – RAT efficacy

Viral haplotype tracking





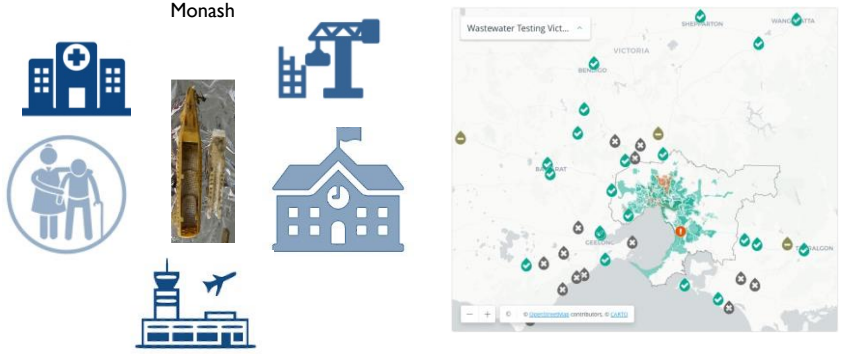
- Improved Sublineage tracking
- Novel variant detection
- Tracking recombinants



Karthikeyan et al, Nature, 2022

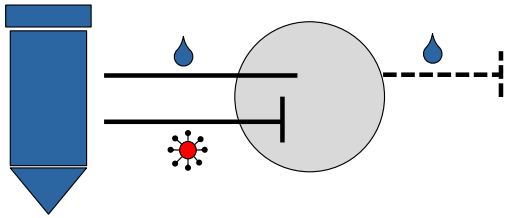
How our AmpSeq method works

A/Prof David McCarthy, Monash



Passive + Grab sampling across Victoria

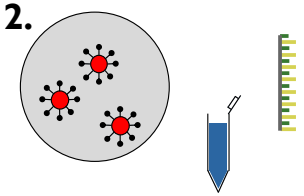
1. Viral concentration



2 x 250mL WWTP Influent

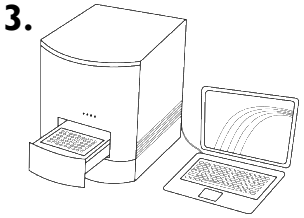
-ve membrane (SW method)

2. RNA extraction



PowerWater Kit (Qiagen) + others

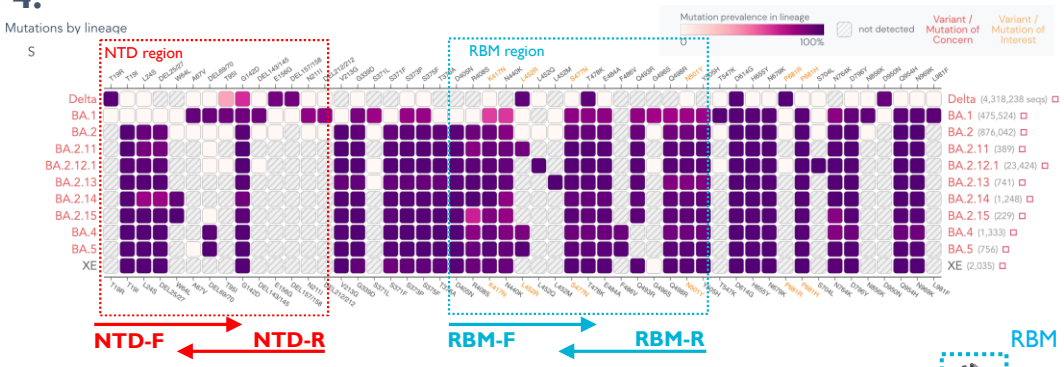
3. PCR detection

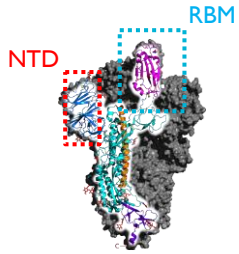


Perkin Elmer SARS-CoV-2 kit

Routine viral screening – Monash/ALS

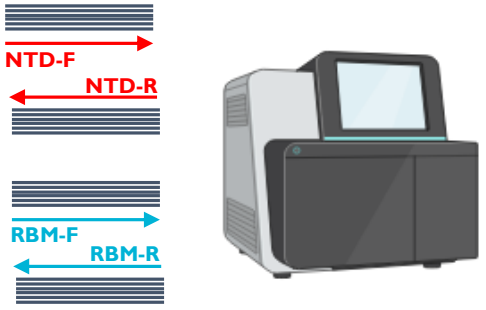
4.





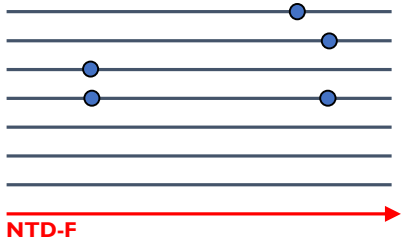
- Spike regions amplified in four pieces
  - NTD F and R
  - RBM F and R

5.

