Interpretation of mutations (FluSurver)

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Outline

I. Intro
II. Tutorial for using FluSurver in EpiFlu
III. Quick Reference for Browsing FluSurver Results
IV. Example Findings with FluSurver
V. Full Reference for Browsing FluSurver Results

From the **sequence** and **structure** we can partially deduce important **properties** of the virus

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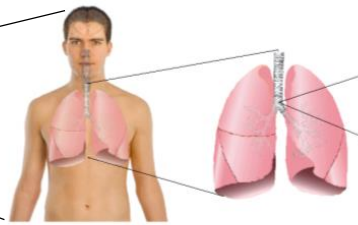
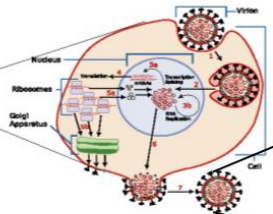
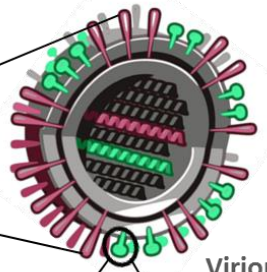
- Infect different hosts
- More or less severe
- Spread more easily
- Antigenic drift, drug resistance

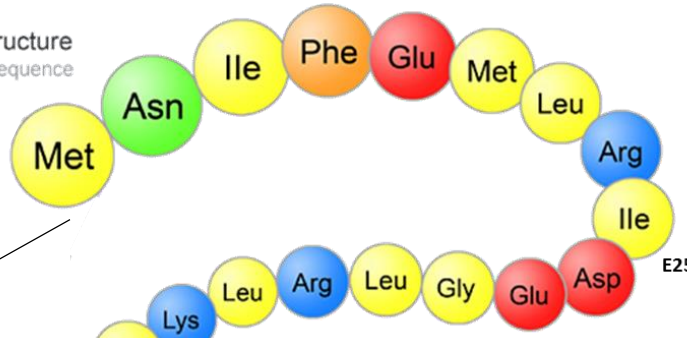




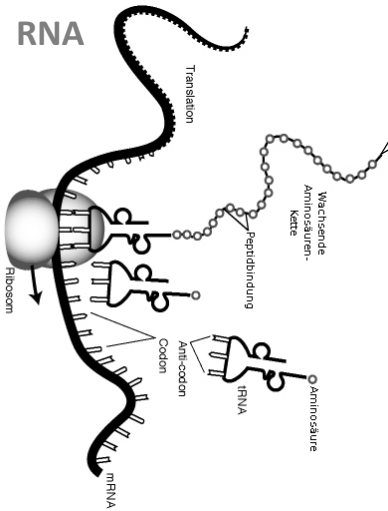


Virion
Viral Particle

Primary Structure
Amino Acid Sequence



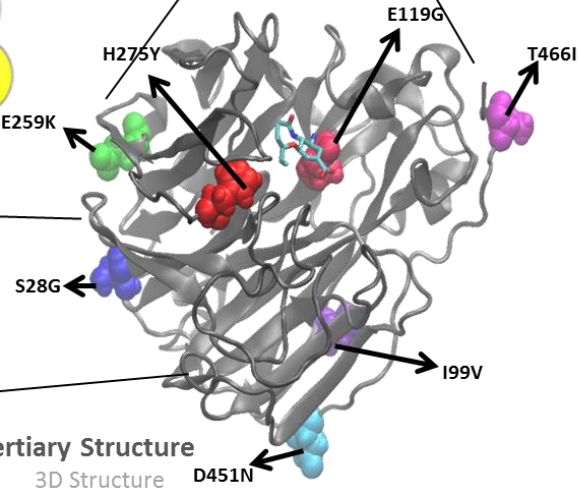
RNA



Secondary Structure
Regular Sub-Structures

α-helix

β-sheet



Protein Tertiary Structure
3D Structure

Simply paste/upload your sequence(s):

http://flusurver.bii.a-star.edu.sg

…or analysis menu from GISAID's EpiFlu

Get list of identified mutations

Result for comparison with reference selection: H1N1_Human_2009_California07 | Back to Reference Selection

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
HA_H1N1_Human_2009_Norway3206-3 gi|269978854|gb|ACZ56080.1|	HA A/California/07/2009(H1N1) find closest related sequences	99.117	100.000	5	P100S, P154S, S220T, D239G, I338V show in structure

Get exhaustive annotation for each mutation

The FluSurver in a nutshell

Geographic

Alternative numberings

Structure models

Temporal

Structural interactions

Effect with Literature links

HA D239G

Key to alternative position numbering:

239	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
HA1 225	Classical H3N2 strain numbering
HA1 222	Classical H1N1 strain numbering
Chosen reference:	
Position in reference:	
AA in reference:	D
AA in query:	

Mutation HA D239G already occurred 194 times (1.37% of all samples with HA sequence) in 28 countries. The first strain with this mutation, collected in April 2009, was A/Texas/11/2009(H1N1). The mutation most recently occurred in strain A/New York/09/2014(H1N1), collected in April 2014. (see map)

See detailed global statistics for this position

A mutation at the position equivalent to **HA 239** has been reported in the literature to be related to host specificity shift.

A combination of mutations including the position equivalent to **HA 239** has been reported in the literature to be related to host specificity shift.

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

See all interactions for this position

PubMed search for this mutation (including alternative numbering)

Known effect(s) of mutations at position equivalent to your mutation:

Protein: HA
Influenza type: Avian, Human H1N1 (2009)
Mutation (as in paper): D222G or D225G
neutral AA: D
neg. eff. AA: G
Effect: host specificity shift

Comment:

HA D239G is also referred to in the literature as D222G or D225G using alternative (e.g. seasonal H1/H3) numberings. It has been found to alter host cell receptor specificity from human alpha-2,6 to also include avian-like alpha-2,3 [illegible] which are common in ciliated human cells of the lower respiratory tract. While this mutation has been found in higher proportions in severe cases, it is IMPORTANT to note that it also can occur as a [illegible] its effect would only be relevant for surveillance if the mutation is also found in the original clinical sample.

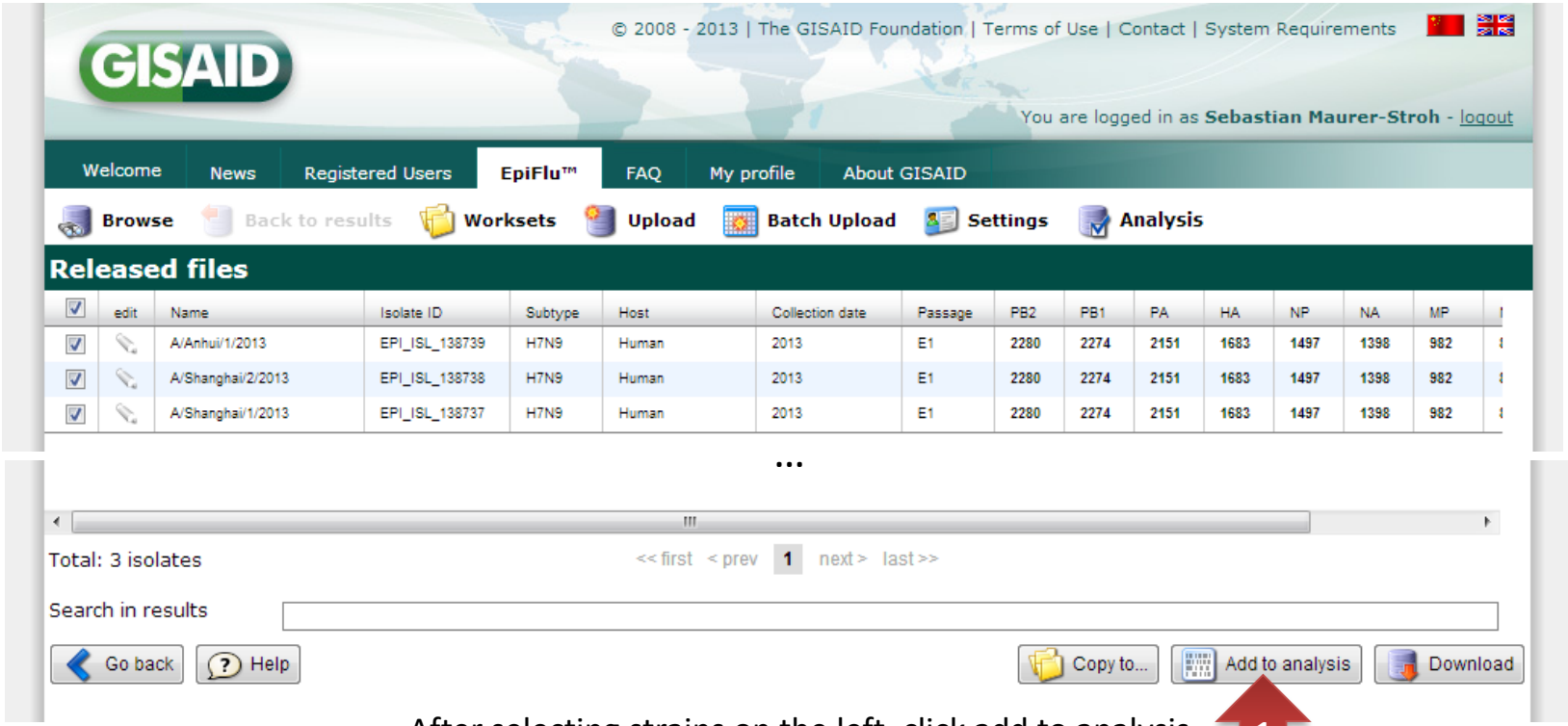
Literature reference

(Mutation D222G or D225G in the paper is at an equivalent position of the mutation in your query)

Section II

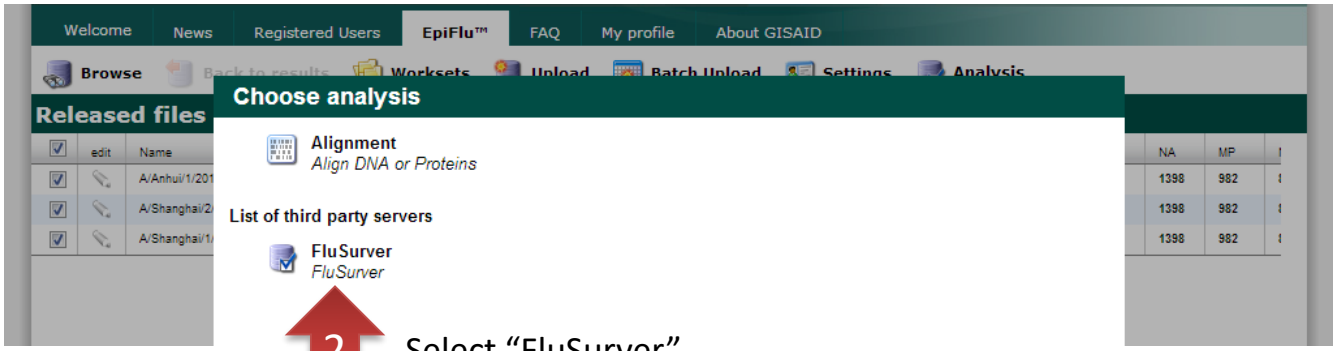
TUTORIAL FOR USING FLUSURVER IN EPIFLU

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



After selecting strains on the left, click add to analysis

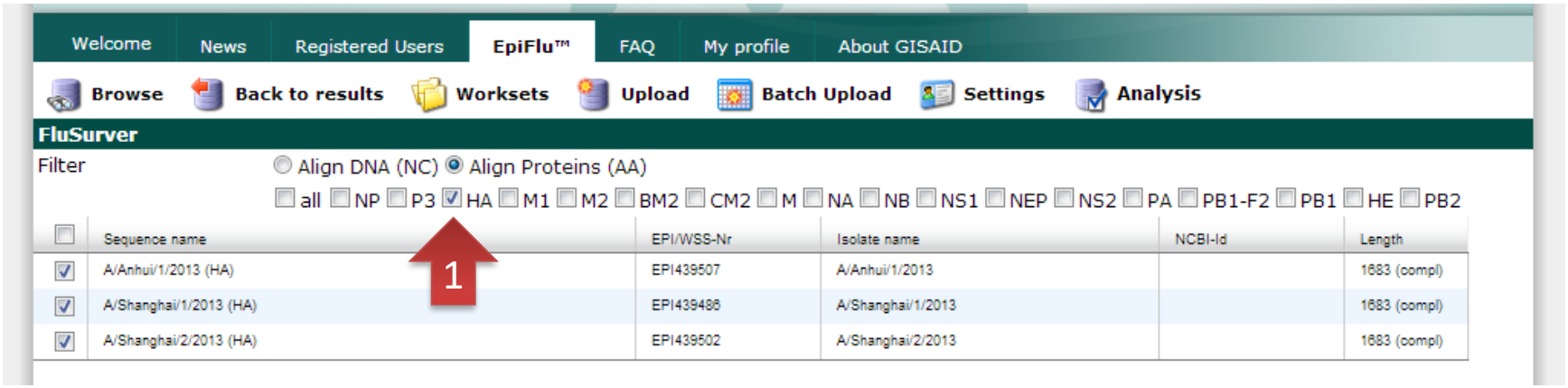
1

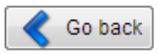


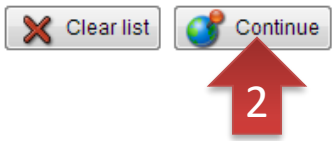
2

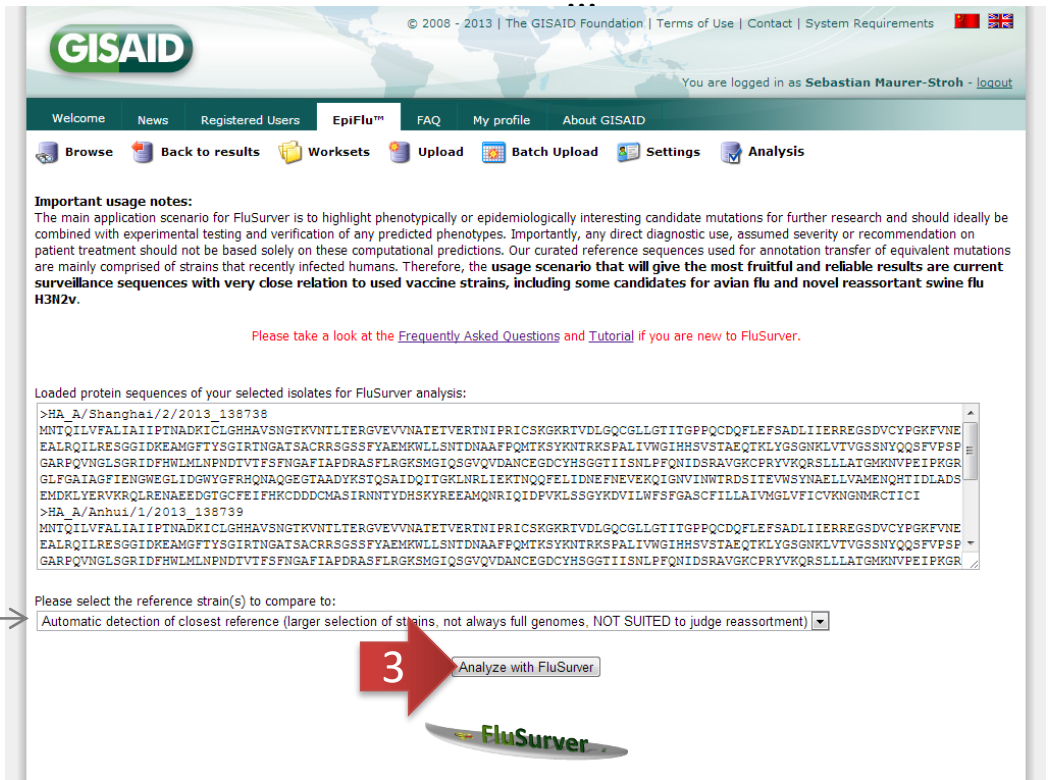
Select “FluSurver”

Next steps: Select proteins to analyze[1] , e.g. HA, then click on continue [2], wait for submission form to load and then click “Analyze with FluSurver” [3].



