

Egg-passaging mutations in influenza H3N2

Lab Meeting
Katie Kistler
February 20, 2018

Background: influenza vaccine
production

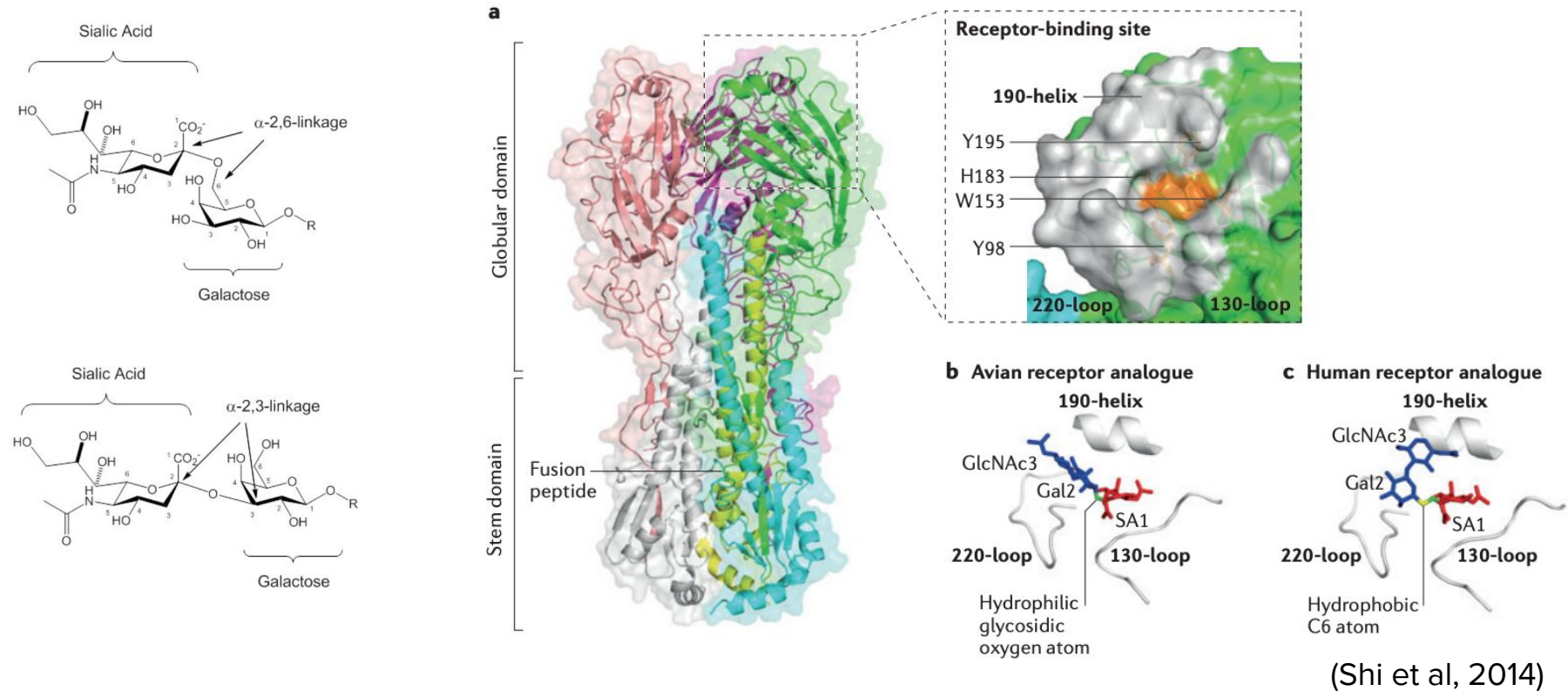
Influenza vaccine

- Seasonal flu burden: millions of cases, hundreds of thousands of deaths
- Prevention via vaccine
- Vaccine continually updated to combat antigenic drift

Influenza vaccine production

- Hundreds of millions of doses yearly
- Mass-produced in embryonated chicken eggs
 - a. Make candidate vaccine virus (CVV)
 - b. Manufacturing facilities grow CVVs
 - c. Purification and inactivation of virus

Egg-adapted mutations during vaccine production



Low vaccine effectiveness may be attributed to egg-adapted mutations

2012-2013:

- 30-40% H3N2 vaccine effectiveness
- HA H156Q, G186V, S219Y

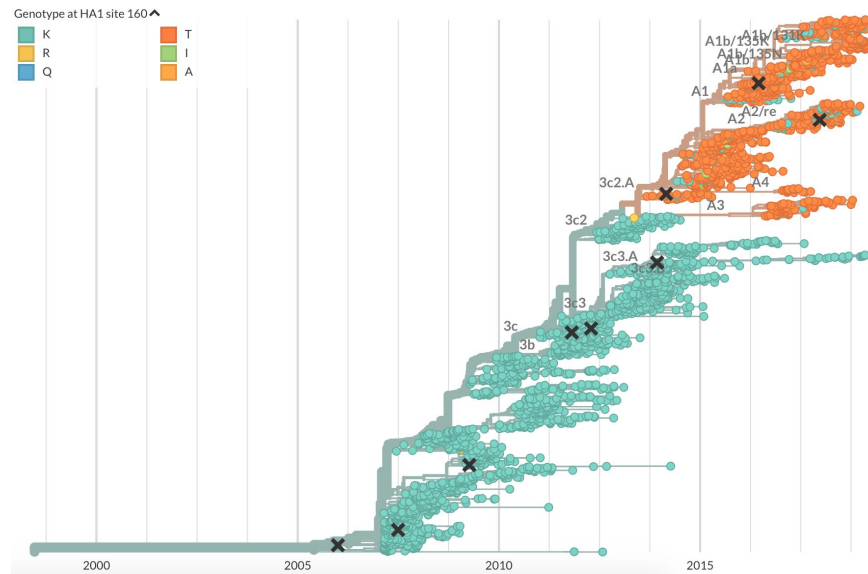
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2016-2017:

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- HA T160K reversion



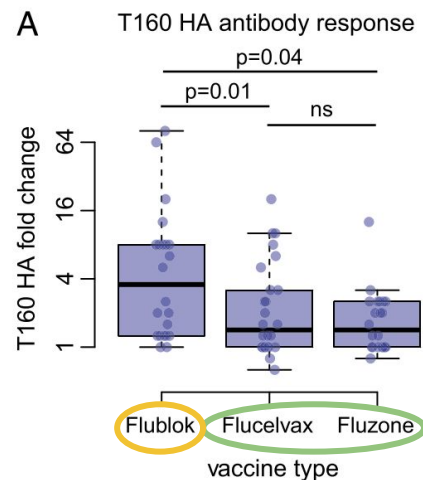
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recombinant T160 egg-adapted K160

(Zost et al, 2017)

How to avoid egg-adapted mutations?

1. Egg-free vaccine production
2. Choose vaccine strains that will not mutate

Predict egg-adapted mutations
and their effect on vaccine effectiveness

Published reports of egg mutations & their effects

Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains

Seth J. Zost^a, Kaela Parkhouse^a, Megan E. Gumina^a, Kangchon Kim^b, Sebastian Diaz Perez^a, Patrick C. Wilson^c, John J. Treanor^d, Andrea J. Sant^e, Sarah Cobey^b, and Scott E. Hensley^{a,1}

of studies to determine whether the difference in glycosylation of HA antigenic site B of H3N2 vaccine strains and circulating strains contributed to a previously unrecognized vaccine mismatch during the 2016–2017 influenza season.

Single amino acid substitutions in the hemagglutinin of influenza A/Singapore/21/04 (H3N2) increase virus growth in embryonated chicken eggs

Bin Lu*, Helen Zhou, Winnie Chan, George Kemble, Hong Jin

Effects of egg-adaptation on receptor-binding and antigenic properties of recent influenza A (H3N2) vaccine viruses

Lauren Parker,^{1,2} Stephen A. Wharton,^{1,2} Stephen R. Martin,^{1,3} Karen Cross,^{1,2} Yipu Lin,^{1,2} Yan Liu,⁴ Ten Feizi,⁴ Rodney S. Daniels^{1,2} and John W. McCauley^{1,2}

acterise the correlation between receptor-binding and the antigenic properties of these viruses. Using the vaccine virus Vic361e, recommended for use in vaccines in 2012 and 2013, as a prototype, a panel of viruses was generated by

Low 2012–13 Influenza Vaccine Effectiveness Associated with Mutation in the Egg-Adapted H3N2 Vaccine Strain Not Antigenic Drift in Circulating Viruses

Danuta M. Skowronski^{1,2*}, Naveed Z. Janjua^{2,3}, Gaston De Serres^{4,5}, Suzana Sabaiduc¹, Alireza Eshaghi⁶, James A. Dickinson⁷, Kevin Fonseca^{8,9}, Anne-Luise Winter¹⁰, Jonathan B. Gubbay^{11,12,13}, Mel Krajden^{1,3}, Martin Petric^{1,3}, Hugues Charest^{14,15}, Nathalie Bastien¹⁶, Trijntje L. Kwindt², Salaheddin M. Mahmud¹⁷, Paul Van Caeseele^{18,19}, Yan Li^{16,19}

