Introduction to Mutation Interpretation with FluSurver

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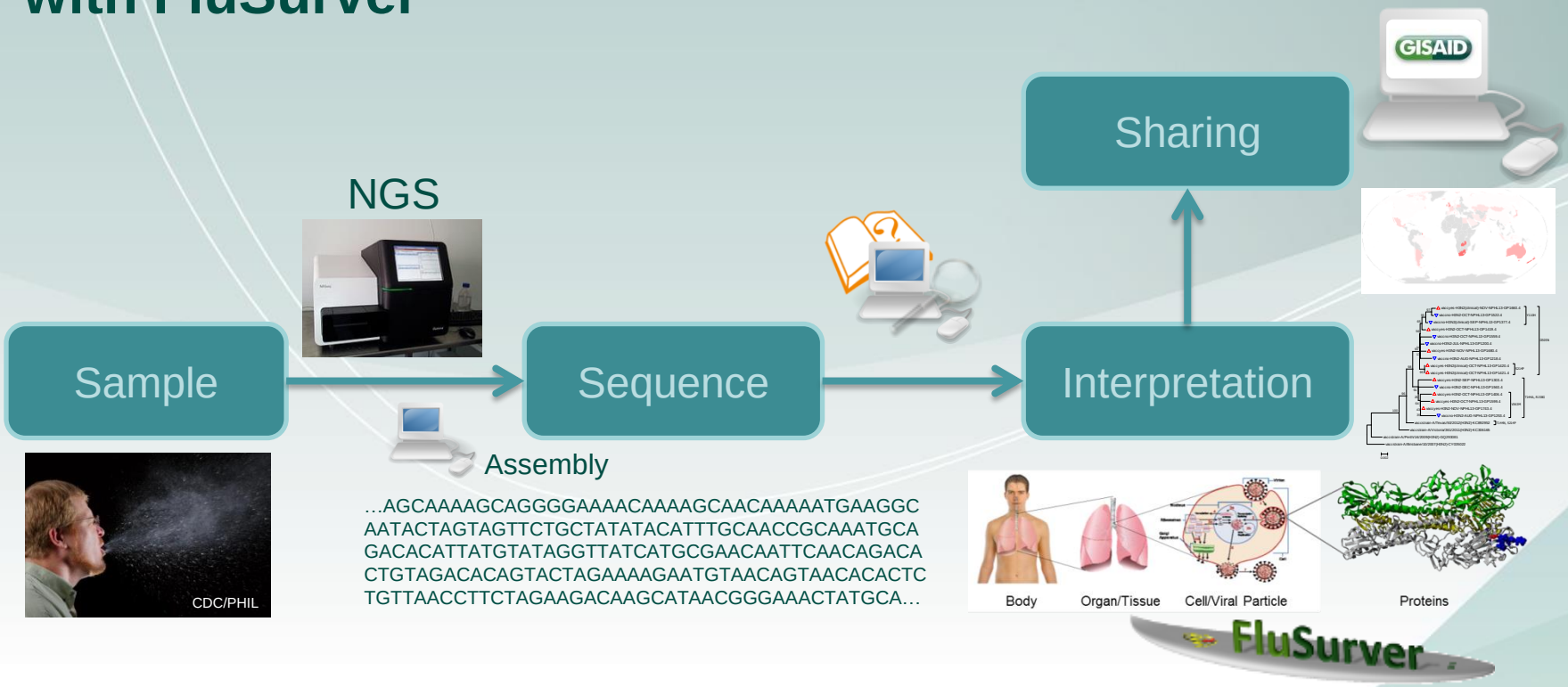
GISAID Database Technical Group

Making full use of influenza sequences with FluSurver



Reduced cost of and easier access to sequencing gives us:

- More sequences (also complete genomes)
- More detail of genetic evolution
- More questions on how to use/interpret/analyze sequences

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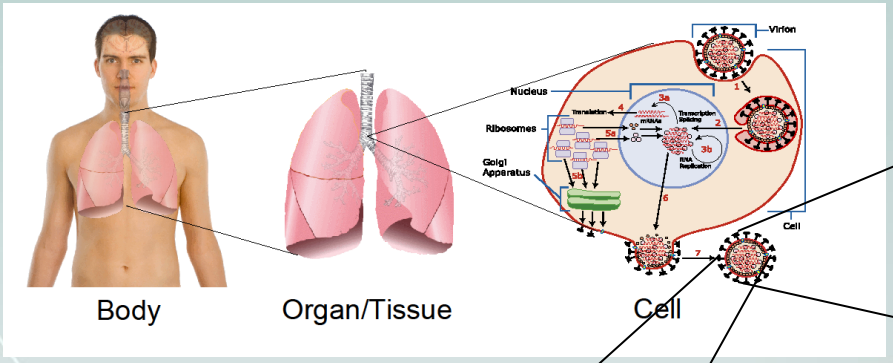
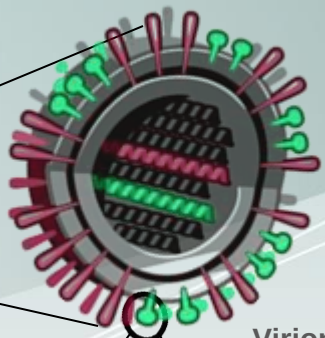
Flu viruses evolve through Reassortment and Mutations

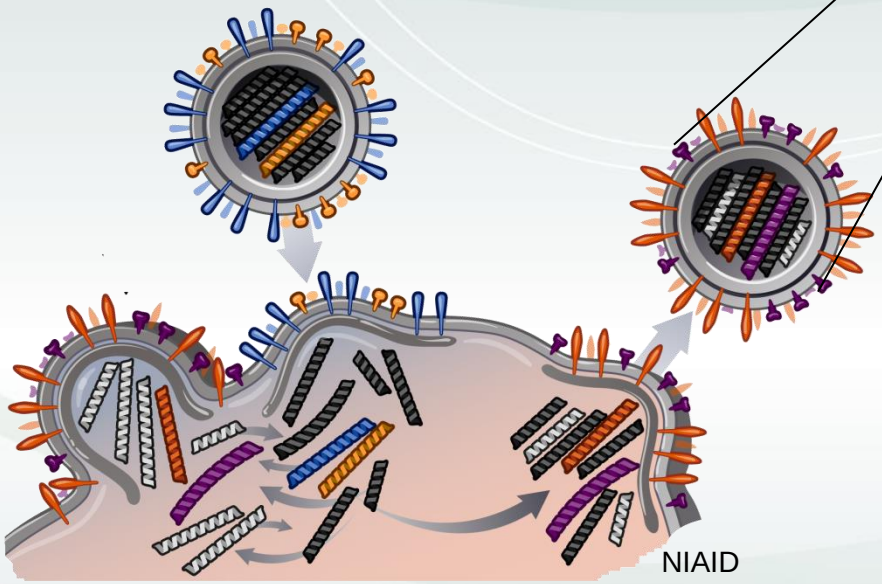
New properties can be:

- Infect new hosts
- More or less severe
- Spread more easily
- Immune escape
- Drug resistance