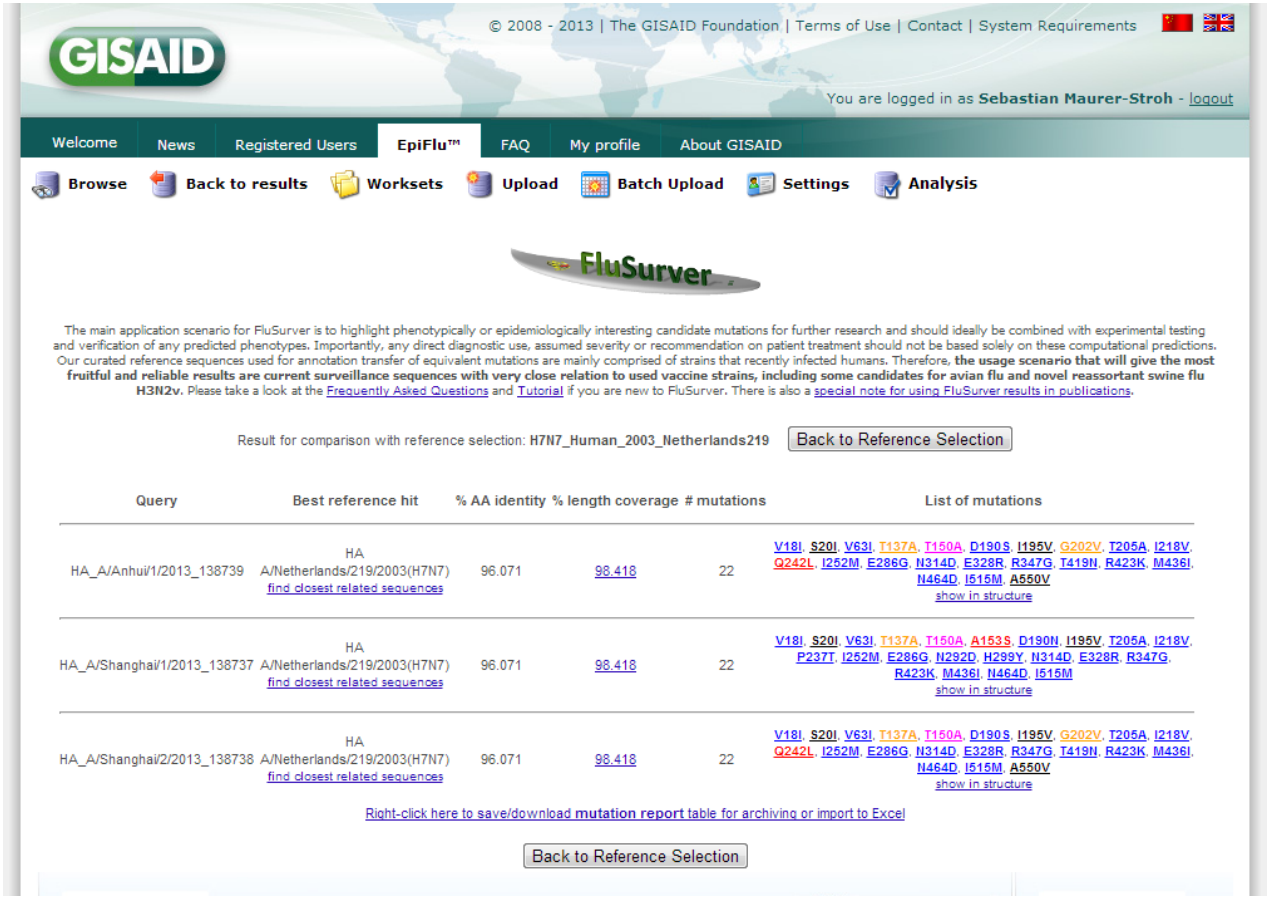






Note: unless one wants to compare to a specific selected reference strain, the drop-down option can be left at default which is an automatic detection of the closest reference.

Warning:
In the current EpiFlu version, there can be a server time-out if too many sequences are selected, <20 sequences for analysis should work fine.



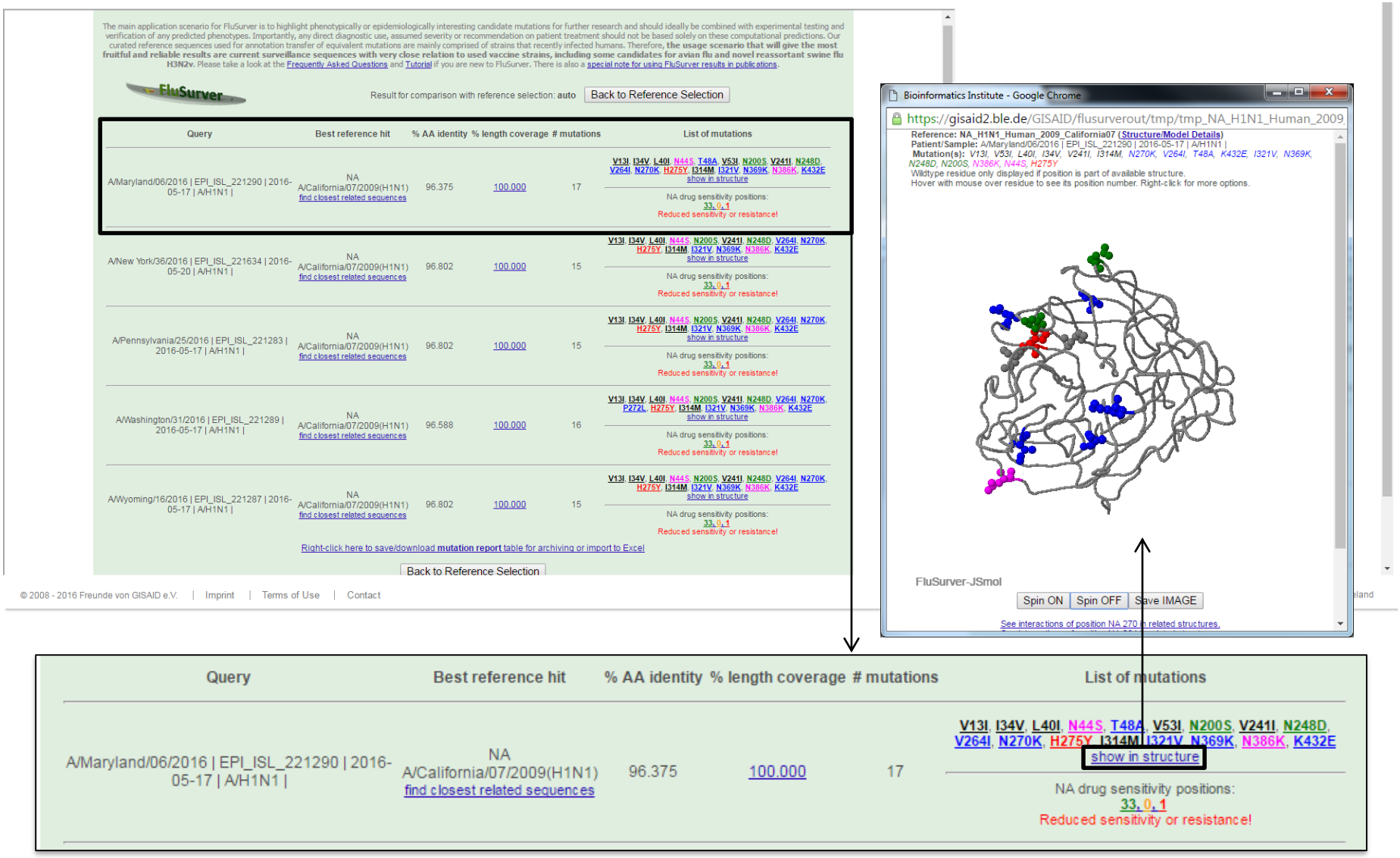
For each of the query sequences, there are six columns of information generated in the result summary page. From here, users may proceed to look at the query sequence's alignment to the reference strain, get more information on each mutation, generate a structural view of all the mutations in the query sequence ("show in structure") or view a summary of the mutations in a table to download (at end of results).

More details on browsing the results further can be found online at:
http://flusurver.bii.a-star.edu.sg/help/tutorialpage.html#part2

Section III

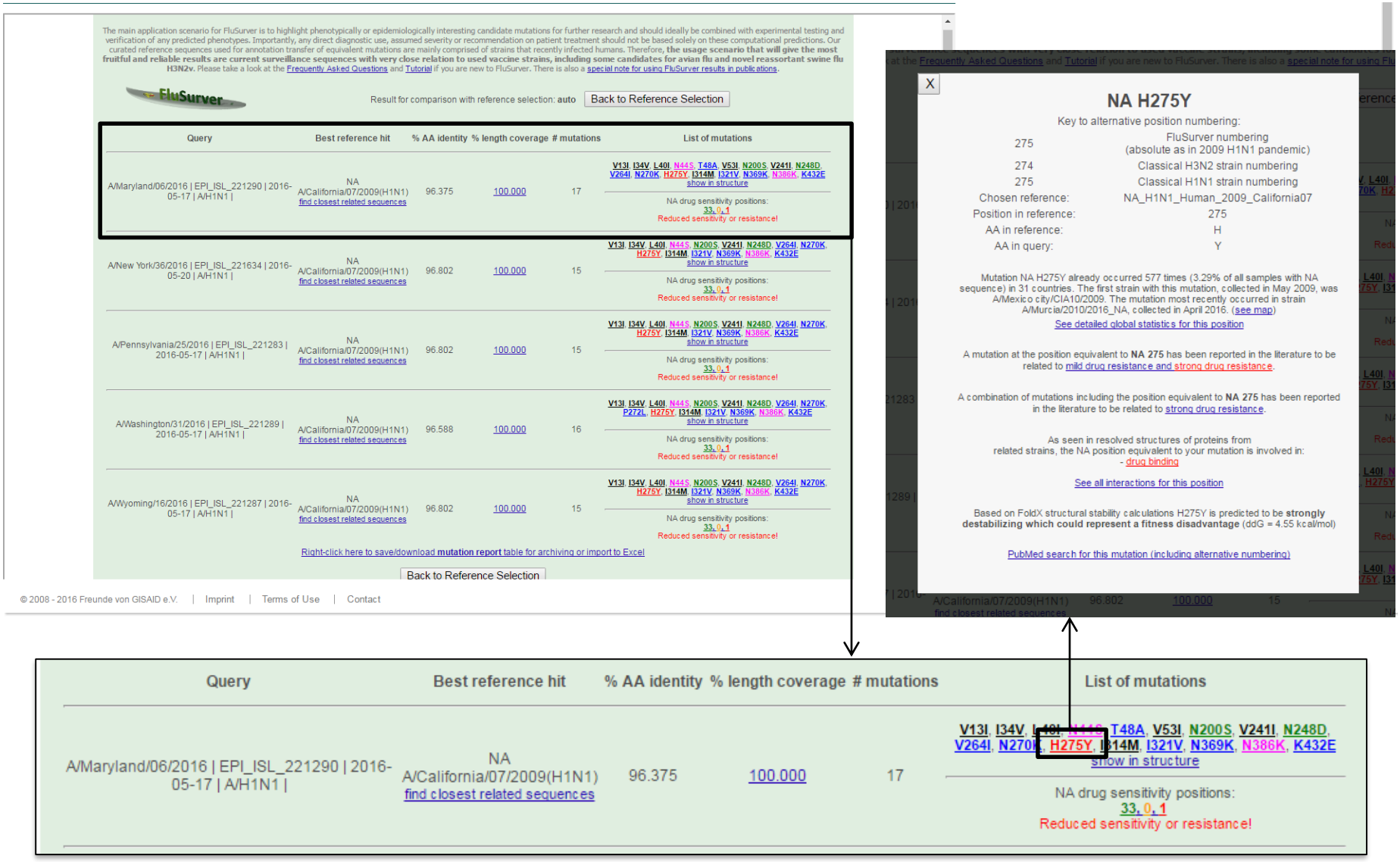
QUICK REFERENCE FOR BROWSING FLUSURVER RESULTS