Poor Immunogenicity, Not Vaccine Strain Egg Adaptation, May Explain the Low H3N2 Influenza Vaccine Effectiveness in 2012–2013

Sarah Cobey,¹ Sigrid Gouma,² Kaela Parkhouse,² Benjamin S. Chambers,^{2,a} Hildegund C. Ertl,² Kenneth E. Schmader,³ Rebecca A. Halpin,⁴ Xudong Lin,⁴ Timothy B. Stockwell,⁴ Suman R. Das,^{4,b} Emily Landon,⁵ Vera Tesic,⁶ Ilan Youngster,^{7,8} Benjamin A. Pinsky,^{9,10} David E. Wentworth,^{4,c} Scott E. Hensley,² and Yonatan H. Grad^{11,12}

Immunodominance of Antigenic Site B over Site A of Hemagglutinin of Recent H3N2 Influenza Viruses

Lyubov Popova^{1,2}, Kenneth Smith³, Ann H. West², Patrick C. Wilson^{3,4,5}, Judith A. James³, Linda F. Thompson⁴, Gillian M. Air^{1*}

recent H3 HAs. We mapped the binding of two human monoclonal antibodies to wild type A/Oklahoma/309 HA and mutant HAs derived from it, and we tested the reactivity of polyclonal antibodies in human plasma samples after seasonal vaccination in 2006 (H3N2 2006–07 component A/Wisconsin/67/05) and/or after vaccination in 2008 (H3N2 2008–09 component A/Uruguay/716/2007), to wild type HA and mutants in antigenic sites A and B. Our results indicate that most

Predict egg-adapted mutations and their effect on vaccine effectiveness

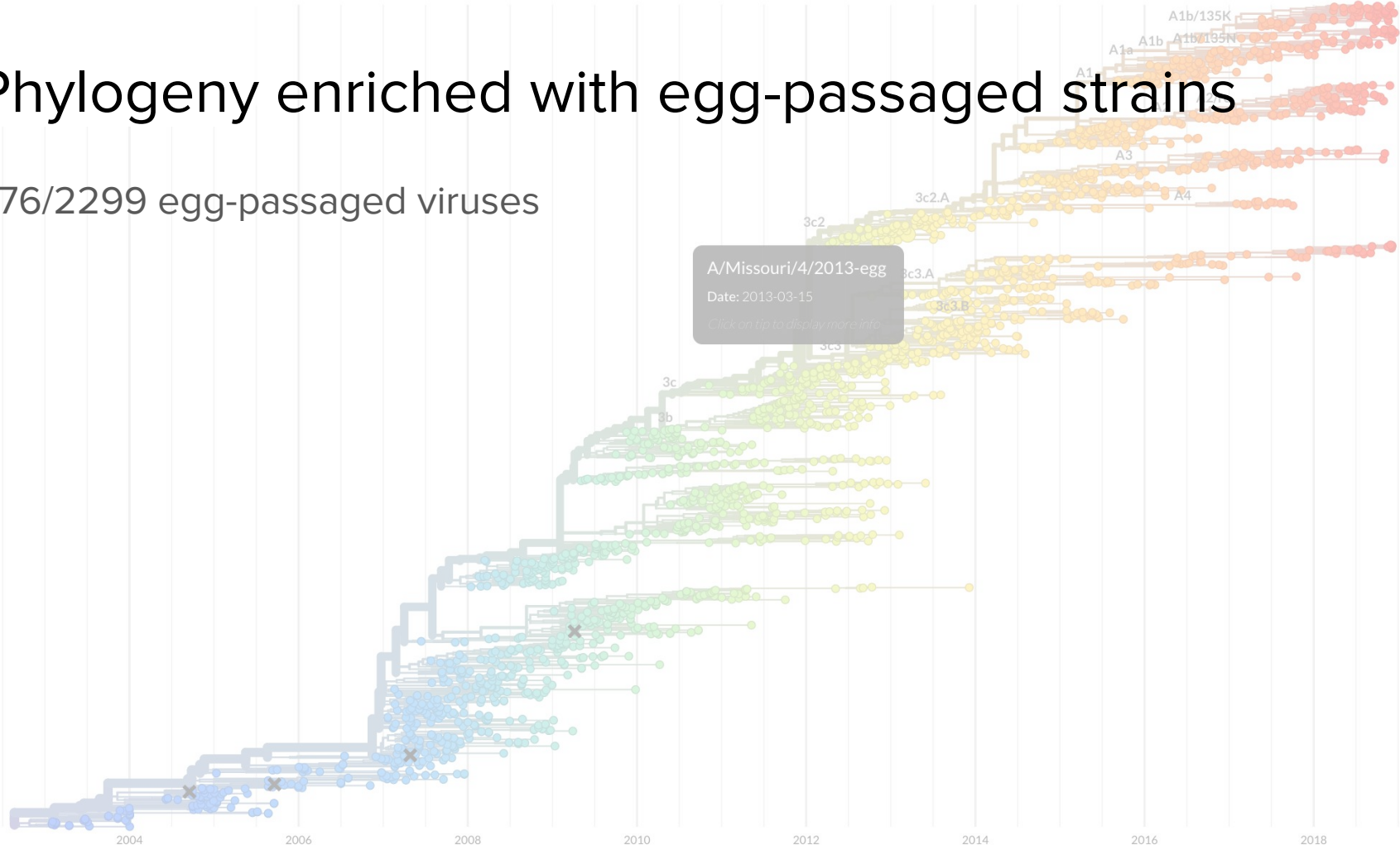
Existing literature- individual studies on specific egg-adapted mutations in specific strains

My goal- use a phylogeny to identify:

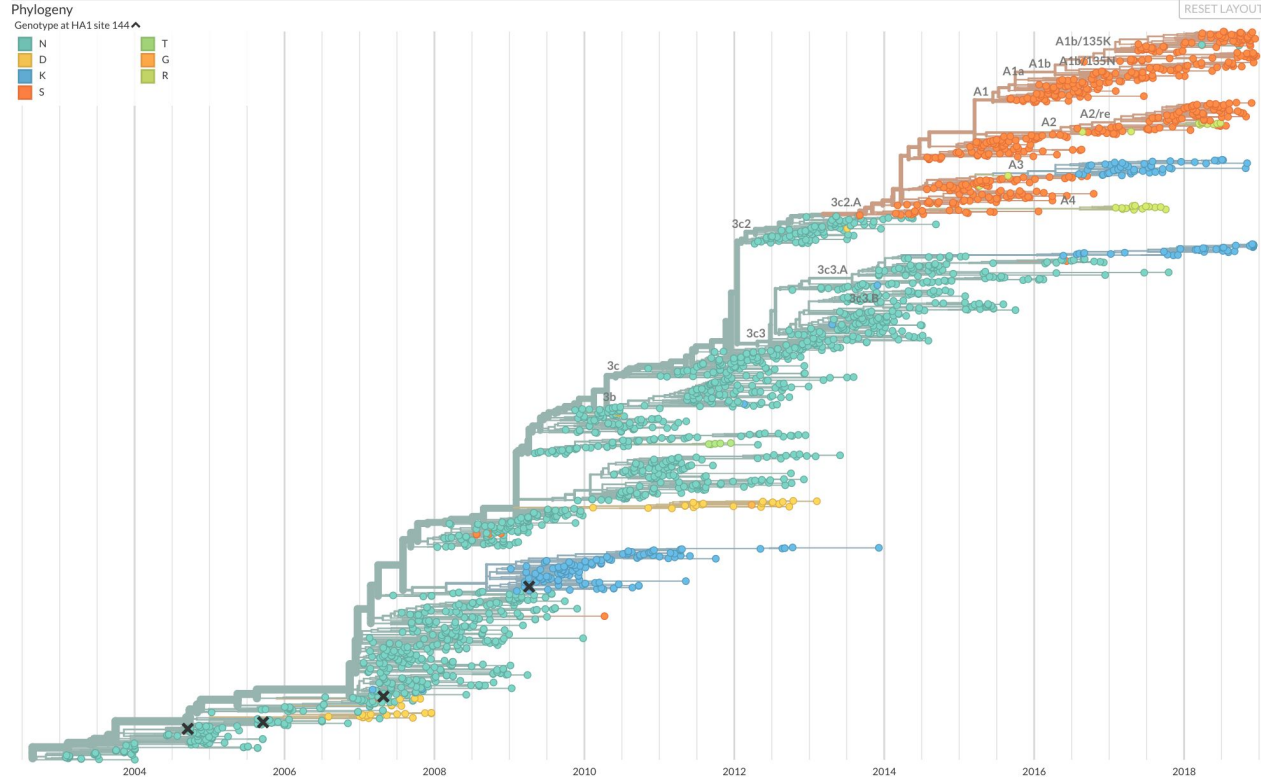
- Common egg-adapted mutations
- Background specificity
- Epistasis between egg-adapted mutations
- Antigenic effect of egg-adapted mutations (titers model)