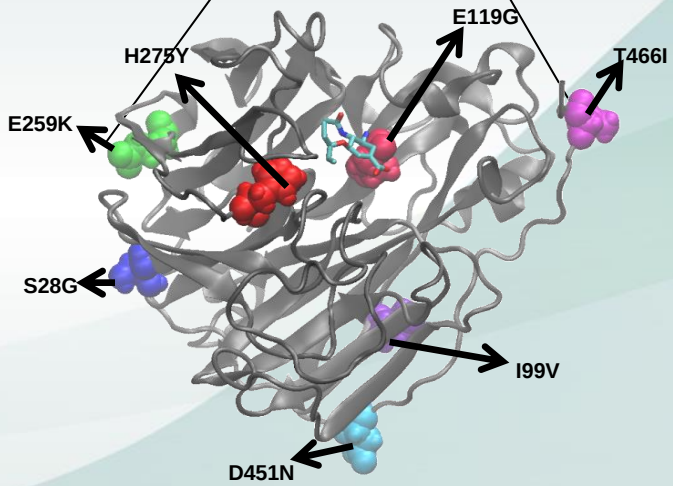




Virion
Viral Particle



NIAID



Reassortment = genome segment mixing
e.g. H1N1 to H3N1

Mutations, e.g. NA H275Y

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FluSurver for Mutation Interpretation

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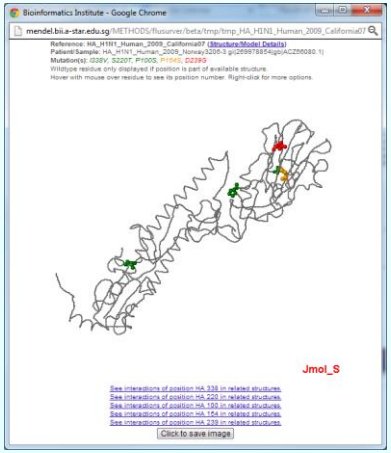
Structure

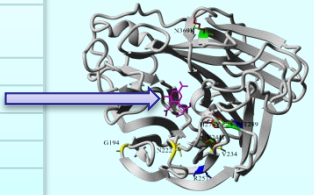


Map mutations to structure

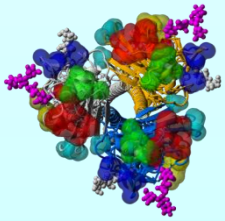
250+ reference homology models

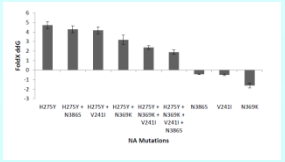
1568	self/oligomerization
975	other small ligand
268	antibody
188	host protein
182	antigen-presenting MHC molecule
132	other viral protein
46	drug
45	nucleic acids
13	host cell receptor
3417	**total interactions for 2062 positions**

Interactions



Glycosylation site changes





FoldX stability calculations

(for high frequency mutations in N1pdm)

Literature

Mutation **numbering scheme conversion** (e.g. H3, H1, H1pdm) and direct **PubMed** search link

Passage bias (egg/cell adaptation) for ~1300 mutations

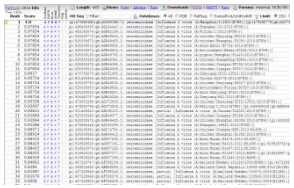
Literature-curated mutation effect database

~250 entries

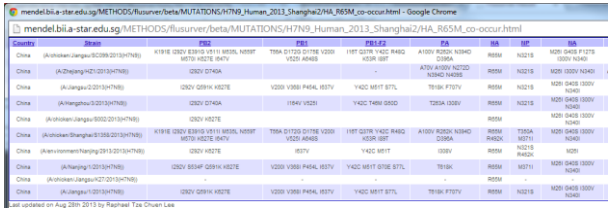
mild drug resistance	19
strong drug resistance	30
virulence	68
antigenic drift / escape mutant	74
host specificity shift	21
other	12