Mutation identification in sequence and 3D structure



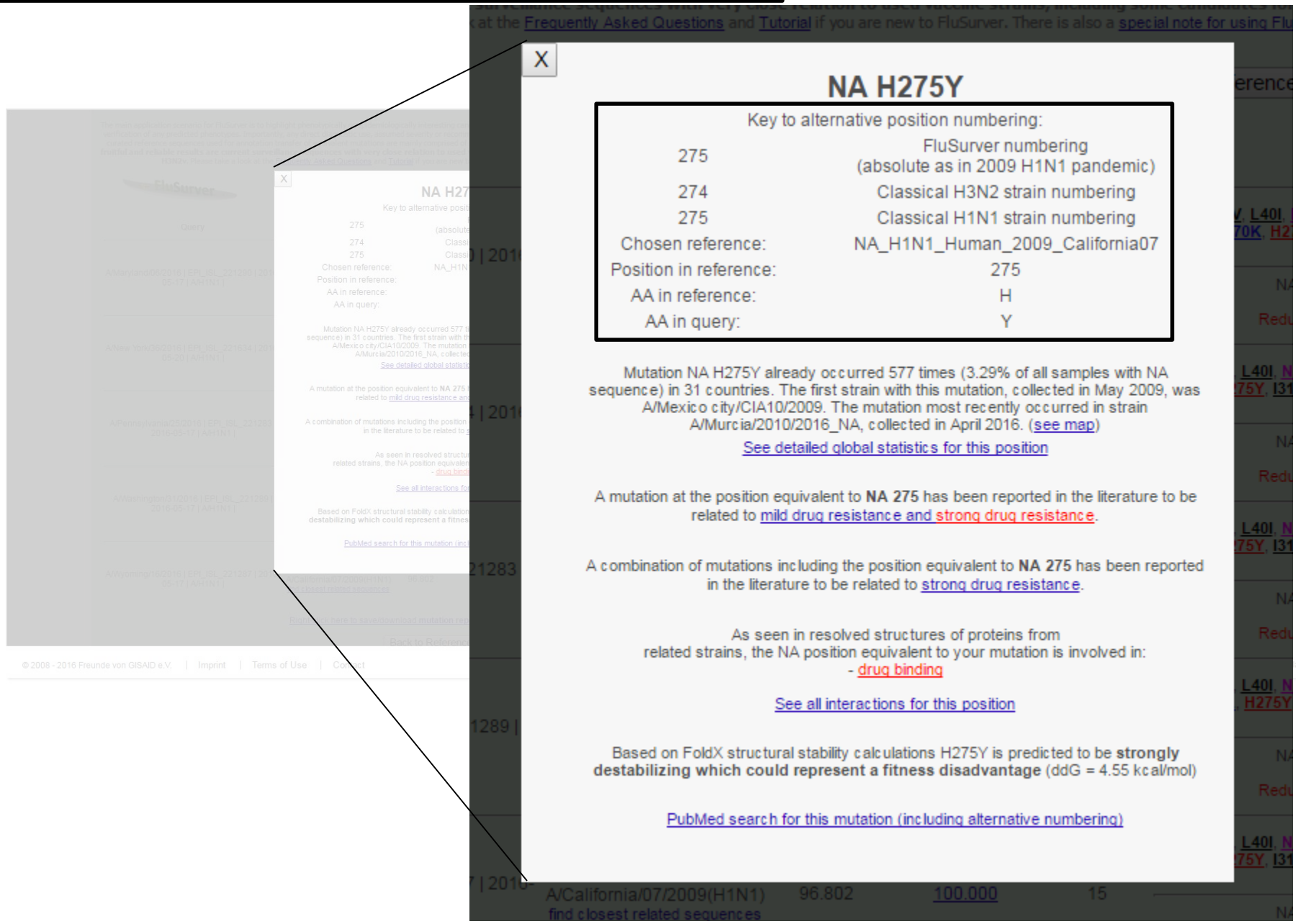
Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
A/Maryland/06/2016 | EPI_ISL_221290 | 2016-05-17 | A/H1N1 |	NA A/California/07/2009(H1N1) find closest related sequences	96.375	100.000	17	V13I, I34V, L40I, N44S, T48A, V53I, N200S, V241I, N248D, V264I, N270K, H275Y, I314M, I321V, N369K, N386K, K432E show in structure NA drug sensitivity positions: 33, 0, 1 Reduced sensitivity or resistance!

Detailed mutation information



Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
A/Maryland/06/2016 | EPI_ISL_221290 | 2016-05-17 | A/H1N1 |	NA A/California/07/2009(H1N1) find closest related sequences	96.375	100.000	17	V13I, I34V, L40I, N44S, T48A, V53I, N200S, V241I, N248D, V264I, N270K, H275Y, I314M, I321V, N369K, N386K, K432E show in structure NA drug sensitivity positions: 33, 0, 1 Reduced sensitivity or resistance!

Alternative numbering information

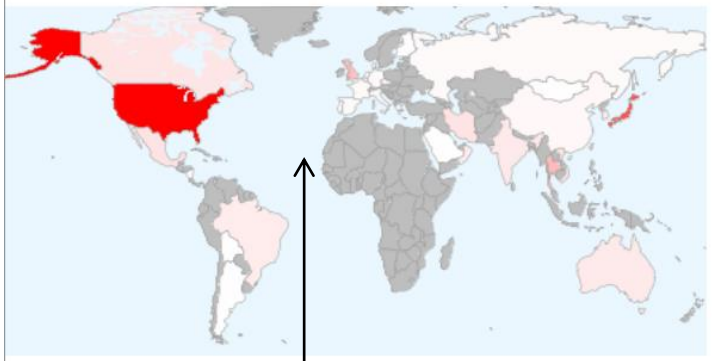


Geospatial and temporal occurrence

The city with **red** label indicates first appearance of the mutation. City with **yellow** label indicate later appearance of the mutation. The city with the most recent appearance of the mutation has the **green** label. Number in the label indicates frequency of occurrence of the mutation in that city. A do in the label indicates that there are 10 or more occurrences in that city.

As there are too many cities with viral isolates carrying this mutation, cities with number of occurrences below 7 are not labeled in the map above.

Map of countries with the NA H275Y mutation



Number of occurences

NA H275Y

Key to alternative position numbering:

275	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
274	Classical H3N2 strain numbering
275	Classical H1N1 strain numbering
Chosen reference:	NA_H1N1_Human_2009_California07
Position in reference:	275
AA in reference:	H
AA in query:	Y

Mutation NA H275Y already occurred 577 times (3.29% of all samples with NA sequence) in 31 countries. The first strain with this mutation, collected in May 2009, was A/Mexico city/CIA10/2009. The mutation most recently occurred in strain A/Murcia/2010/2016_NA, collected in April 2016. (see map)

See detailed global statistics for this position

A mutation at the position equivalent to NA 275 has been reported in the literature to be

A combination

related

Based on F
destabilizing

Pub

A/California/07/200
find closest related s

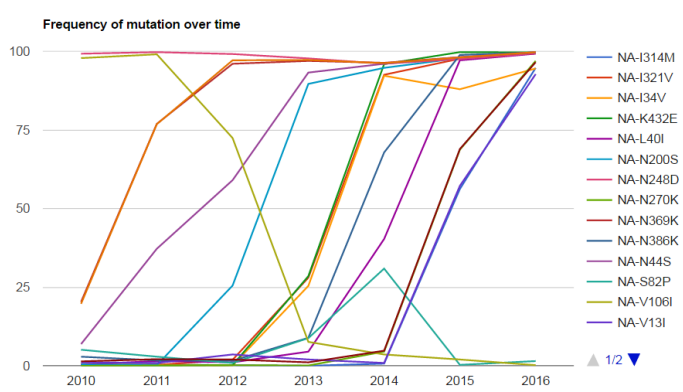
Mutation statistics for NA at position 275

AA	# Occ.	%	Geo Distribution	Co-occurrences
X	57	0.32		
Y	577	3.29	(geo)	(co-occur)
-	17	0.10		
H	16891	96.28	reference aa	reference aa
R	1	0.01		(co-occur)
ALL	17543	100.00		

Sequences were compared to reference strain A/California/07/2009 ACQ63272.
Last updated on Jun 25th 2016 by Raphael Tze Chuen Lee

Above are the occurrences of all amino acid residues at position 275 in NA. For statistics of all position in NA in this subtype click here: H1N1_NA_mutations_table

https://gisaid2.ble.de/GISAID/flusurverout/MUTATIONS/H1N1_Human_2009_California07/H1N1_NA_new_mutations_table.html



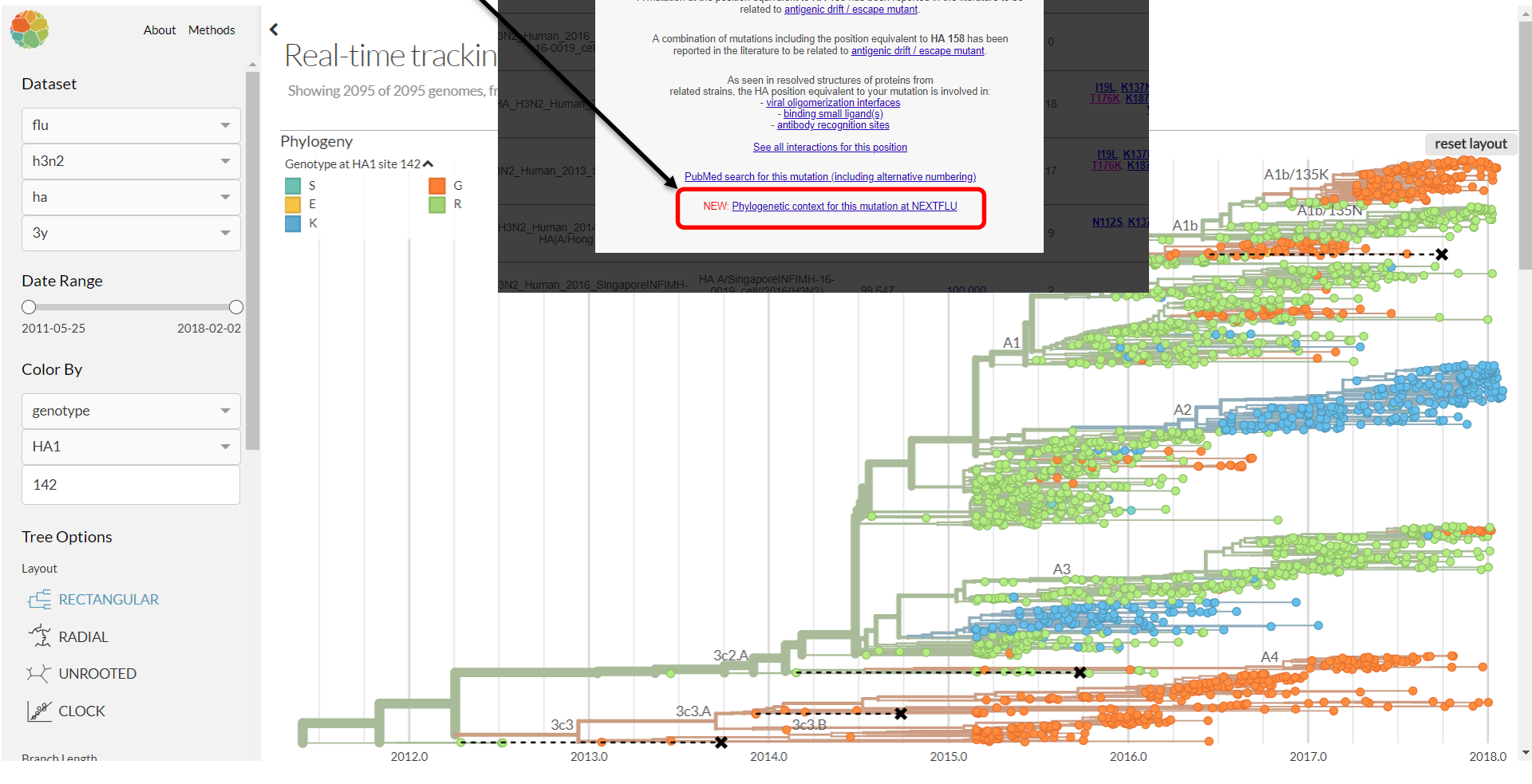
The line chart above shows the average yearly frequency of mutations in NA. Only mutations that were present in more than 30 percent of circulating strains in any of the years were represented in t will appear to be empty if there are no mutations that met this threshold in the protein.

Accession	Protein	Strain	WildtypeAA	Position	MutatedAA	Frequency	Date_of_collection(YYYYMMDD)	Remarks
EPI_ISL_61168	NA	A/Italy/137/2009	M	1	L	1	20090701	(co-occur)
EPI_ISL_105930	NA	A/Kenya/071/2010	N	2	H	2 (geo)	20101129	(co-occur)
EPI_ISL_61372	NA	A/Poland/303/2009	N	2	I	4 (geo)	20090710	(co-occur)
EPI_ISL_103740	NA	2012702607_209232_v1_6	N	2	D	1	20111212	(co-occur)
EPI_ISL_30173	NA	A/New_York/3099/2009	N	2	S	1	20090429	(co-occur)

New: Direct link to phylogenetic context of mutation in NEXTFLU

nextflu

By Trevor Bedford & Richard Neher



Phenotype information

Protein: NA

Influenza type: Human H1N1 (2006)

Mutation (as in paper): H274Y

neutral AA: H

neg. eff. AA: Y

Effect: strong drug resistance (drug name in comments)

Comment:

Tamiflu but not Relenza resistance (Table 3).

Literature reference

(Mutation H274Y in the paper is at an equivalent position of the mutation in your query)

NA H275Y

Key to alternative position numbering:

275	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
274	Classical H3N2 strain numbering
275	Classical H1N1 strain numbering
Chosen reference:	NA_H1N1_Human_2009_California07
Position in reference:	275
AA in reference:	H
AA in query:	Y

Mutation NA H275Y already occurred 577 times (3.29% of all samples with NA sequence) in 31 countries. The first strain with this mutation, collected in May 2009, was A/Mexico city/CIA10/2009. The most recently occurred in strain A/Murcia/2010/2016_NA, collected in April 2016. (see map)

See detailed global statistics for this position

A mutation at the position equivalent to **NA 275** has been reported in the literature to be related to mild drug resistance and strong drug resistance.

A combination of mutations including the position equivalent to **NA 275** has been reported in the literature to be related to strong drug resistance.