Phylogeny enriched with egg-passaged strains

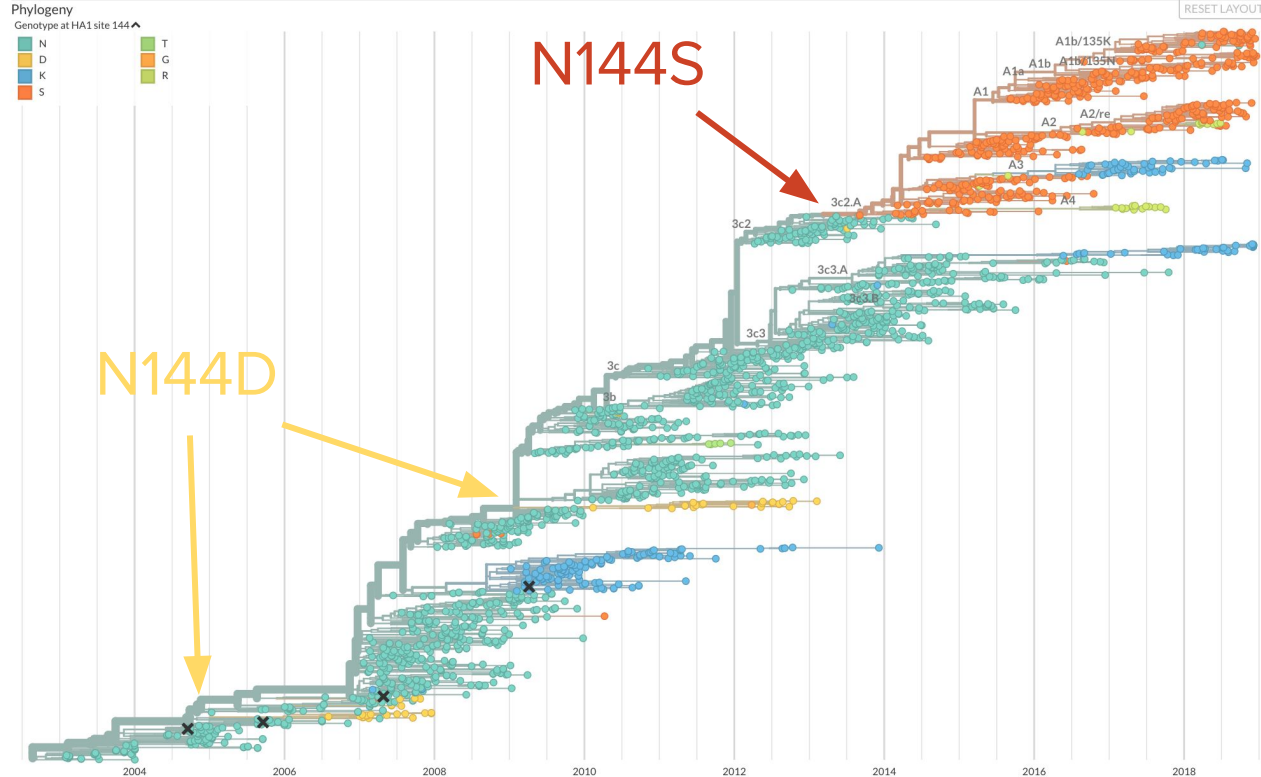
276/2299 egg-passaged viruses



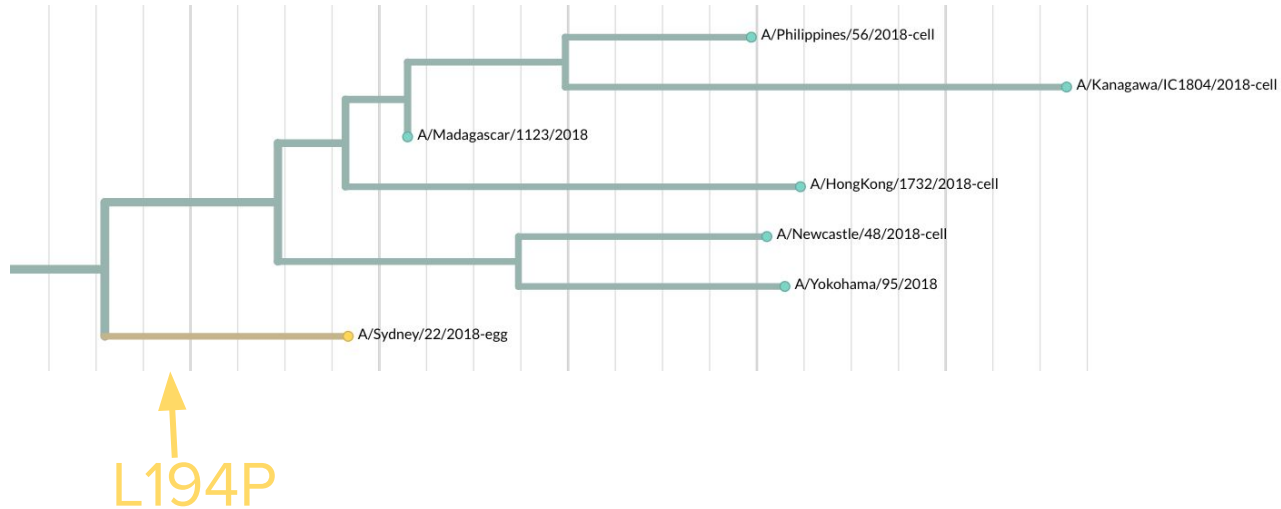
Identify egg-passaging mutations



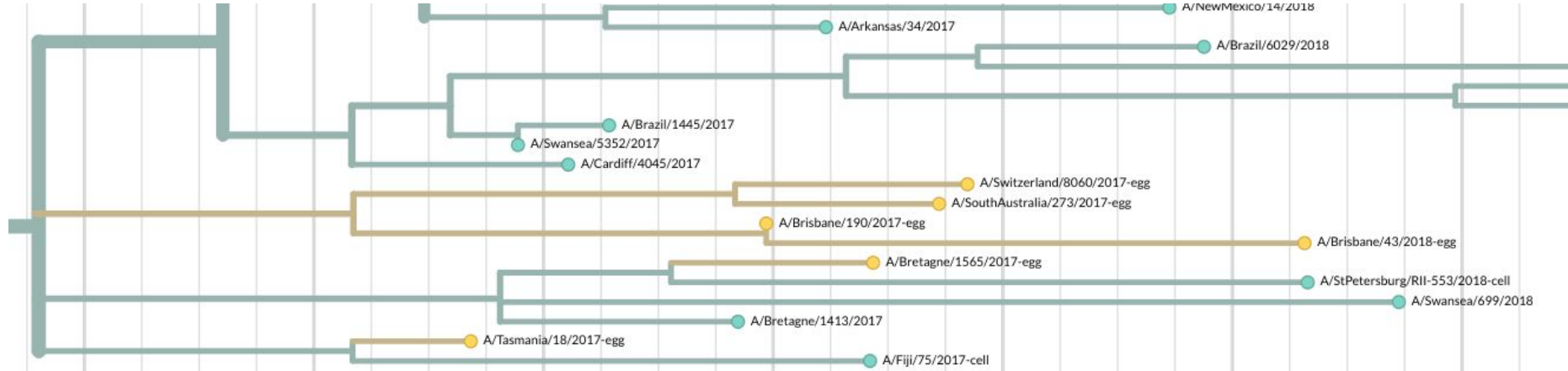
Identify egg-passaging mutations



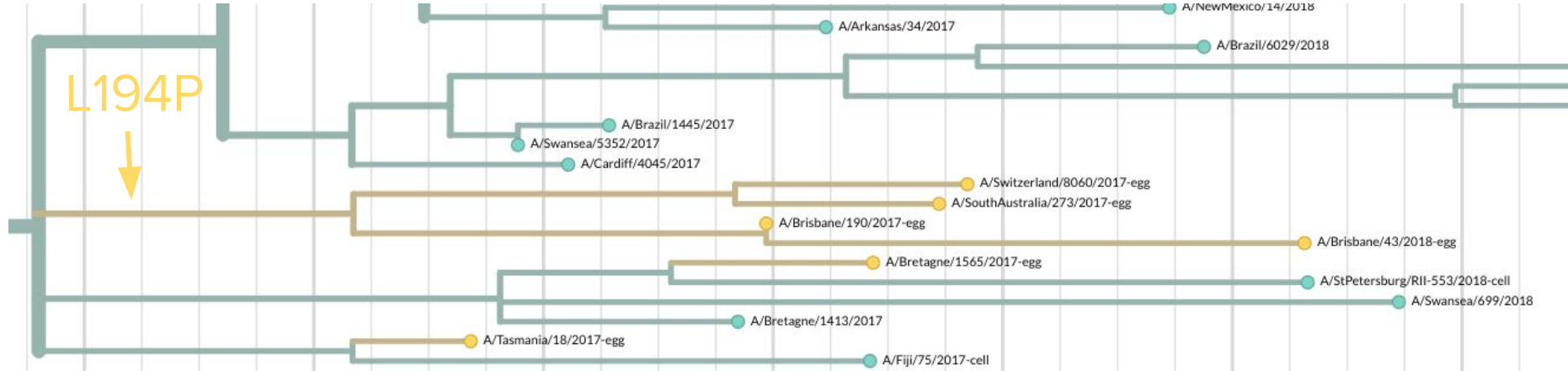
Identify egg-passaging mutations



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Identify egg-passaging mutations