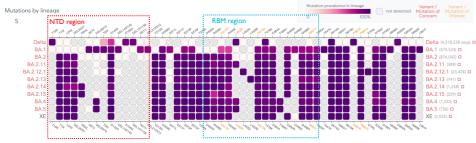


- Each Spike region barcoded and sequenced

6.

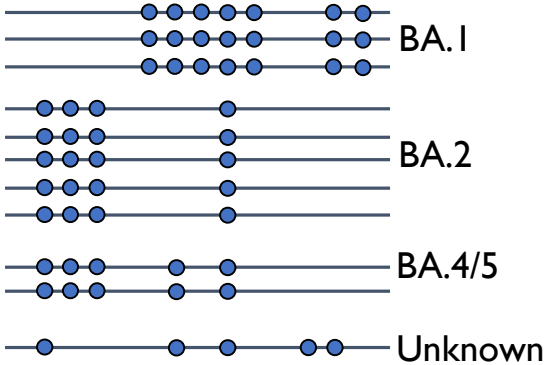


- Mutations identified
- Variants Classified
- VOCs quantified

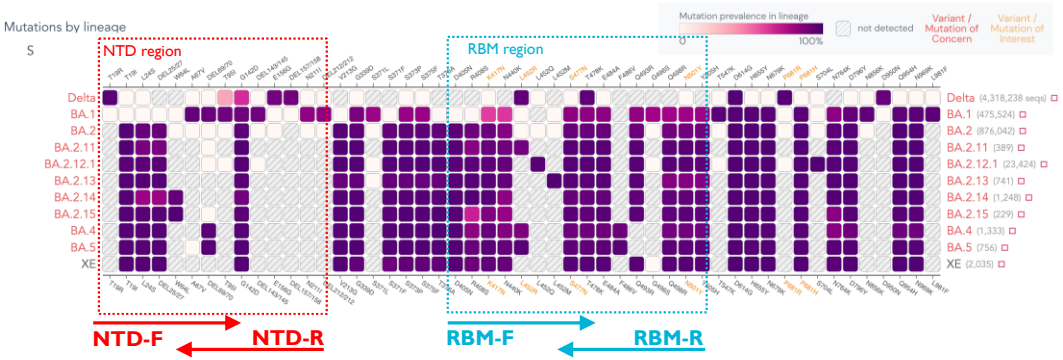


Variant classification and quantification

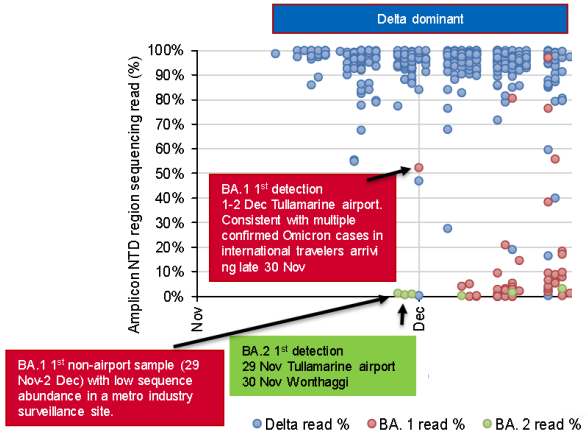
4. By counting how many reads belong to each variant



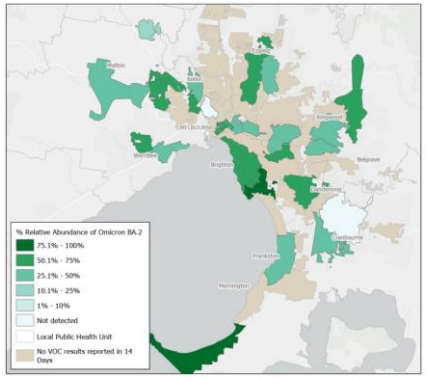
5. And averaging across all for amplicon fragments