As seen in resolved structures of proteins from related strains, the NA position equivalent to your mutation is involved in:
- drug binding

See all interactions for this position

Based on FoldX structural stability calculations H275Y is predicted to be **strongly destabilizing which could represent a fitness disadvantage** (ddG = 4.55 kcal/mol)

PubMed search for this mutation (including alternative numbering)

PubMed | influenza AND (neuraminidase OR NA) AND (H275Y OR H274Y)

Create RSS Create alert Advanced

Format: Summary Sort by: Most Recent Send to

Search results

Items: 1 to 20 of 323 << First < Prev Page 1 of 17 Next > Last >>

1. Competitive Fitness of **Influenza** B Viruses Possessing E119A and **H274Y Neuraminidase** Inhibitor Resistance-Associated Substitutions in Ferrets.
Pascua PN, Marathe BM, Burnham AJ, Vogel P, Webby RJ, Webster RG, Govorkova EA.
PLoS One. 2016 Jul 28;11(7):e0159847. doi: 10.1371/journal.pone.0159847. eCollection 2016.
PMID: 27466813 Free Article
Similar articles

2. **Influenza** A(H1N1)pdm09 virus exhibiting enhanced cross-resistance to oseltamivir and peramivir due to a dual **H275Y**/G147R substitution, Japan, March 2016.
Takashita E, Fujisaki S, Shirakura M, Nakamura K, Kishida N, Kuwahara T, Shimazu Y, Shimomura T, Watanabe S, Odagiri T; **Influenza** Virus Surveillance Group of Japan.
Euro Surveill. 2016 Jun 16;21(24). doi: 10.2807/1560-7917.ES.2016.21.24.30258.
PMID: 27336226
Similar articles

Literature-curated genotype to phenotype effect annotations

Effect Type	# Annotations
host specificity shift	136
virulence	106
antigenic drift / escape mutant	84
strong drug sensitivity change	40
mild drug sensitivity change	30
other	23
total (2019)	**419**

Example:

Known effect(s) of mutations at position equivalent to your mutation:

Protein: HA
Influenza type: Avian, Human H1N1 (2009)
Mutation (as in paper): D222G or D225G
neutral AA: D
neg. eff. AA: G
Effect: host specificity shift

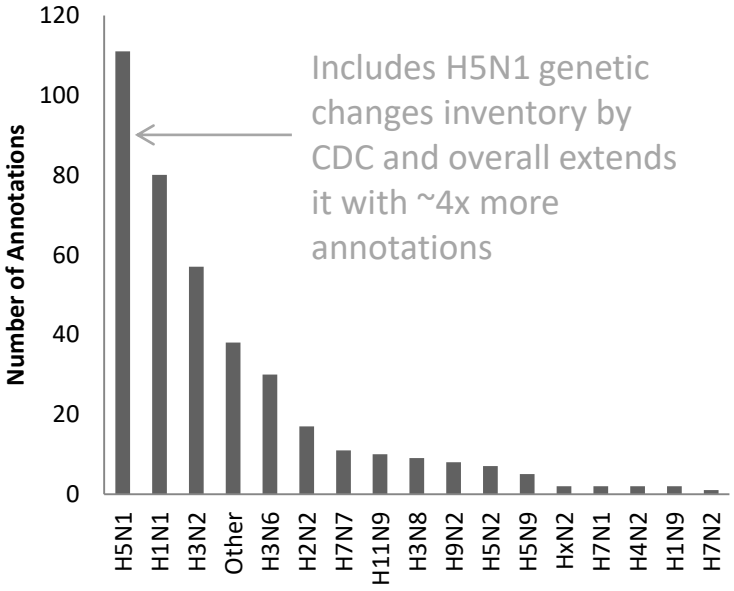
Comment:

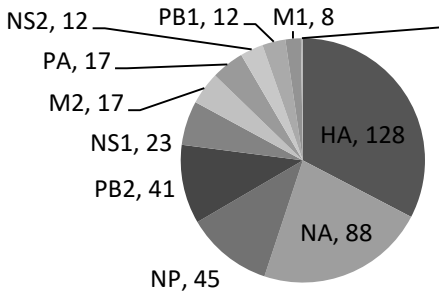
HA D239G is also referred to in the literature as D222G or D225G using alternative (e.g. seasonal H1/H3) numberings. It has been found to alter host cell receptor specificity from human alpha-2,6 to also include avian-like alpha-2,3 sialic acid which is more common in ciliated human cells of the lower respiratory tract. While this mutation has been found in higher proportions in severe cases, it is IMPORTANT to note that it also can occur as egg or cell culture adaptation. Therefore, its effect would only be relevant for surveillance if the mutation is also found in the original clinical sample.

Literature reference

(Mutation D222G or D225G in the paper is at an equivalent position of the mutation in your query)

Annotations per Subtype



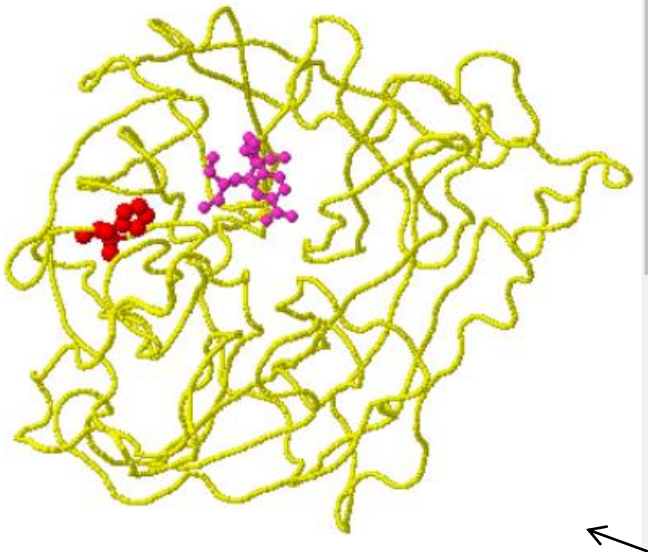


Annotations per Protein Type

Structural interaction and stability information

NA_275_1B9V_A_273 - Google Chrome

https://gisaid2.ble.de/GISAID/flusurverout/INTERACTIONS/NA/27!



Spin ON | Spin OFF | Save IMAGE

Description:
The mutation position (red atoms) corresponds to position 273 on viral A (yellow backbone) and is within 5 A from drug RA2 (pink atoms).

NA H275Y

Key to alternative position numbering:

275	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
274	Classical H3N2 strain numbering
275	Classical H1N1 strain numbering
Chosen reference:	NA_H1N1_Human_2009_California07
Position in reference:	275
AA in reference:	H
AA in query:	Y

Mutation NA H275Y already occurred 577 times (3.29% of all samples with NA sequence) in 31 countries. The first strain with this mutation, collected in May 2009, was A/Mexico city/CIA10/2009. The mutation most recently occurred in strain A/Murcia/2010/2016_NA, collected in April 2016. (see map)

See detailed global statistics for this position

A mutation at the position equivalent to **NA 275** has been reported in the literature to be related to mild drug resistance and strong drug resistance.

A combination of mutations including the position equivalent to **NA 275** has been reported in the literature to be related to strong drug resistance.

As seen in resolved structures of proteins from related strains, the NA position equivalent to your mutation is involved in:
- drug binding

See all interactions for this position

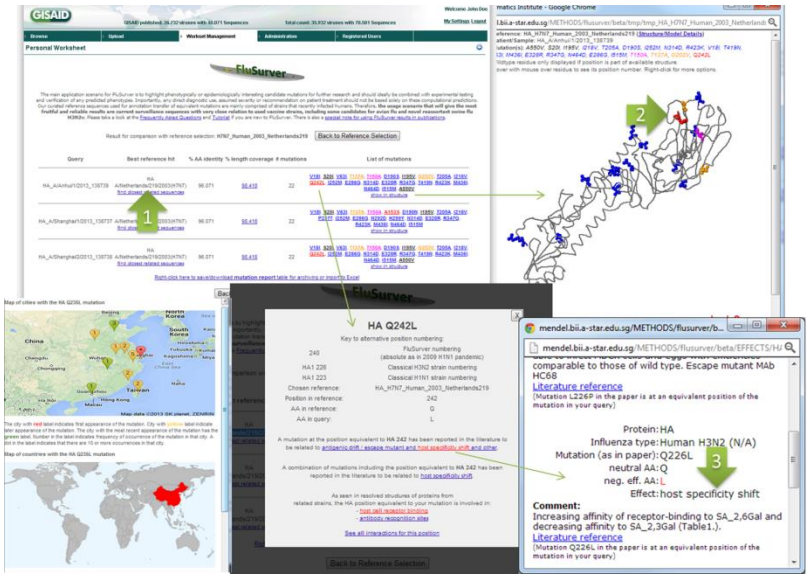
Based on FoldX structural stability calculations H275Y is predicted to be **strongly destabilizing which could represent a fitness disadvantage** (ddG = 4.55 kcal/mol)

PubMed search for this mutation (including alternative numbering)

A/California/07/2009(H1N1) 96.802 100.000 15

find closest related sequences

Analysis – FluSurver for Mutation Interpretation



Important disclaimer:

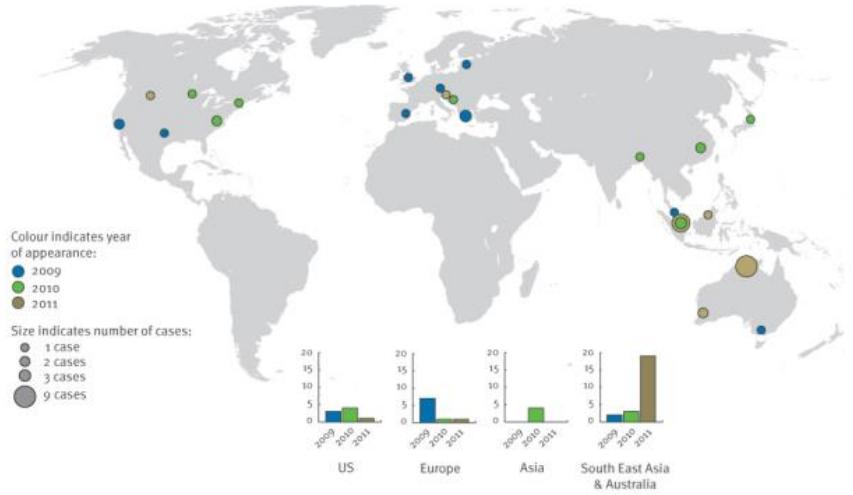
FluSurver makes it very easy to link mutations with prior literature and potential phenotypic effects.

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.

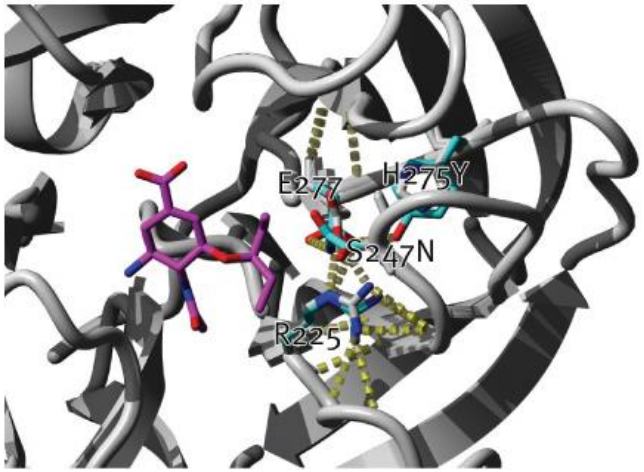
Section IV

EXAMPLE FINDINGS WITH FLUSURVER

New drug sensitivity altering mutation NA S247N

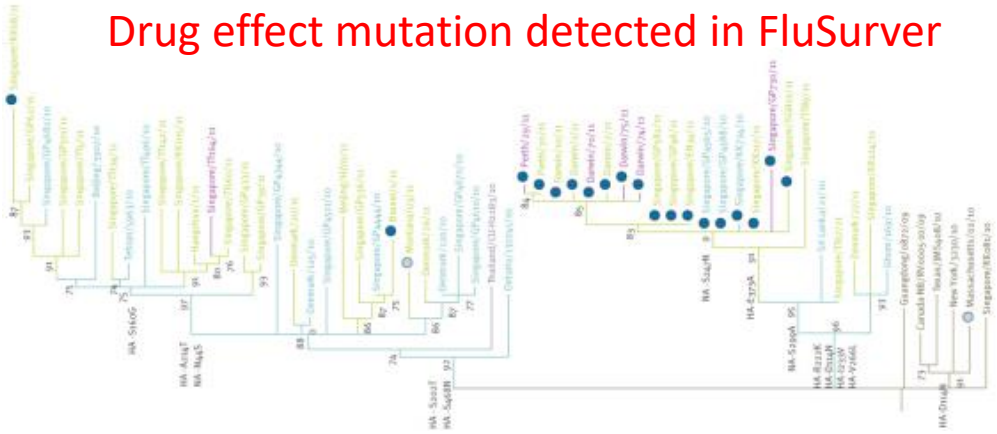


Global occurrence of new variant



Structural context of mutation

Drug effect mutation detected in FluSurver



Phylogenetic context of new variant

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

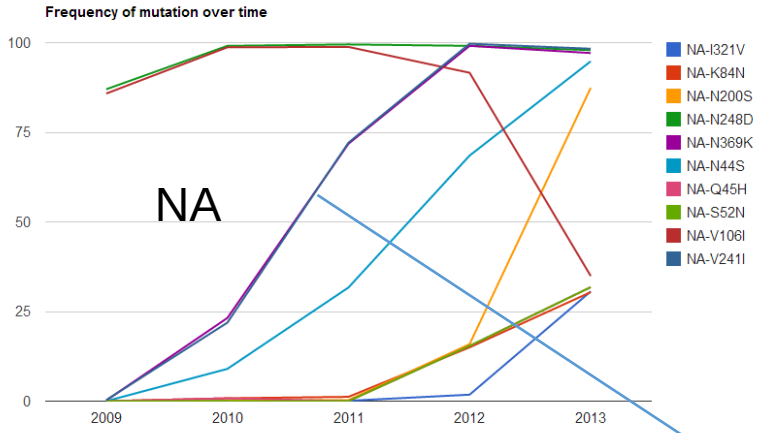
Experimentally measured increase of IC50 for Tamiflu by 6-fold and Relenza by 3-fold but normally administered dose of drugs still sufficient.

Hurt AC, Lee RT, Leang SK, Cui L, Deng YM, Phuah SP, Caldwell N, Freeman K, Komadina N, Smith D, Speers D, Kelso A, Lin RT, Maurer-Stroh S, Barr IG. *Increased detection in Australia and Singapore of a novel influenza A(H1N1)2009 variant with reduced oseltamivir and zanamivir sensitivity due to a S247N neuraminidase mutation.* Euro Surveill. 2011 Jun 9;16(23). pii: 19884.

Bioinformatics Institute
A*STAR

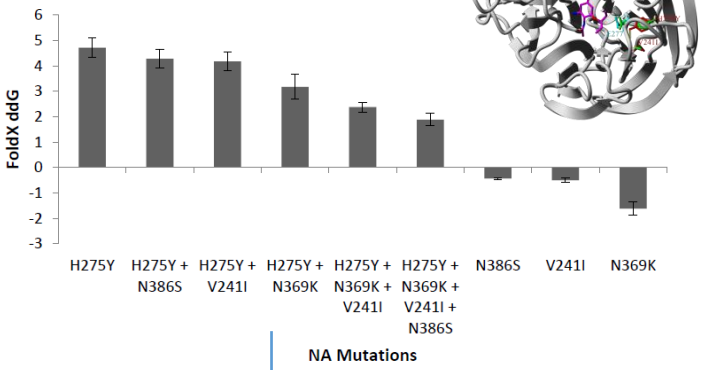
Collaboration with NPHL/Ministry of Health Singapore and WHO Collaborating Centre for Reference and Research on Influenza.