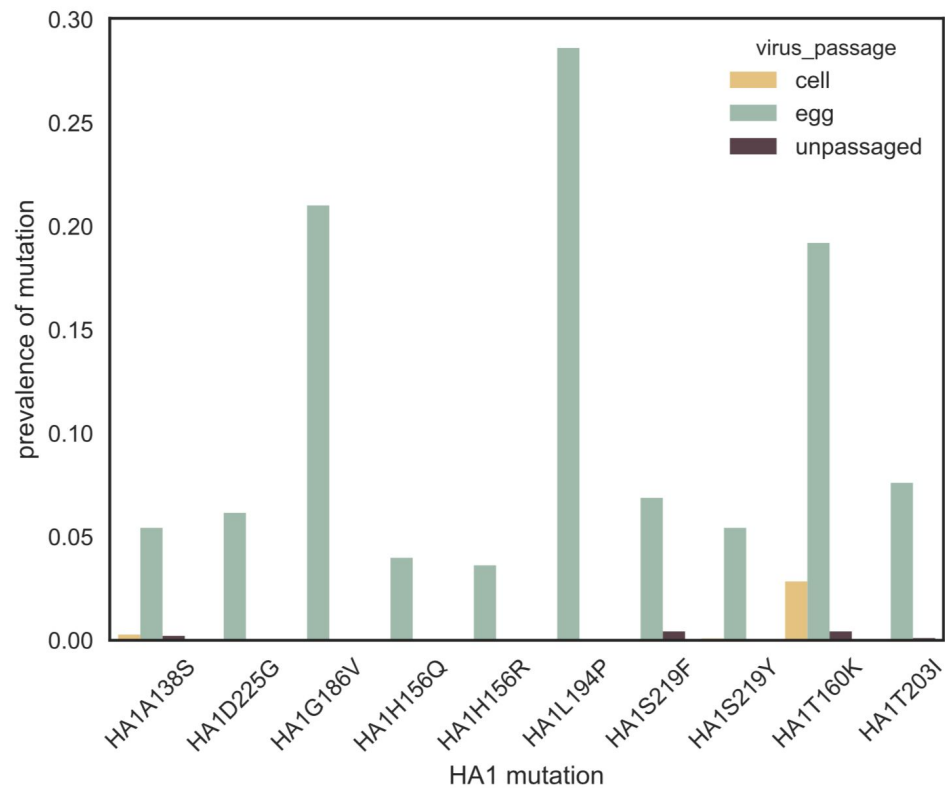


Common egg-passaging HA mutations

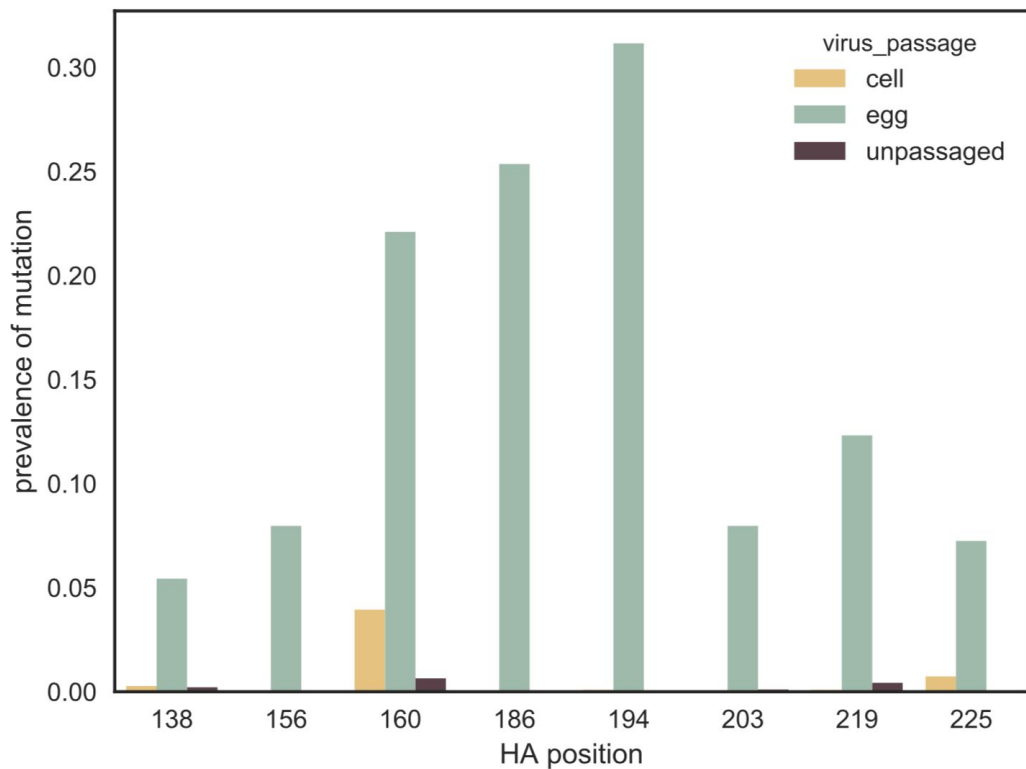
- HA1 L194P
- HA1 G186V
- HA1 T160K
- HA1 T203I
- HA1 S219F
- HA1 D225G
- HA1 S219Y
- HA1 A138S
- HA1 H156Q
- HA1 H156R