Epidemiology

Closest DB hits



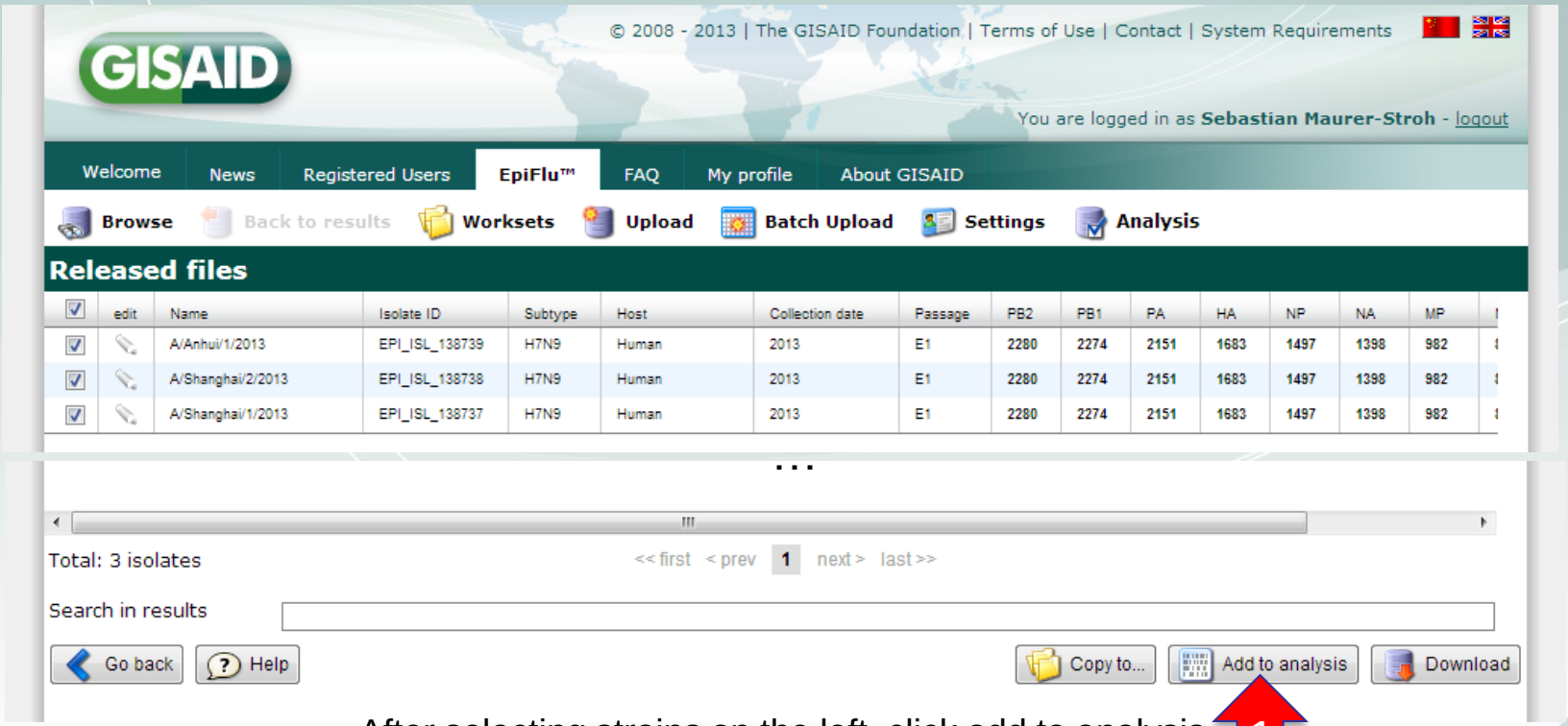
Temporal pattern



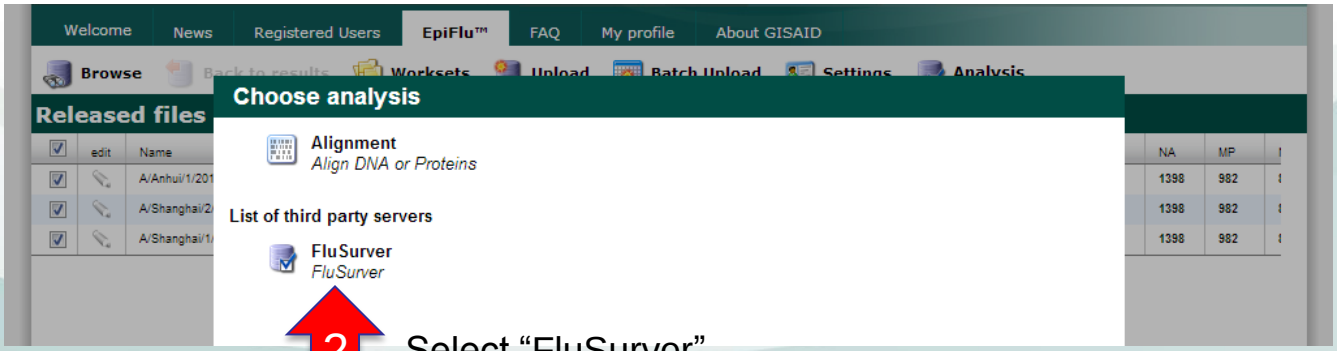
Genomic co-occurrence

Global occurrence