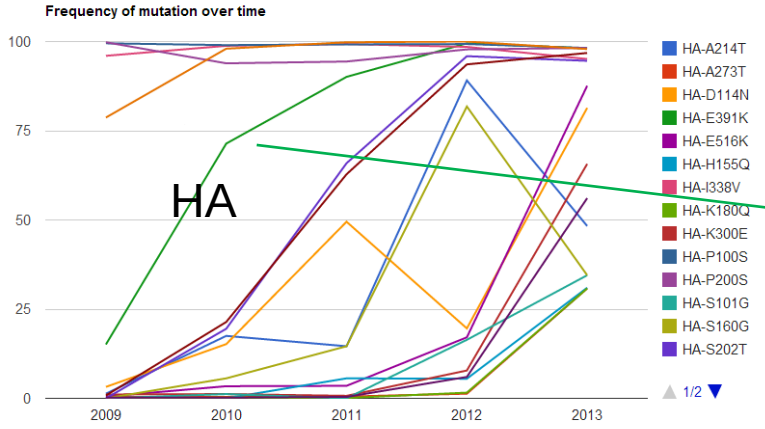
Mutation frequency pattern highlights relevant changes



Temporal frequency plot in FluSurver

FoldX stability for N1pdm in FluSurver



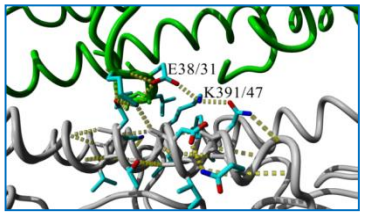


New H275Y permissive mutations

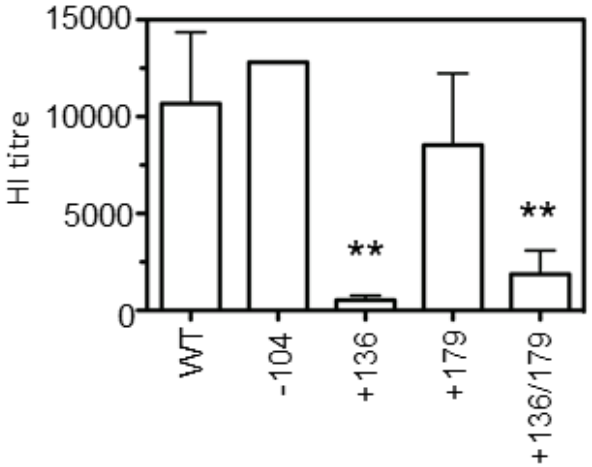
Hurt *et al.* J Infect Dis. 2012 Jul 15;206(2):148-57.
Butler *et al.* PLoS Pathog. 2014 Apr 3;10(4):e1004065.

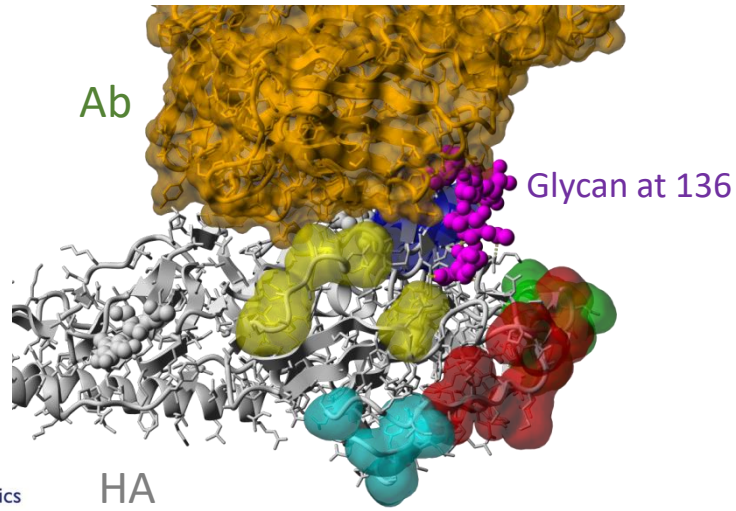
Change in pH-dependency of fusion

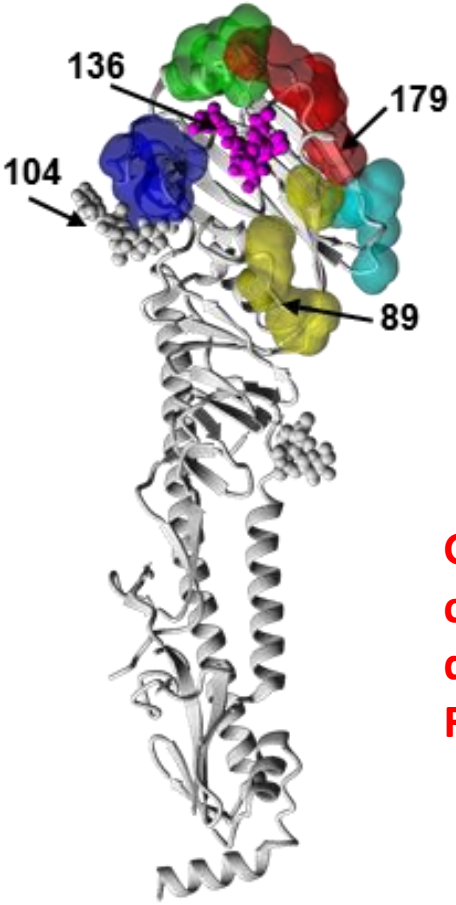
Maurer-Stroh *et al.* PLoS Curr. 2010 Jun 1;2:RRN1162.
Cotter *et al.* PLoS Pathog. 2014 Jan;10(1):e1003831.



Addition of Glycosylation to Influenza A Virus Hemagglutinin Modulates Antibody-Mediated Recognition of H1N1 2009 Pandemic Viruses. Job ER, Deng YM, Barfod KK, Tate MD, Caldwell N, Reddiex S, Maurer-Stroh S, Brooks AG, Reading PC.
J Immunol. 2013 Mar 1;190(5):2169-77.







Glycosylation change detection in FluSurver

Addition of a glycan to A(H1N1)pdm HA through a K136N mutation was associated with resistance to neutralizing Abs and showed enhanced growth in A(H1N1)pdm-vaccinated mice, consistent with evasion of Ab-mediated immunity in vivo.

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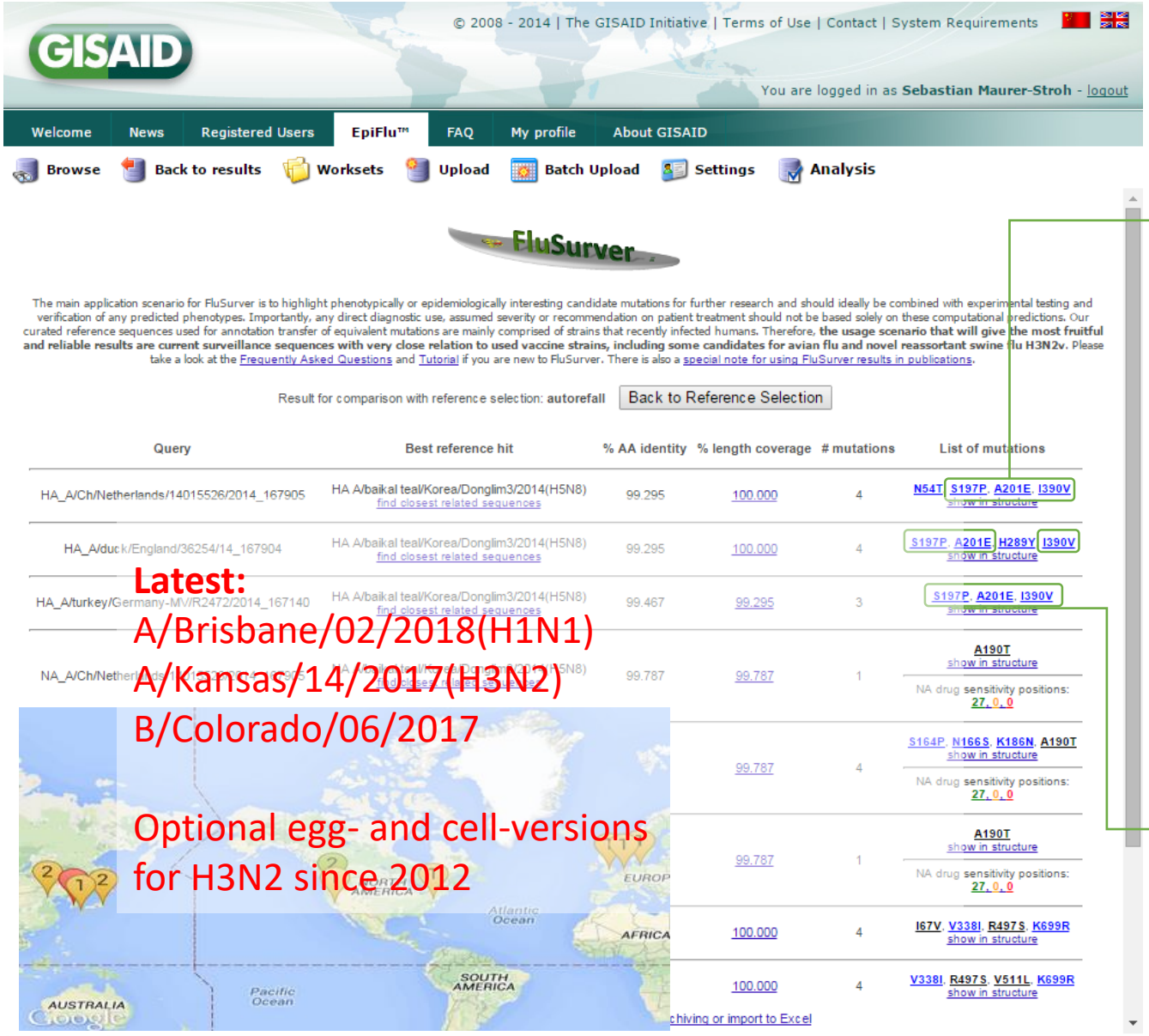
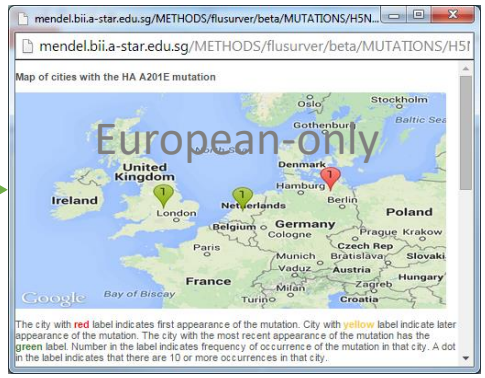
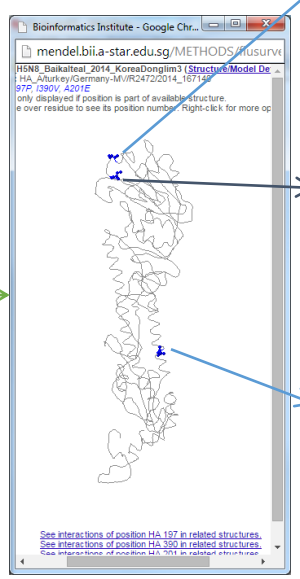
New reference strains (e.g. H7N9, H5N8) constantly added to FluSurver

E.g. H5N8 Emerg Infect Dis. 2015 May;21(5):860-3.





E.g. 3 HA mutations relative to Korean H5N8 shared among European cases



A201E (A189E)
Strong surface change at common epitope (antibody binding site)

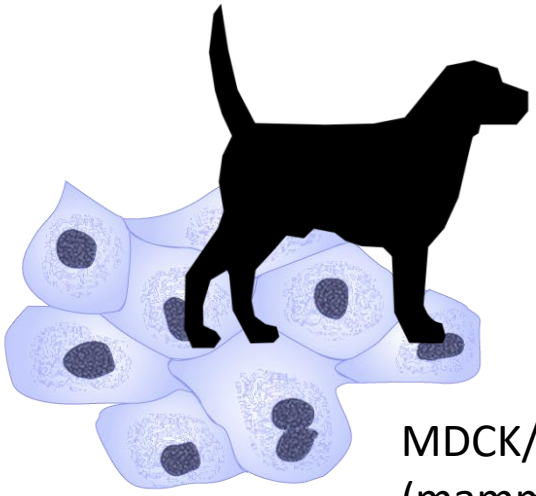
S197P (S185P)
Structural change near receptor binding site but effect not yet reported in literature

I390V (I45V)
Conservative change at site recognized by "universal" stem antibodies

H5N8 analysis available on GISAID platform

Virus culture/passage bias is a common problem for flu vaccine production!

Original clinical sample



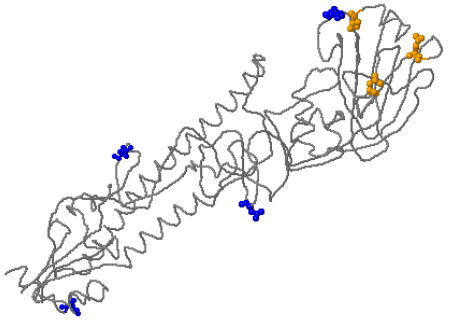
MDCK/SIAT cells
(mammalian host)

Often like original

Eggs (avian host)
also used for vaccine production!

Egg adapted

+ H172Q, G202V, S235Y



Chen H, Deng Q, Ng SH, Lee RT, Maurer-Stroh S, Zhai W. *Dynamic Convergent Evolution Drives the Passage Adaptation across 48 Years' History of H3N2 Influenza Evolution.* Mol Biol Evol. 2016 Sep 7. pii: msw190.

New: you can choose egg-derived reference H3N2s (default) or cell-derived versions!

Comparison of current and last 3 H3N2 vaccine strains in cell-derived vs egg-derived versions:

The main application scenario for FluSurver is to highlight phenotypically or ep
verification of any predicted phenotypes. Importantly, any direct diagnostic us
curated reference sequences used for annotation transfer of equivalent mut
fruitful and reliable results are current surveillance sequences with very close relation to used vaccine strains, including some candidates for avian flu and novel reassortant swine flu H3N2v. Please take a look at the Frequently Asked Questions and Tutorial if you are new to FluSurver. There is also a special note for using FluSurver results in publications.

FluSurver

Result for comparison with reference selection: **autorefall** Back to Reference Selection

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
cell_HA_H3N2_Human_2012_Texas50_cell	HA A/Texas/50/2012(H3N2) find closest related sequences	99.647	100.000	2	V202G, F235S show in structure
cell_HA_H3N2_Human_2013_Switzerland9715293	HA A/Switzerland/9715293/2013(H3N2) find closest related sequences	99.470	100.000	3	R156I, V202G, X235S show in structure
cell_HA_H3N2_Human_2014_HongKong4801_cell HA|A/Hong	HA A/HongKong/4801/2014(H3N2) find closest related sequences	99.470	100.000	3	S112N, K176T, P210L show in structure
cell_HA_H3N2_Human_2016_SingaporeINFIMH-16-0019_cell	HA A/SingaporeINFIMH-16-0019//2016(H3N2) find closest related sequences	99.647	100.000	2	K176T, P210L show in structure

Right-click here to save/download **mutation report** table for archiving or import to Excel

Warning: this reference selection includes sequences of strains without complete genomes, e.g. only HA and NA available/included. Therefore, hits of other segments to different reference strains do not represent reassortments. Please see also this help section regarding reassortments.

