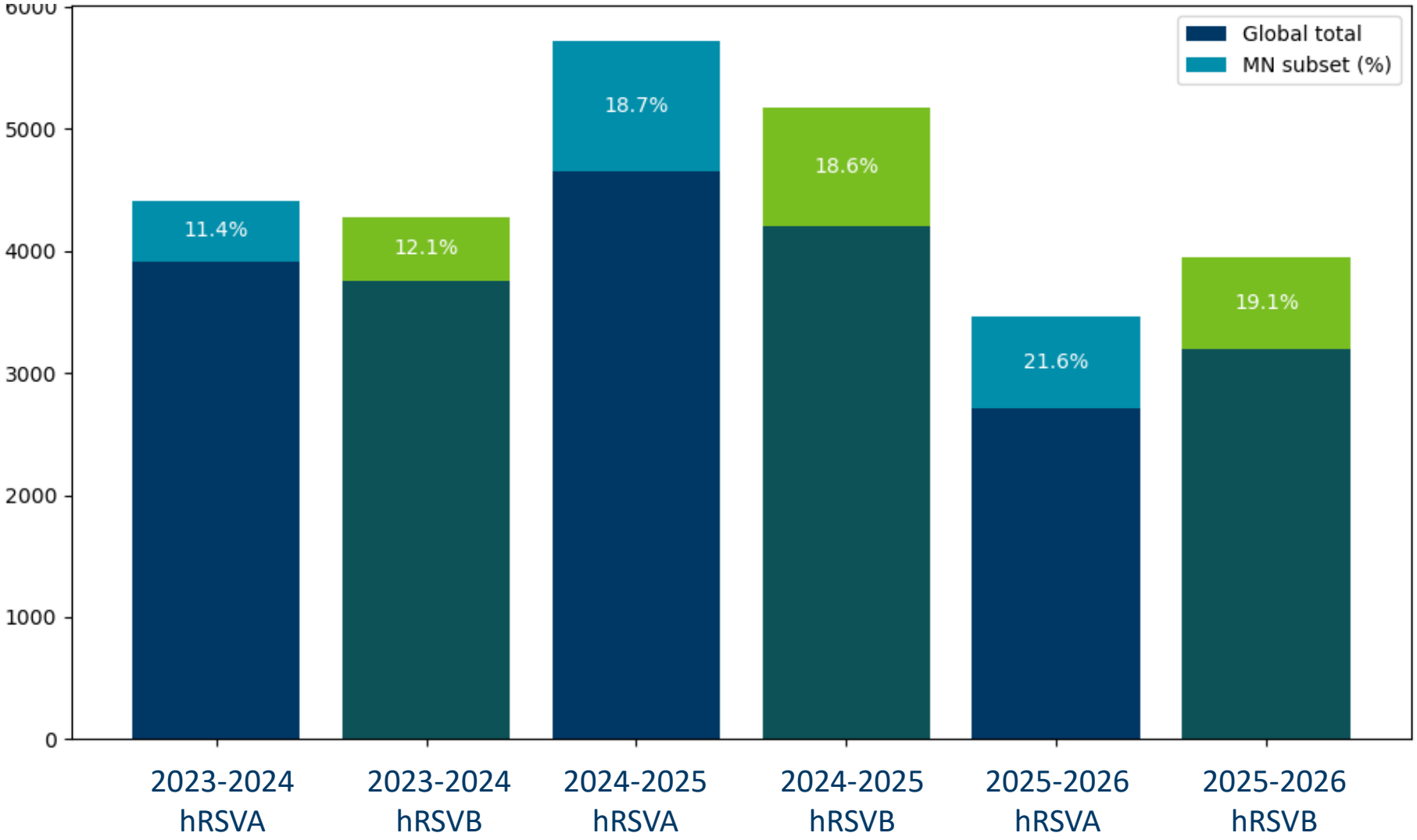


Genomic Surveillance of Human Respiratory Syncytial Virus in Minnesota – Statewide Efforts, Global Impacts

Henry Kunerth

PGCoE Network Tools Seminar – October 27th, 2026

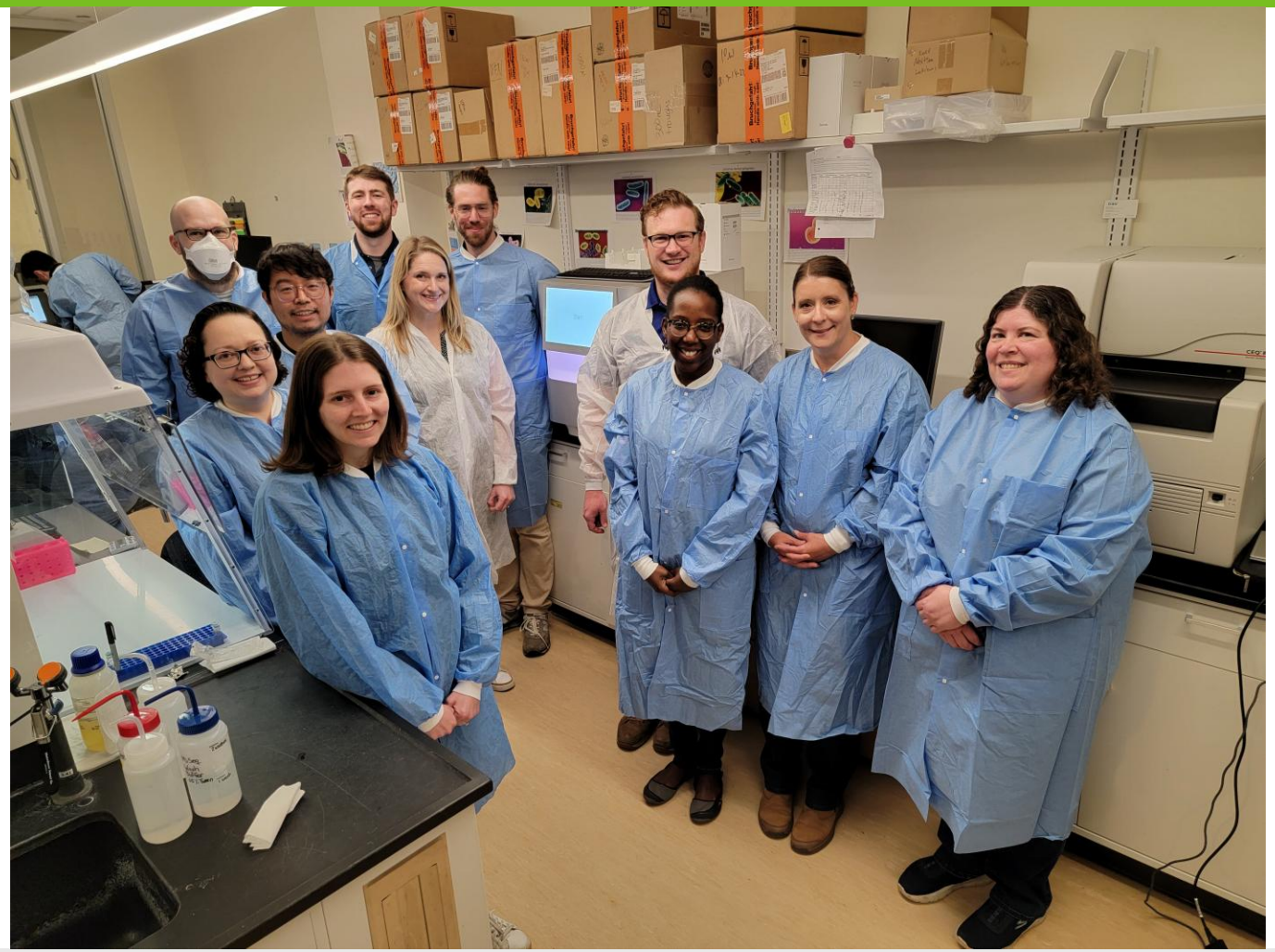
Since 2023, we've been sequencing a lot of hRSV



*complete (>90% breadth) genomes

Data from GISAID

How did we get here?

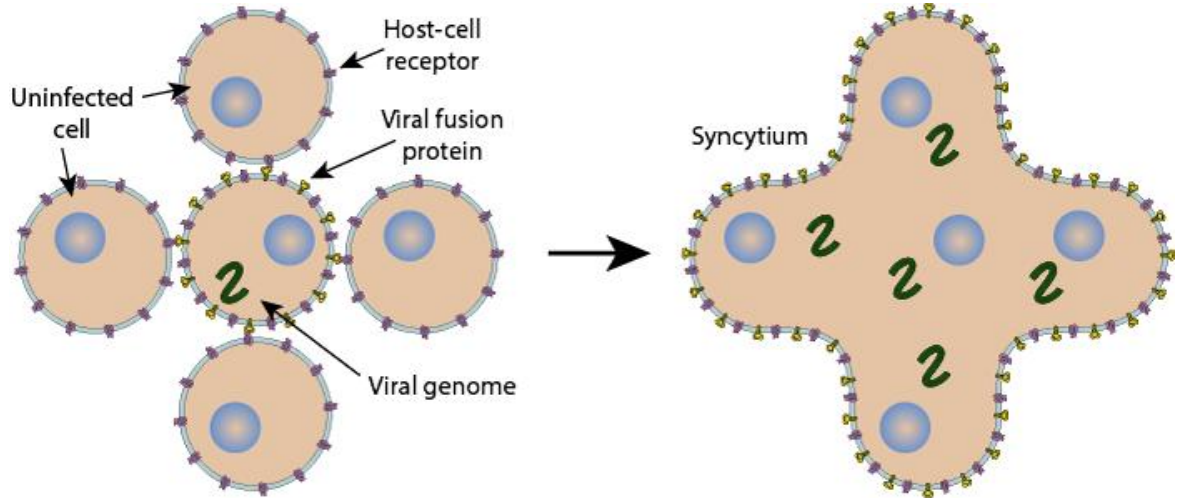


hRSV roadmap:

1. Robust network of submitters
2. Existing framework of laboratory testing + new development
3. Collaboration between bioinformatics and genomic epidemiology

Human respiratory syncytial virus (hRSV)

- Risk of severe disease higher in young children, the elderly, and immunocompromised persons
- ~3,000 hRSV-associated inpatient hospitalizations each season in MN
- Single-stranded RNA genome ~15,000 nucleotides long
- Two major viral subgroups: hRSV-A and hRSV-B



Shading shows >70% identity of amino acid alignments

ViralZone: "RSV"; data derived from CDC RSV-NET
GBKviz alignment of Minnesota RSV genomes

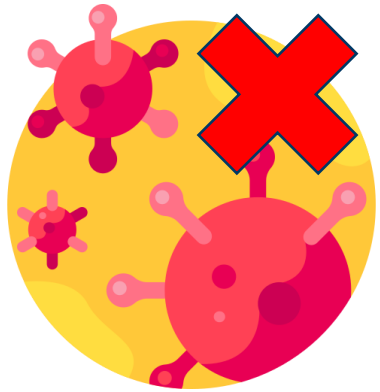
hRSV surveillance in Minnesota BEFORE genomic epi

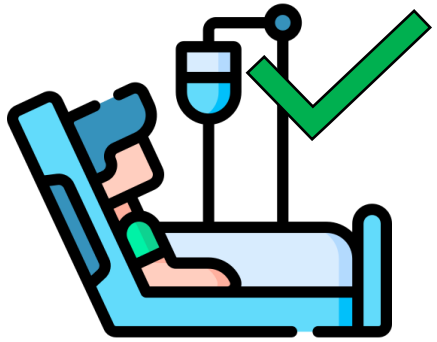
Not all laboratory-confirmed hRSV cases must be reported to MDH.

Hospitalized cases and hRSV-associated deaths **ARE reportable (RSV-NET)**.

Epis rarely receive A/B subgroup data.

Outbreak response is limited, with **no genomic analyses** involved.





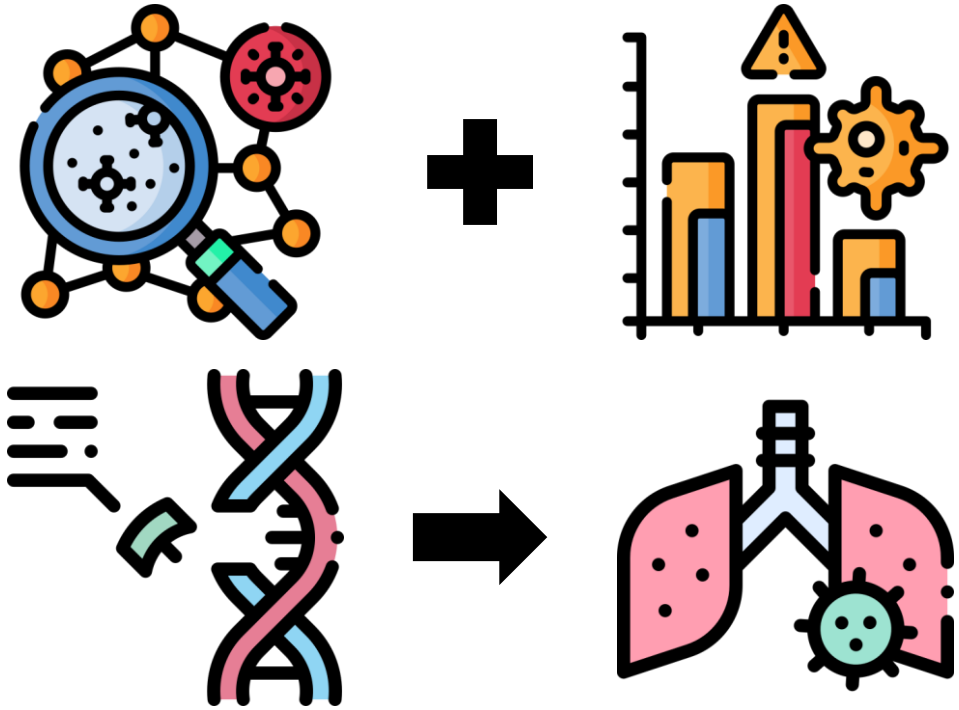


Flaticon: Freepik
CDC RSV-NET

Our objective – genomic epidemiology of hRSV in MN

Can we collaborate across the "lab-epi interface" to strengthen hRSV surveillance by using whole-genome sequencing technology?

- More precise surveillance
- Detection of clusters and outbreaks
- Emergence of new variants / lineages
- Associate mutations or subgroups with disease risk

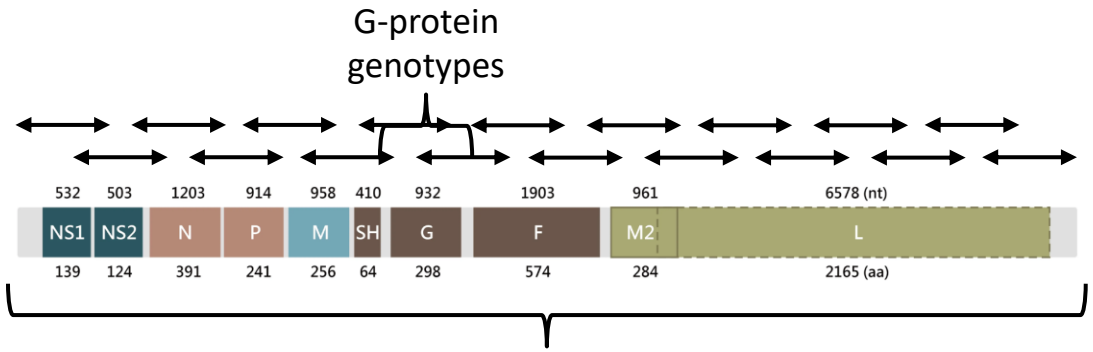


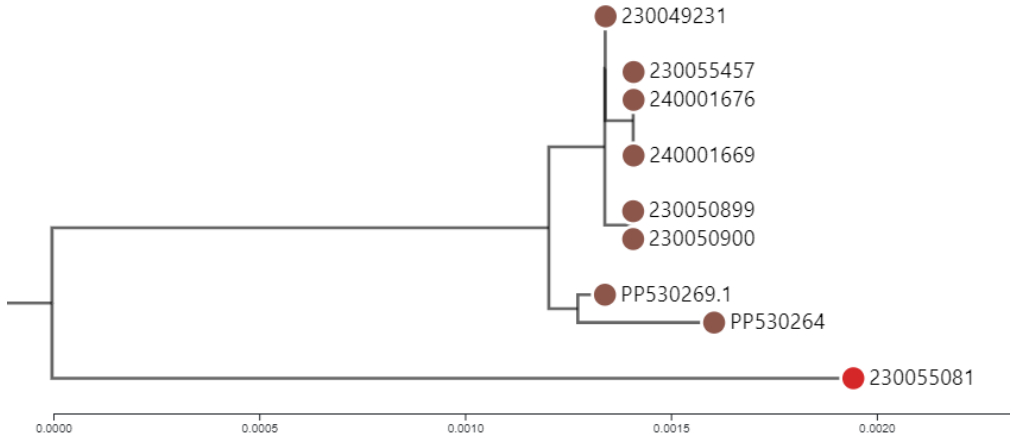
Flaticon: Freepik
CDC RSV-NET

We adapted our SARS-CoV-2 laboratory protocols to sequence and analyze hRSV genomes

- hRSV-positive specimens from >**30 healthcare facilities** in MN
- **"Tiled amplicon"** sequencing
- Genome assembly, QC, and typing using Viralrecon and Nextclade
  - **Subgroups** (A and B)
  - **Genotypes** (glycoprotein gene)
  - **Lineages** (whole-genome)
- Constructed **phylogenetic trees** with Nextstrain's Augur pipeline

Goya et al. 2020 and Goya et al. 2024





Primer scheme credit to Daniel Maloney et al.