First steps: find, select and add isolates to analyze from the EpiFlu™ database

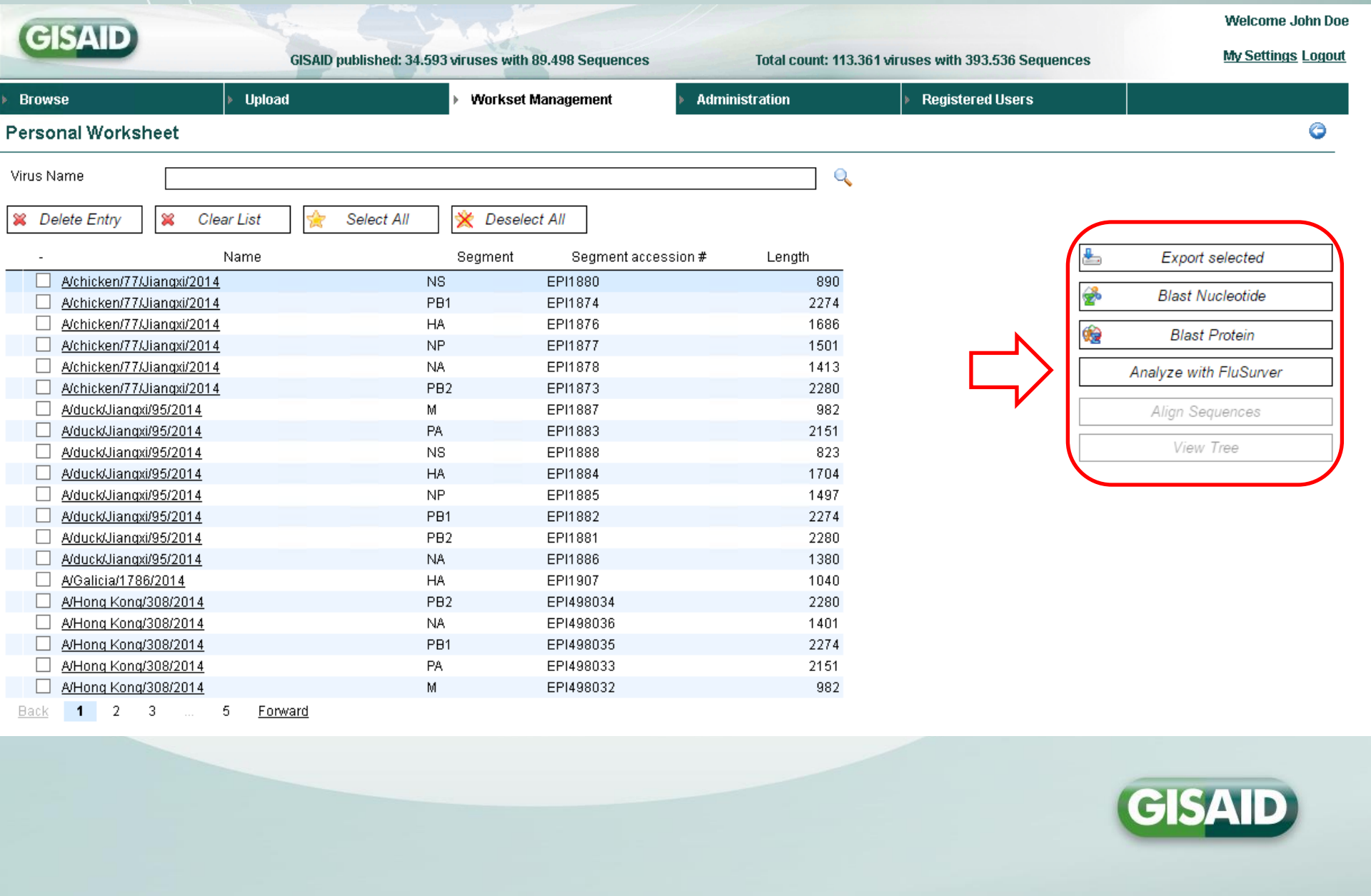


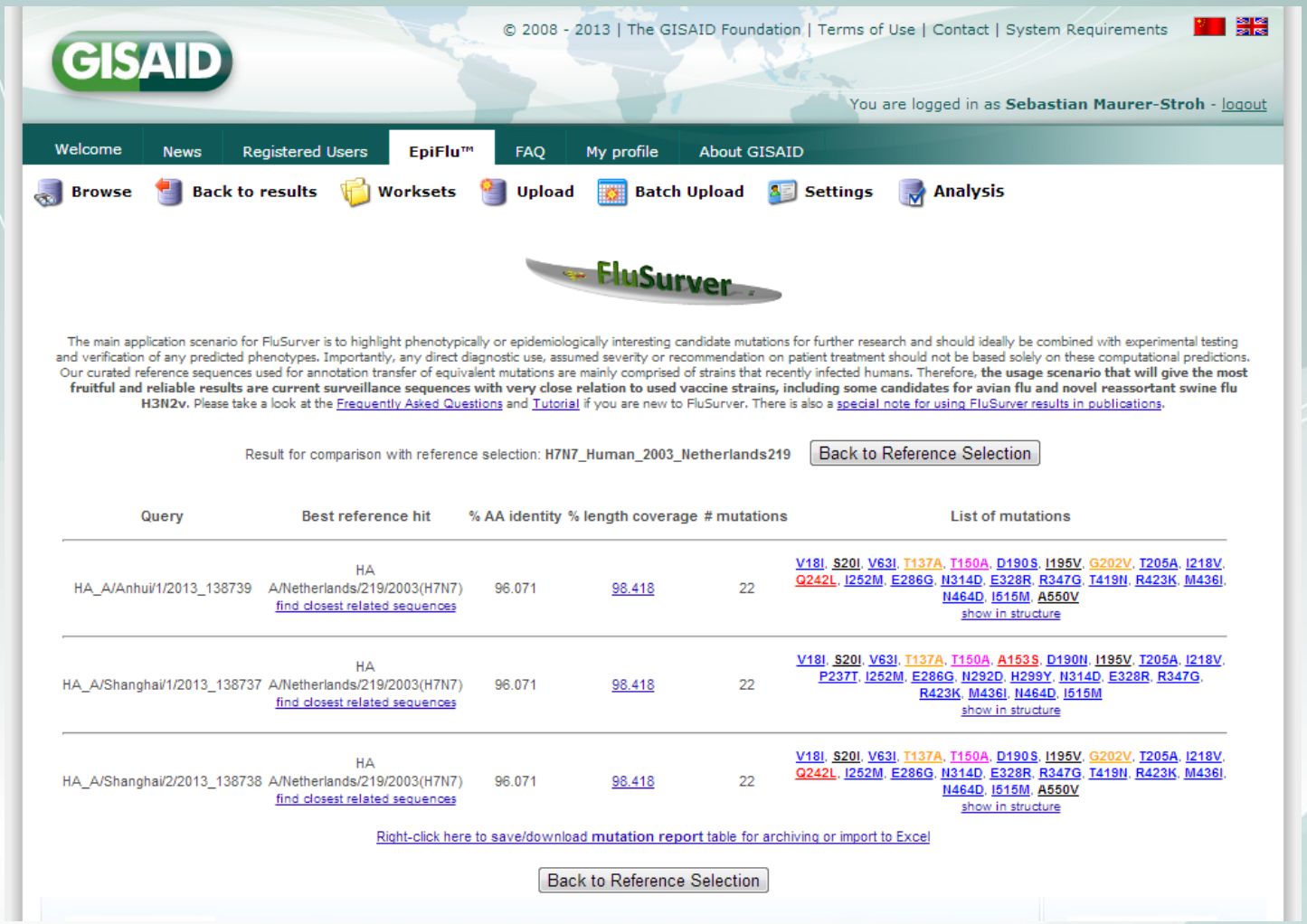
After selecting strains on the left, click add to analysis 1