Mutations are egg-passaging-specific



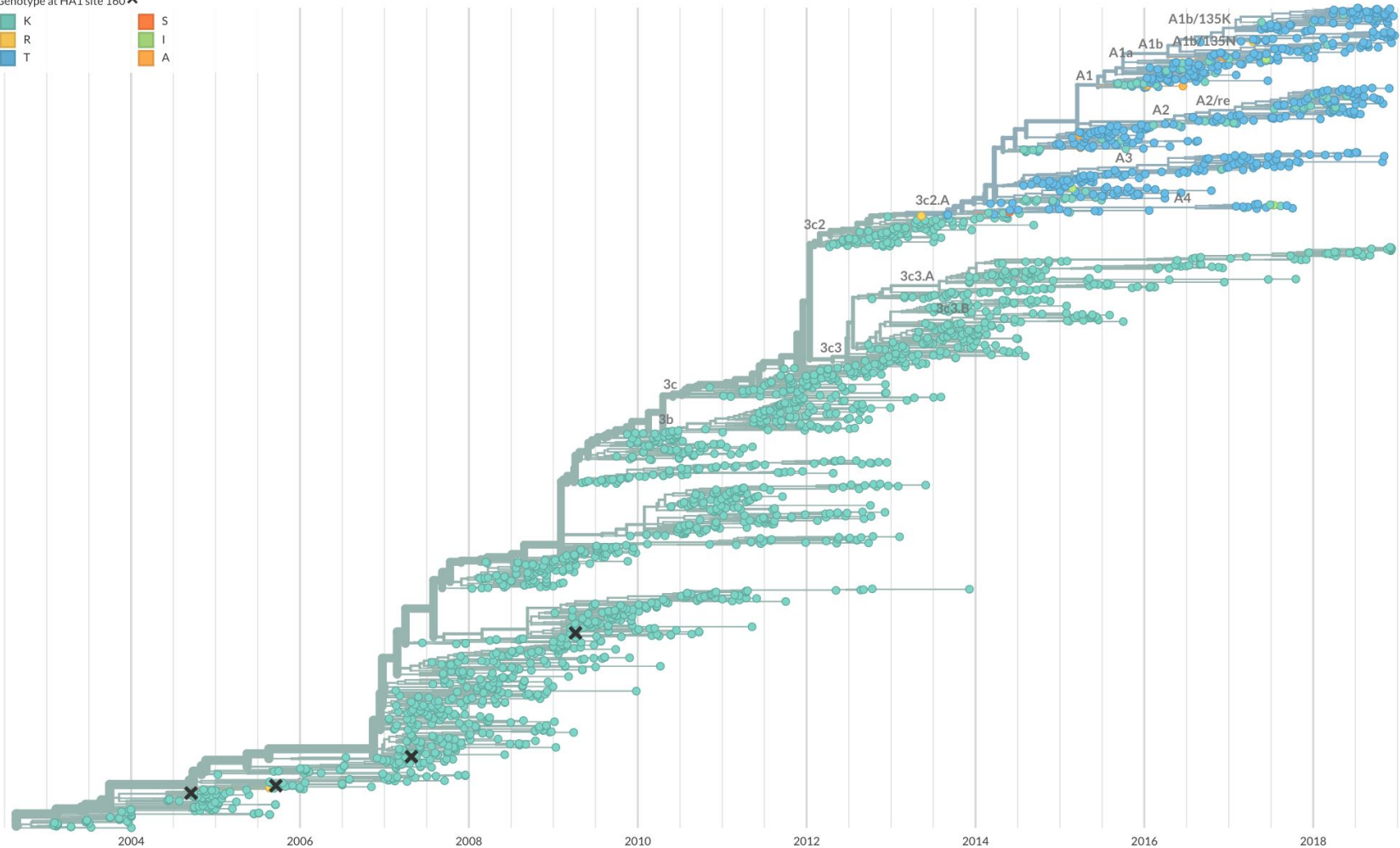
Mutations are egg-passaging-specific



Background specificity of mutations

T160K

Phylogeny
Genotype at HA1 site 160 ^

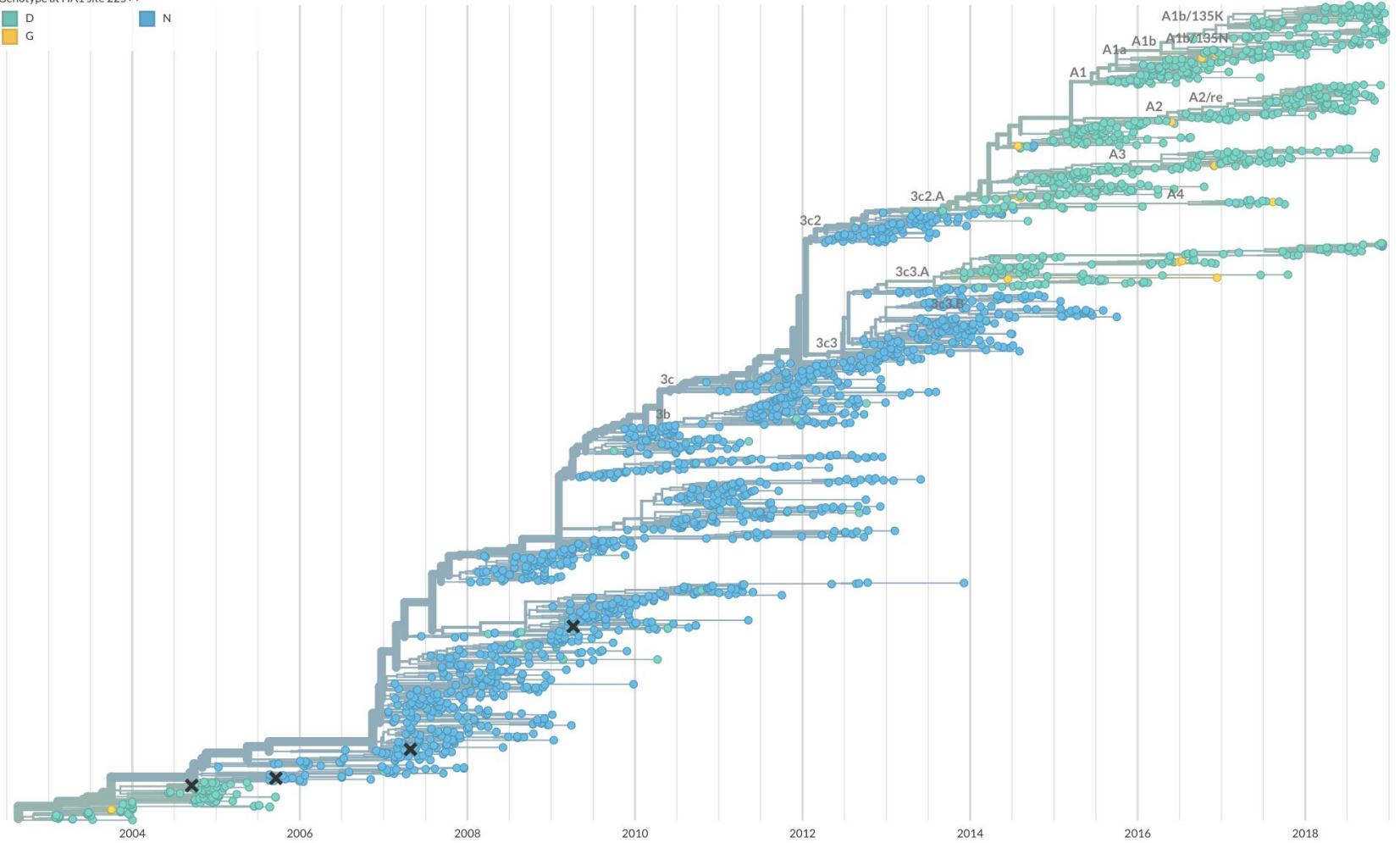


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D225G

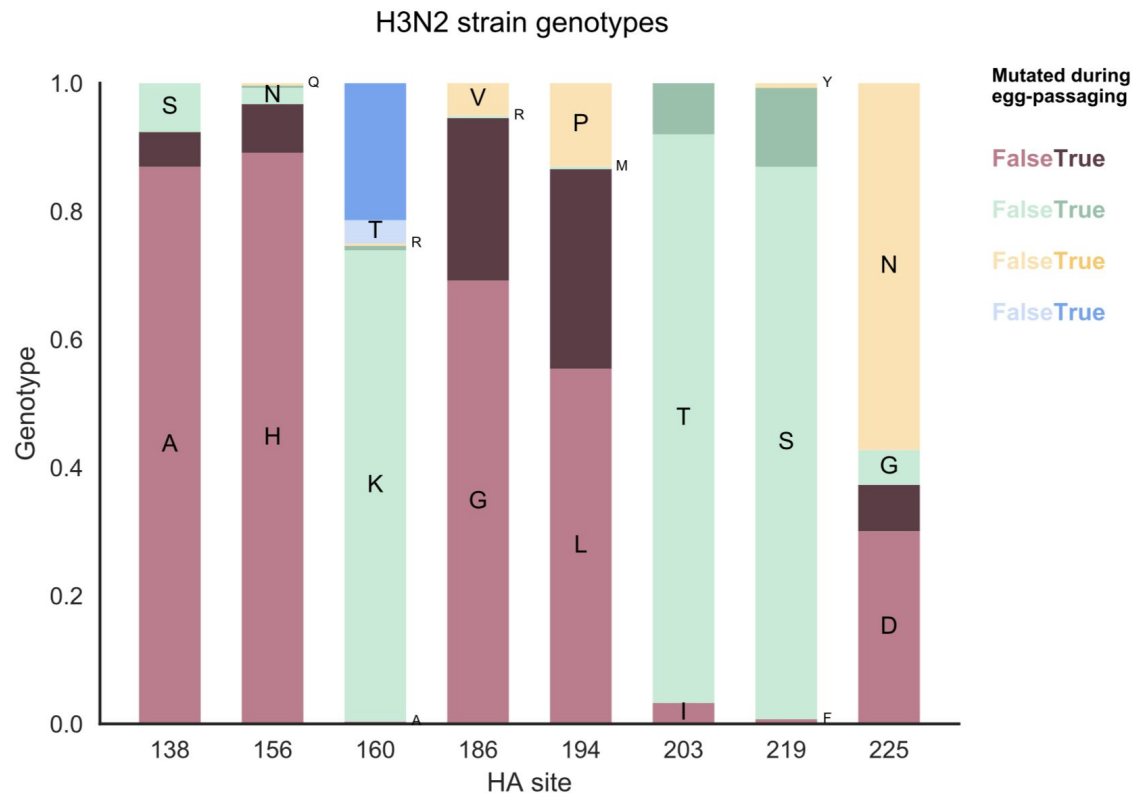
Phylogeny
Genotype at HA1 site 225 ^
D
G
N

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Background specificity of egg mutations

Genotype of virus
influences whether it will
mutate at that position
during egg-passaging

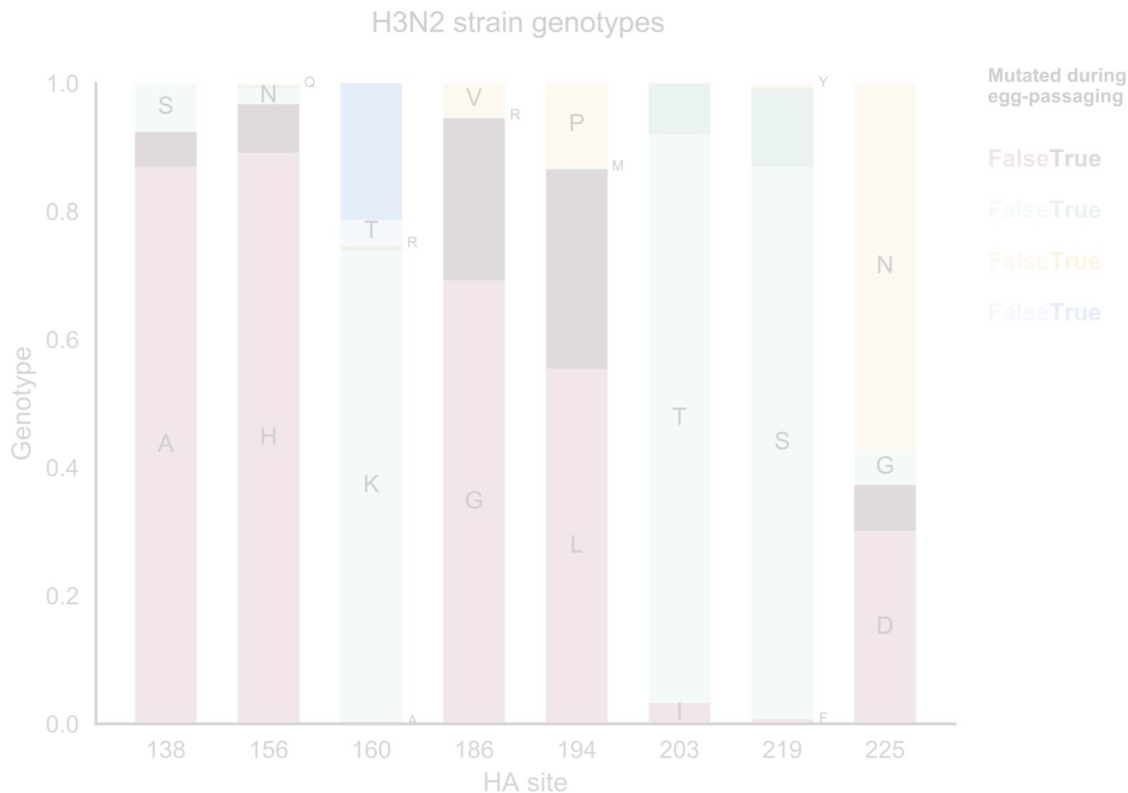


Background specificity of egg mutations

Genotype of virus

influences whether it will
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In the works: does HA
genotype (proxy: clade) of
virus determine whether it
mutate during
egg-passaging?