Preliminary Results from two novel ARTIC-style amplicon based sequencing approaches for RSV A and RSV B

RSV

dmaloney 3 Dec 2022

Preliminary Results from two novel ARTIC-style amplicon based sequencing approaches for RSV A and RSV B

Daniel M Maloney1,2*; Goncalo Fernandes1*; Rebecca Dewar1; Aine O'Toole2; Chenge Mphanga3; Thomas C Williams3; Martin McHugh1; Andrew Rambaut2; Kate E Templeton1

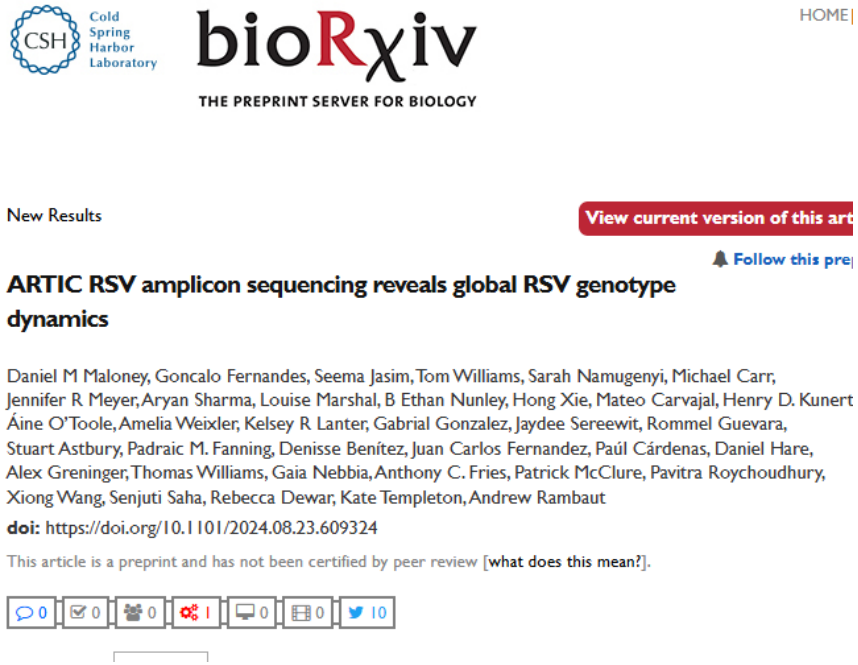
** These authors contributed equally*

1 - Viral Sequencing Service, Royal Infirmary of Edinburgh, UK
2 - Institute of Ecology and Evolution, University of Edinburgh, UK
3 - Child Life and Health, University of Edinburgh, UK

Contact: daniel.maloney@nhslothian.scot.nhs.uk

Introduction:

Dec 2022
1 / 2
Dec 2022
Dec 2023



CSH Cold Spring Harbor Laboratory

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ARTIC RSV amplicon sequencing reveals global RSV genotype dynamics

Daniel M Maloney, Goncalo Fernandes, Seema Jasim, Tom Williams, Sarah Namugenyi, Michael Carr, Jennifer R Meyer, Aryan Sharma, Louise Marshal, B Ethan Nunley, Hong Xie, Mateo Carvajal, Henry D. Kunert Áine O'Toole, Amelia Weixler, Kelsey R Lanter, Gabrial Gonzalez, Jaydee Sereewit, Rommel Guevara, Stuart Astbury, Padraic M. Fanning, Denisse Benítez, Juan Carlos Fernandez, Paúl Cárdenas, Daniel Hare, Alex Greninger, Thomas Williams, Gaia Nebbia, Anthony C. Fries, Patrick McClure, Pavitra Roychoudhury, Xiong Wang, Senjuti Saha, Rebecca Dewar, Kate Templeton, Andrew Rambaut

doi: https://doi.org/10.1101/2024.08.23.609324

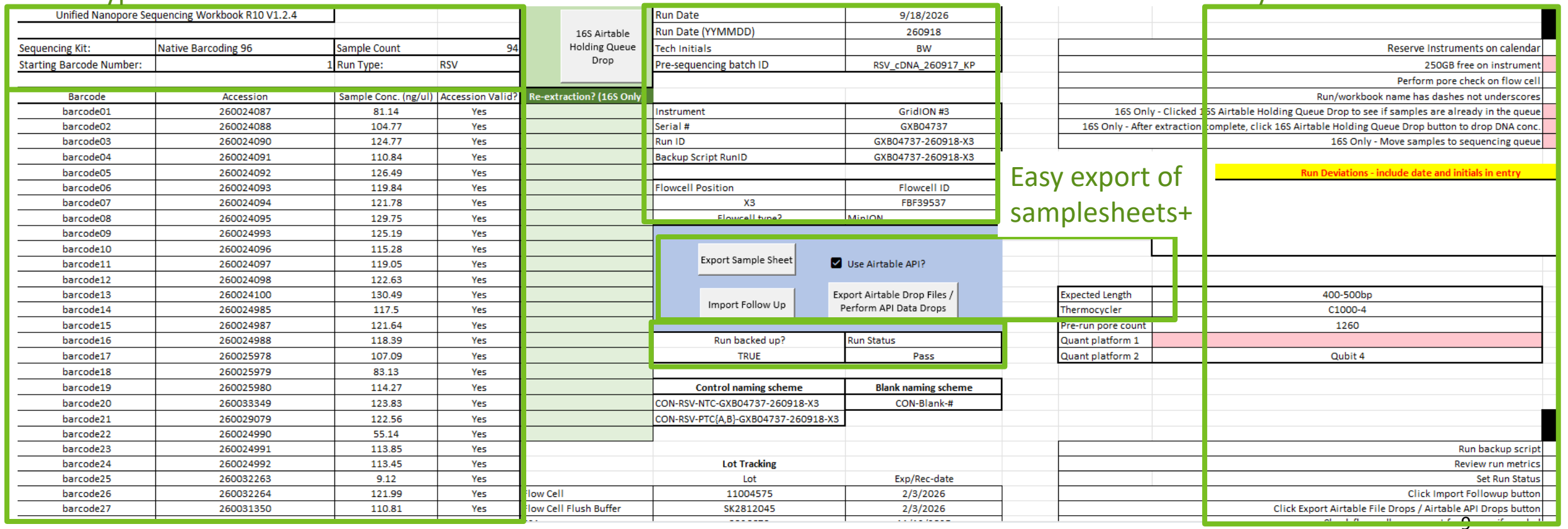
This article is a preprint and has not been certified by peer review [what does this mean?].

0 0 0 1 0 0 10

Maloney et al. 2022, 2024

Sequencing conducted on ONT GridIONs

Organized with dynamic workbook templates:



Run type

More run metrics

Dynamic checks

Easy export of samplesheets+

Sample IDs and concentrations

Unified Nanopore Sequencing Workbook R10 V1.2.4			
Sequencing Kit:	Native Barcoding 96	Sample Count	94
Starting Barcode Number:	1	Run Type:	RSV

Barcode	Accession	Sample Conc. (ng/ul)	Accession Valid?
barcode01	260024087	81.14	Yes
barcode02	260024088	104.77	Yes
barcode03	260024090	124.77	Yes
barcode04	260024091	110.84	Yes
barcode05	260024092	126.49	Yes
barcode06	260024093	119.84	Yes
barcode07	260024094	121.78	Yes
barcode08	260024095	129.75	Yes
barcode09	260024993	125.19	Yes
barcode10	260024096	115.28	Yes
barcode11	260024097	119.05	Yes
barcode12	260024098	122.63	Yes
barcode13	260024100	130.49	Yes
barcode14	260024985	117.5	Yes
barcode15	260024987	121.64	Yes
barcode16	260024988	118.39	Yes
barcode17	260025978	107.09	Yes
barcode18	260025979	83.13	Yes
barcode19	260025980	114.27	Yes
barcode20	260033349	123.83	Yes
barcode21	260029079	122.56	Yes
barcode22	260024990	55.14	Yes
barcode23	260024991	113.85	Yes
barcode24	260024992	113.45	Yes
barcode25	260032263	9.12	Yes
barcode26	260032264	121.99	Yes
barcode27	260031350	110.81	Yes

16S Airtable Holding Queue Drop

Re-extraction? (16S Only

Run Date	9/18/2026
Run Date (YYMMDD)	260918
Tech Initials	BW
Pre-sequencing batch ID	RSV_cDNA_260917_KP
Instrument	GridION #3
Serial #	GXB04737
Run ID	GXB04737-260918-X3
Backup Script RunID	GXB04737-260918-X3
Flowcell Position	Flowcell ID
X3	FBF39537
Flowcell type?	MinION

Export Sample Sheet

Use Airtable API?

Import Follow Up

Export Airtable Drop Files / Perform API Data Drops

Run backed up?	Run Status
TRUE	Pass

Control naming scheme	Blank naming scheme
CON-RSV-NTC-GXB04737-260918-X3	CON-Blank-#
CON-RSV-PTC{A,B}-GXB04737-260918-X3	

Lot Tracking	Lot	Exp/Rec-date
Flow Cell	11004575	2/3/2026
Flow Cell Flush Buffer	SK2812045	2/3/2026

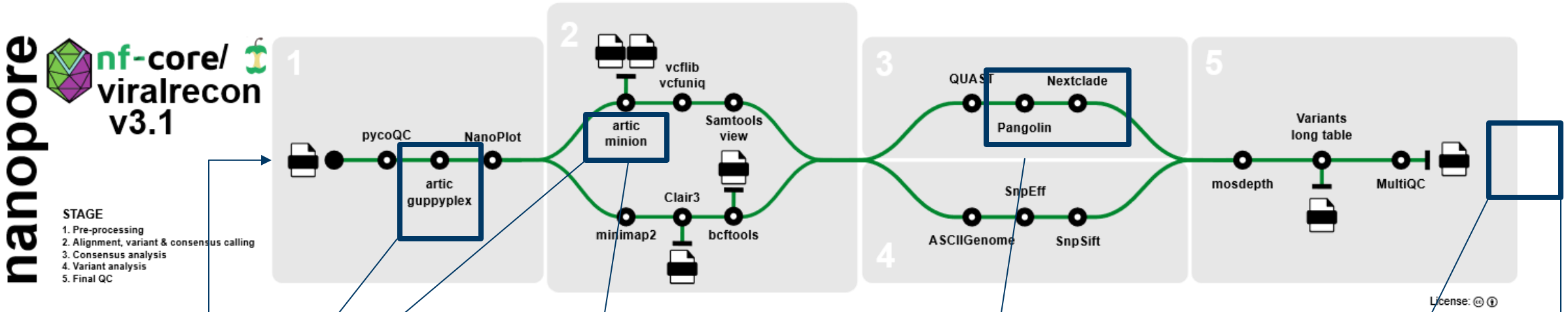
Reserve Instruments on calendar	
250GB free on instrument	
Perform pore check on flow cell	
Run/workbook name has dashes not underscores	
16S Only - Clicked 16S Airtable Holding Queue Drop to see if samples are already in the queue	
16S Only - After extraction complete, click 16S Airtable Holding Queue Drop button to drop DNA conc.	
16S Only - Move samples to sequencing queue	

Run Deviations - include date and initials in entry

Expected Length	400-500bp
Thermocycler	C1000-4
Pre-run pore count	1260
Quant platform 1	
Quant platform 2	Qubit 4

Run backup script	
Review run metrics	
Set Run Status	
Click Import Followup button	
Click Export Airtable File Drops / Airtable API Drops button	

nf-core/viralrecon 3.0.0 performs QC, assembly, and typing



Minor modifications:

1. Update artic container to match basecalling model

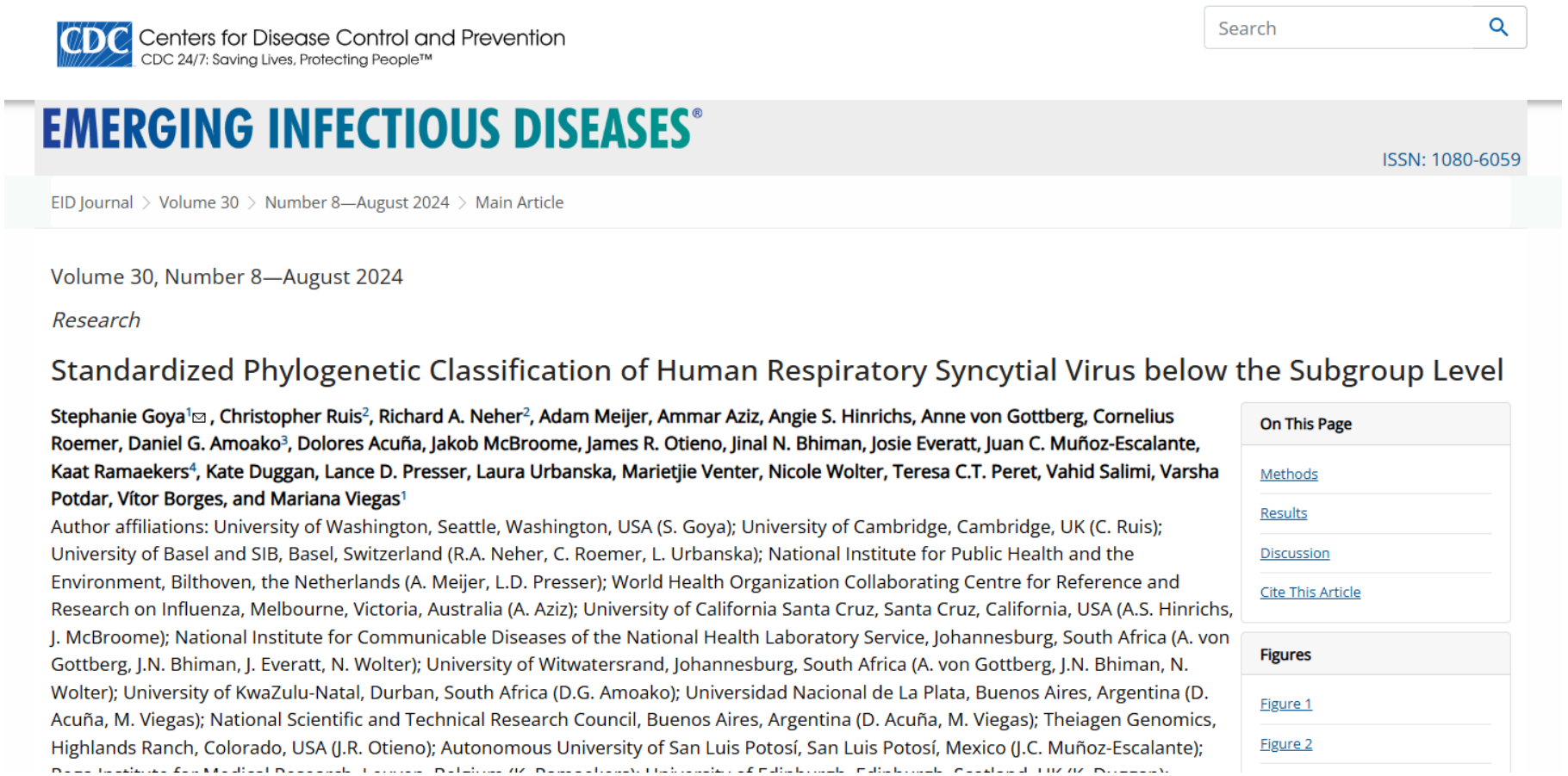
2. Adjust minimum depth cutoff to 40x

3. Turn off Pangolin, supply updated Nextclade lineage data for hRSV

4. Loop through to analyze hRSVA and hRSVB

5. Perform additional QC (90% breadth of genome cutoff, VADR) and submit data to NCBI

Lineages are defined based on Goya et al. 2024 and community updates



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ISSN: 1080-6059

EID Journal > Volume 30 > Number 8—August 2024 > Main Article

Volume 30, Number 8—August 2024

Research

Standardized Phylogenetic Classification of Human Respiratory Syncytial Virus below the Subgroup Level