2 Select “FluSurver”

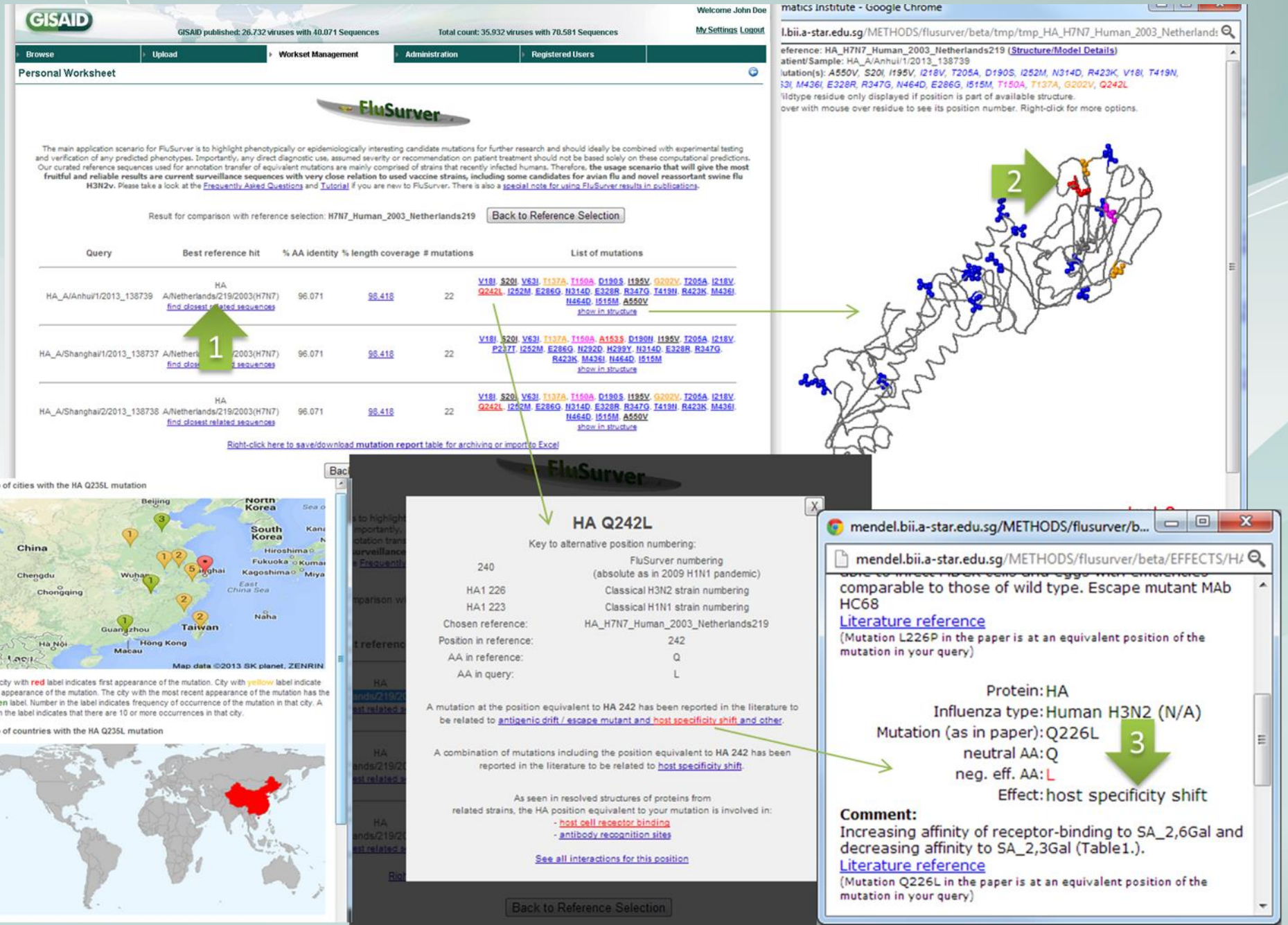
EpiFlu™ 2.0 – Analysis Tools



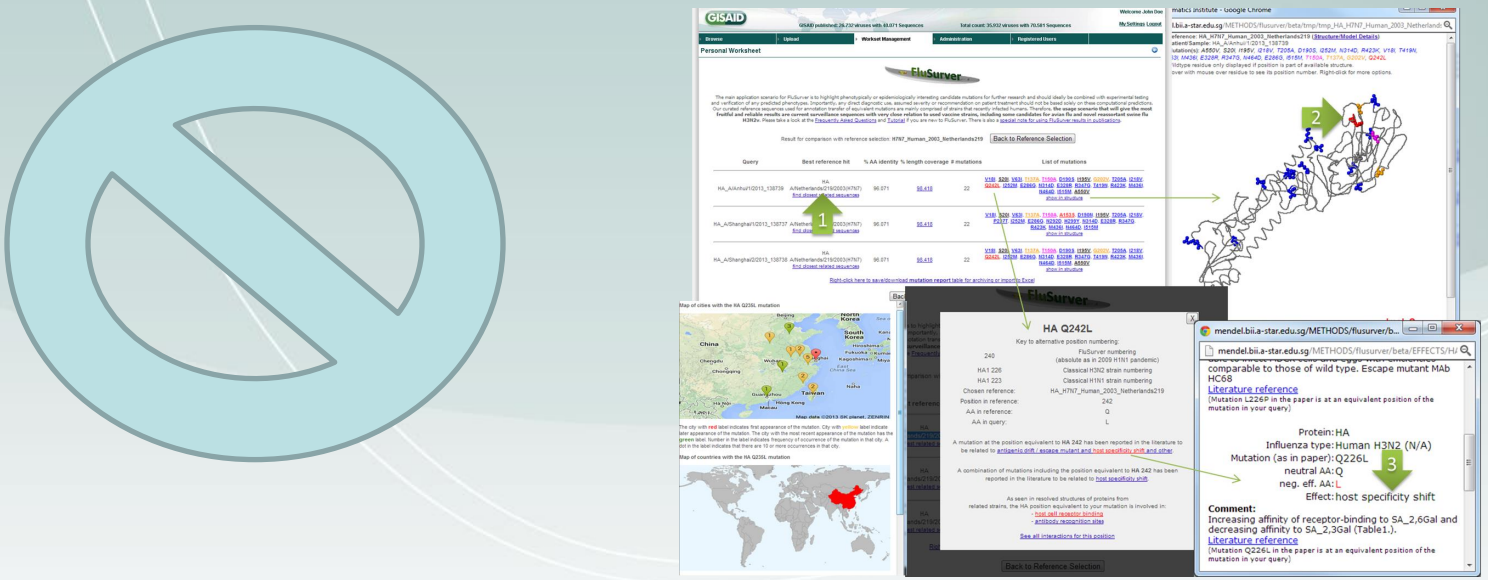


For each of the query sequences, users may proceed to look at the alignment to the reference strain, get more information on each mutation, generate a structural view of all the mutations ("show in structure")…

Analysis – FluSurver for Mutation Interpretation



Analysis – FluSurver for Mutation Interpretation

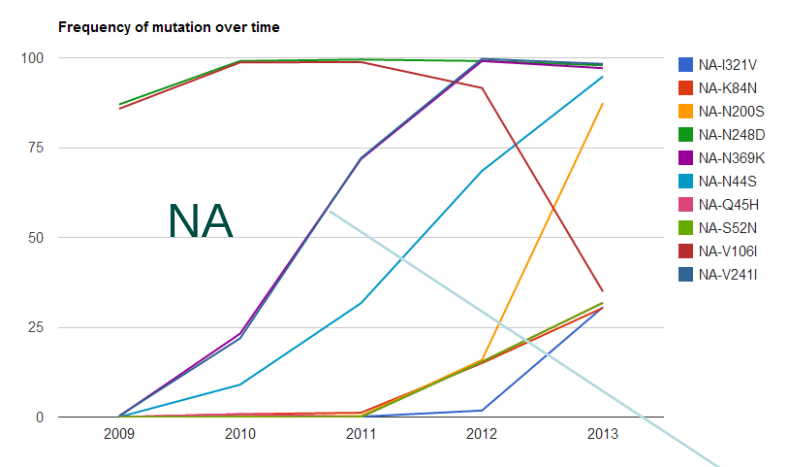


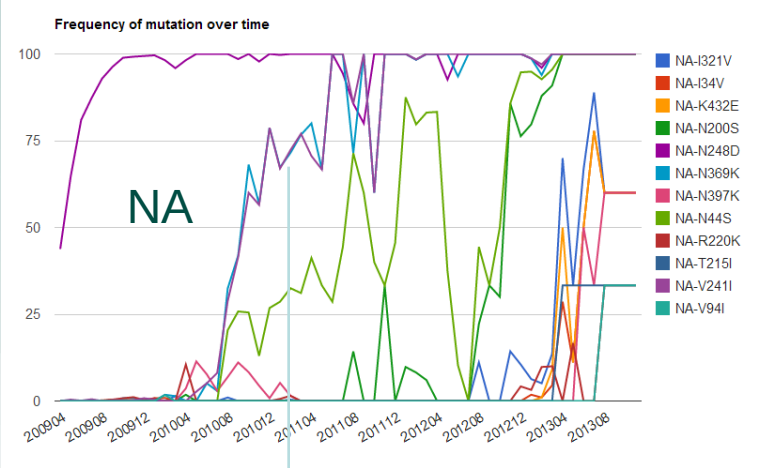
Important disclaimer:

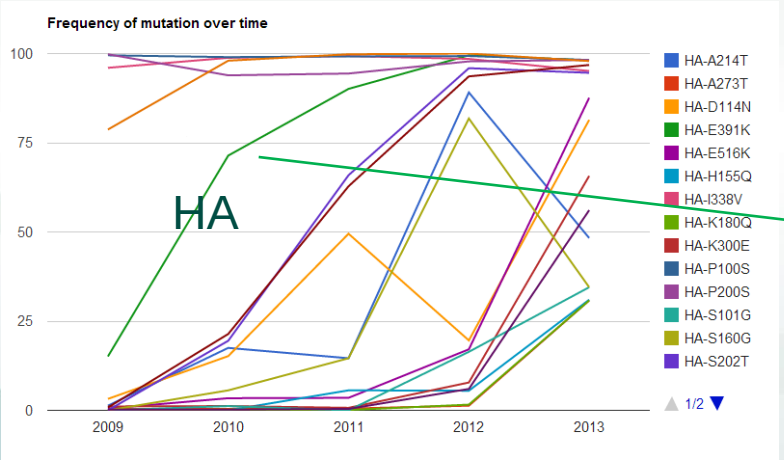
FluSurver makes it very easy to link mutations with prior literature and potential phenotypic effects.

While we have placed great emphasis on avoiding false positive alerts and provide tutorials, one still needs to read the associated papers and interpret the provided evidence carefully to judge any effect realistically.

Mutation frequency pattern highlights relevant changes







New H275Y permissive mutations

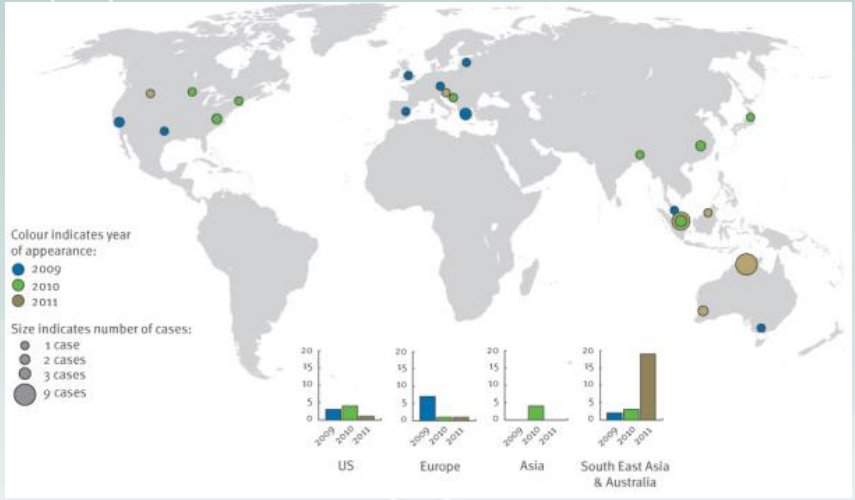
Hurt *et al.* J Infect Dis. 2012 Jul 15;206(2):148-57.
Butler *et al.* PLoS Pathog. 2014 Apr 3;10(4):e1004065.