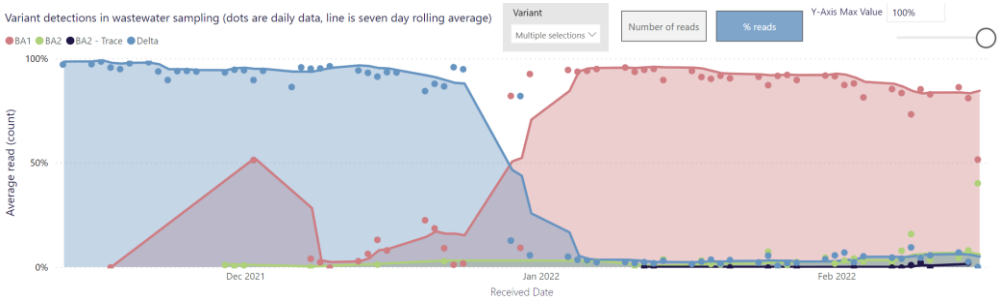
6. We can track incursions …



7. Map the distribution of…



8. And quantify changes in abundance of each VOC …



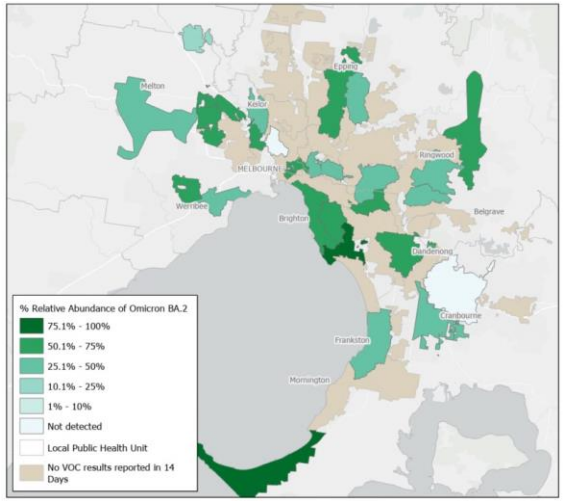
For hundreds of samples across all of Victoria, multiple times per week

WW prefaces clinical data and informs modelling

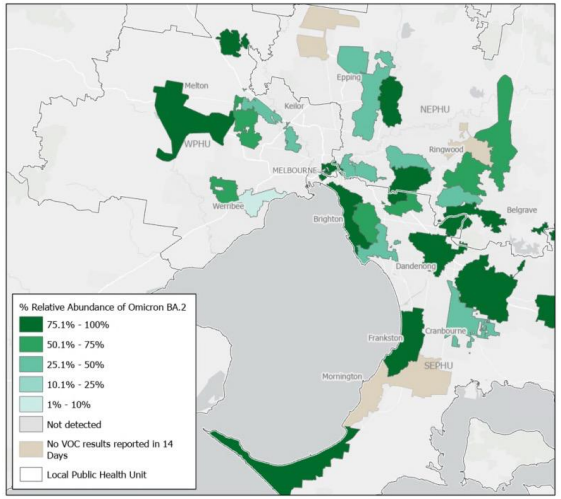
WW variant surveillance:

- 1st BA.1 detection same day as 1st known case in Vic
- 1st BA.2 and BA.5 detections in Australia before clinic
- BA.2.75 detection within 2 days of its first global report
- Identified I68T+ BA.2 before clinic in Vic

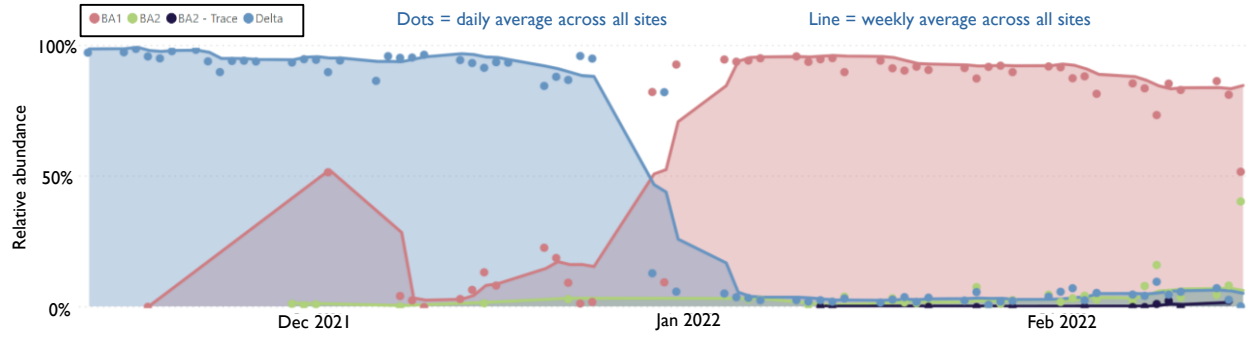
- Identified BA.1, BA2 and BA5 dominance before clinic
- Provides low-cost state-wide surveillance
- 4-day TAT from sample to result
- Critical role in state-based COIVD modelling
- Adopted in NZL, SA and NT (national discussion now)