Stephanie Goya[1], Christopher Ruis[2], Richard A. Neher[2], Adam Meijer, Ammar Aziz, Angie S. Hinrichs, Anne von Gottberg, Cornelius Roemer, Daniel G. Amoako[3], Dolores Acuña, Jakob McBroome, James R. Otieno, Jinal N. Bhiman, Josie Everatt, Juan C. Muñoz-Escalante, Kaat Ramaekers[4], Kate Duggan, Lance D. Presser, Laura Urbanska, Marietjie Venter, Nicole Wolter, Teresa C.T. Peret, Vahid Salimi, Varsha Potdar, Vítor Borges, and Mariana Viegas[1]

Author affiliations: University of Washington, Seattle, Washington, USA (S. Goya); University of Cambridge, Cambridge, UK (C. Ruis); University of Basel and SIB, Basel, Switzerland (R.A. Neher, C. Roemer, L. Urbanska); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (A. Meijer, L.D. Presser); World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Victoria, Australia (A. Aziz); University of California Santa Cruz, Santa Cruz, California, USA (A.S. Hinrichs, J. McBroome); National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa (A. von Gottberg, J.N. Bhiman, J. Everatt, N. Wolter); University of Witwatersrand, Johannesburg, South Africa (A. von Gottberg, J.N. Bhiman, N. Wolter); University of KwaZulu-Natal, Durban, South Africa (D.G. Amoako); Universidad Nacional de La Plata, Buenos Aires, Argentina (D. Acuña, M. Viegas); National Scientific and Technical Research Council, Buenos Aires, Argentina (D. Acuña, M. Viegas); Theiagen Genomics, Highlands Ranch, Colorado, USA (J.R. Otieno); Autonomous University of San Luis Potosí, San Luis Potosí, Mexico (J.C. Muñoz-Escalante);

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Methods

Results

Discussion

Cite This Article

Figures

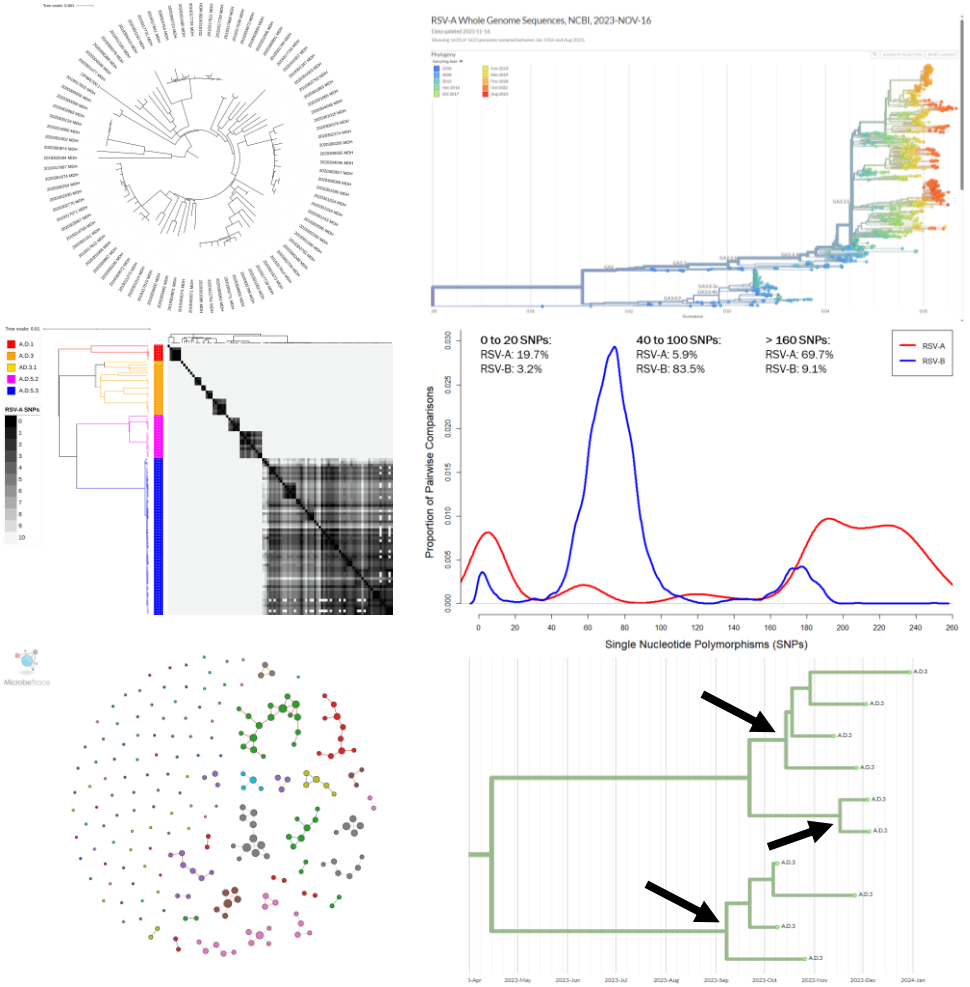
Figure 1

Figure 2

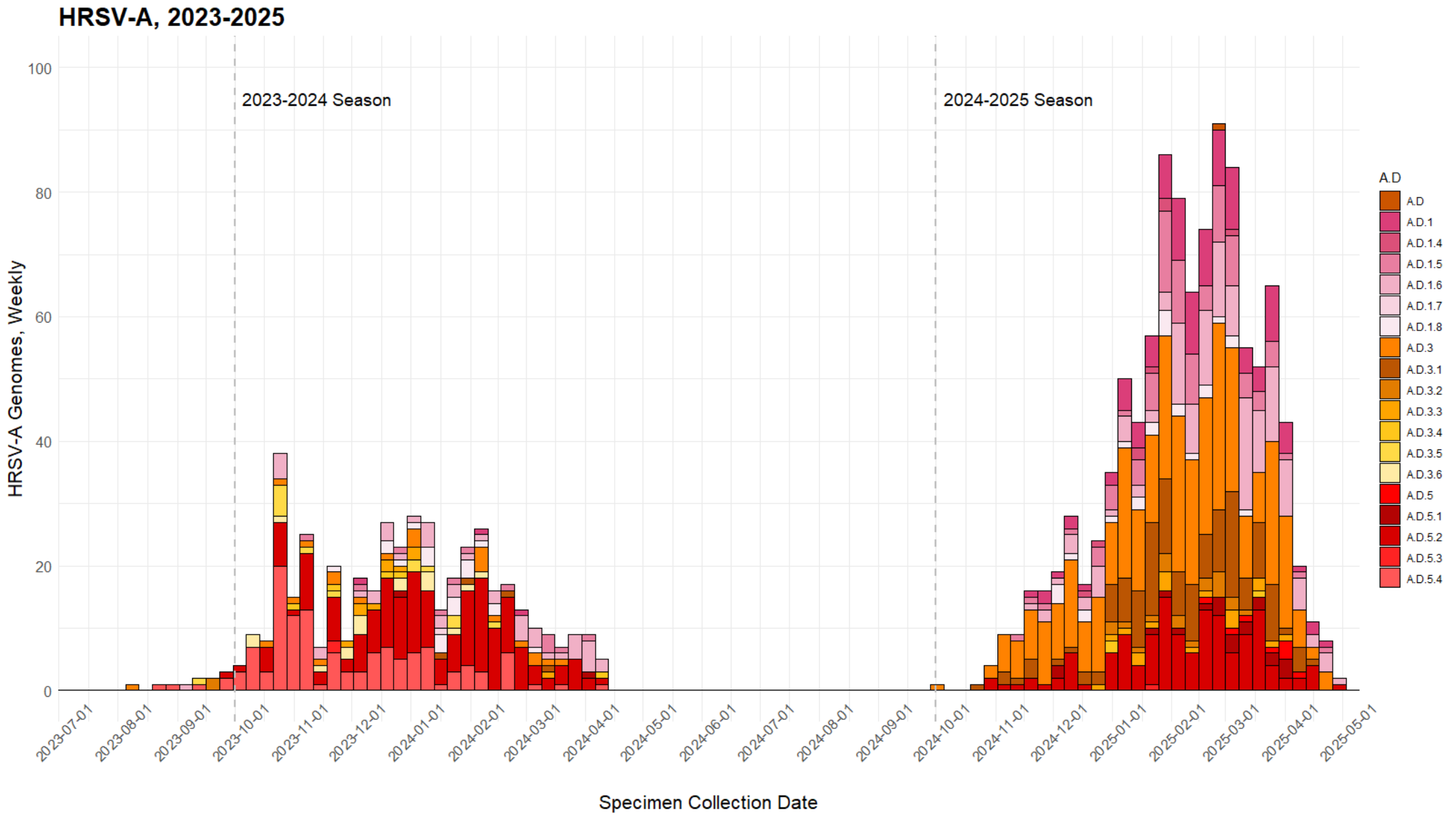
Goya et al. 2024

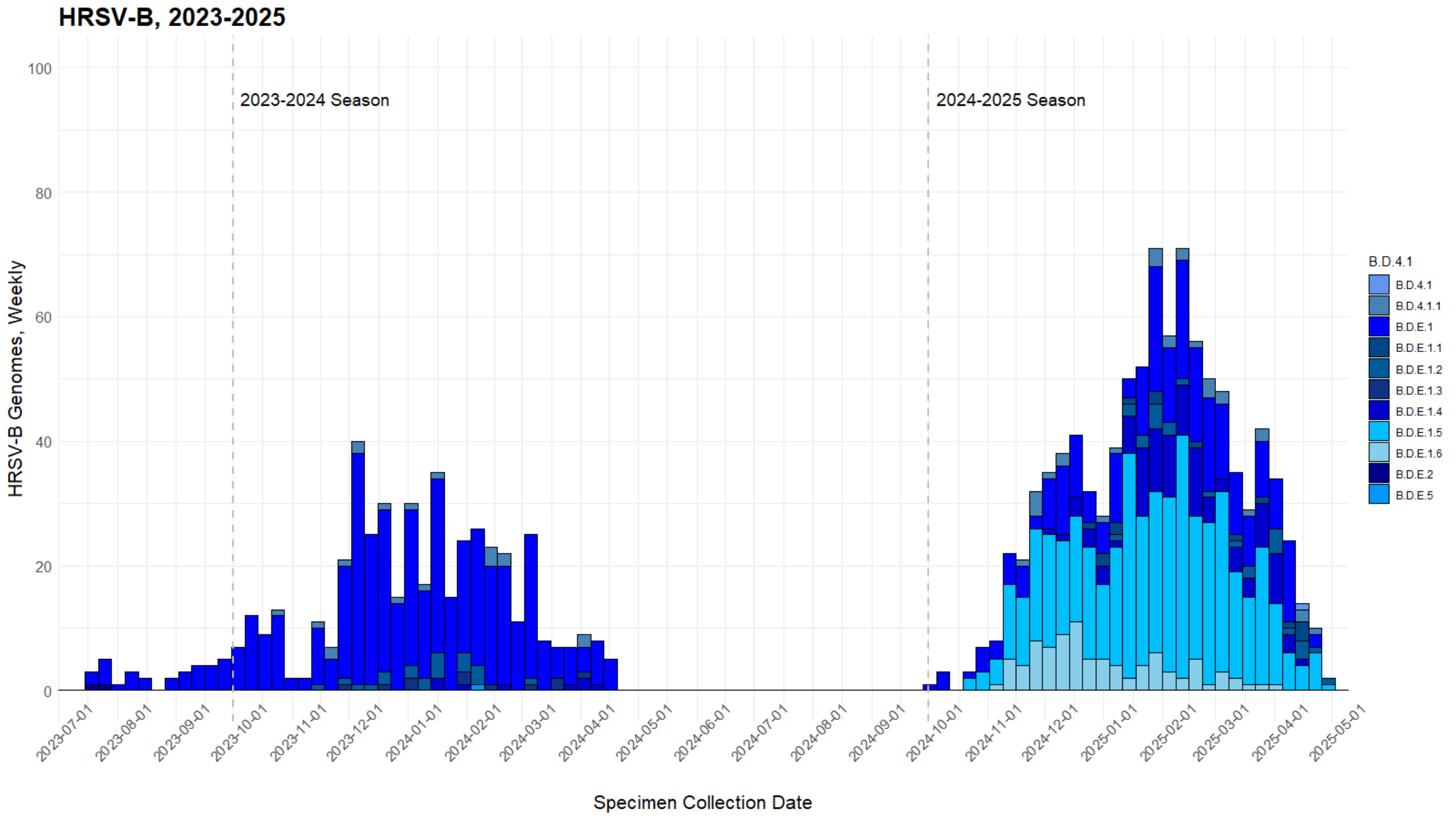
What did we find?