Change in pH-dependency of fusion

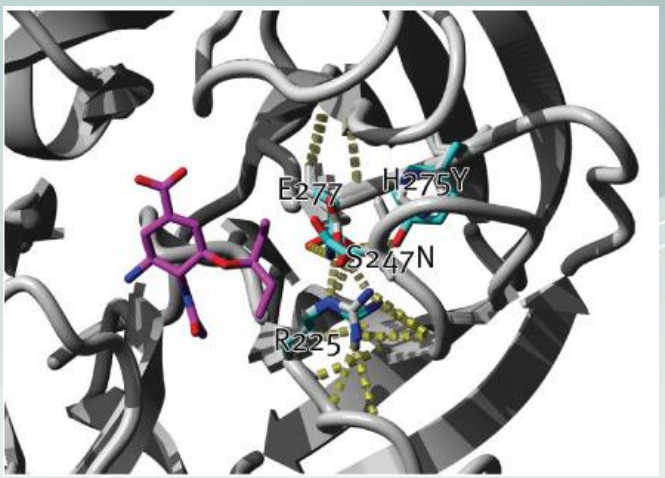
Maurer-Stroh *et al.* PLoS Curr. 2010 Jun 1;2:RRN1162.
Cotter *et al.* PLoS Pathog. 2014 Jan;10(1):e1003831.

Example H1N1pdm in FluSurver

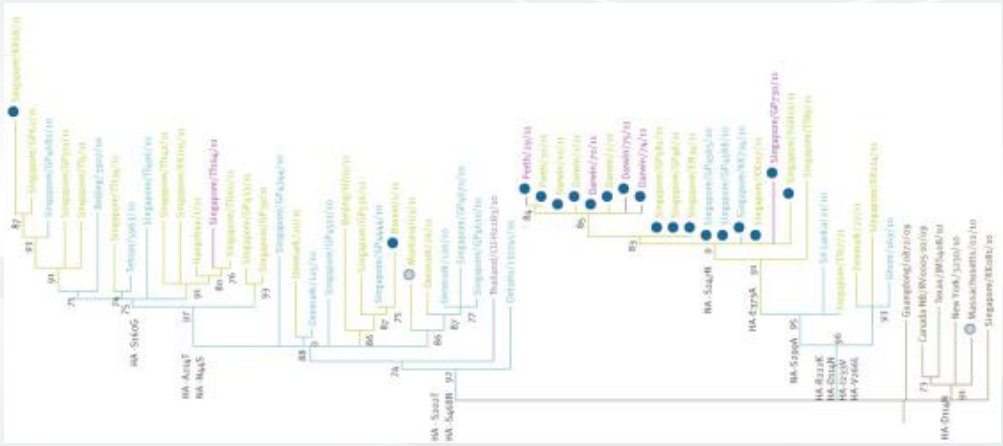
New drug sensitivity altering mutation NA S247N



Global occurrence of new variant



Structural context of mutation



Phylogenetic context of new variant

Found circulating in 10% of samples in Singapore and 30% of samples in Northern Australia in early 2011.

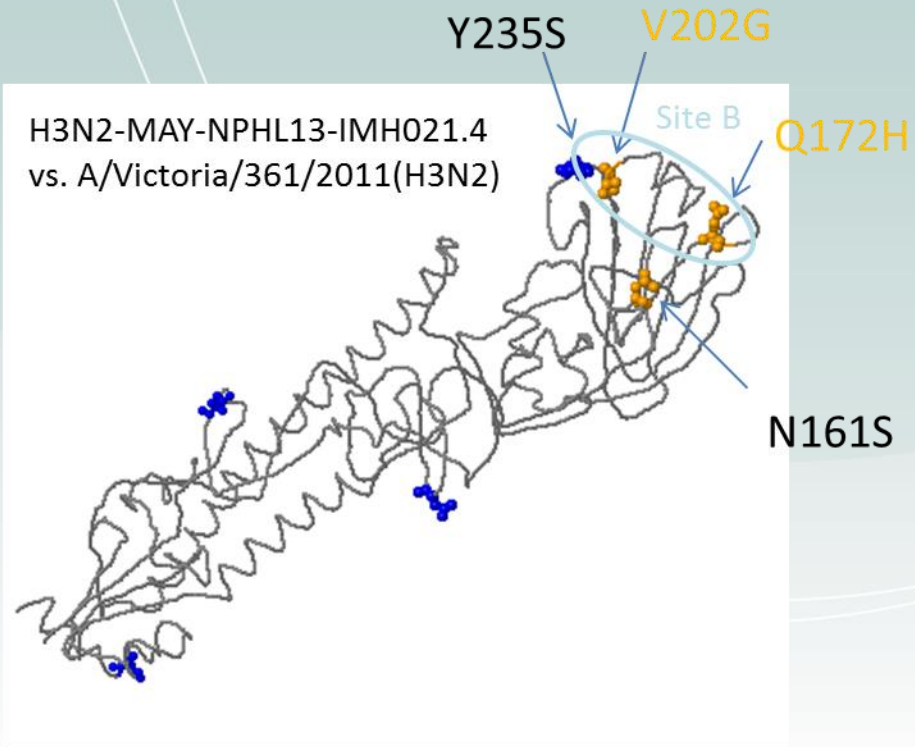
Experimentally measured increase of IC50 for Tamiflu by 6-fold and Relenza by 3-fold but **normally administered dose of drugs still sufficient.**

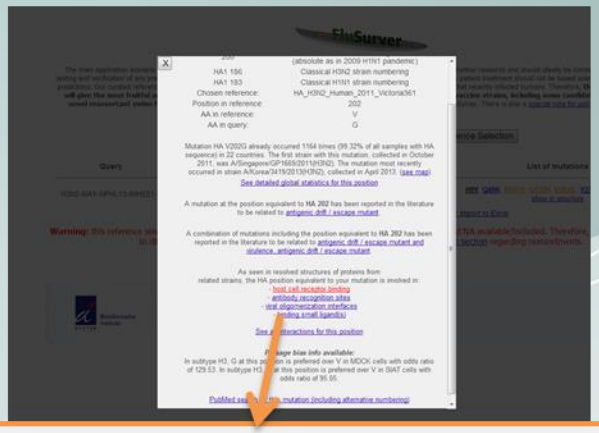
*Collaboration between Bioinformatics Institute, A*STAR with NPHL/Ministry of Health Singapore and WHO Collaborating Centre for Reference and Research on Influenza.*

Euro Surveill. 2011;16(23):pii=19884.

GISAID

Current H3N2 strains have HA passage bias mutations in antigenic sites





As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:
- host cell receptor binding
- antibody recognition sites
- viral oligomerization interfaces
- binding small ligand(s)

V202G

See all interactions for this position

Passage bias info available:
In subtype H3, G at this position is preferred over V in MDCK cells with odds ratio of 129.53. In subtype H3, G at this position is preferred over V in SIAT cells with odds ratio of 95.05.