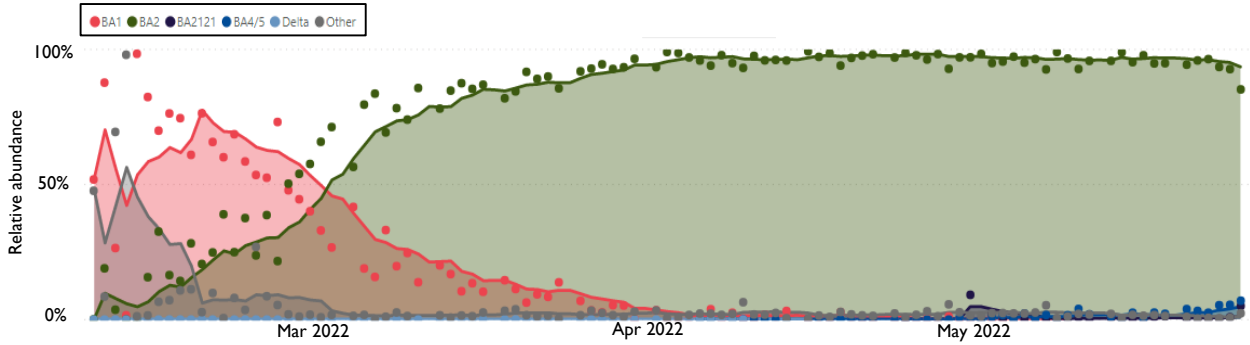


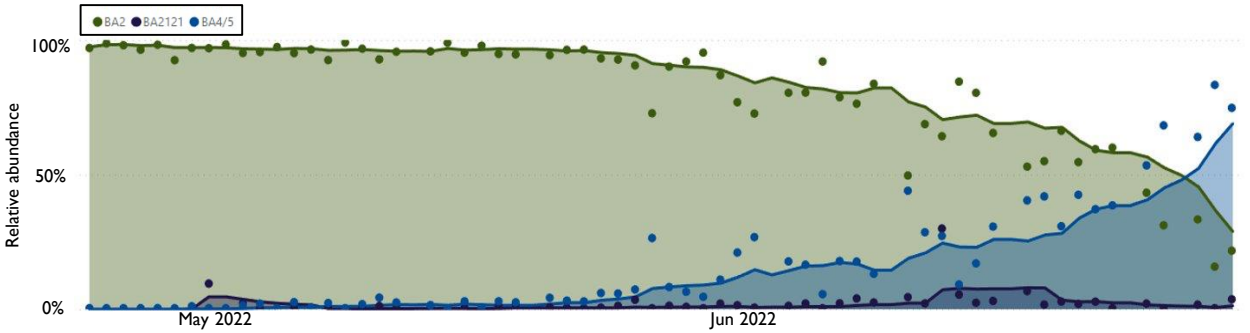
Samples retrieved in 14-day period : 19 Feb – 4 Mar 2022