- July 2023 – June 2026: **4856 genomes** (n = 2465 hRSV-A; n = 2391 hRSV-B)
- 24+ potential **coinfections** of A and B
- Year by year surveillance of lineages and mutations of interest
- Linking epidemiological data to genomic data helps us track resistance mutations

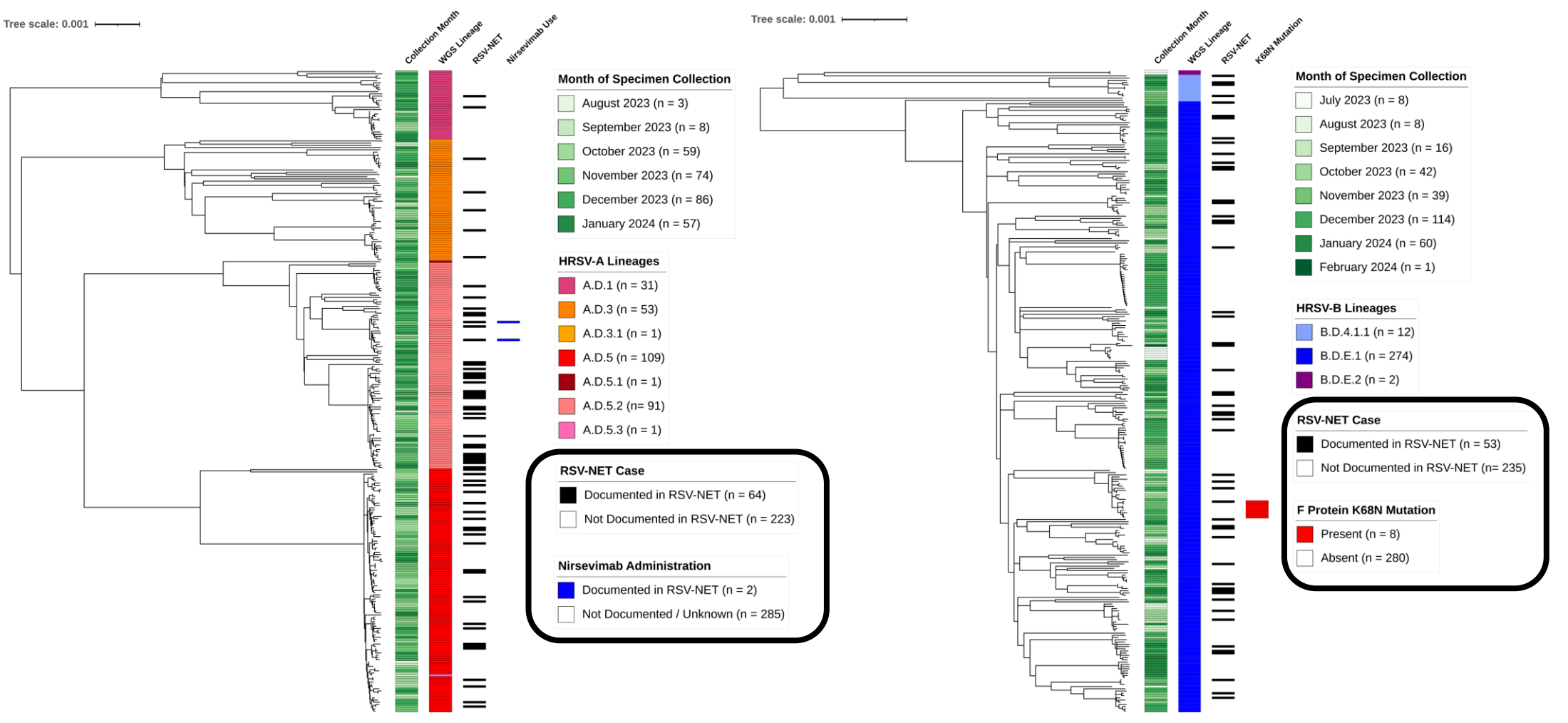


Sequencing data visualized using Nextstrain, ITOL, MicrobeTrace, and R


HRSV-A, 2023-2025
2023-2024 Season
2024-2025 Season
HRSV-A Genomes, Weekly
Specimen Collection Date
A.D
A.D
A.D.1
A.D.1.4
A.D.1.5
A.D.1.6
A.D.1.7
A.D.1.8
A.D.3
A.D.3.1
A.D.3.2
A.D.3.3
A.D.3.4
A.D.3.5
A.D.3.6
A.D.5
A.D.5.1
A.D.5.2
A.D.5.3
A.D.5.4

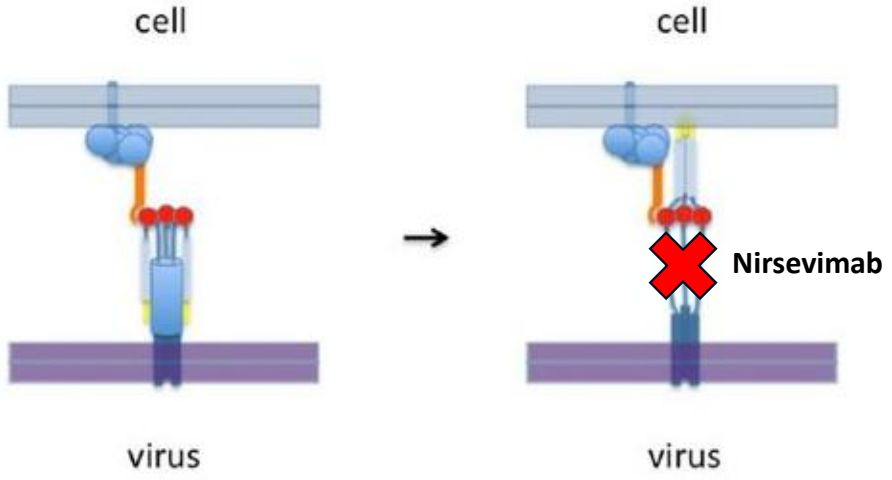

HRSV-B, 2023-2025
2023-2024 Season
2024-2025 Season
HRSV-B Genomes, Weekly
Specimen Collection Date
B.D.4.1
B.D.4.1
B.D.4.1.1
B.D.E.1
B.D.E.1.1
B.D.E.1.2
B.D.E.1.3
B.D.E.1.4
B.D.E.1.5
B.D.E.1.6
B.D.E.2
B.D.E.5

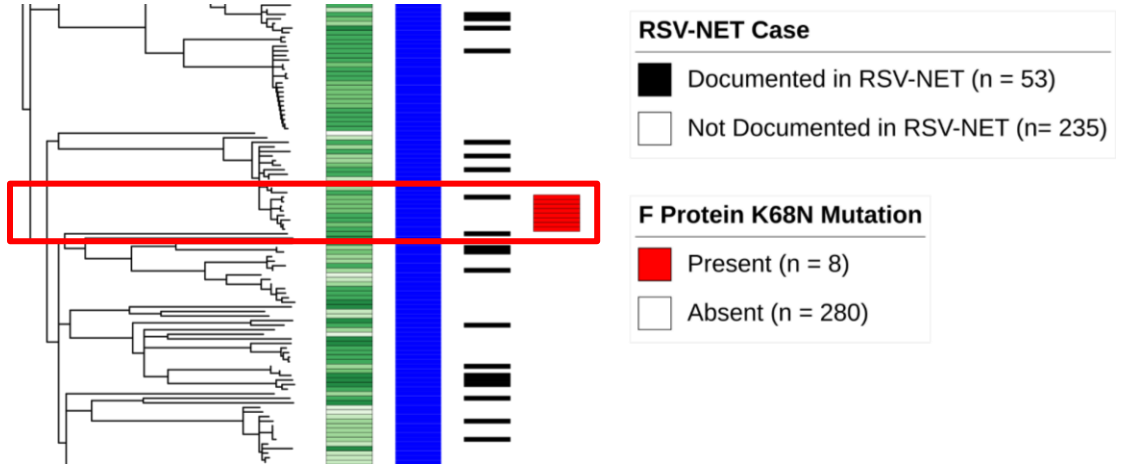
We can link useful epidemiologic data to RSV genomes



Nirsevimab breakthrough cases and resistance mutations in Minnesota RSV cases

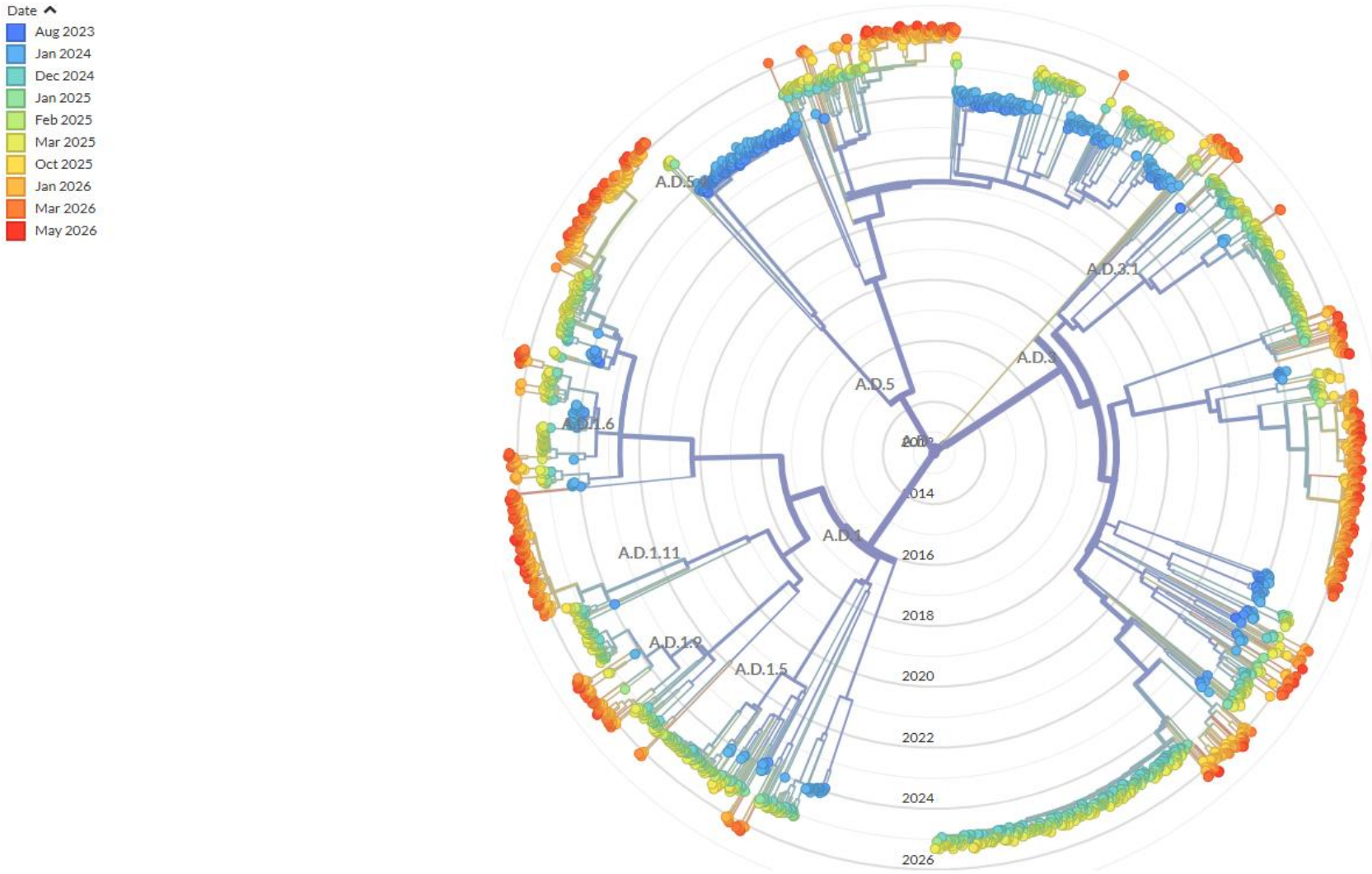
- Nirsevimab: monoclonal antibody administered for **short-term immune boost** against RSV infection
- Mutations in RSV genome known to **confer resistance** to nirsevimab
- Two RSV-A hospitalizations occurred **after** administration of nirsevimab
- "Clade" of RSV-B genomes shared the F-protein **"K68N" mutation**





Mastrangelo and Hegele, Viruses 2013
Phylogenetic build of RSV-B genomes from Minnesota healthcare facilities

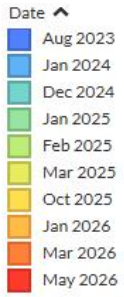
Three seasons of data allows us to track trends