Epistasis between mutations

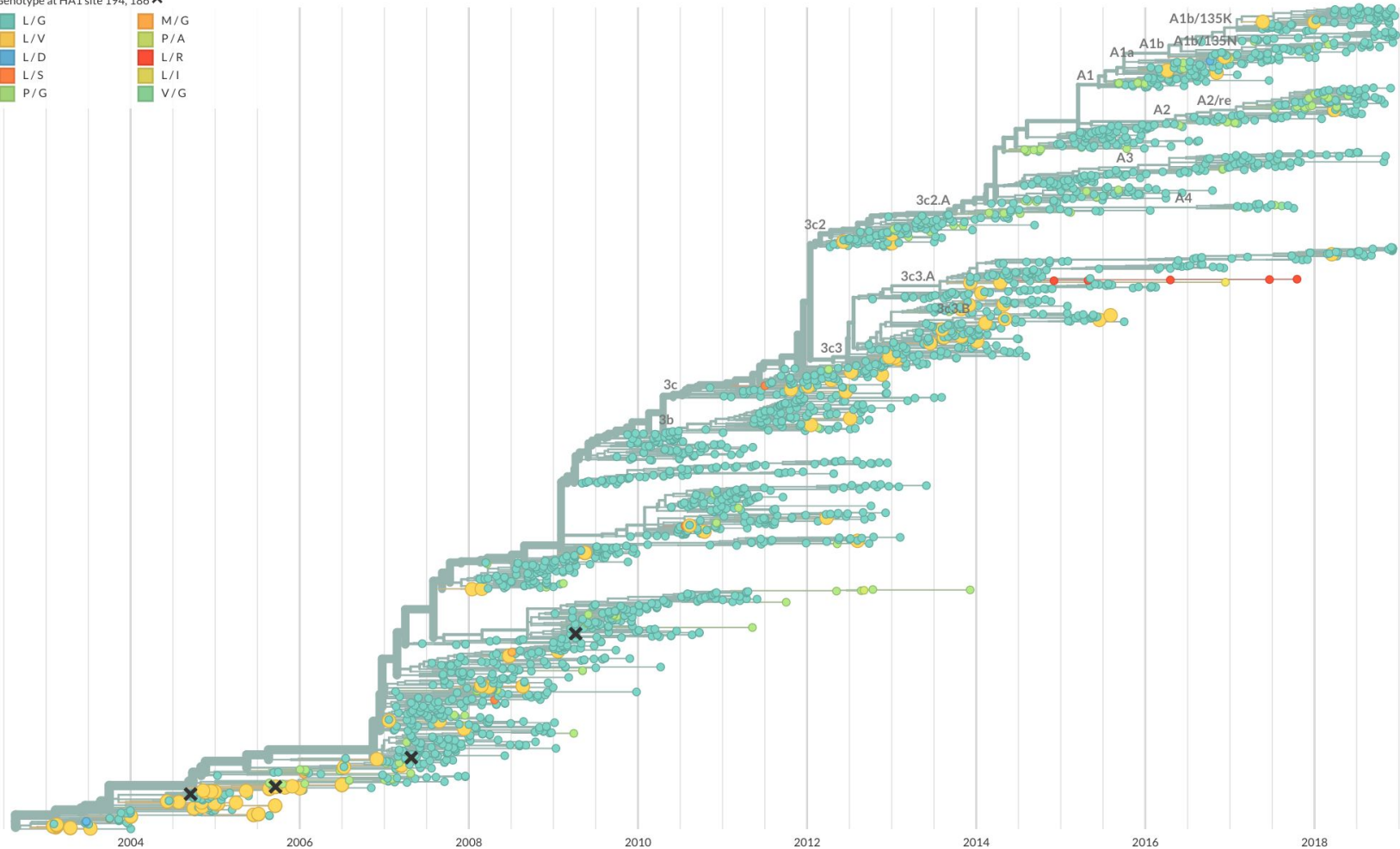
L194P
OR
G186V

Phylogeny

Genotype at HA1 site 194, 186 ^



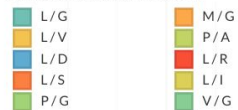
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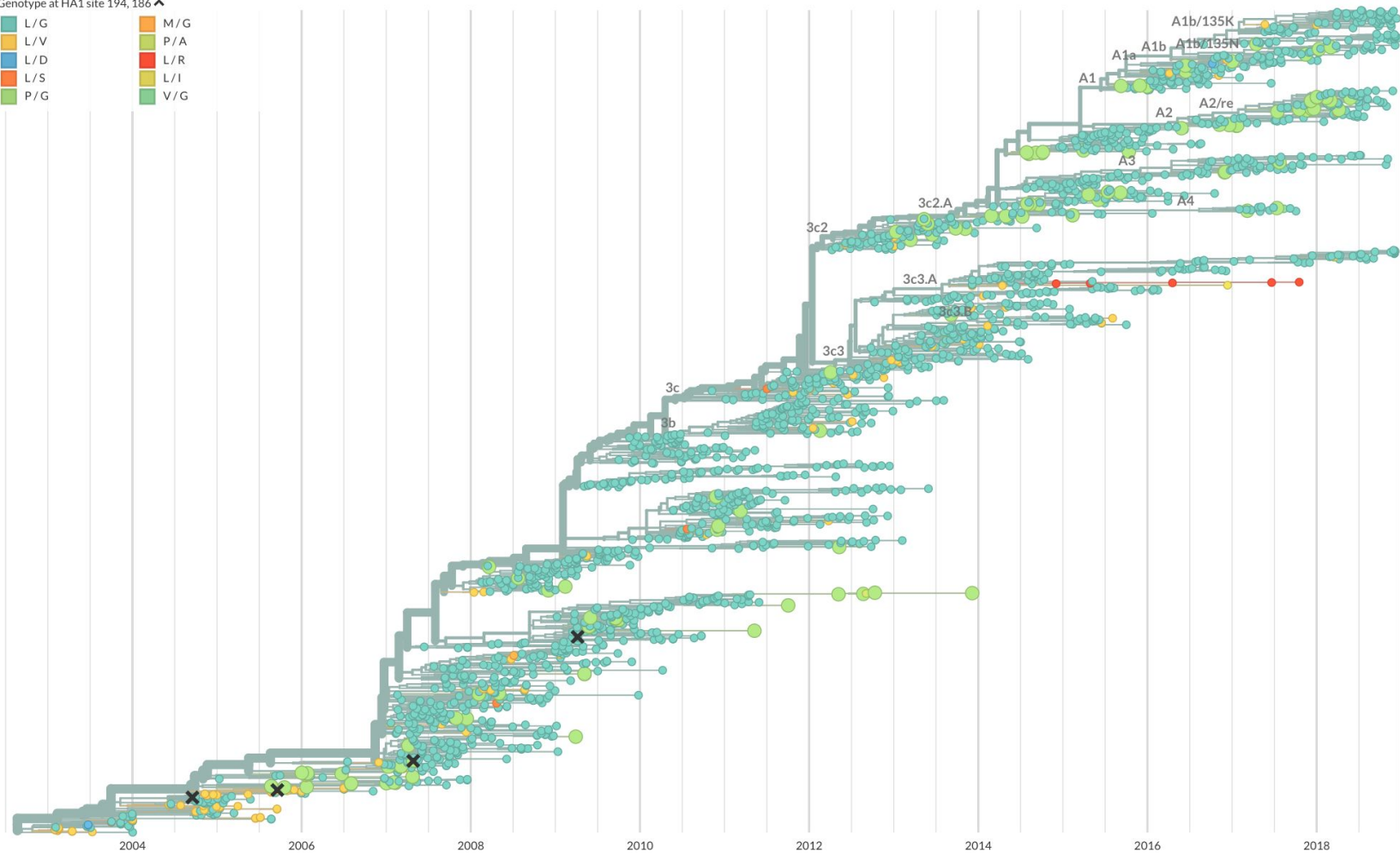
L194P
OR
G186V