Samples retrieved in 14-day period : 2- 15 Mar 2022

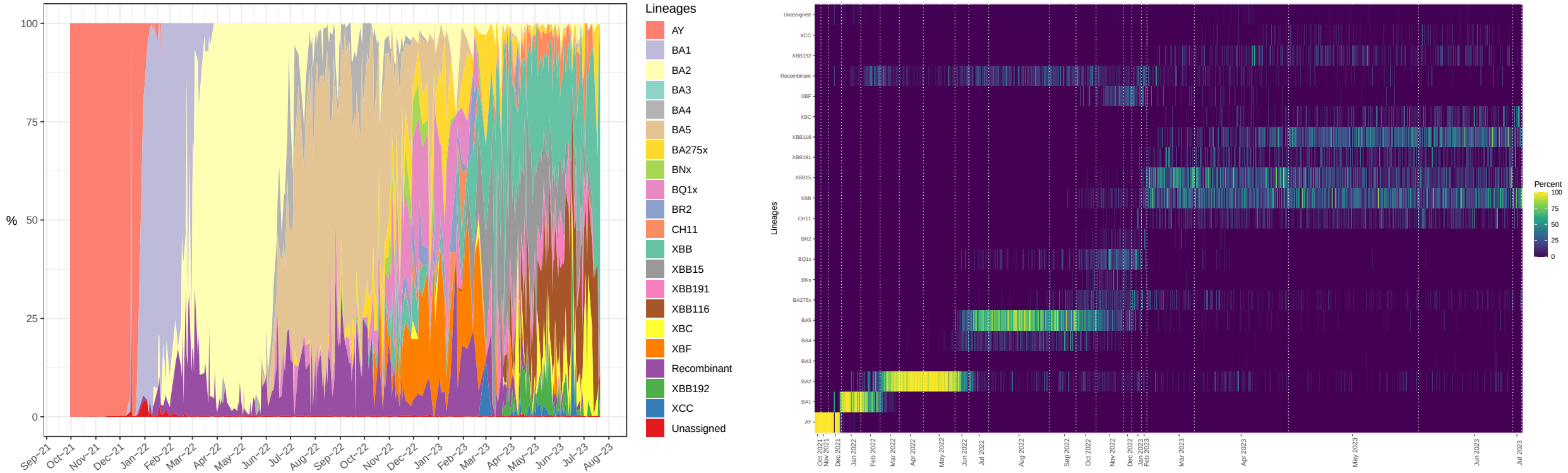






Figures and Trend analysis courtesy of Victorian DoH

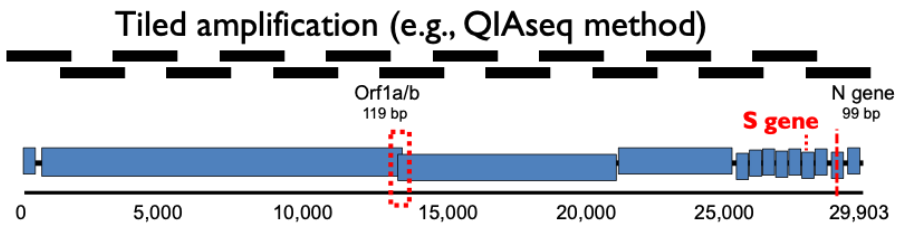
Variant detection and tracking

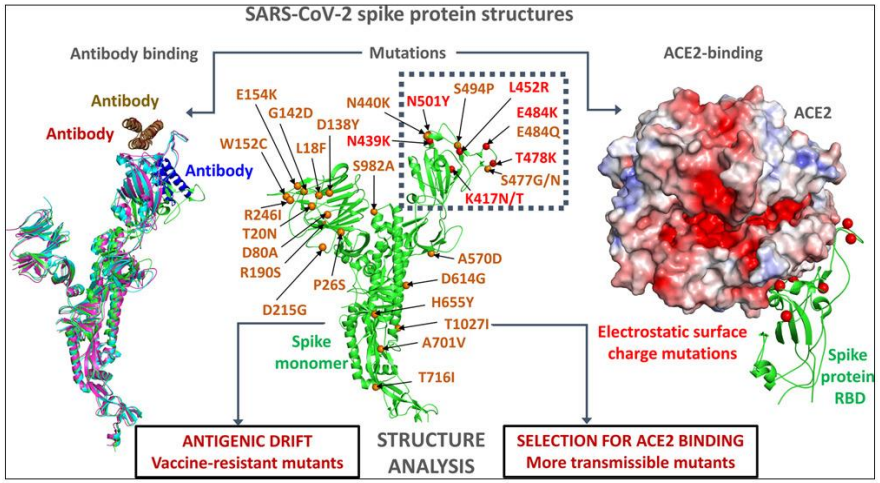


- QIAseq WGS as the frontline method since Feb 2023
- Both methods show excellent agreement – wastewater vs clinical data (not shown)

Freyja: Karthikeyan et al Nature, 2022

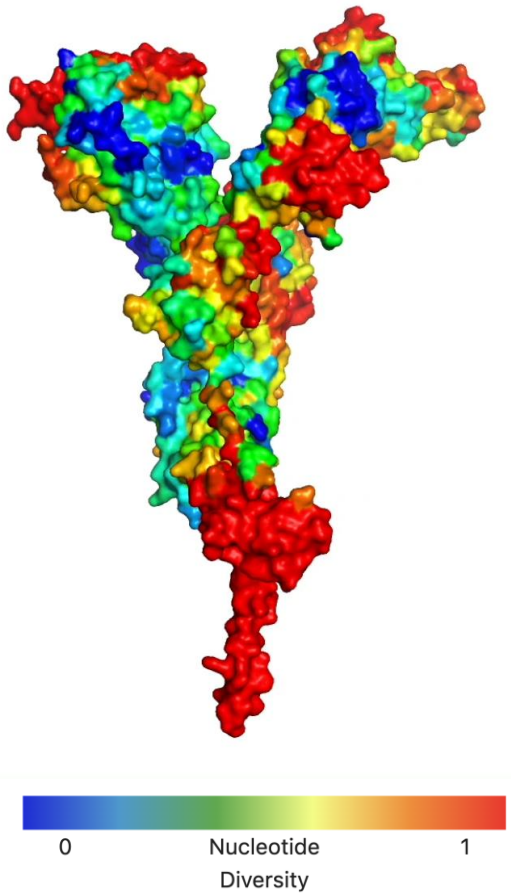
Composite evolution in Spike protein



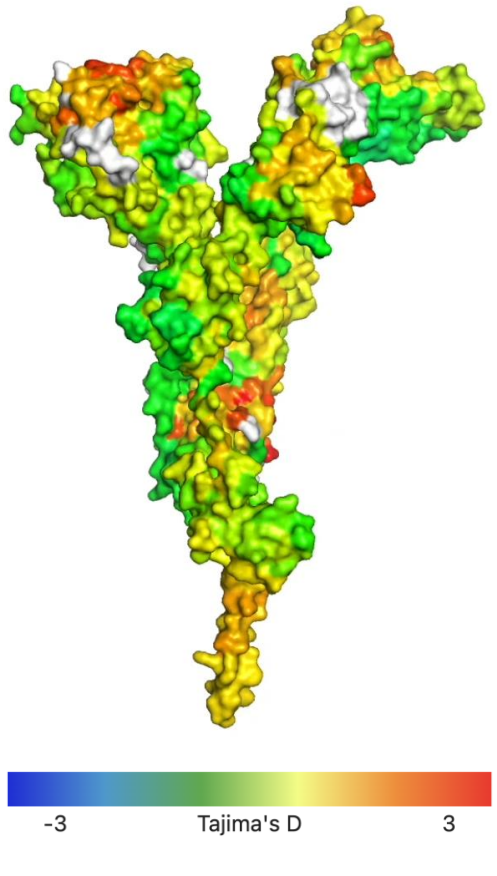


Source: [Mehra et al, ACS Inf Dis, 2022]