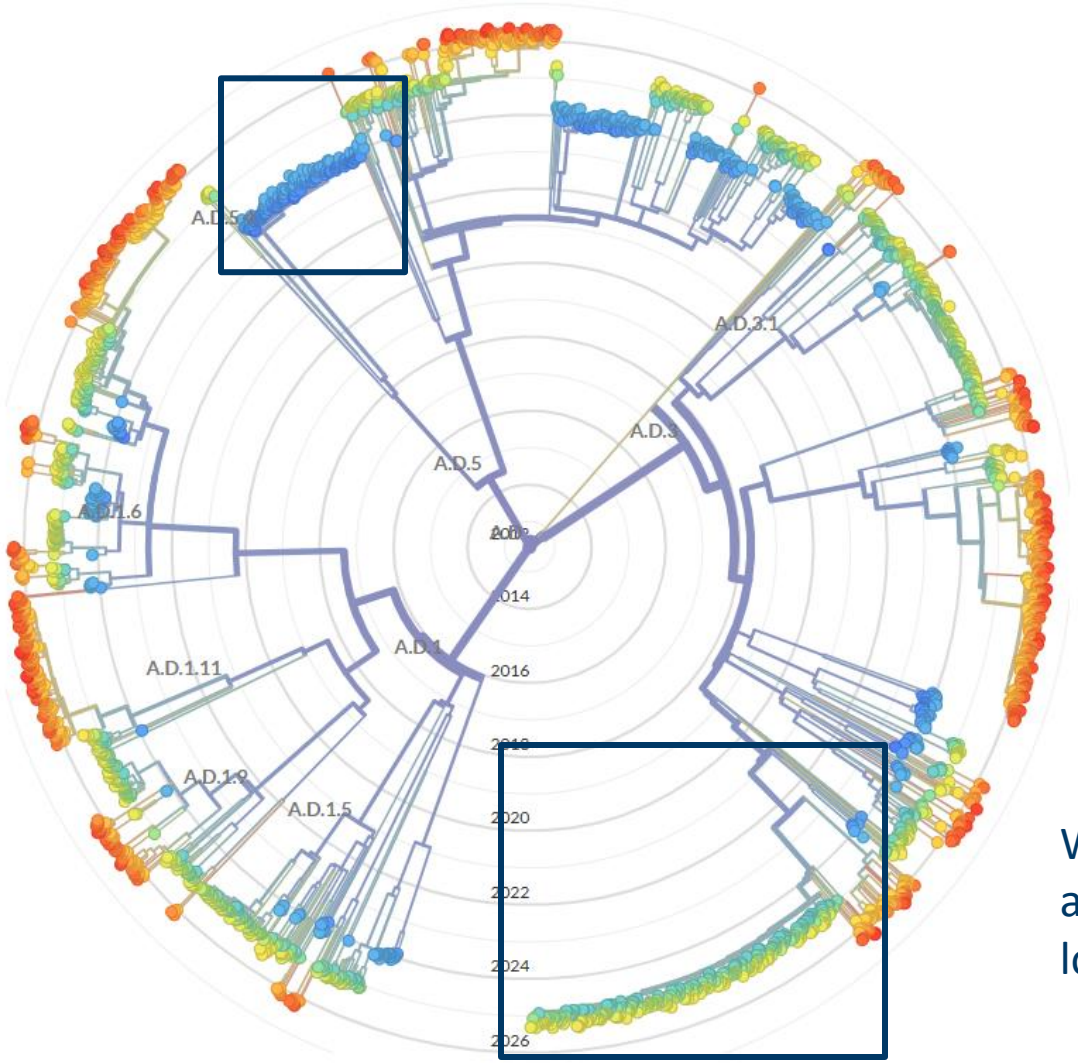


hRSVA – Minnesota genomes

Colored by collection date

Three seasons of data allows us to track trends



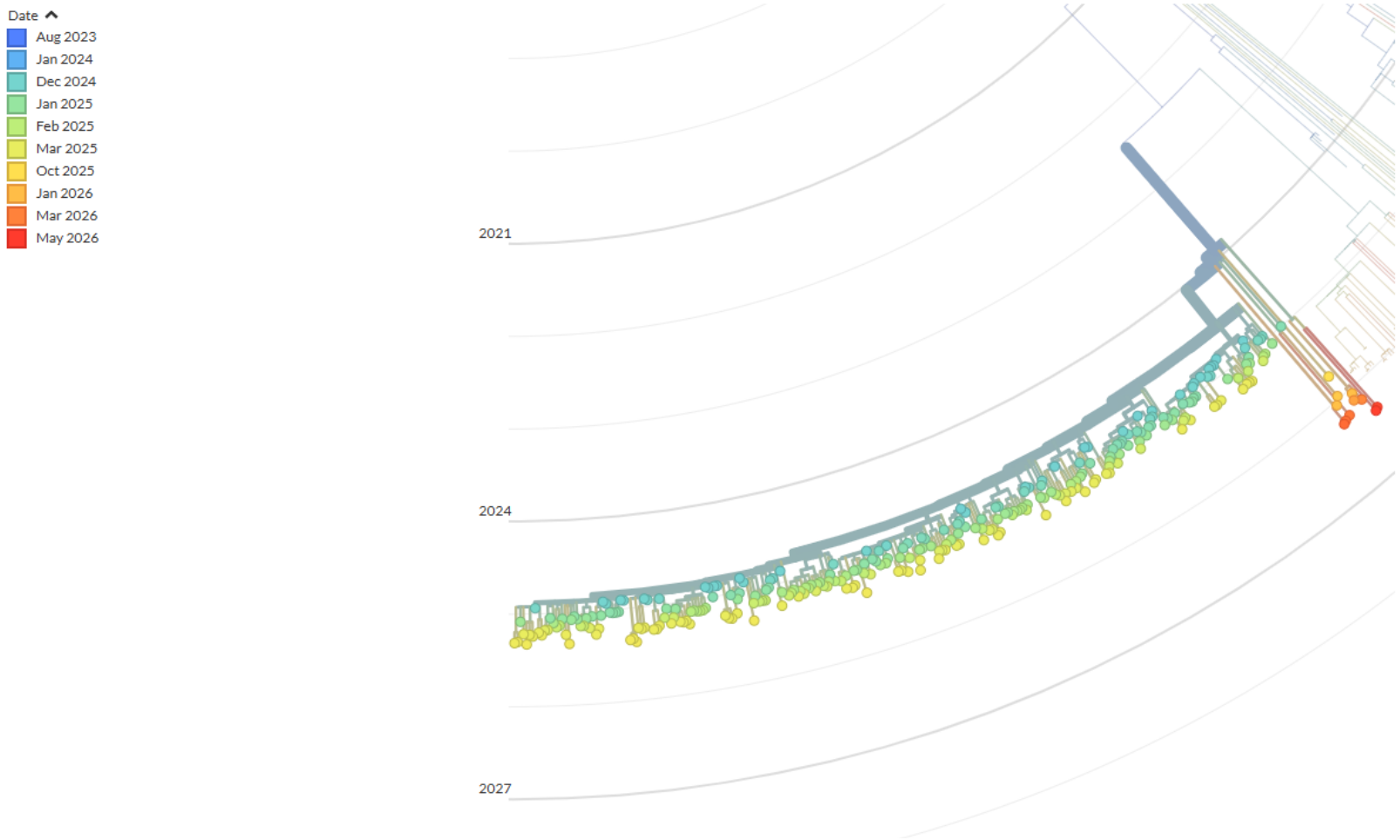


hRSVA – Minnesota genomes

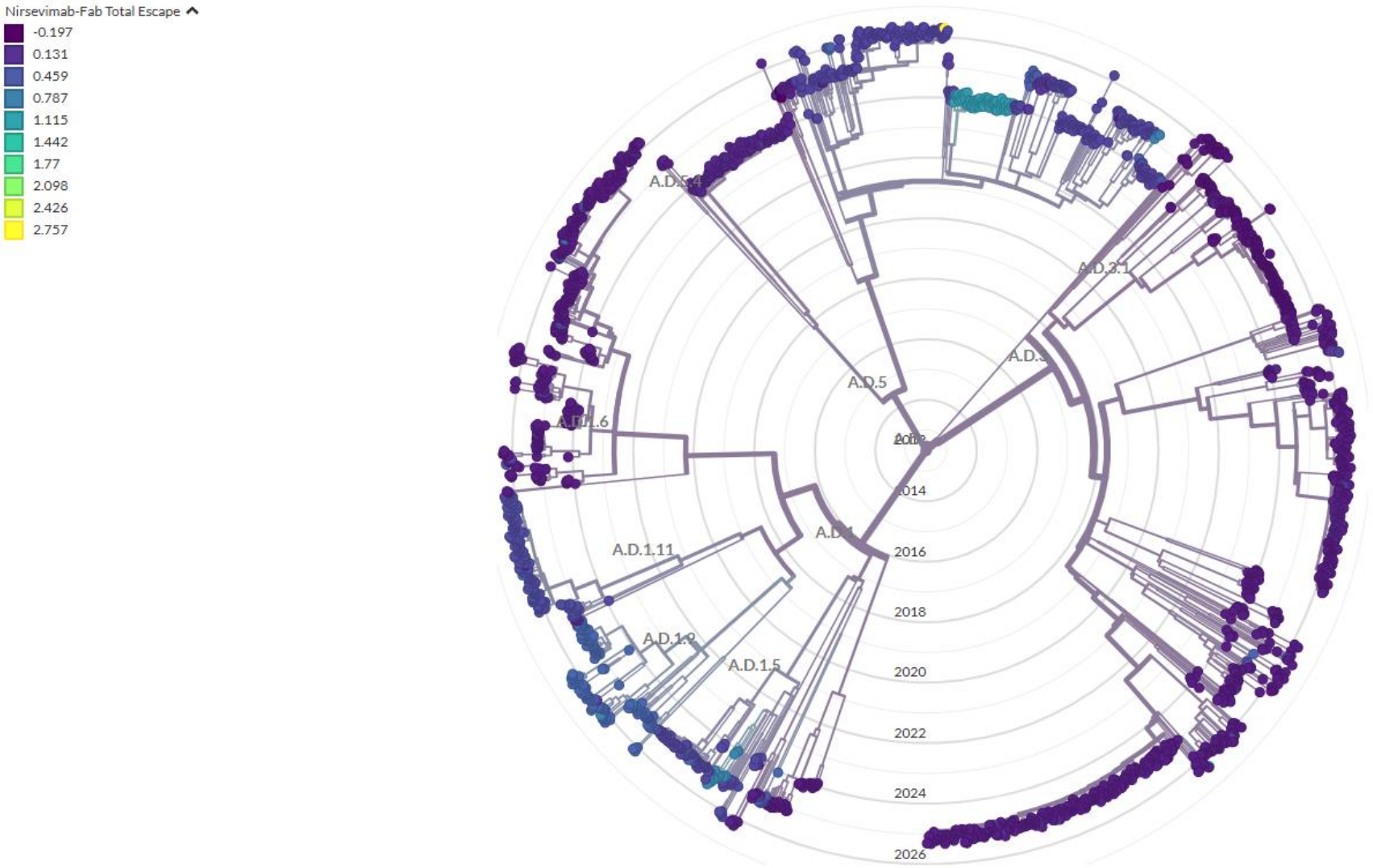
Colored by collection date

Why are some clades abundant one year and lost the next?

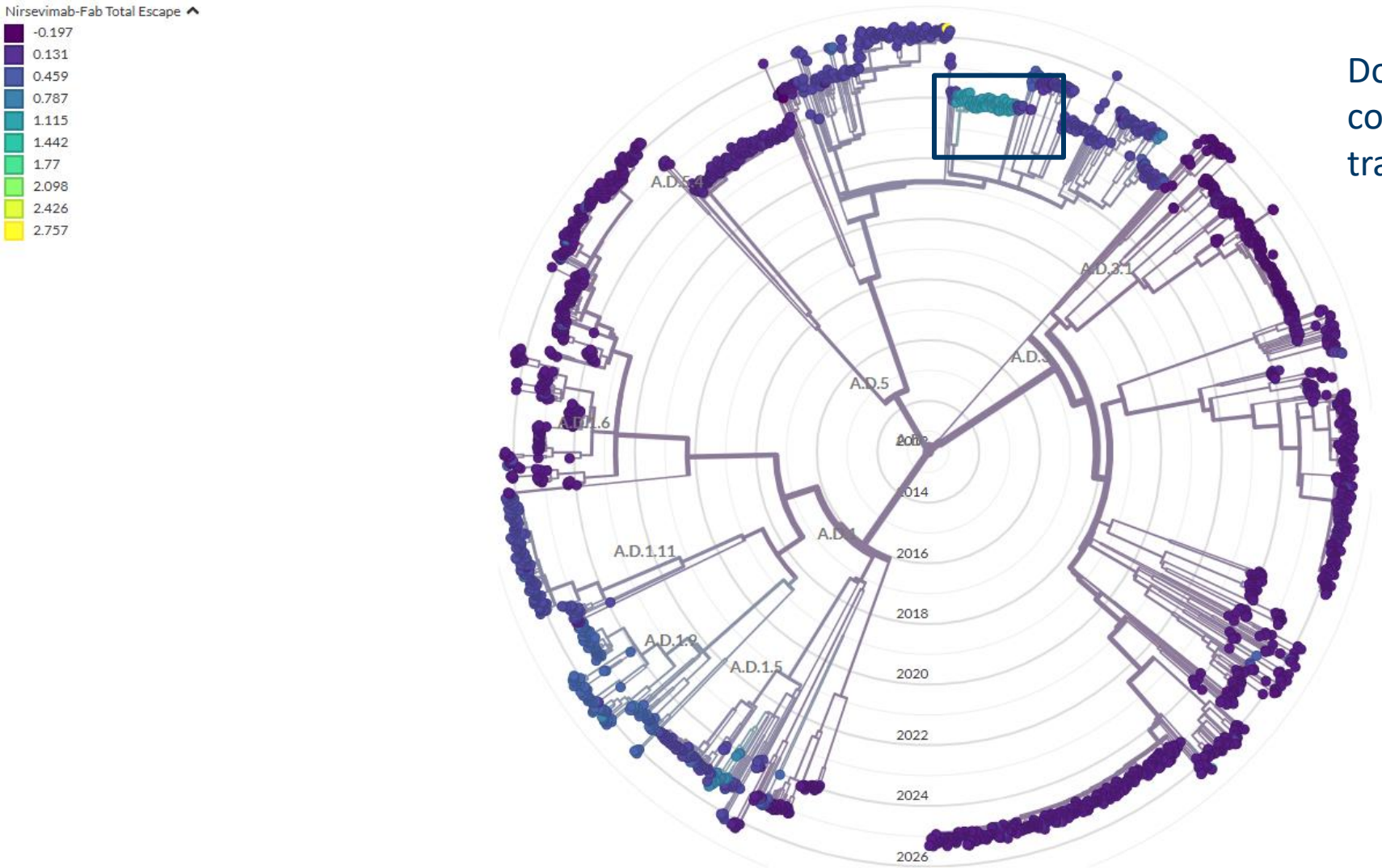
Common clades are seasonally lost



We can also track antiviral escape

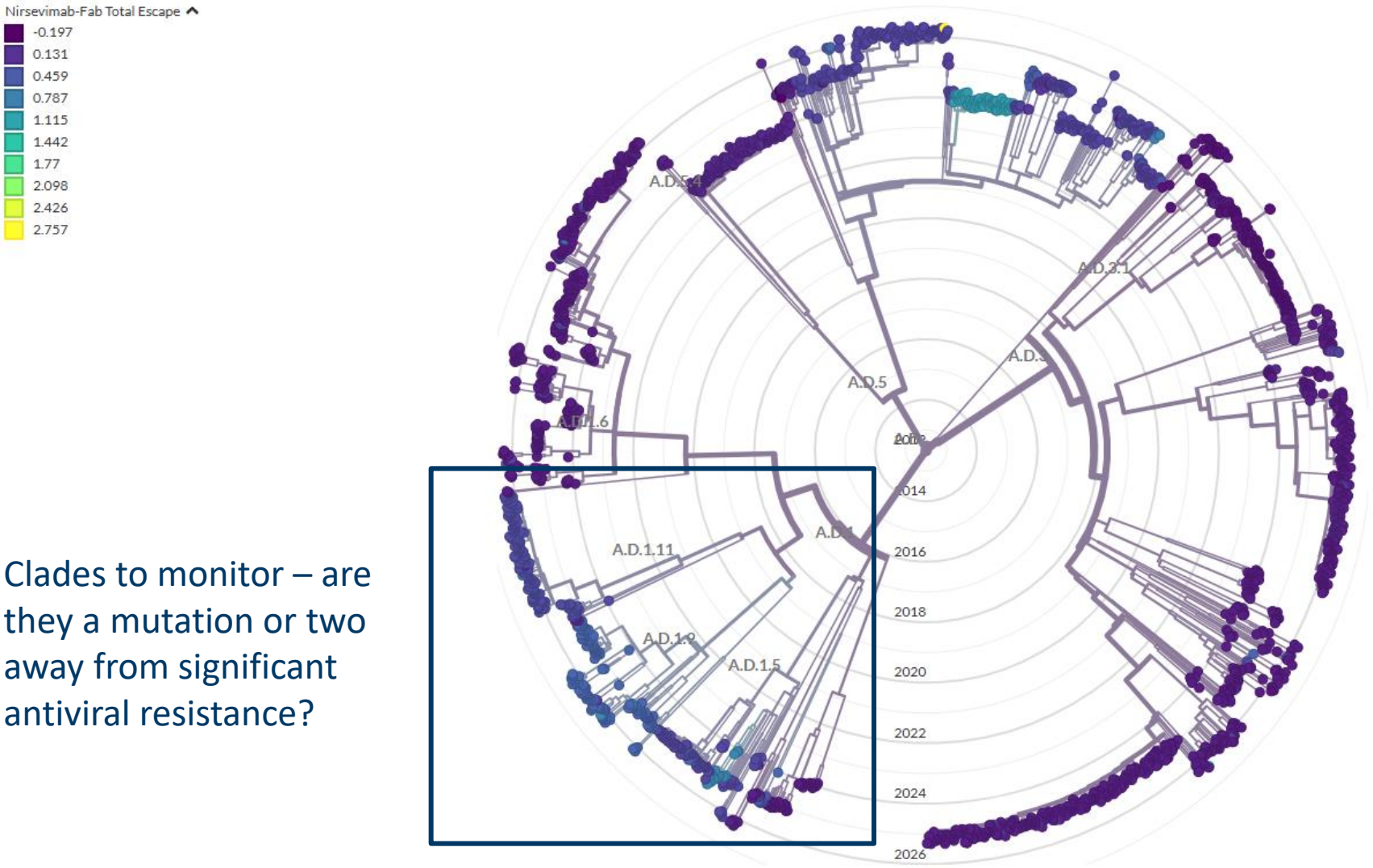


We can also track antiviral escape



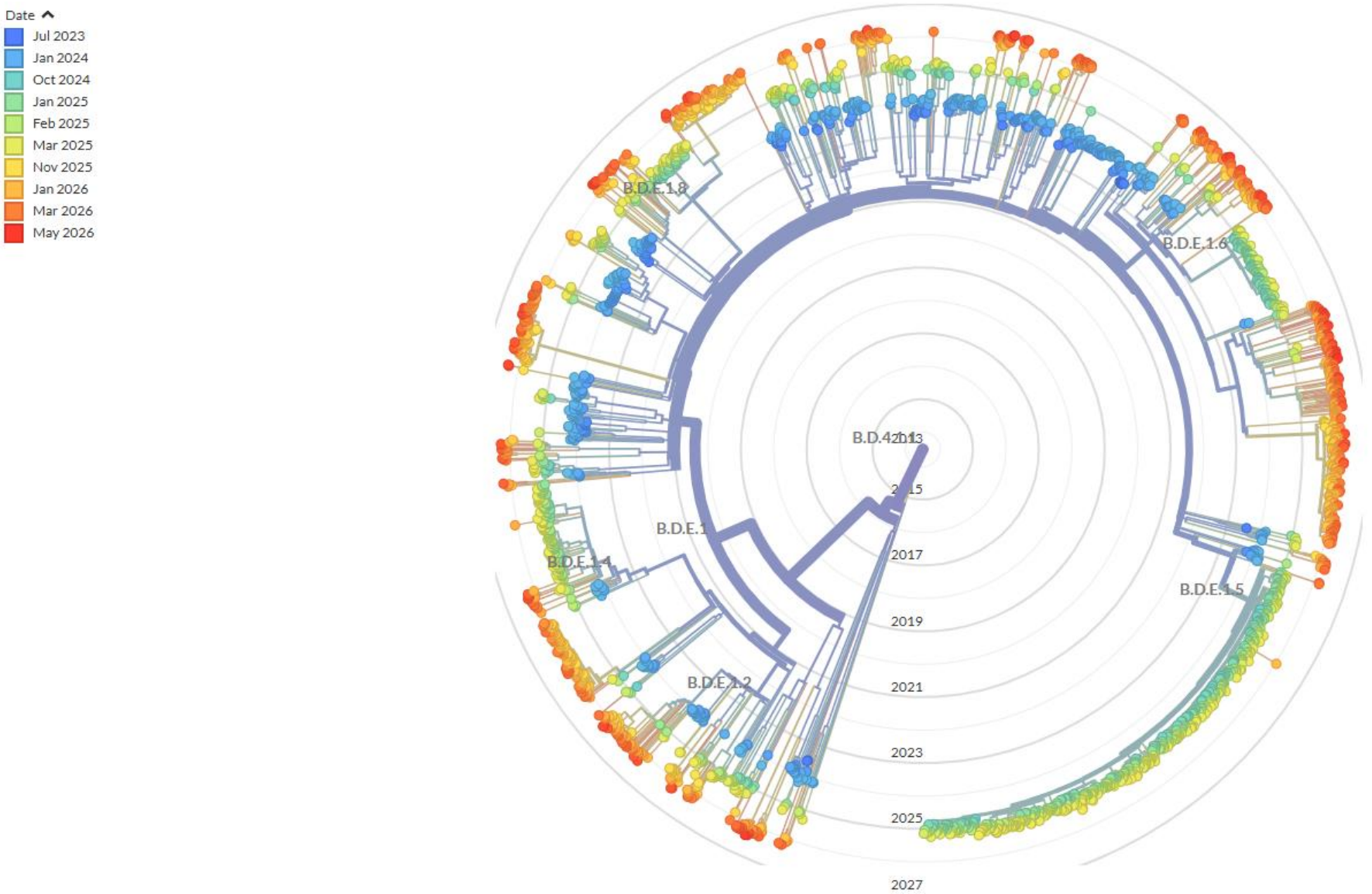
Does antiviral resistance come with fitness tradeoffs?