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:
- host cell receptor binding
- antibody recognition sites
- binding small ligand(s)
- is involved in binding host protein(s)
- viral oligomerization interfaces

Q172H

See all interactions for this position

Passage bias info available:
In subtype H3, H at this position is preferred over Q in SIAT cells with odds ratio of 67.59.

Same isolate but different passage
(A/SINGAPORE/22/2012 NPHL: GP1187-2012)

GISAID ID	Submitter	Passage	Mutations relative to Victoria/361
EPI_ISL_128750	WHO CC Melbourne via NPHL	**MDCK0, MDCK1**	H9Y, Q49R, N161S, **Q172H, V202G, Y235S**, N294K
EPI_ISL_135838	US CDC via WHO CC Melbourne	**E4/E1**	H9Y, Q49R, N161S, N294K

FluSurver Acknowledgements

Many current and former colleagues from the A*STAR Bioinformatics Institute (BII) contribute(d) critically to its development and maintenance, including:

Sebastian Maurer-Stroh, Raphael Tze Chuen Lee, Vithiagaran Gunalan, Vachiranee Limviphuvadh, Fernanda L Sirota, Biruhalem Taye, Jianmin Ma, Swe Swe Thet Paing, Narumol Doungpan, Joy Xiang and Frank Eisenhaber.

The FluSurver would be nothing without the valuable feedback and interaction with the influenza research and surveillance community, including especially and in chronological order:

- Genome Institute of Singapore (GIS), Singapore
- INMEGEN Mexico City, Mexico
- Experimental Therapeutics Centre (ETC), Singapore
- Tan Tock Seng Hospital (TTSH), Singapore
- National Public Health Laboratory (NPHL) of the Ministry of Health, Singapore
- IAL Sao Paulo, Brazil
- WHO Collaborating Centre for Reference and Research on Influenza, Australia
- Duke-NUS Emerging Infectious Disease Programme, Singapore
- University of Melbourne, Australia
- Global Initiative for Sharing All Influenza Data (GISAID)
- Federal Office for Agriculture and Food (BLE), Germany
- Health Protection Agency of Canada

Fishing for Flu Mutations since 2009!

Bioinformatics Institute
A*STAR

Contact: flusurver@gisaid.org
(sebastianms@bii.a-star.edu.sg)

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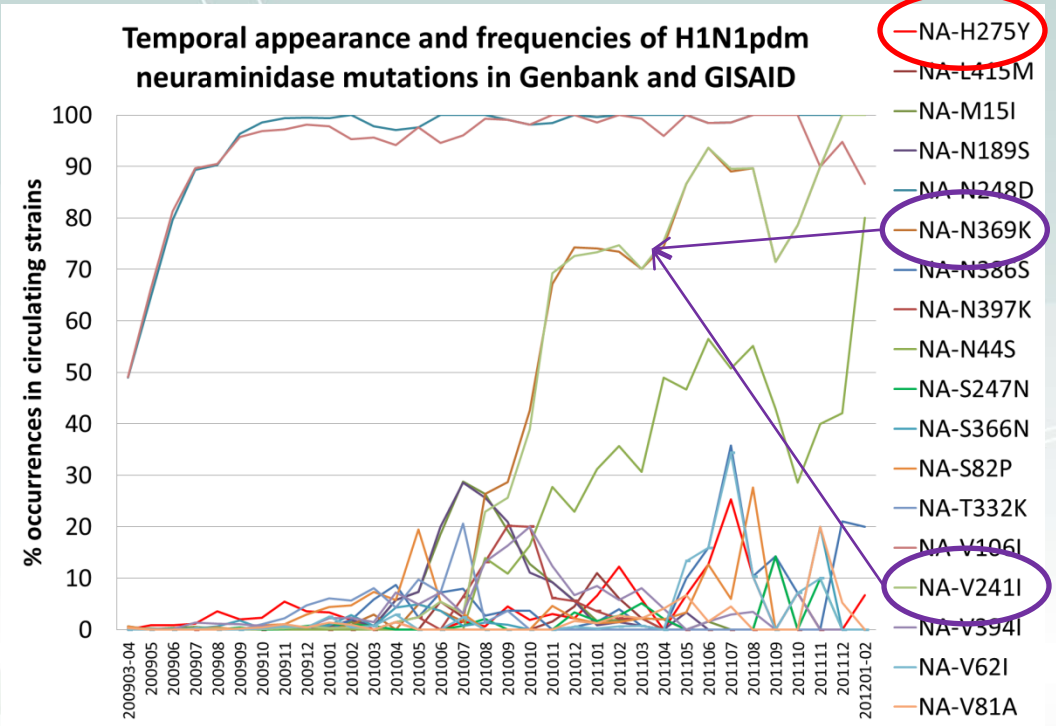
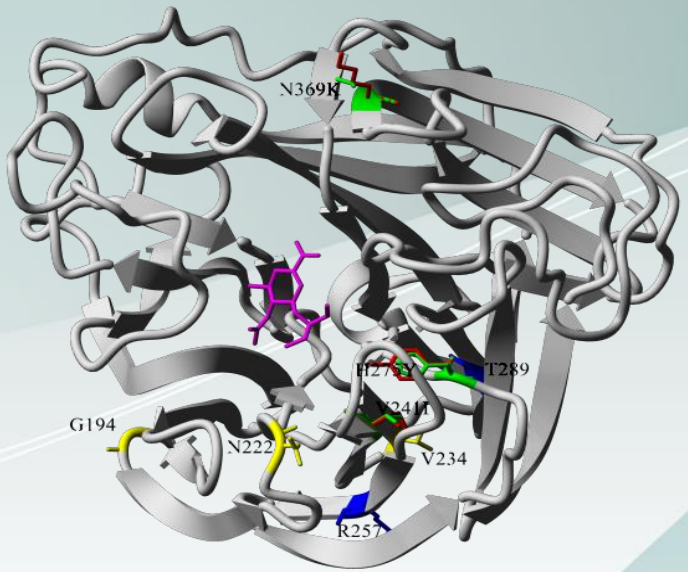
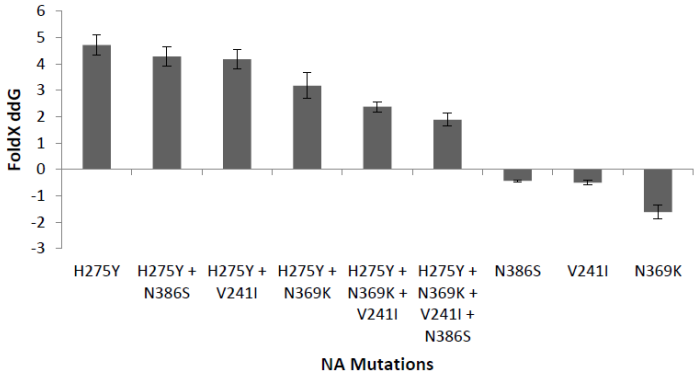
Optional back-up slides for questions…

Frequency rise points to role of permissive mutations



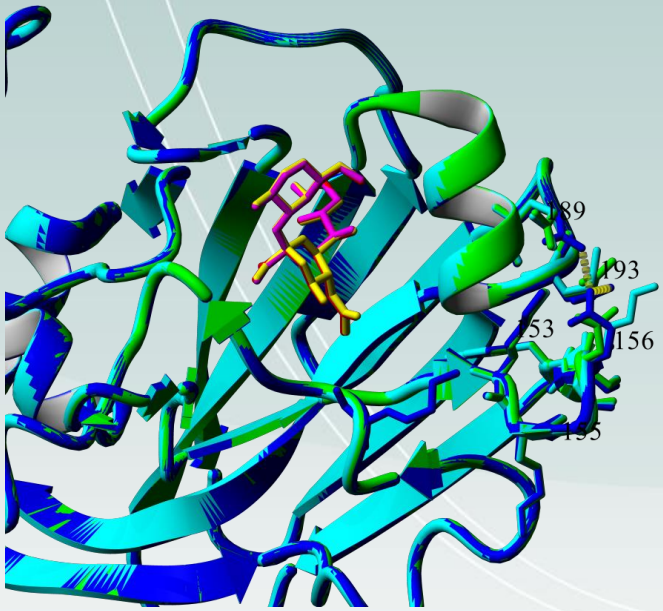




FoldX predicts increase in structural stability for mutations that were increasing in frequency and were fixed in Newcastle strains.