Back to Reference Selection

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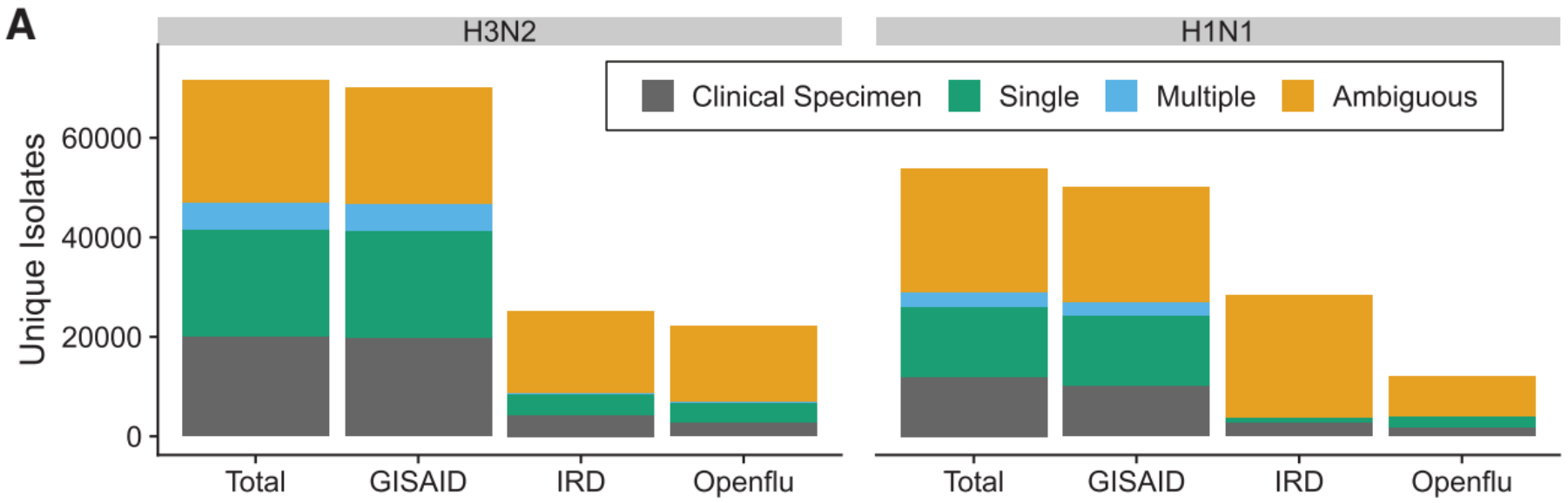
FluSurver

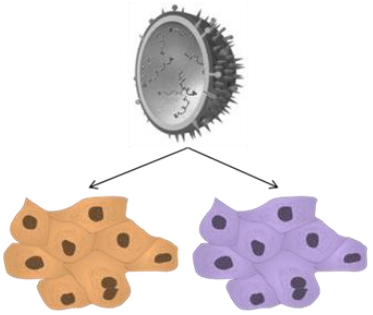
Comparison of database content with passage annotation



DuPai CD, McWhite CD, Smith CB, Garten R, Maurer-Stroh S, Wilke CO. *Influenza passaging annotations: what they tell us and why we should listen.* Virus Evol. 2019 Jun 30;5(1):vez016.

Passage bias sites and host specificity mutations

(A) Same virus (H3, H1s, H1p)
passaged in different cell types

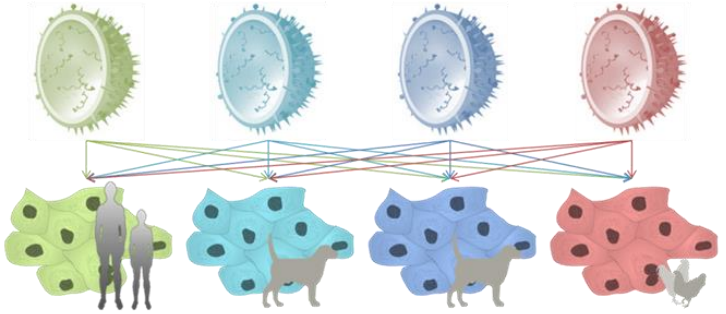


Direct passage bias evidence

~800 strains

4 Viruses (H3, H1s, H1p, H5)
4 cell types (ORIGINAL, SIAT, MDCK, EGG)

(B)



Statistical passage bias evidence

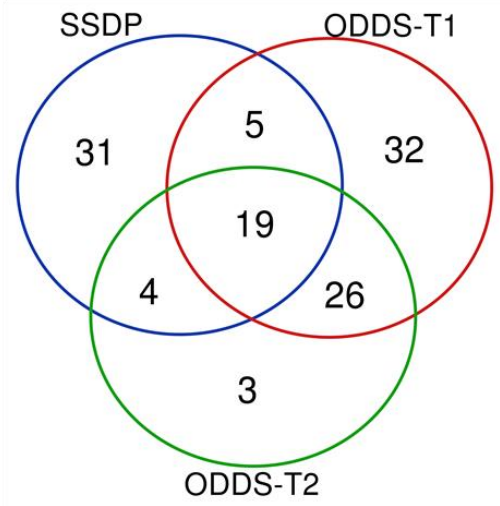
~80000 sequences
2 time periods

SSDP

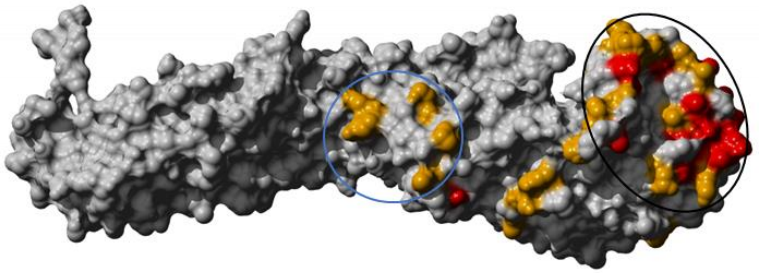
Overlap

ODDS

(C)



(D)



19 sites shared by all 3 sets (red),
35 sites shared by 2 sets (orange)

Lee RTC, Chang HH, Russell CA, Lipsitch M, Maurer-Stroh S. *Influenza A Hemagglutinin Passage Bias Sites and Host Specificity Mutations.* Cells. 2019 Aug 22;8(9). pii: E958.

Influenza quasi-species and FluSurver

Q: What is a quasi-species?
A: A group of viruses that are closely related and co-exist in the host but with a twist or two ...

... TIME & PRESSURE

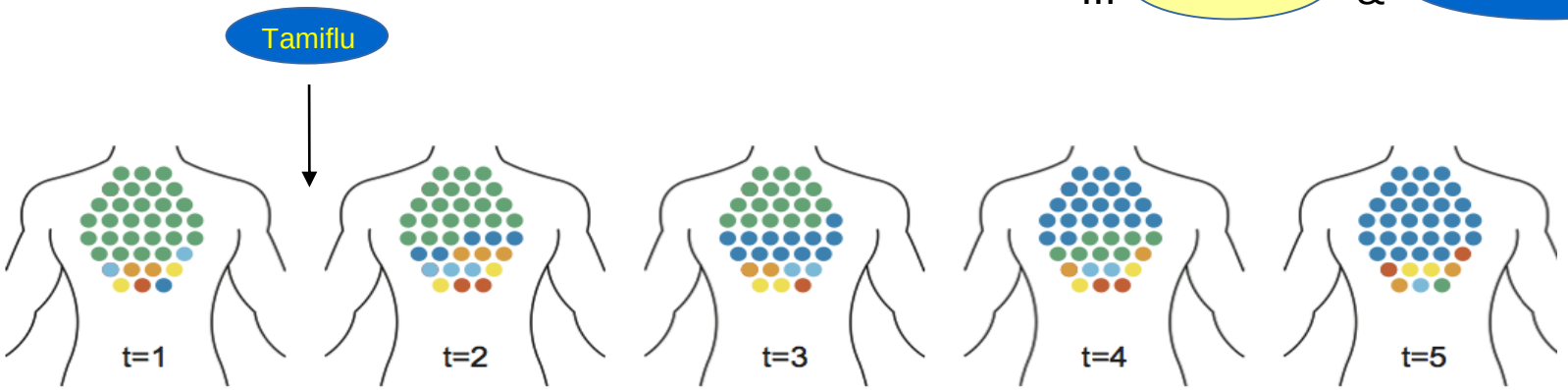
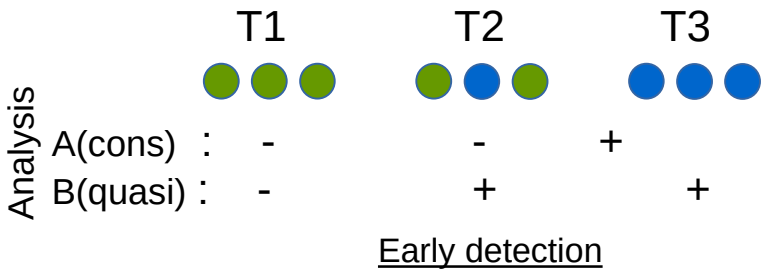
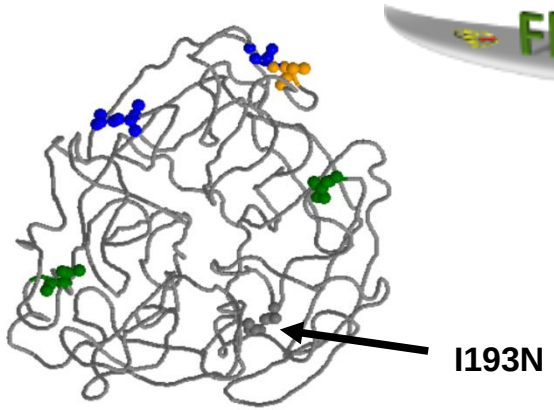


Illustration by Bjorn Koel (Amsterdam UMC)



Early detection



FluSurver

Summary of FluSurver annotations

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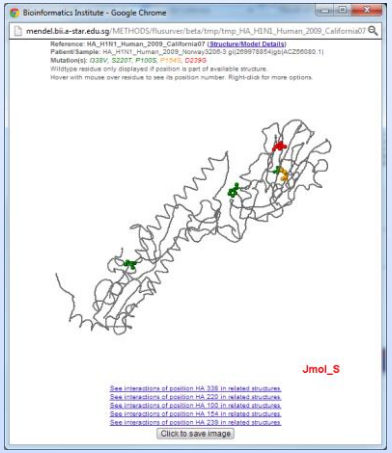
GISAID

Structure

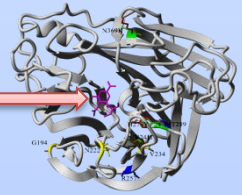


Map mutations to structure

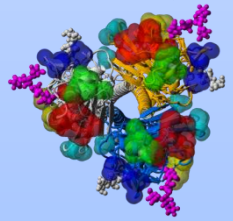
300+ reference homology models

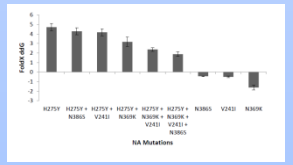
1568	self/oligomerization
975	other small ligand
268	antibody
188	host protein
182	antigen-presenting MHC molecule
132	other viral protein
46	drug
45	nucleic acids
13	host cell receptor
3417	**total interactions for 2062 positions**

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

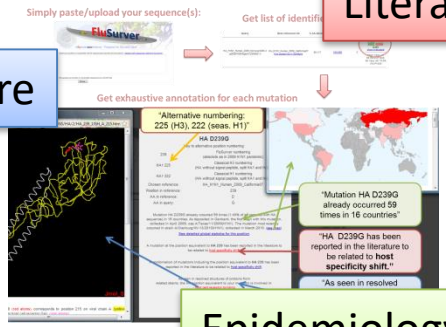
Updated

Literature-curated mutation effect database

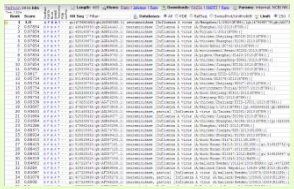
400+ entries

mild drug resistance	30
strong drug resistance	40
virulence	106
antigenic drift / escape mutant	84
host specificity shift	136
other	23

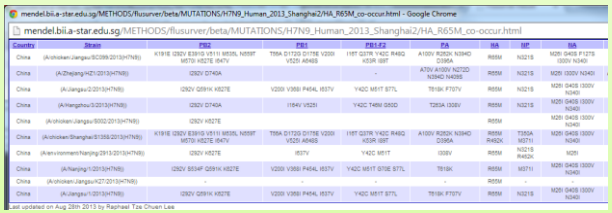
Epidemiology



Closest DB hits



Temporal pattern



Genomic co-occurrence

Regional & global occurrence

FluSurver acknowledgements

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

Sebastian Maurer-Stroh, **Raphael Tze Chuen Lee, Vithiagaran Gunalan, Vachiranee Limviphuvadh, Fernanda L Sirota, Biruhalem Taye, Alvin Han, Han Hao, Dimitar Kenanov, 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:

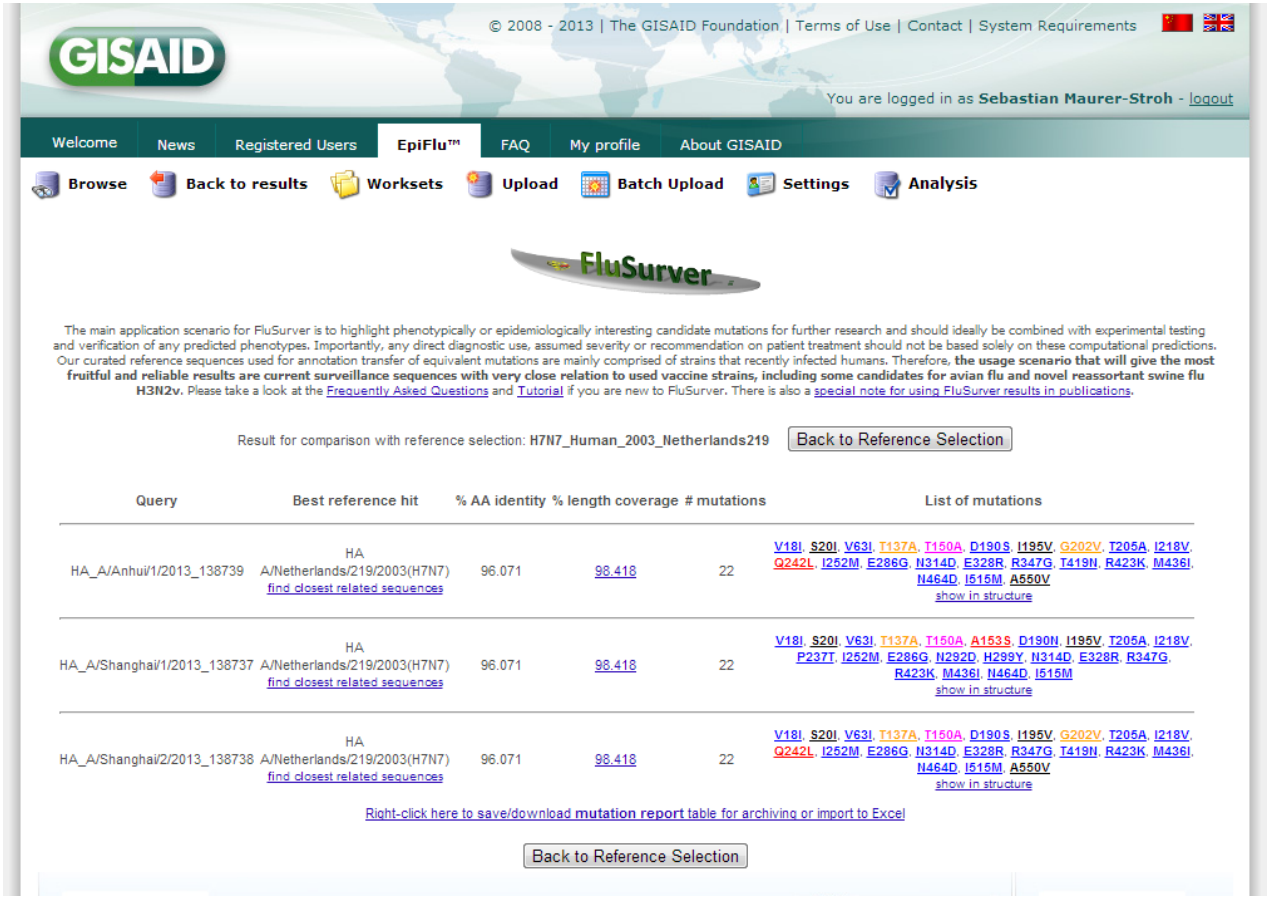
- Genome Institute of Singapore (GIS), Singapore
- INMEGEN Mexico City, Mexico
- Experimental Therapeutics Centre (ETC), Singapore
- Tan Tock Seng Hospital (TTSH), Singapore
- National Public Health Laboratory (NPHL) of the Ministry of Health, Singapore
- IAL Sao Paulo, Brazil
- WHO Collaborating Centre 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
- Centers for Disease Control (CDC) Atlanta, USA
- Research and Policy for Infectious Disease Dynamics (RAPIDD)
- Health Protection Agency of Canada
- Friedrich Loeffler Institute, Germany
- NEXTFLU T. Bedford and R. Neher

... and thank all of you!

Fishing for Flu Mutations since 2009!

Section IV

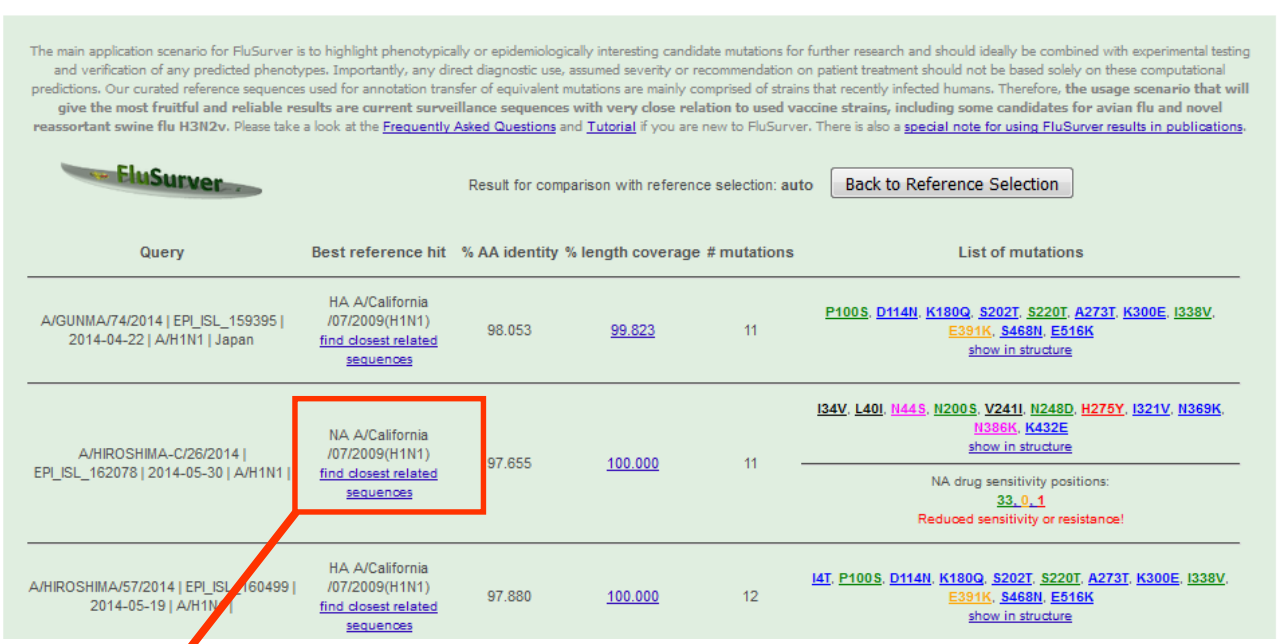
FULL REFERENCE FOR BROWSING FLUSURVER RESULTS



For each of the query sequences, there are six columns of information generated in the result summary page. From here, users may proceed to look at the query sequence's alignment to the reference strain, get more information on each mutation, generate a structural view of all the mutations in the query sequence ("show in structure") or view a summary of the mutations in a table to download (at end of results).

More details on browsing the results further can be found online at:
http://flusurver.bii.a-star.edu.sg/help/tutorialpage.html#part2

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



The main application scenario for FluSurver is to highlight phenotypically or epidemiologically interesting candidate mutations for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Importantly, any direct diagnostic use, assumed severity or recommendation on patient treatment should not be based solely on these computational predictions. Our curated reference sequences used for annotation transfer of equivalent mutations are mainly comprised of strains that recently infected humans. Therefore, **the usage scenario that will give the most fruitful and reliable results are current surveillance sequences with very close relation to used vaccine strains, including some candidates for avian flu and novel reassortant swine flu H3N2v.** Please take a look at the Frequently Asked Questions and Tutorial if you are new to FluSurver. There is also a special note for using FluSurver results in publications.

FluSurver

Result for comparison with reference selection: auto | Back to Reference Selection

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
A/GUNMA/74/2014 | EPI_ISL_159395 | 2014-04-22 | A/H1N1 | Japan	HA A/California /07/2009(H1N1) find closest related sequences	98.053	99.823	11	P100S, D114N, K180Q, S202T, S220T, A273T, K300E, I338V, E391K, S468N, E516K show in structure
A/HIROSHIMA-C/26/2014 | EPI_ISL_162078 | 2014-05-30 | A/H1N1 |	NA A/California /07/2009(H1N1) find closest related sequences	97.655	100.000	11	I34V, L40I, N44S, N200S, V241I, N248D, H275Y, I321V, N369K, N386K, K432E show in structure NA drug sensitivity positions: 33, 0, 1 Reduced sensitivity or resistance!
A/HIROSHIMA/57/2014 | EPI_ISL_160499 | 2014-05-19 | A/H1N1 |	HA A/California /07/2009(H1N1) find closest related sequences	97.880	100.000	12	I4T, P100S, D114N, K180Q, S202T, S220T, A273T, K300E, I338V, E391K, S468N, E516K show in structure

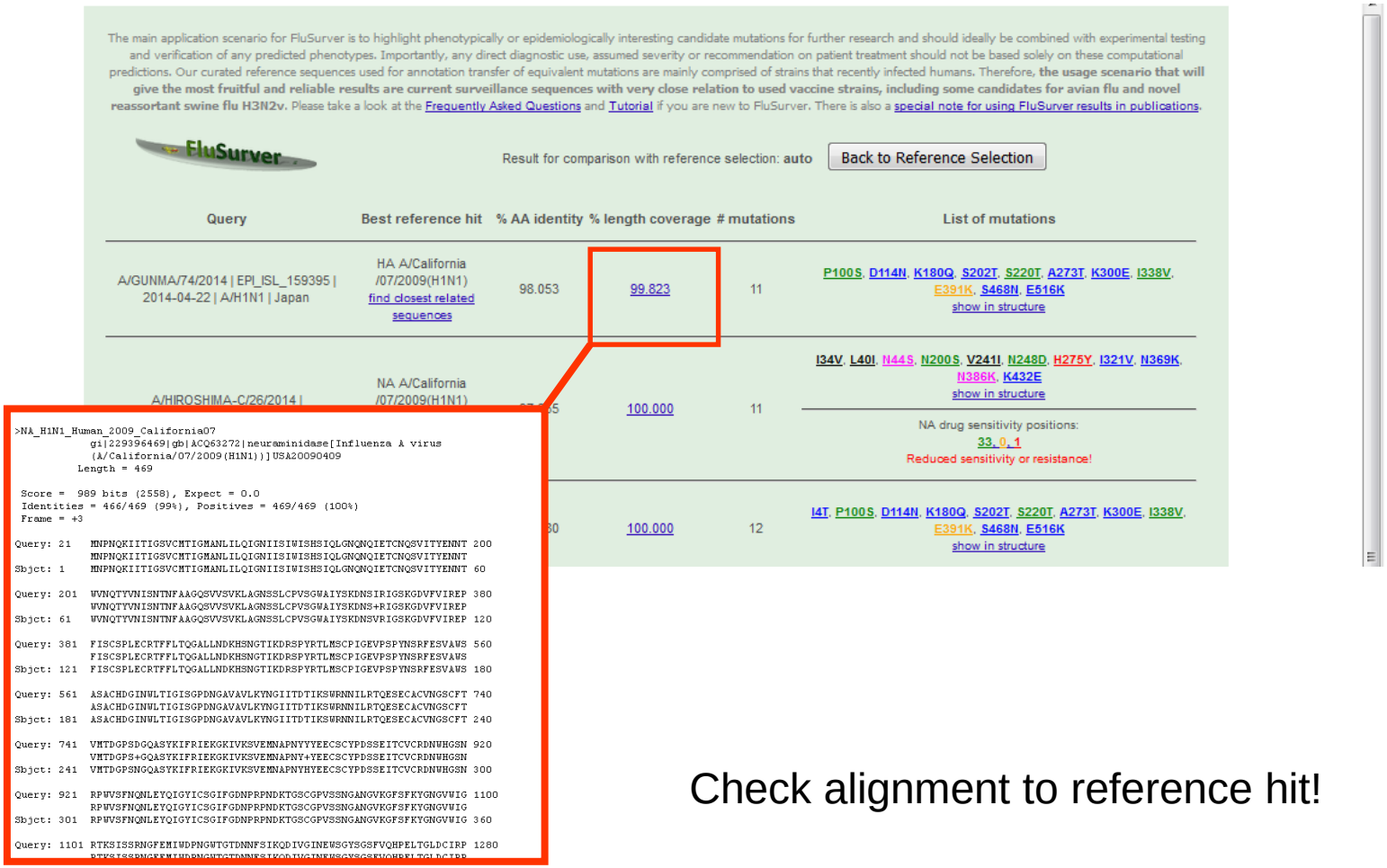
Tachyon 11364 hits (Time: 15.854s) | GenBank FASTA BLink ANNIE Taxonomy PDB UniProt | Length: 469 | Views: Plain | Jalview | Raw | Downloads: FASTA | MAFFT | Raw | Params: internal, NCBI NR-24070523 sequer

Hit Seq | Filter: | Databases: All PDB RefSeq SwissProt/UniProtKB | Limit: 250 1000 None

Rank	Score	Hit Seq
1	1.0	gi|251748198|gb|ACT10319.1| neuraminidase [Influenza A virus (A/Hong Kong/2369/2009(H1N1))]gi|254548844|gb|ACT67256.1| neura
2	0.9914	gi|300117086|gb|ADJ67981.1| neuraminidase, partial [Influenza A virus (A/Perth/262/2009(H1N1))]
3	0.98718	gi|326320245|gb|ADZ53143.1| neuraminidase [Influenza A virus (A/Hong Kong/FFD/2009(H1N1))]
4	0.98294	gi|291219999|gb|ADD84685.1| neuraminidase [Influenza A virus (A/Mexico/InDRE797/2010(H1N1))]
5	0.97872	gi|251833646|gb|ACT22016.1| neuraminidase [Influenza A virus (A/Osaka/180/2009(H1N1))]
6	0.97872	gi|294544923|gb|ADF10109.1| neuraminidase [Influenza A virus (A/Ontario/25913/2009(H1N1))]gi|307071058|gb|ADN24730.1| neuram
7	0.97872	gi|294544441|gb|ADF10049.1| neuraminidase [Influenza A virus (A/Ontario/10016/2009(H1N1))]gi|294544523|gb|ADF10059.1| neuram
8	0.97872	gi|299781814|gb|ADJ40477.1| neuraminidase [Influenza A virus (A/Netherlands/2445b/2009(H1N1))]
9	0.97872	gi|325451706|gb|ADZ13521.1| neuraminidase [Influenza A virus (A/Lyon/48.49/2009(H1N1))]
10	0.97872	gi|294611208|gb|ADF27356.1| neuraminidase [Influenza A virus (A/Taiwan/6663/2009(H1N1))]
11	0.97872	gi|326320207|gb|ADZ53124.1| neuraminidase [Influenza A virus (A/Hong Kong/23369/2009(H1N1))]
12	0.97872	gi|425786025|gb|AFX96841.1| neuraminidase [Influenza A virus (A/Viet Nam/12032005/2009(H1N1))]
13	0.97872	gi|316986112|gb|ADU76312.1| neuraminidase [Influenza A virus (A/England/00380009/2009(H1N1))]gi|316986114|gb|ADU76313.1| neu
14	0.97872	gi|295147036|gb|ADF80503.1| neuraminidase [Influenza A virus (A/Seoul/1870/2009(H1N1))]
15	0.97872	gi|307071034|gb|ADN24718.1| neuraminidase, partial [Influenza A virus (A/Canada-AB/RV2828/2009(H1N1))]

Find closest reference strain and database hits!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



The main application scenario for FluSurver is to highlight phenotypically or epidemiologically interesting candidate mutations for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Importantly, any direct diagnostic use, assumed severity or recommendation on patient treatment should not be based solely on these computational predictions. Our curated reference sequences used for annotation transfer of equivalent mutations are mainly comprised of strains that recently infected humans. Therefore, **the usage scenario that will give the most fruitful and reliable results are current surveillance sequences with very close relation to used vaccine strains, including some candidates for avian flu and novel reassortant swine flu H3N2v.** Please take a look at the Frequently Asked Questions and Tutorial if you are new to FluSurver. There is also a special note for using FluSurver results in publications.