We can also track antiviral escape

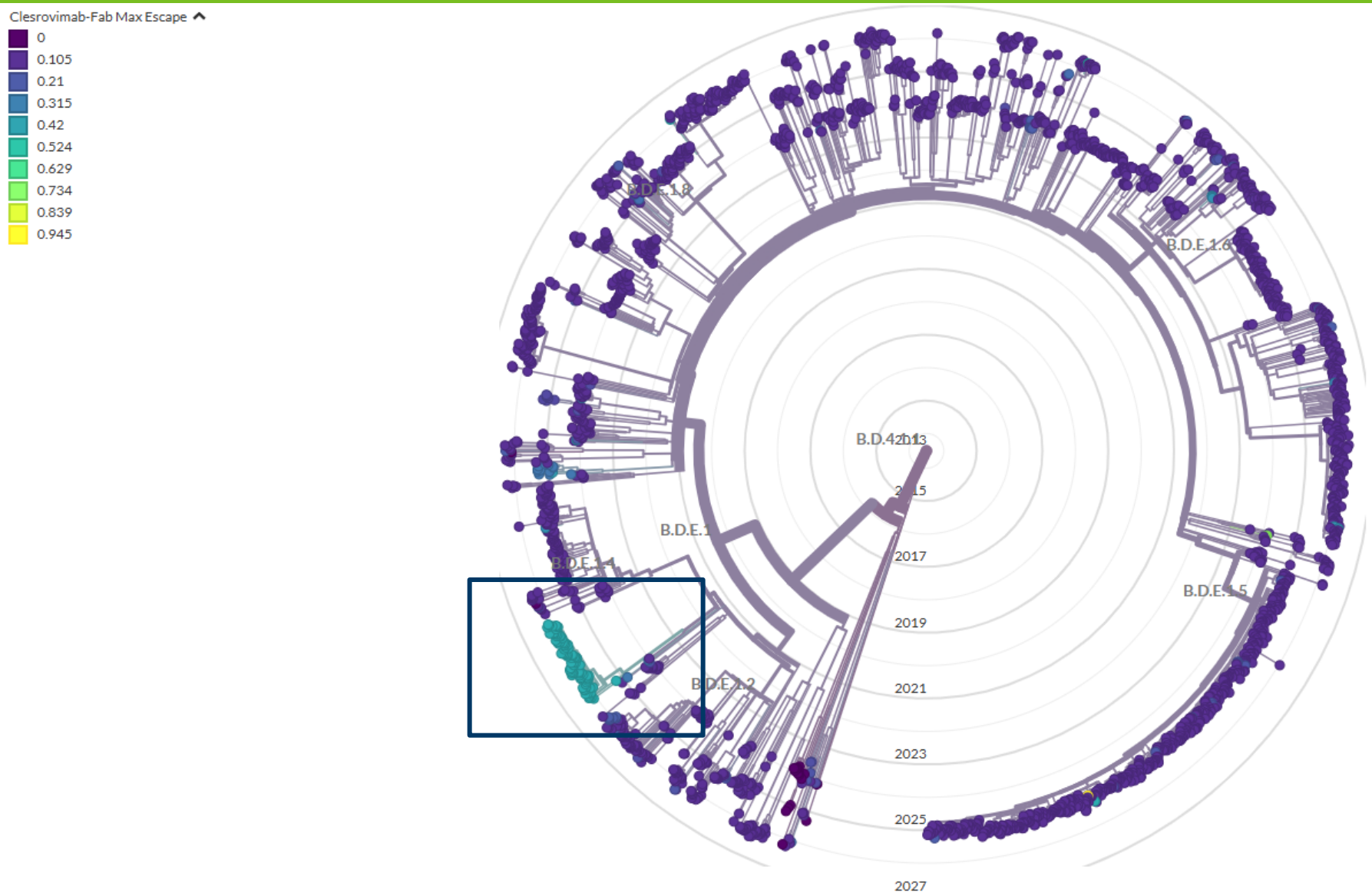


Clades to monitor – are they a mutation or two away from significant antiviral resistance?

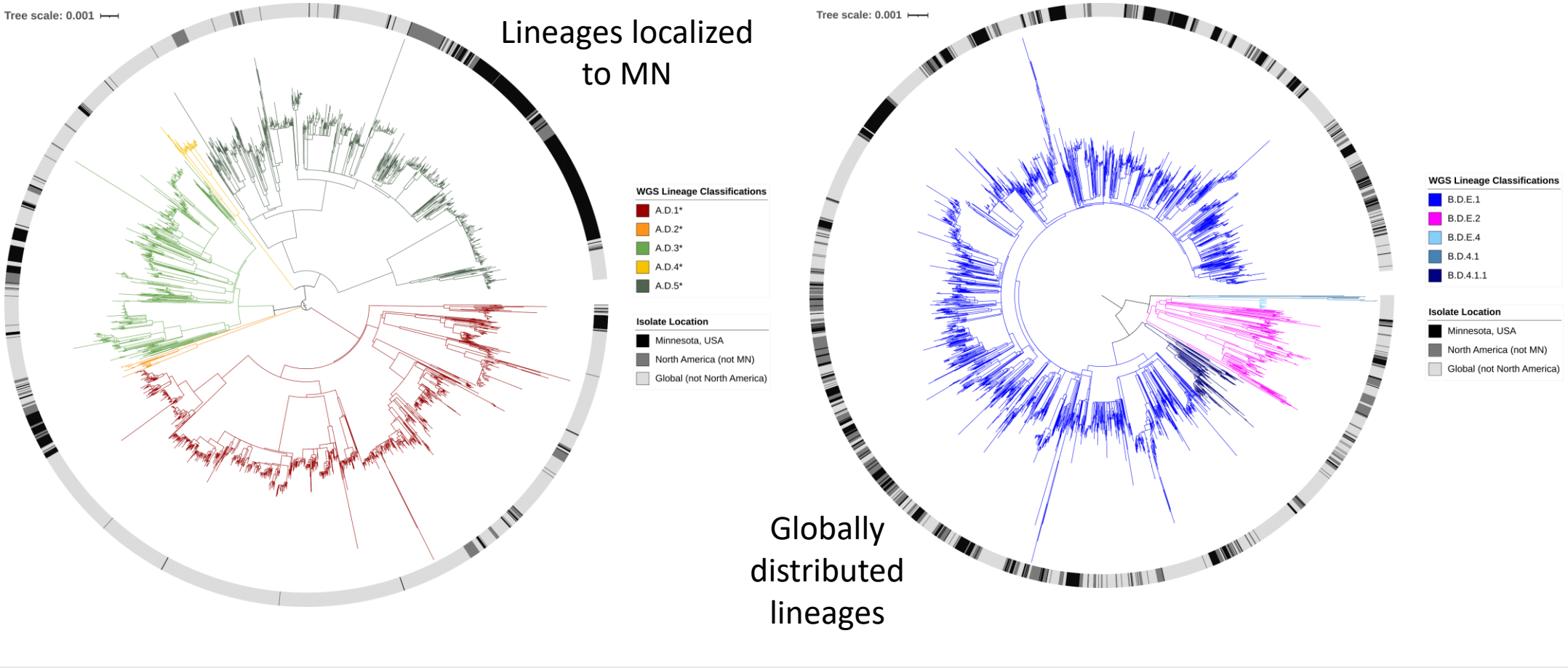
hRSVB statewide tree



hRSVB statewide tree – Clesrovimab resistance



We can compare MN genomes to global datasets

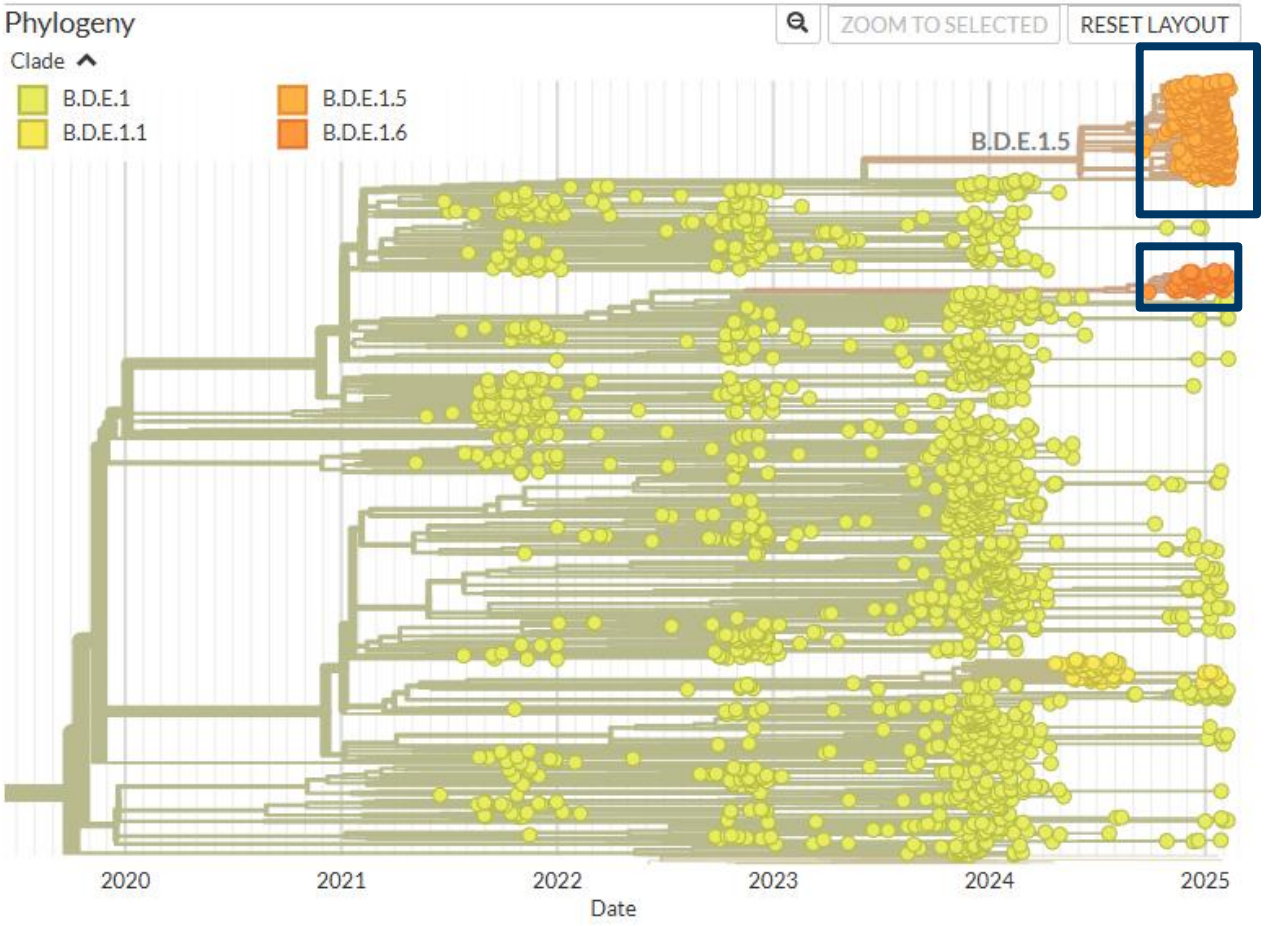


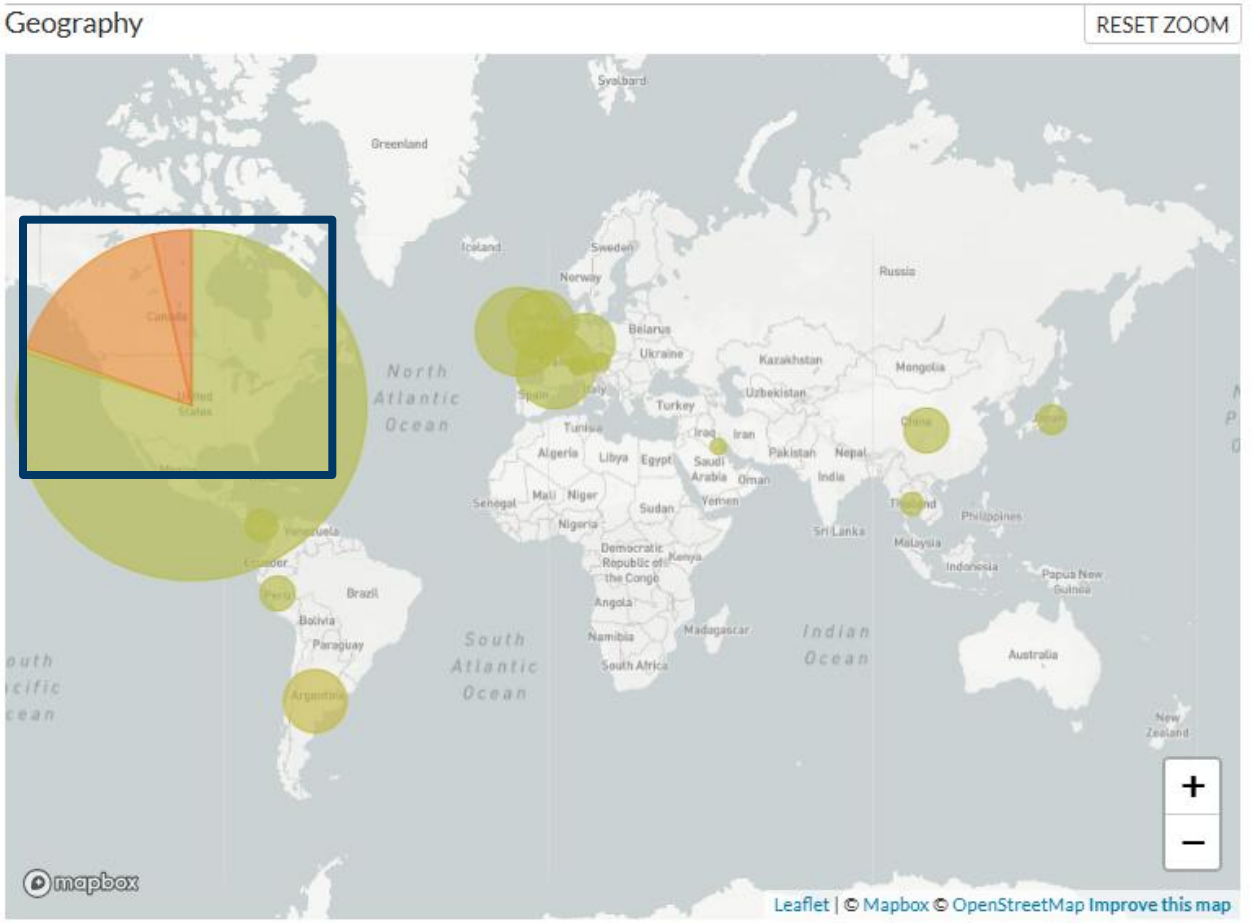
Our work shapes the global picture

RSV-B phylogeny

Built with nextstrain/rsv. Maintained by Nextstrain team and Richard Neher. Data updated 2025-03-11. Enabled by data from GenBank.