Feb-22

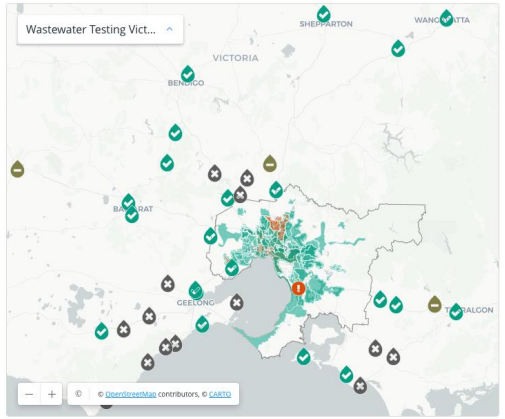


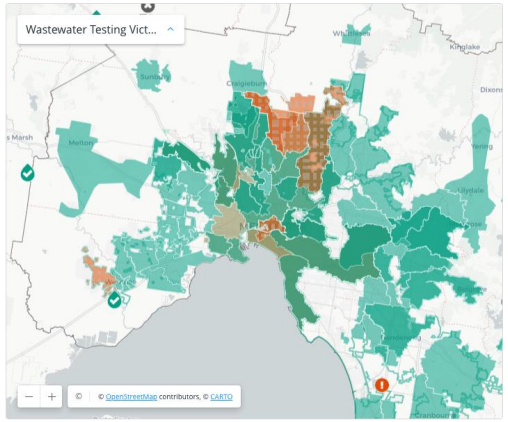
Feb-22

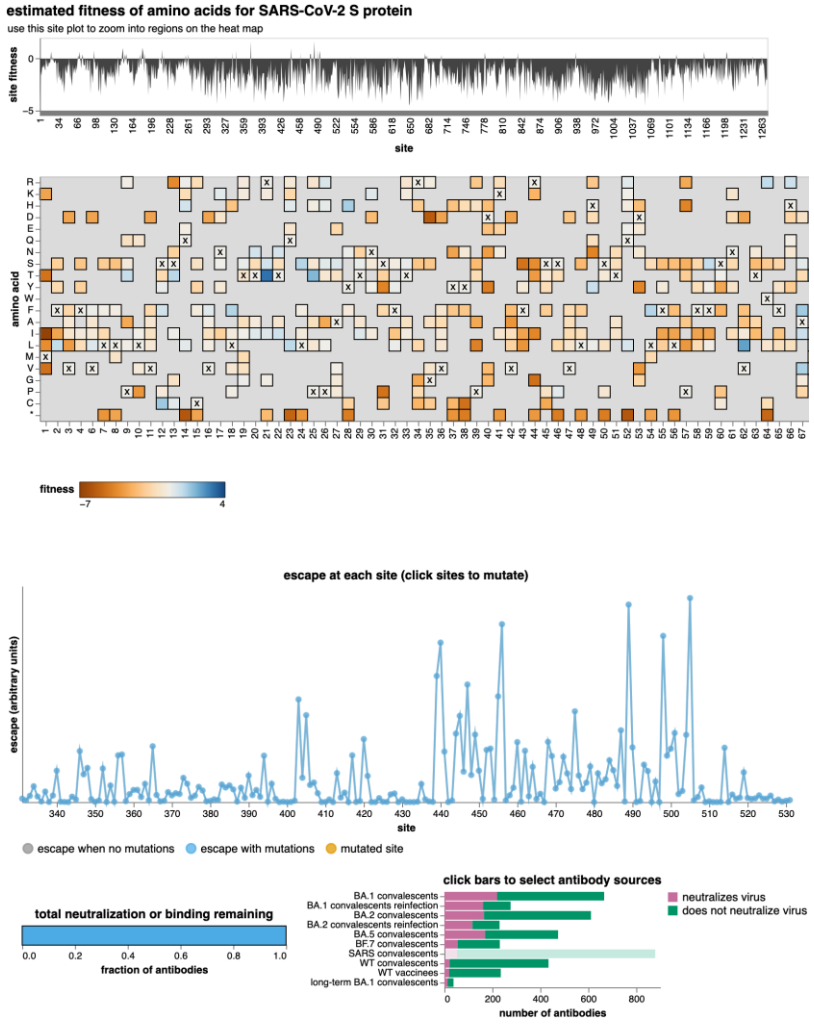


VIVID: Tichkule at al, Mol Biol Evol, 2022

Future directions







Bloom Lab – Fred Hutchins Cancer Center

Current program running until end of 2023

18 routine sampling sites + fortnightly sequencing

Focus on identifying major variant changes and emergence

Incorporating complex modelling

- Burnet Modelling – variant emergence and infection wave forecasting
- WEHI modelling:
  - best sampling sites
  - patterns in variant spread
  - determining which variant wins