Hurt AC, Hardie K, Wilson NJ, Deng YM, Osbourn M, Leang SK, Lee RT, Iannello P, Gehrig N, Shaw R, Wark P, Caldwell N, Givney R, Xue L, Maurer-Stroh S, Dwyer DE, Wang B, Smith DW, Levy A, Booy R, Dixit R, Merritt T, Kelso A, Dalton C, Durrheim D, Barr IG.
Characteristics of a widespread community cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza in Australia.
J Infect Dis. 2012 Jul 15;206(2):148-57.

A “stealth” antigenic drift mutation due to passage bias



A new mutation causing vaccine escape in ferret model cannot be found by classical virus culture plus sequencing because it always reverts to wildtype under culture conditions.

HA receptor with bound ligand (pink/yellow) and passage dependent mutations with number labels

Analysis for Ian Barr WHO CC

				Passage history Odds Ratio (mutant vs wildtype)			
Mutation	No. reported[a]	No. isolates (mutant/ wildtype)[b]	Total frequency (%)[c]	Egg isolate	MDCK cell isolate	MDCK-SIAT1 cell isolate	Original clinical sample
N125D	581	321/7730	3.99	2.05	0.96	0.57	1.04
K153E	19	16/8018	0.20	1.59	6.55	-	-
G155E	133	103/7827	1.30	0.46	4.63	0.68	0.05
N156D	31	22/7980	0.27	-	4.32	0.52	0.22
N156K	22	12/7980	0.15	-	0.43	1.00	3.28
L191I	47	25/8008	0.31	55.31	0.20	-	-
Q223R	133	71/7966	0.88	564.42	0.02	0.00	0.00

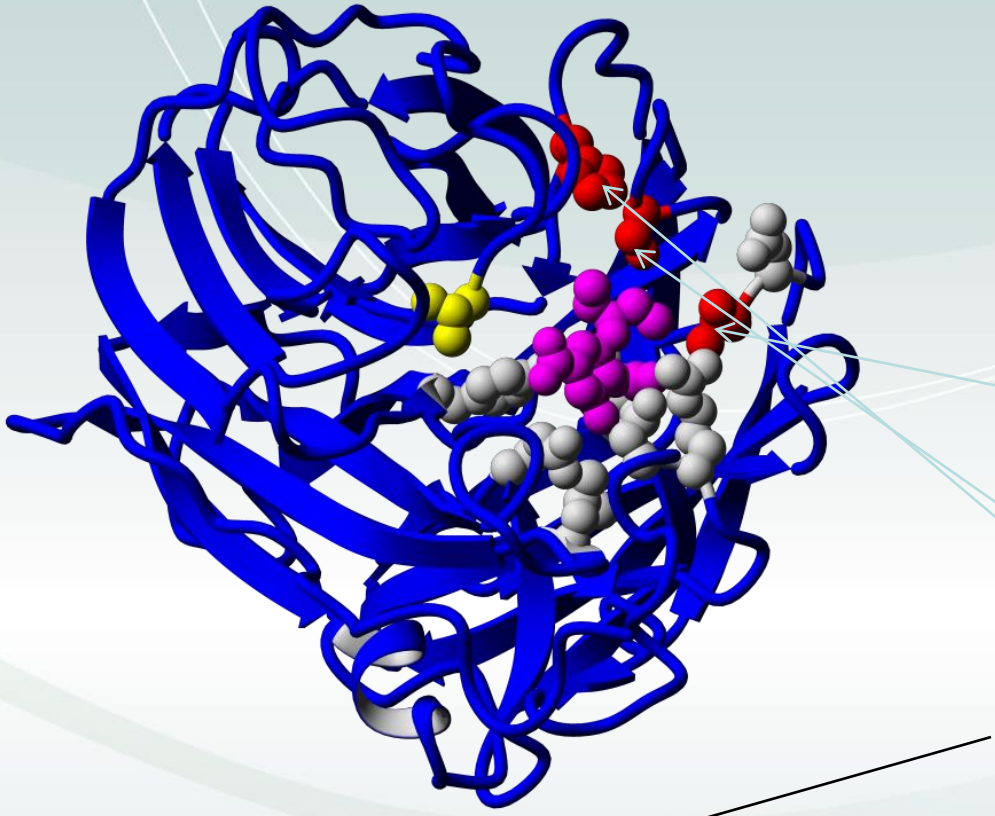
The odds ratio, indicating strength of association to passage history, for mutant versus wildtype virus is indicated. Mutations with <10 samples with any passage information were omitted (e.g. K156E). (-) indicates that 10–30 records with passage information were available, and no reports were indicated in this passage history.
[a]Occurrence of mutation in all 16740 A(H1N1)pdm09 sequences on GISAID and/or Genbank, regardless of passage history up to December 2012.
[b]Occurrence of mutant or wildtype in all A(H1N1)pdm09 sequences on GISAID with passage history information.
[c]% Occurrence of mutant in all A(H1N1)pdm09 sequences on GISAID with passage history information.
doi:10.1371/journal.ppat.1003354.t004

Guarnaccia T, Carolan LA, Maurer-Stroh S, Lee RTC, et al. (2013)
Antigenic Drift of the Pandemic 2009 A(H1N1) Influenza Virus in a Ferret Model.
PLoS Pathog 9(5): e1003354. doi:10.1371/journal.ppat.1003354

GISAID

New drug sensitivity altering mutations

Neuraminidase and Tamiflu (pink)



amino acid of NA with distance<5 from G39	position	mutation occurred in H1N1	frequency of the mutation
R	118	-	-
E	119	G	1
		K	1
L	134	S	1
D	151	N	1
R	152	S	1
R	156	-	-
W	179	-	-
S	180	P	1
N	222	K	1
		S	1
I	223	K	2
		V	1
		R	2
L	224	-	-
R	225	-	-
T	226	A	1
E	228	-	-
S	247	N	5
H	275	Y	48
E	277	-	-
E	278	-	-
R	293	-	-
N	295	-	-
G	345	-	-
V	346	I	2
R	368	-	-
Y	402	H	1

Position	# occ mut
275	48
247	5
223	5

H5N1 in Laos:
"S246N, identified in two isolates, reduced the sensitivity of isolate A/chicken/Laos/13/08 to oseltamivir by 24-fold as a single mutation"
J Gen Virol. 2010 Apr;91(Pt 4):949-59.

GISAID