Showing 1803 of 3175 genomes sampled between May 2021 and Feb 2025.



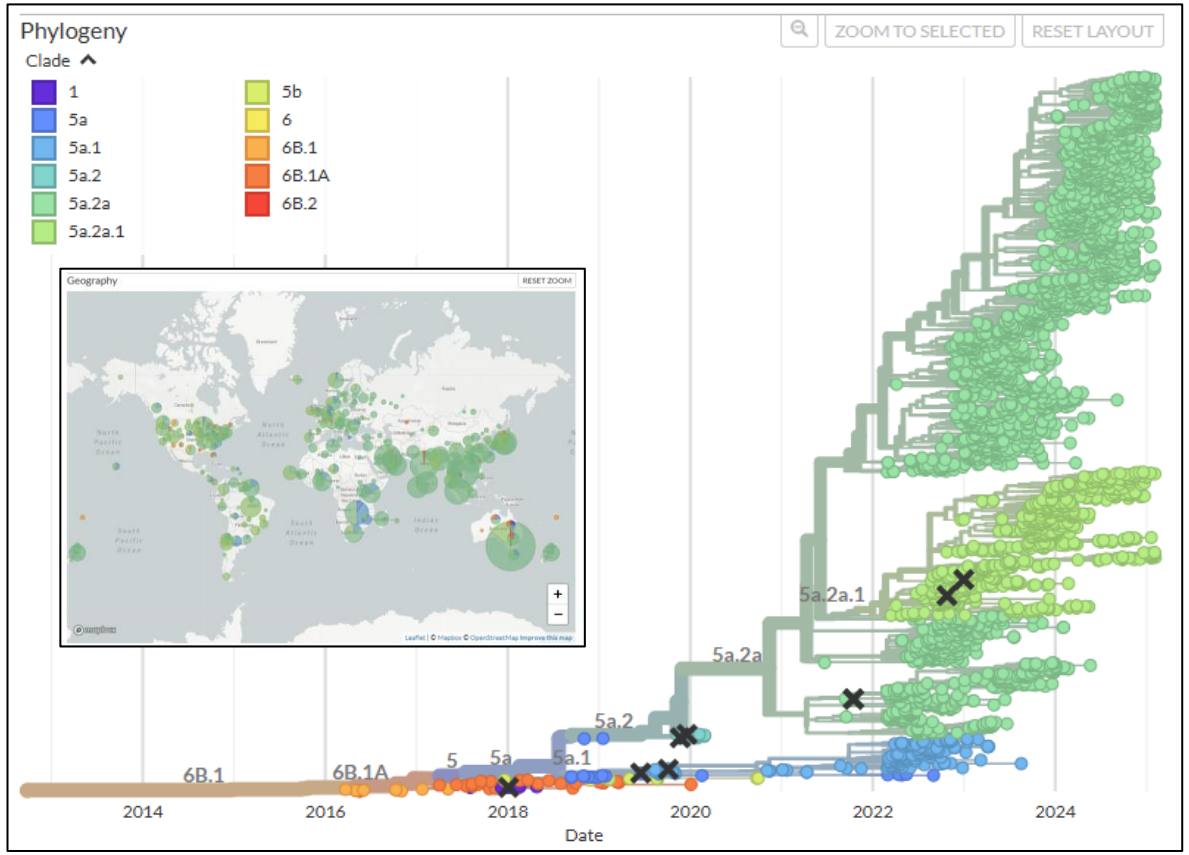


Lessons for Genomic Epidemiology

Use cases for respiratory viral genomic epidemiology will vary by surveillance scale.

National and global scale

- Larger datasets – thousands of genomes
- Trends in predominant genetic groups (clades, subclades, etc.) over time / across regions
- Tracking rare mutations or high-consequence lineages (H5N1)
- Vaccine design

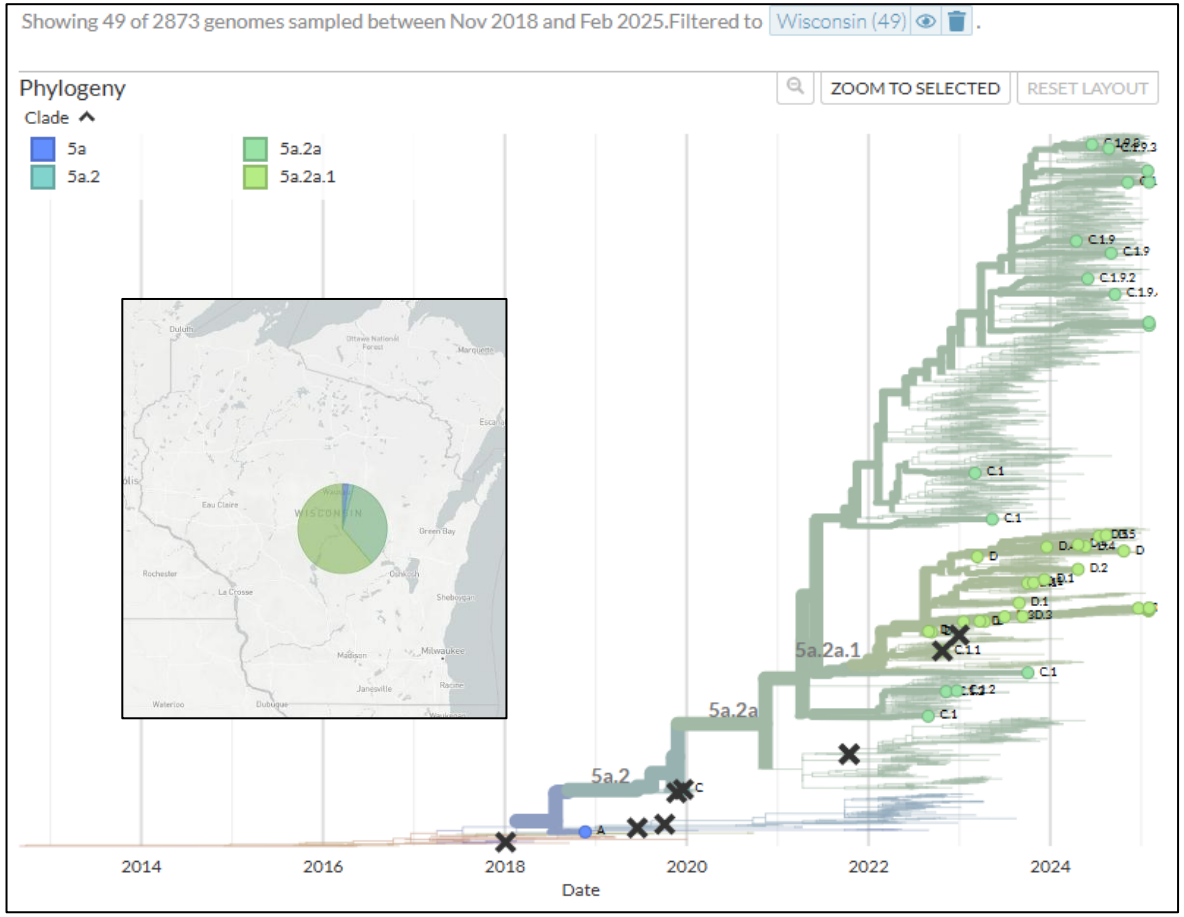


Public Nextstrain build of influenza A/H1N1pdm, enabled by data from GISAID

Use cases for respiratory viral genomic epidemiology will vary by surveillance scale.

Regional/jurisdictional scale

- Dozens to hundreds of genomes
- Cross-reference genomic and epidemiological data, case by case
- Cluster/outbreak investigation of endemic viral lineages
- Localized analyses are highly vulnerable to sampling biases



Public Nextstrain build of influenza A/H1N1pdm, enabled by data from GISAID

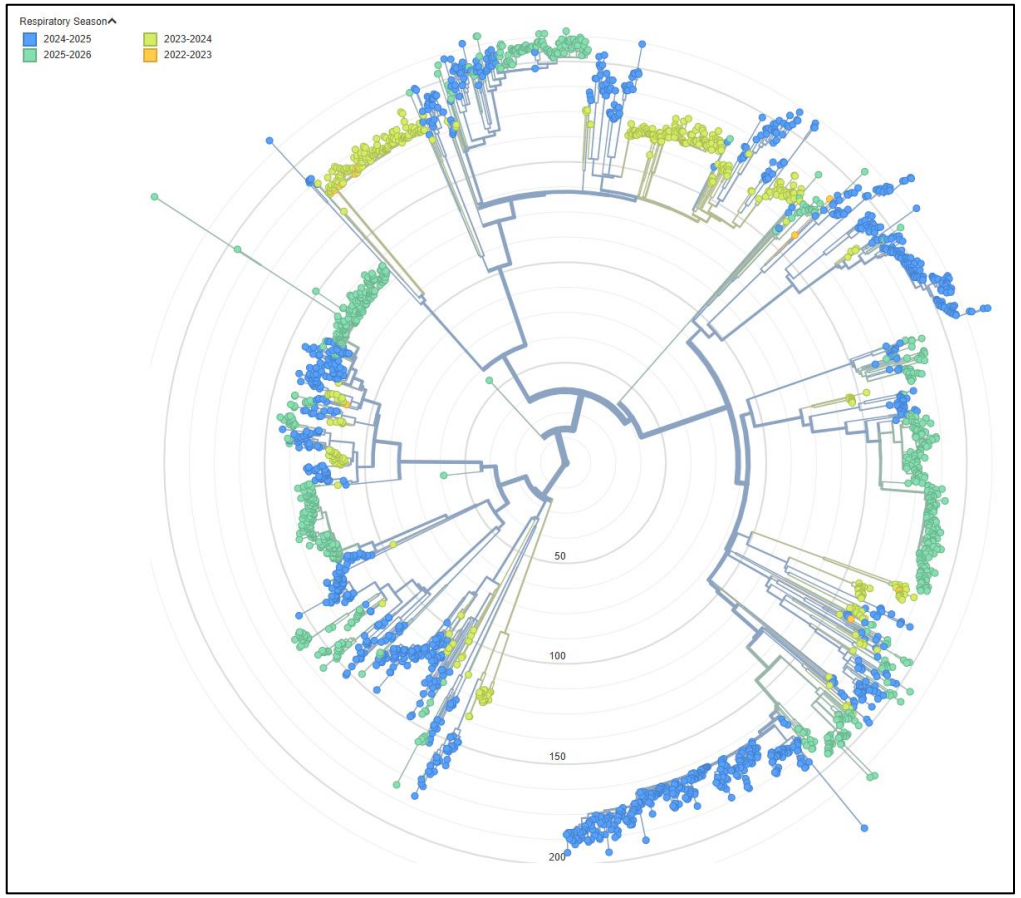
Consider the five "classes" of genomic epidemiology as you develop your strategic surveillance plans.

Descriptive	Detective	Investigative	Analytical	Predictive
				
What variants / genotypes / etc. are in this population? What genetic markers were involved in this known outbreak?	Has a new variant emerged in this population? Are there outbreaks that we cannot detect without genomics?	We are detecting a surge in infections. Is it an outbreak? When, where, how, and why is this outbreak happening?	Is variant X statistically significantly linked to disease outcome Y? How much did our genomic epi analyses help our interventions?	What variant(s) will most likely circulate in the future? How does our model change when we add genomic data?

Good question → appropriate strategy → robust surveillance design → useful data → **effective action**

Flaticon: Magnific

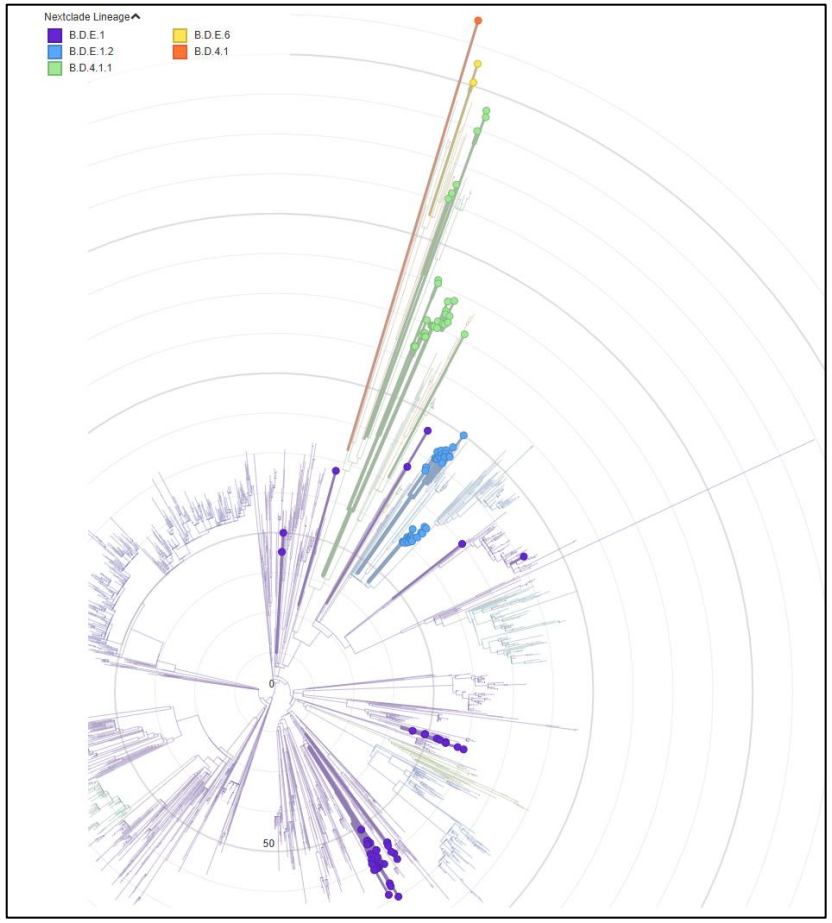
Examples of the “five classes” in action: Descriptive respiratory viral genomic epi



Phylogenetic tree of RSV-A genomes constructed using Nextstrain software

How diverse are respiratory viral lineages over time and geography?