Developing a public facing dashboard

Incorporating into Commonwealth reporting

Acknowledgments

WEHI- Jex lab
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Dr James Merrett
Dr Swapnil Tichkule

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Jennifer Jordan
Caroline Nicolas
Phyo Aung
Jean Paul Mfizi

Monash University- McCarthy's Lab
A/ Prof. David McCarthy
Dr Christelle Schang
Other lab members

University of Melbourne
Prof. Peter Scales
Dr Shane Usher

Melbourne Water
Dr Nick Crosbie

QIAGEN
Gulcan Grassa
Ehsan Nourbakhsh

SA Water (Adelaide)
Dr. Paul Monis
Lau Melody

Peter Doherty (VIDRL)
A/Prof. Bruce Thorley
Dr Julian Druce

WEHI -Genomics Lab
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Prof. Rory Bowden
Dr Daniel Brown

WEHI-Herold Lab/Pellegrini Lab
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A/Prof. Marco Herold
Dr Andrew Kueh
Ashley Lisboa-Pinto

Australian Laboratory Services
Natacha Begue
Dr Steven Myers
Christine Kaucner

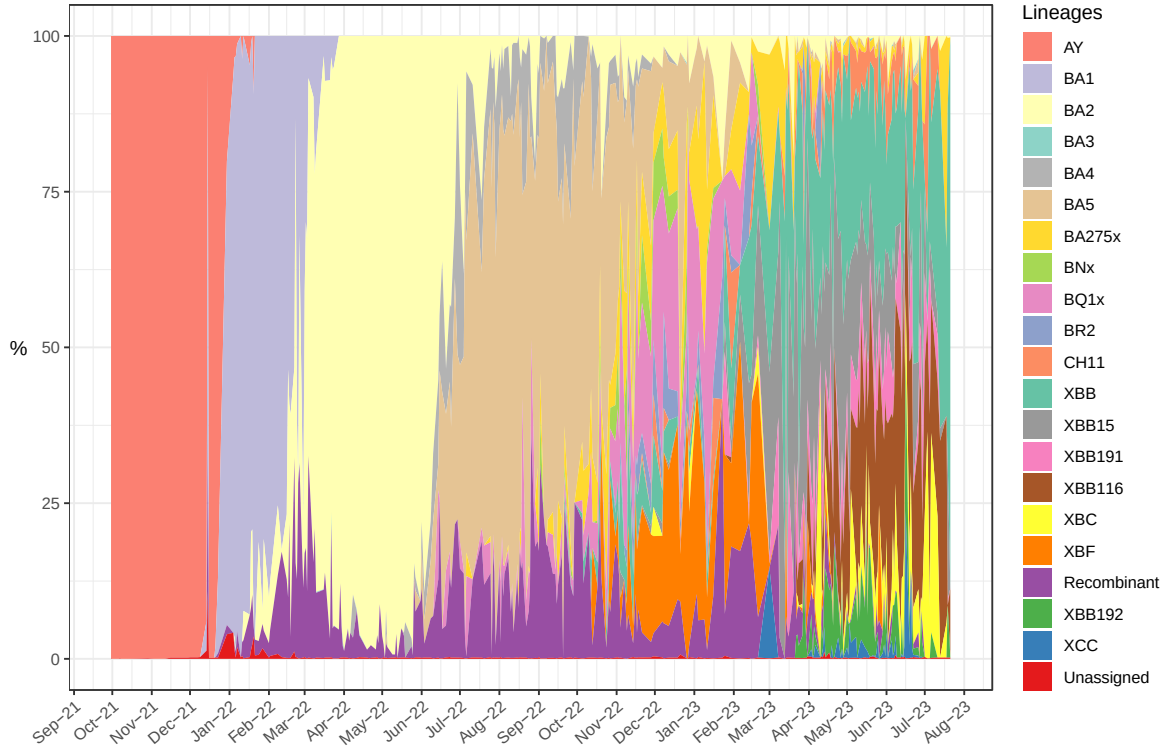
Water Research Australia
Karen Rouse
Dan Deere
Arash Zamyadi