Phylogeny

Genotype at HA1 site 194, 186 ^



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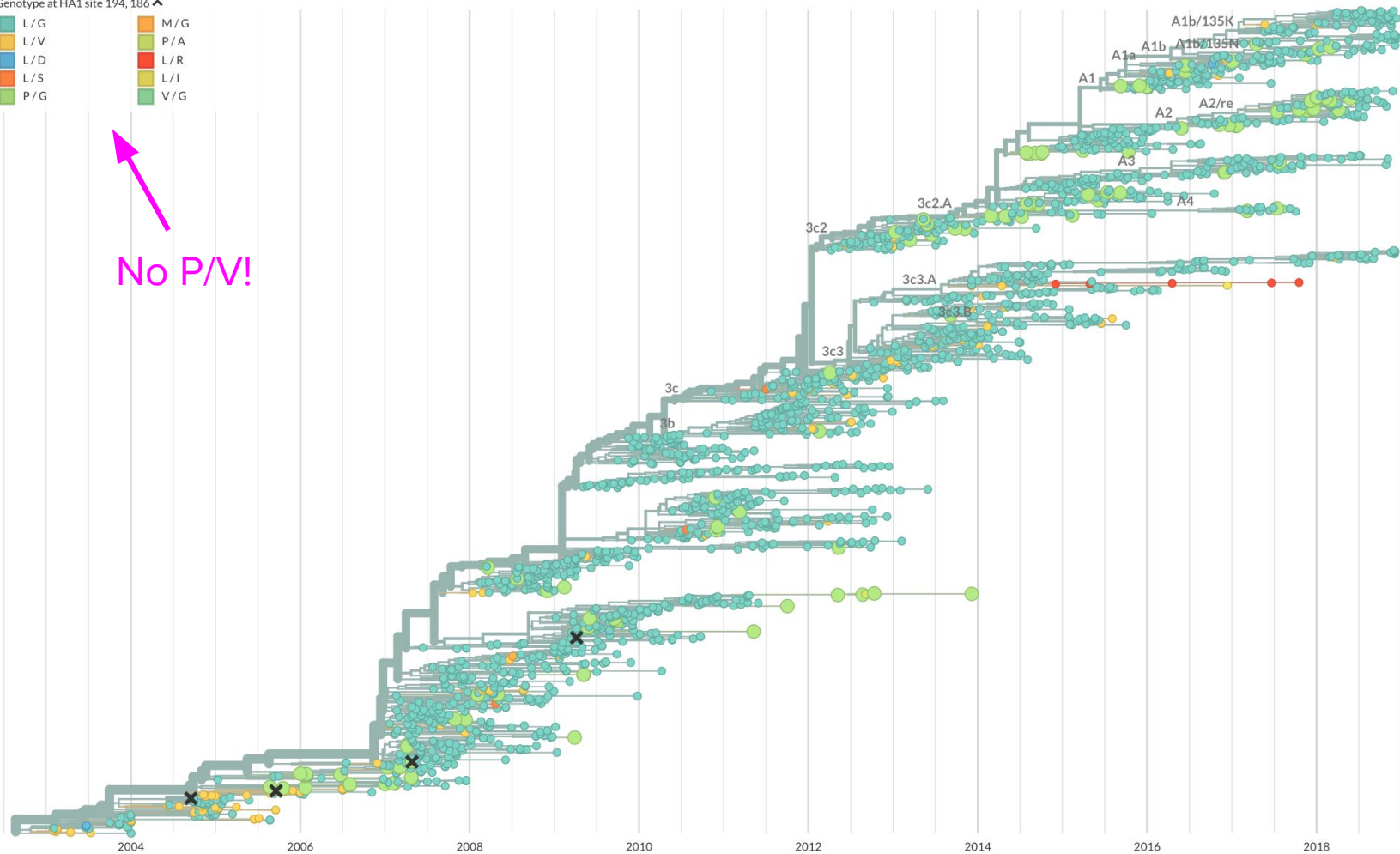
L194P
OR
G186V

Phylogeny

Genotype at HA1 site 194, 186 ^



No P/V!



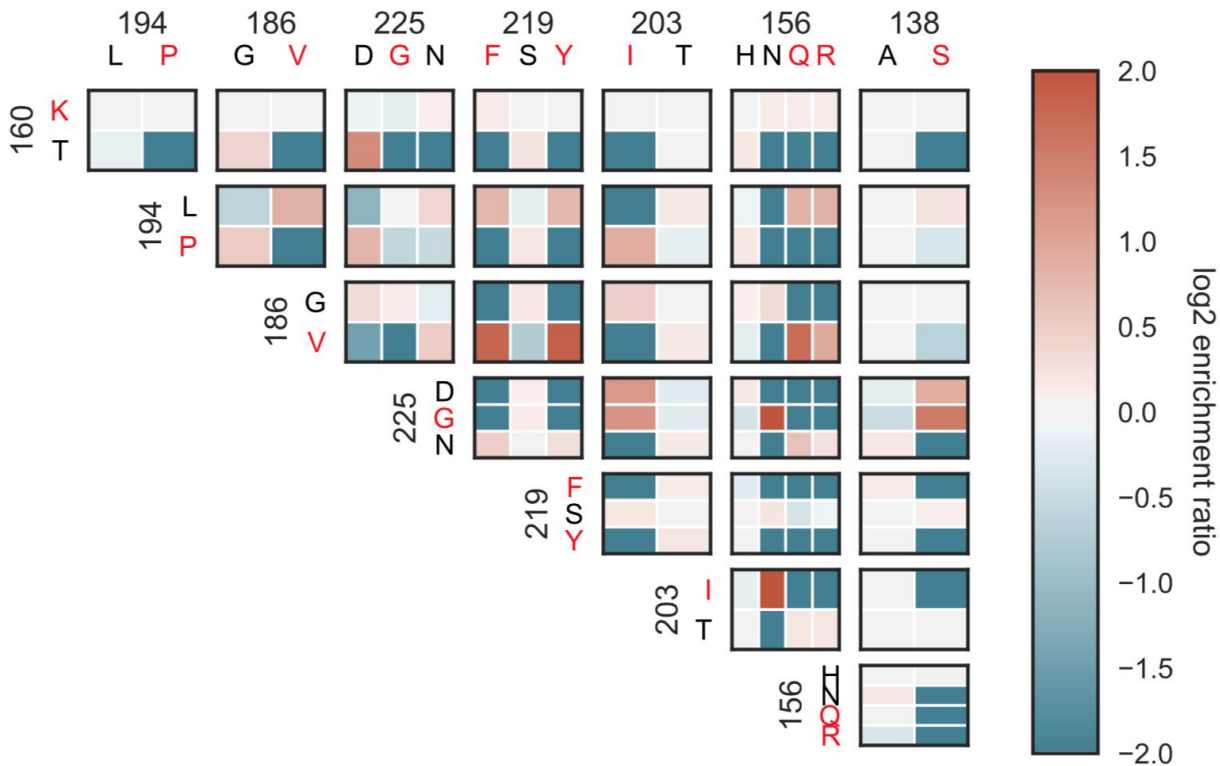
Pairwise epistatic interactions

$$\frac{\textit{observed}}{\textit{expected}} = \log_2 \frac{f_{194P\&186V}}{f_{194P} * f_{186V}}$$

Pairwise epistatic interactions

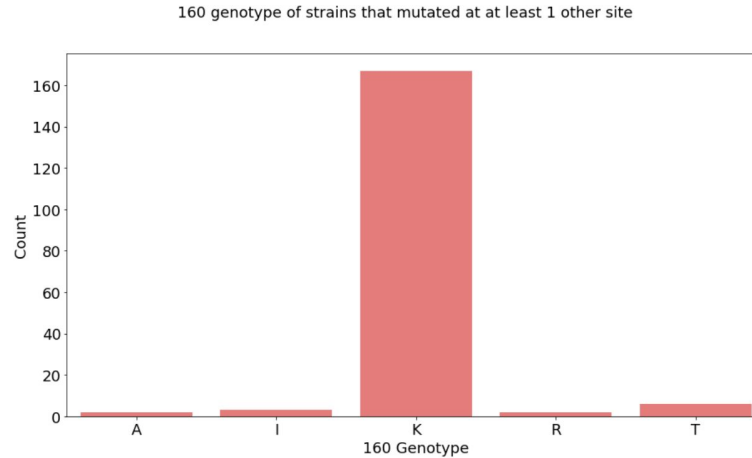
Epistasis between HA sites in egg-passaged influenza H3N2

$$\frac{\text{observed}}{\text{expected}} = \log_2 \frac{f_{194P \& 186V}}{f_{194P} * f_{186V}}$$