Examples of the “five classes” in action: Detective respiratory viral genomic epi



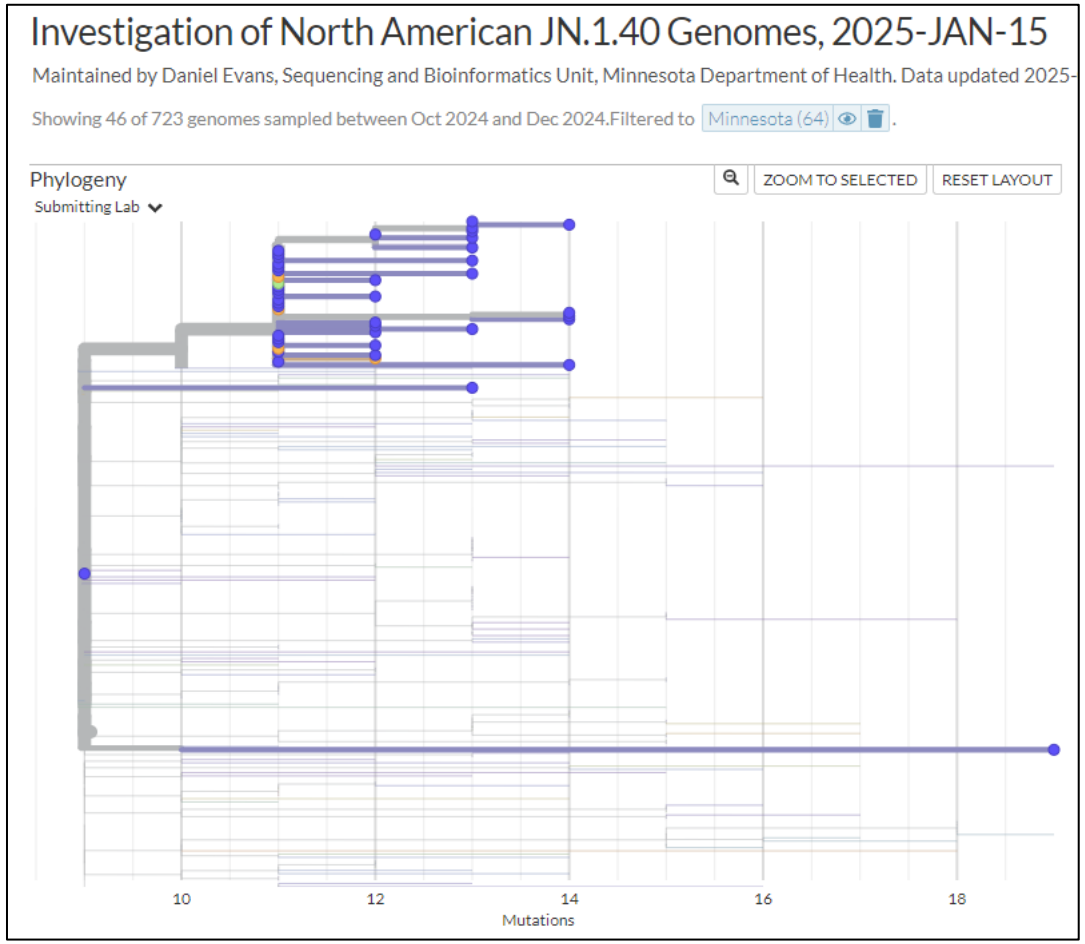
Phylogenetic tree of RSV-A genomes constructed using Nextstrain software

Where, and how frequently, are RSV genomes mutating in ways that could affect the function of monoclonal antibodies?

F Protein AA Position	All Seasons	2023-2024 Season	2024-2025 Season	2025-2026 Season
64	0.31%	0.00%	0.41%	0.38%
68	0.35%	1.50%	0.00%	0.13%
75	0.04%	0.21%	0.00%	0.00%
102	0.48%	0.21%	1.02%	0.00%
103	0.88%	0.64%	1.63%	0.13%
201	0.04%	0.00%	0.10%	0.00%
208	0.18%	0.21%	0.31%	0.00%
209	6.10%	5.58%	3.26%	10.16%
211	0.92%	3.00%	0.41%	0.13%
272	0.09%	0.21%	0.00%	0.13%
394	0.04%	0.00%	0.10%	0.00%
400	0.04%	0.21%	0.00%	0.00%
Summary	9.49%	11.80%	7.23%	11.04%

Examples of the “five classes” in action: Investigative respiratory viral genomic epi

Cases of (variant X) are surging. Is this only happening in our jurisdiction, or are others seeing a surge as well?



Phylogenetic tree of SARS-CoV-2 genomes constructed using Nextstrain software

Examples of the “five classes” in action: Analytical respiratory viral genomic epi

Viral lineages emerge and go extinct every respiratory surveillance season.

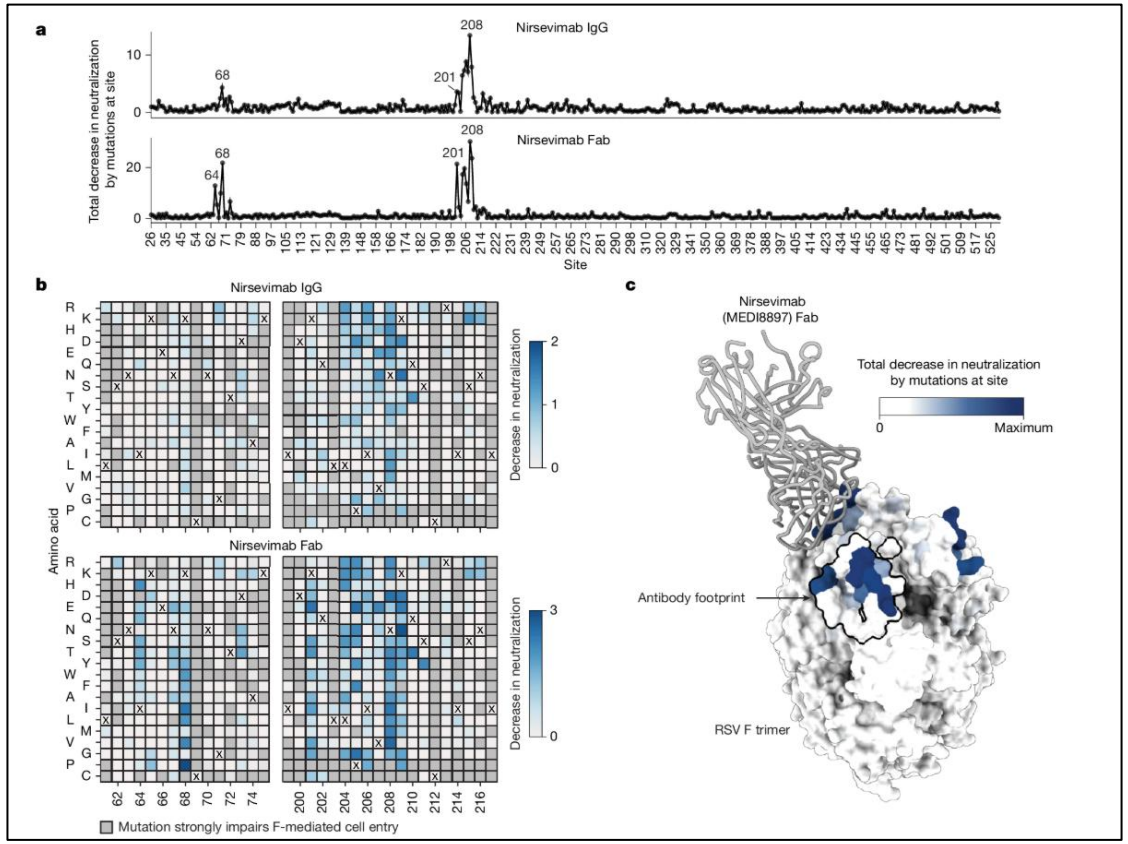
Which mutations in the genome are statistically associated with emergence/extinction?

RSV-A Site/Variant	af	filter-pvalue	lrt-pvalue	beta	beta-std-err	intercept
Site 1, Mutation X-Y	8.27E-02	8.45E-87	1.63E-112	5.96	0.63	-0.65
Site 2, Mutation X-Y	3.10E-02	7.19E-34	3.94E-49	6.54	0.7	-0.45
Site 3, Mutation X-Y	5.56E-02	2.48E-43	9.49E-44	3.18	0.3	-0.64
Site 4, Mutation X-Y	1.99E-02	3.69E-22	2.41E-27	6.08	0.68	-0.4
Site 5, Mutation X-Y	5.39E-02	1.14E-16	4.20E-26	-5.2	0.7	-0.28
Site 6, Mutation X-Y	3.39E-02	3.61E-10	1.95E-22	-4.6	1.19	-0.44
Site 7, Mutation X-Y	2.09E-01	7.94E-09	7.13E-18	-1.3	0.18	-0.24
Site 8, Mutation X-Y	1.49E-01	1.02E-13	4.75E-15	-1.2	0.16	-0.17
Site 9, Mutation X-Y	2.04E-02	1.22E-17	1.46E-14	3.26	0.61	-0.5
Site 10, Mutation X-Y	1.06E-02	2.13E-12	2.63E-14	4.85	0.69	-0.43
Site 11, Mutation X-Y	1.44E-01	1.49E-12	9.13E-14	-1.1	0.16	-0.18
Site 12, Mutation X-Y	1.81E-01	1.78E-06	1.71E-13	-1.2	0.19	-0.28
Site 13, Mutation X-Y	1.91E-01	2.31E-06	4.20E-13	-1.2	0.18	-0.27
Site 14, Mutation X-Y	1.27E-02	8.02E-05	2.84E-08	-4.2	0.68	-0.41
Site 15, Mutation X-Y	2.93E-02	9.26E-01	9.59E-01	0.01	0.27	-0.39

Redacted preliminary output of PySEER GWAS analysis of RSV-A genomes

Examples of the “five classes” in action: Predictive respiratory viral genomic epi

How will mutations in the RSV genome affect the utility of monoclonal antibodies?



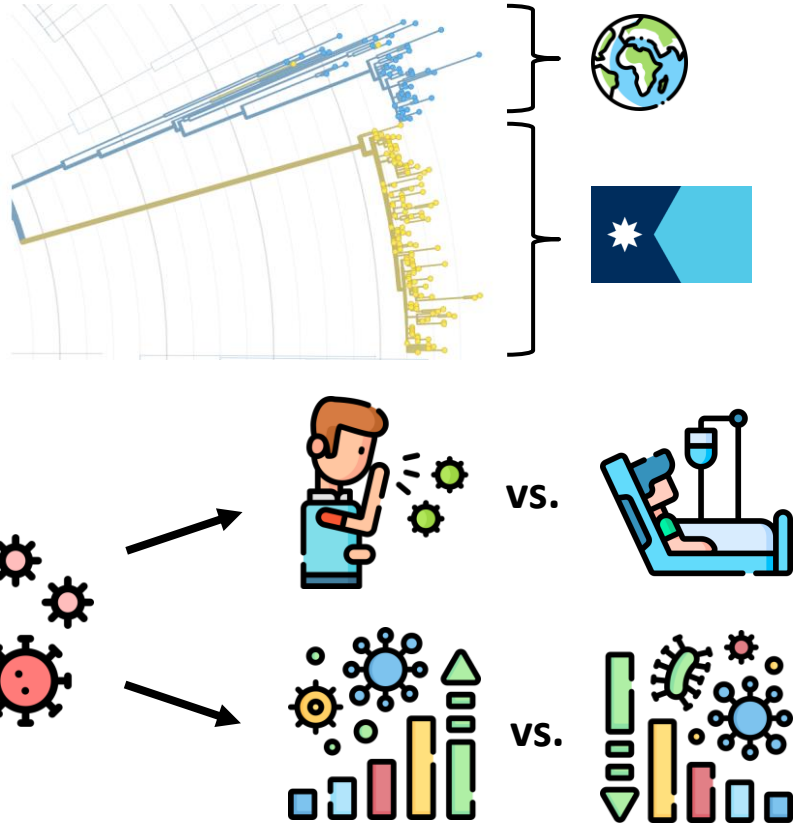


Simonich et al, *Nature* 2026; phylogenetic tree from public Nextstrain build of RSV-B

What does this mean for public health?

Our work in Minnesota has shifted the global landscape of the study of RSV.

- Newly discovered clades of RSV found only through our sequencing
- Pairing with RSV-NET data classifies each infection as severe vs. mild/moderate
- Additional data for genetic studies of RSV mutations during / between infections
- Influencing strategies for studies of other viral pathogens

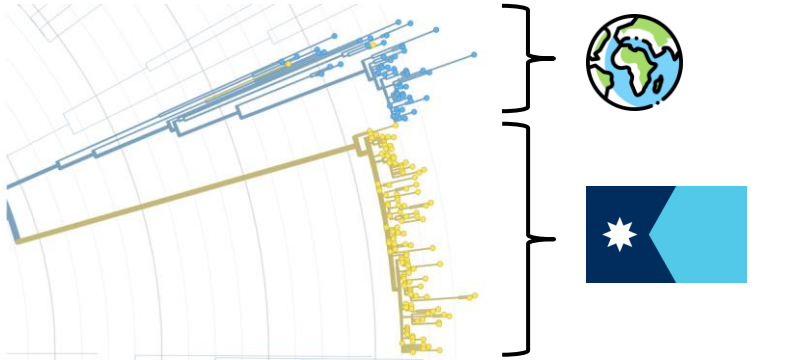


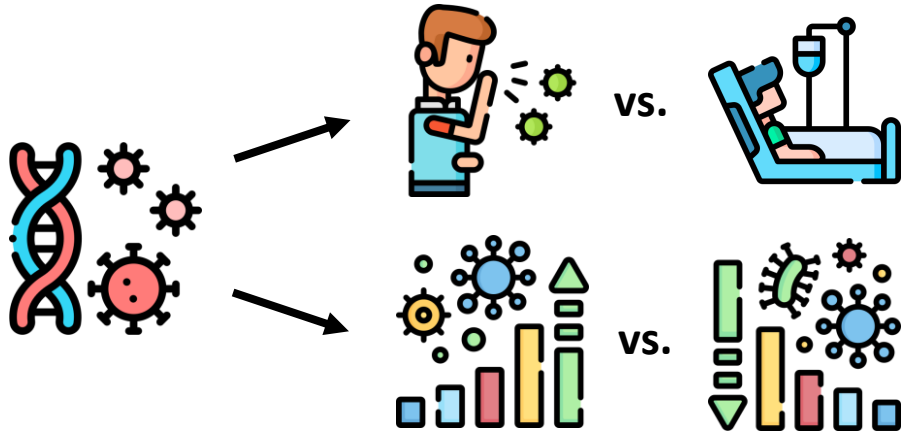
Flaticon: Freepik

What does this mean for public health?

Our work in Minnesota has shifted the global landscape of the study of RSV.

- Our data adds to the global picture
- Phylogenetics with specific goals in minds yields better results
- Mutational level analysis and estimates of natural selection are ongoing – stay tuned!



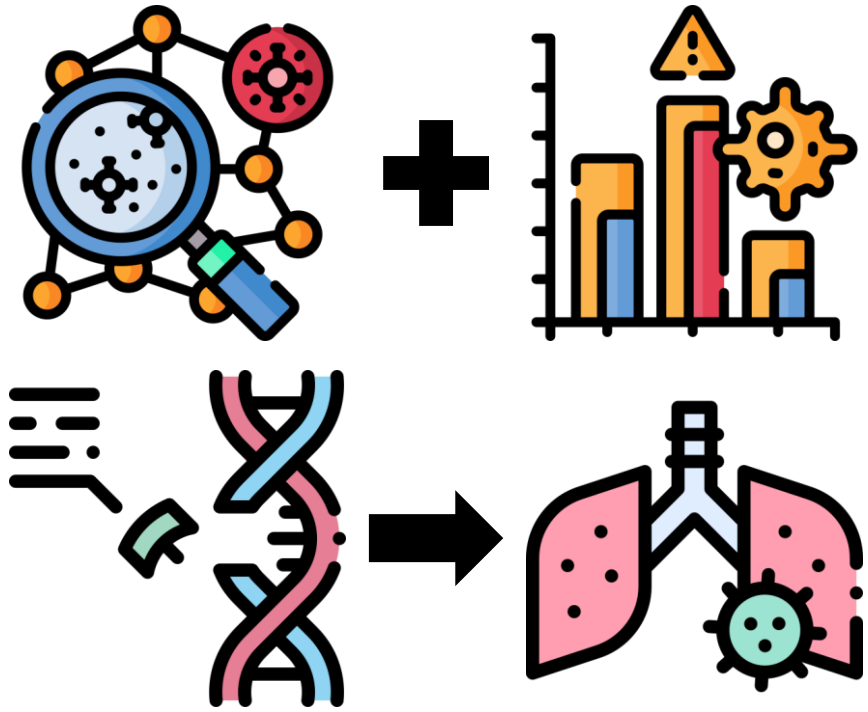


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Our potential future directions, with more data

Thinking strategically about how to use WGS data…

- Can we **prospectively identify and intervene against RSV outbreaks** by collecting WGS data?
- Can we leverage RSV-NET data to find viral lineages or mutations **linked to severe infection**?
- Can we track the emergence of mutations linked to **antibody resistance and/or vaccine escape**?
- *Can we **motivate other states** to expand their viral genomic surveillance programs beyond COVID?*



Flaticon: Freepik

Thank you!

Questions?

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