Thank you

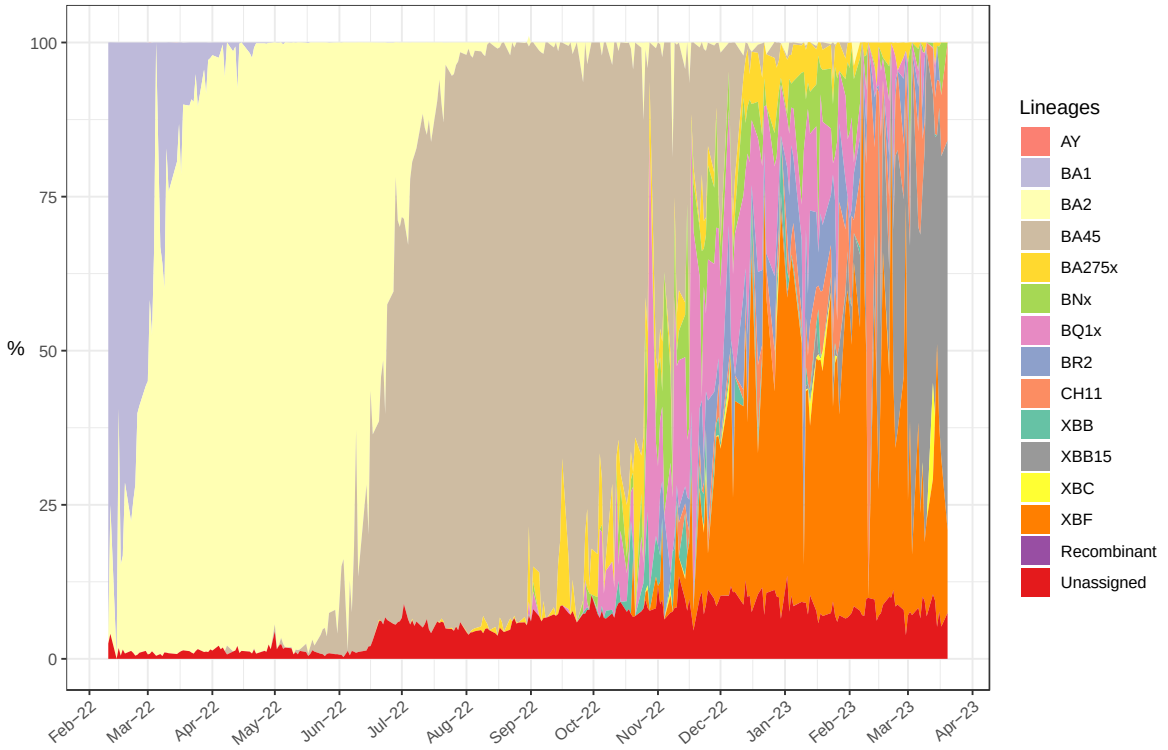
WEHI_research WEHIresearch WEHImovies WEHI_research Walter and Eliza Hall Institute

Variant detection and tracking

QIAseq – Freyja analysis



Spike Amplicon Sequencing



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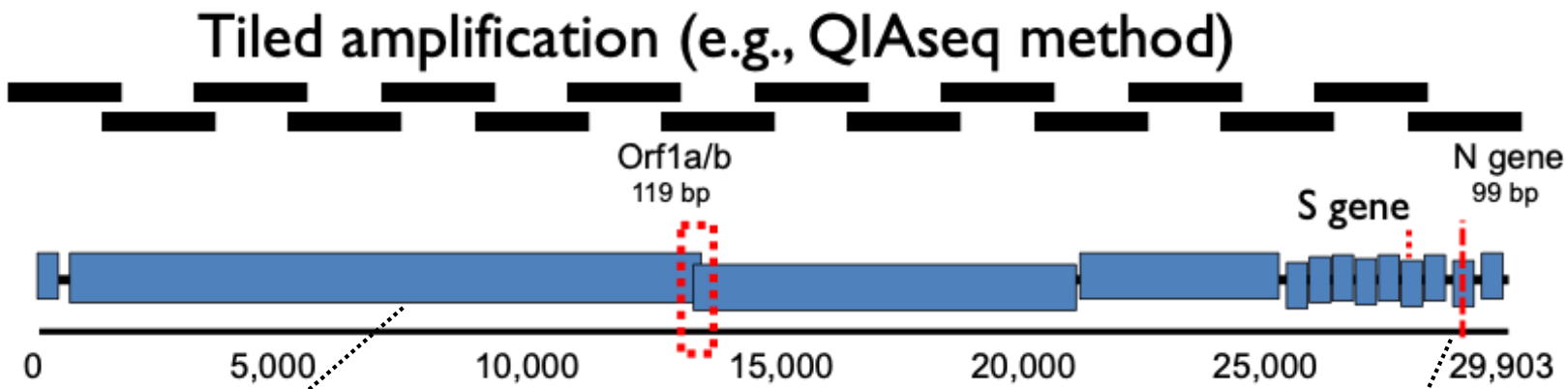
Freyja: Karthikeyan et al Nature, 2022

Composite evolution for drug resistance or RAT escape



nsp5 gene (Paxlovid target)

- nsp5: G15S ~5%
- nsp5: T135I <1%
- nsp5: Q189K ~1%

Caution: small sample size*

N-gene SNPs – RAT escape