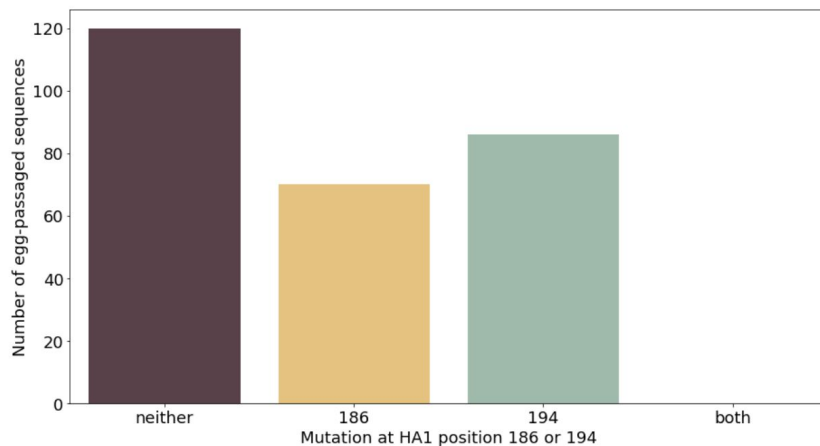
Take aways from epistasis analysis

1. Virus only mutates at other sites during egg-passaging if 160K (pos. epistasis)



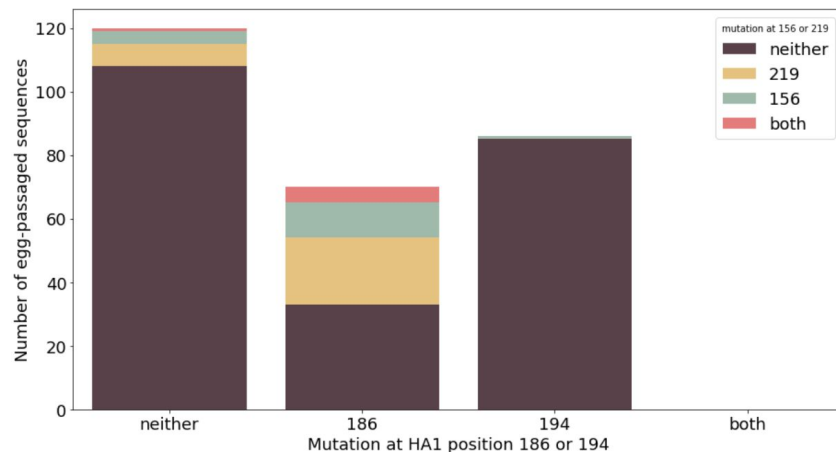
Take aways from epistasis analysis

1. Virus only mutates at other sites during egg-passaging if 160K (pos. epistasis)
2. Mutations at positions 186 and 194 are mutually exclusive (neg. epistasis)



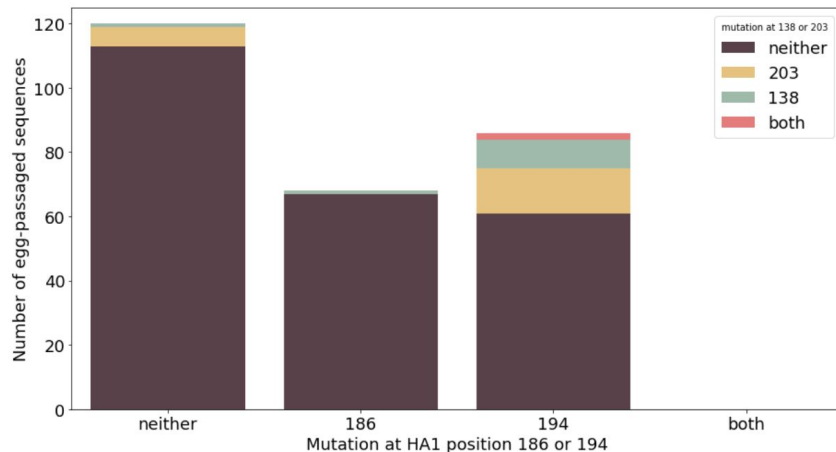
Take aways from epistasis analysis

1. Virus only mutates at other sites during egg-passaging if 160K (pos. epistasis)
2. Mutations at positions 186 and 194 are mutually exclusive (neg. epistasis)
3. Positions 219 and 156 do not mutate if 194 is mutated (neg. epistasis)



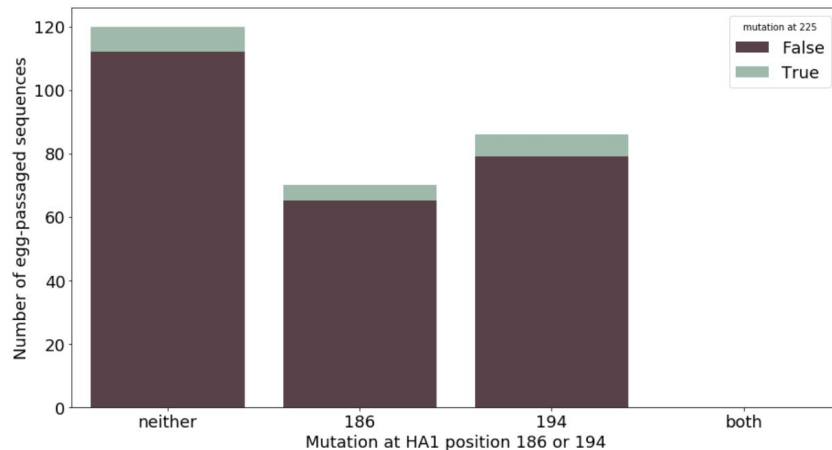
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1. Virus only mutates at other sites during egg-passaging if 160K (pos. epistasis)
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4. Positions 138 and 203 do not mutate if 186 is mutated (neg. epistasis)



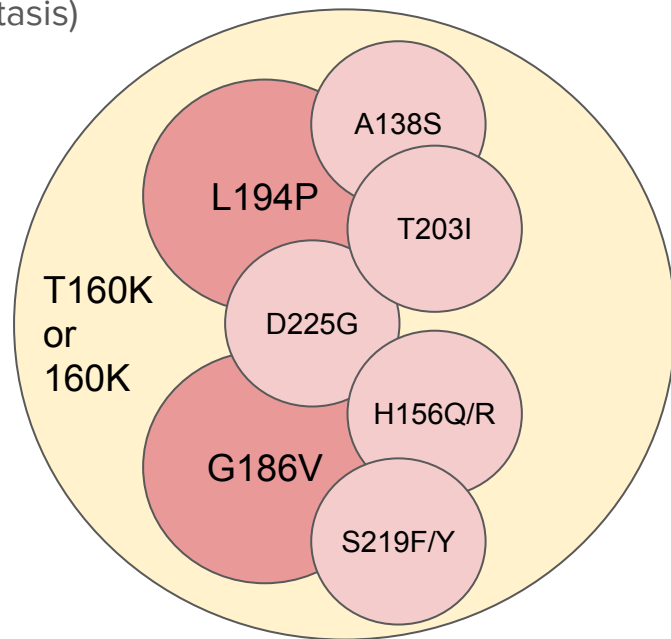
Take aways from epistasis analysis

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4. Positions 138 and 203 do not mutate if 186 is mutated (neg. epistasis)
5. Mutation at 225 does not depend on 186 or 194



Take aways from epistasis analysis

1. Virus only mutates at other sites during egg-passaging if 160K (pos. epistasis)
2. Mutations at positions 186 and 194 are mutually exclusive (neg. epistasis)
3. Positions 219 and 156 do not mutate if 194 is mutated (neg. epistasis)
4. Positions 138 and 203 do not mutate if 186 is mutated (neg. epistasis)
5. Mutation at 225 does not depend on 186 or 194



Temporal order?

And temporal order

Background specificity + epistatic effects

How are mutations at a position influenced by genotype at other positions?

Effects of egg-passaging mutations

Documented phenotypic effects of egg mutations

| | Documented egg mutation? | HA domain | Viral replication in eggs | Antigenicity |
|----------------|--------------------------|------------------------------------|---------------------------|-------------------|
| L194P | | 190-helix, antigenic site B | ↑ | Δ |
| G186V | | Antigenic site B | ↑ | Neutral |
| T160K | | Antigenic site B, glycosylation | ↑ | Δ |
| T203I | sequenced. | | | |
| S219F/Y | | | ↑ | Δ / Neutral |
| D225G | in H1N1 | 220-loop | ↑ (in H1N1) | Neutral (in H1N1) |
| A138S | sequenced. | 130-loop, antigenic site A | | |
| H156Q/R | | Antigenic site B | ↑ (H156Q) | Δ (H156Q) |

Phenotypic effects based on titers model

- Substitution model (Neher et al, 2016): ascribe titer drops to amino acid substitutions

Phenotypic effects based on titers model

- Substitution model (Neher et al, 2016): ascribe titer drops to amino acid substitutions

| | Antigenic units (2-fold decrease in titer) | |
|--------------|---|--------|
| | HI | FRA |
| L194P | 1.556 | 1.4434 |
| G186V | 0.7317 | 0.2806 |
| T160K | 0.2864 | 0.8269 |
| T203I | 0.1125 | None |
| S219F | 0.6432 | None |
| S219Y | 0.2013 | 0.3543 |

| | Antigenic units (2-fold decrease in titer) | |
|--------------|---|--------|
| | HI | FRA |
| D225G | None | 0.7157 |
| A138S | None | 0.2315 |
| H156Q | None | None |
| H156R | None | None |

Still to be done

1. Continuation of these analyses
 - a. Titters model interpretation
 - b. Larger n
 - c. Predict mutation based on background

2. Potential additional analyses
 - a. Synonymous mutations
 - b. NA mutations
 - c. H1N1 mutations