FluSurver

Result for comparison with reference selection: **auto** [Back to Reference Selection]

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
A/GUNMA/74/2014 | EPI_ISL_159395 | 2014-04-22 | A/H1N1 | Japan	HA A/California /07/2009(H1N1) find closest related sequences	98.053	99.823	11	P100S, D114N, K180Q, S202T, S220T, A273T, K300E, I338V, E391K, S468N, E516K show in structure
A/HIROSHIMA-C/26/2014 |	NA A/California /07/2009(H1N1)	[illegible]	100.000	11	I34V, L40I, N44S, N200S, V241I, N248D, H275Y, I321V, N369K, N386K, K432E show in structure NA drug sensitivity positions: 33, 0, 1 Reduced sensitivity or resistance!
		[illegible]	100.000	12	I4T, P100S, D114N, K180Q, S202T, S220T, A273T, K300E, I338V, E391K, S468N, E516K show in structure

```
>NA_H1N1_Human_2009_California07
          gi|229396469|gb|ACQ63272|neuraminidase[Influenza A virus
          (A/California/07/2009(H1N1))]USA20090409
        Length = 469

 Score =  989 bits (2558), Expect = 0.0
 Identities = 466/469 (99%), Positives = 469/469 (100%)
 Frame = +3

Query: 21   MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNT 200
            MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNT
Sbjct: 1    MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNT 60

Query: 201  WVNQTYVNISNTNFAAGQSVVSVKLAGNSSLCPVSGWAIYSKDNSIRIGSKGDVFVIREP 380
            WVNQTYVNISNTNFAAGQSVVSVKLAGNSSLCPVSGWAIYSKDNS+RIGSKGDVFVIREP
Sbjct: 61   WVNQTYVNISNTNFAAGQSVVSVKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVFVIREP 120

Query: 381  FISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRTLMSCPIGEVPSPYNSRFESVAWS 560
            FISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRTLMSCPIGEVPSPYNSRFESVAWS
Sbjct: 121  FISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRTLMSCPIGEVPSPYNSRFESVAWS 180

Query: 561  ASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFT 740
            ASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFT
Sbjct: 181  ASACHDGINWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFT 240

Query: 741  VMTDGPSDGQASYKIFRIEKGKIVKSVEMNAPNYYYEECSCYPDSSEITCVCRDNWHGSN 920
            VMTDGPS+GQASYKIFRIEKGKIVKSVEMNAPNY+YEECSCYPDSSEITCVCRDNWHGSN
Sbjct: 241  VMTDGPSNGQASYKIFRIEKGKIVKSVEMNAPNYHYEECSCYPDSSEITCVCRDNWHGSN 300

Query: 921  RPWVSFNQNLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIG 1100
            RPWVSFNQNLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIG
Sbjct: 301  RPWVSFNQNLEYQIGYICSGIFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIG 360

Query: 1101 RTKSISSRNGFEMIWDPNGWTGTDNNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRP 1280
            RTKSISSRNGFEMIWDPNGWTGTDNNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRP
```

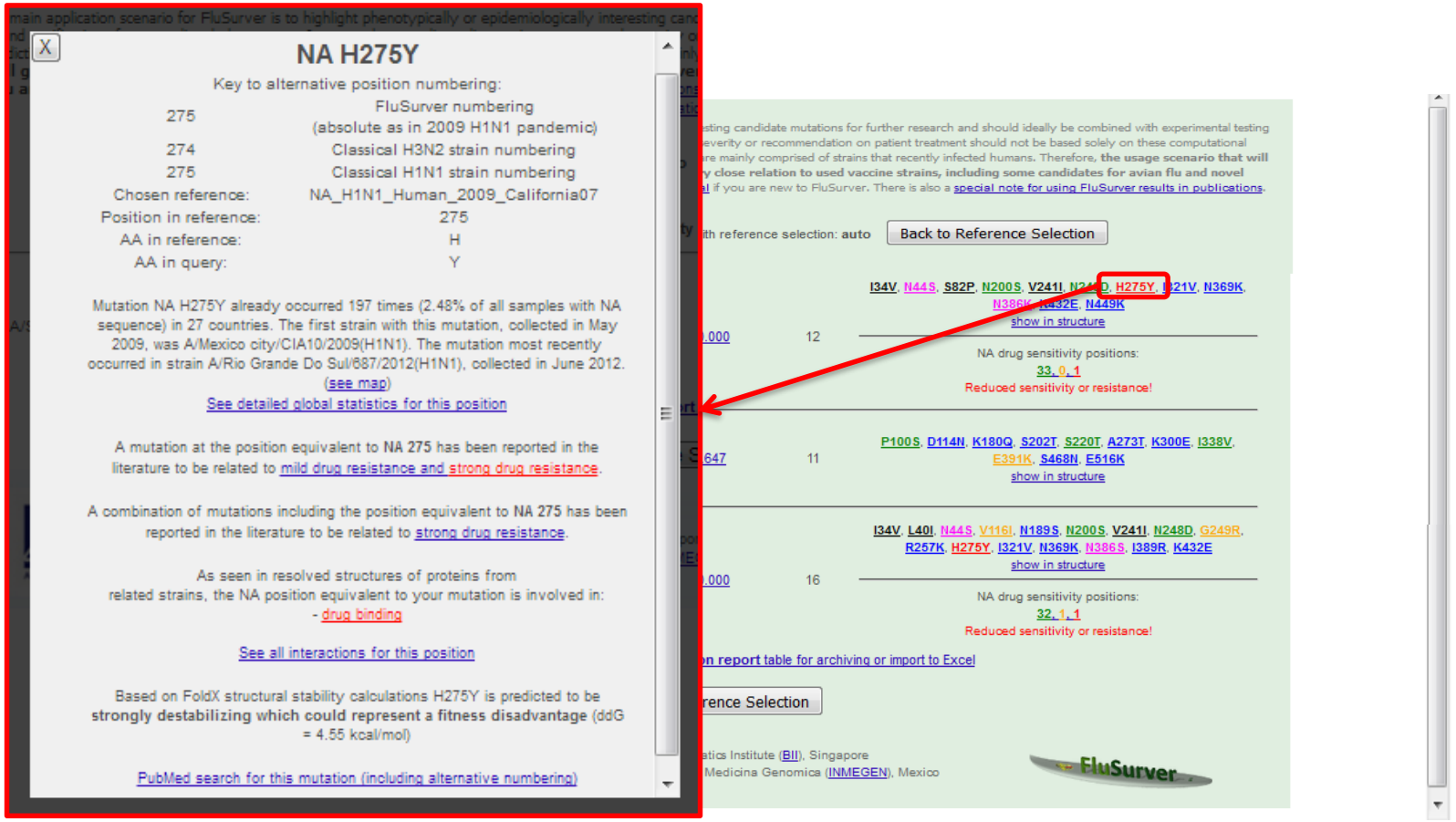
Check alignment to reference hit!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

Color	Interest level	Remarks
Black	0 (least significant)	No known effects
Green	0	Common
Blue	1	At site of interaction
Magenta	2	Addition or removal of N-glycosylation sites
Orange	2	At site known to involved in drug-binding, alter host-specificity.
Red	3 (most significant)	At site known to alter virulence, cause drug resistance, reverses premature STOP codon in PB1-F2.

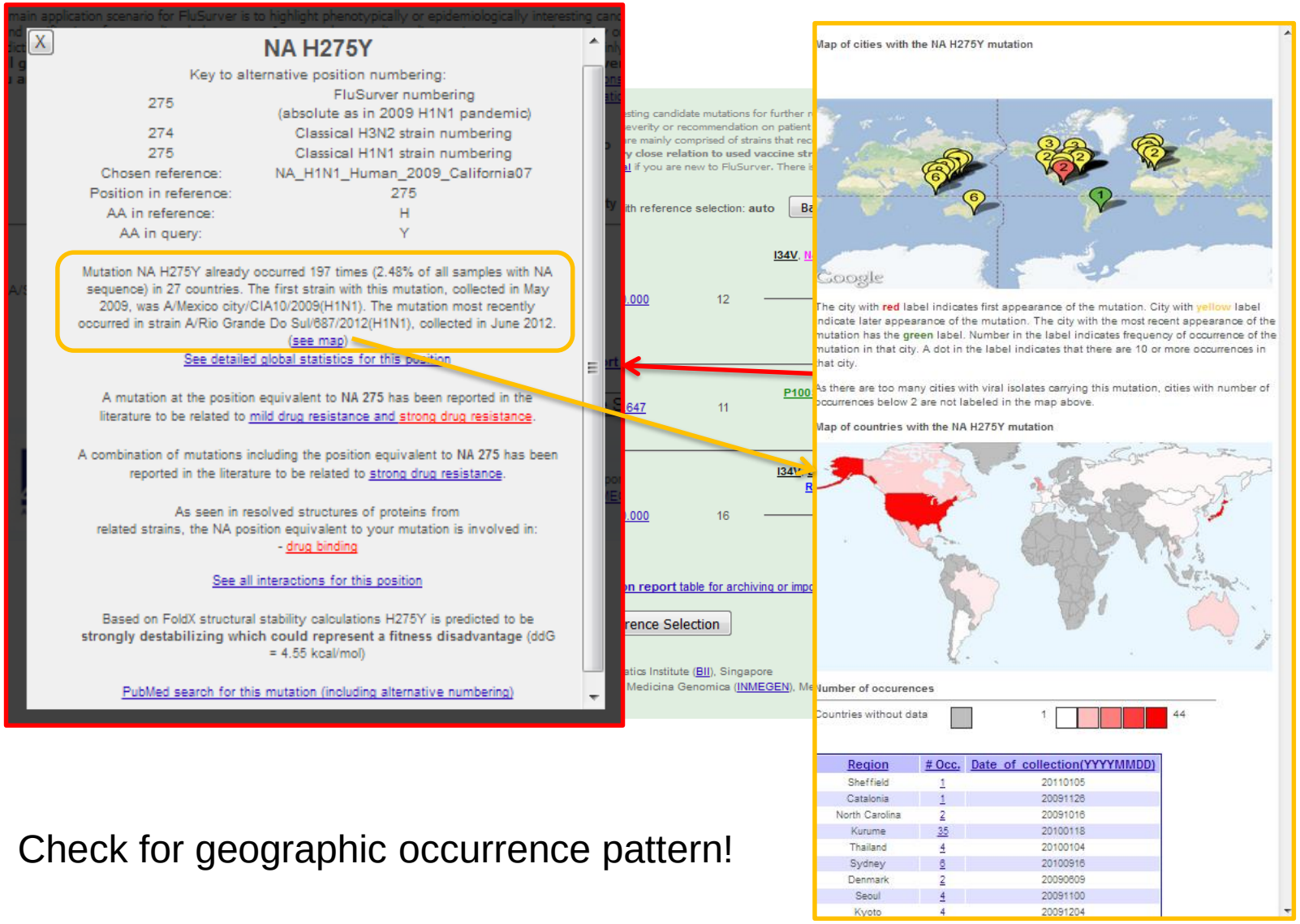
Check list of mutations!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



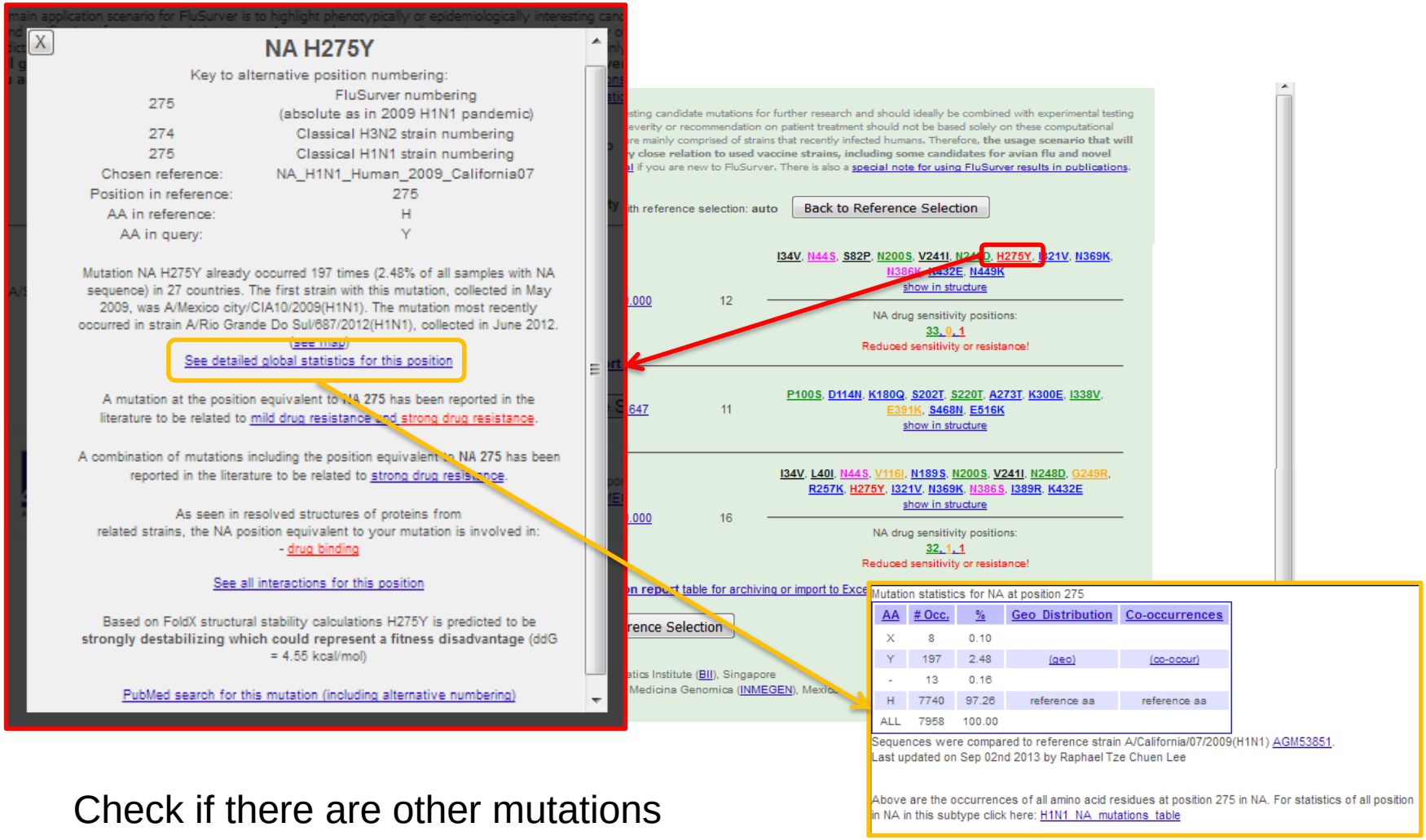
Click on mutation of interest for details!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



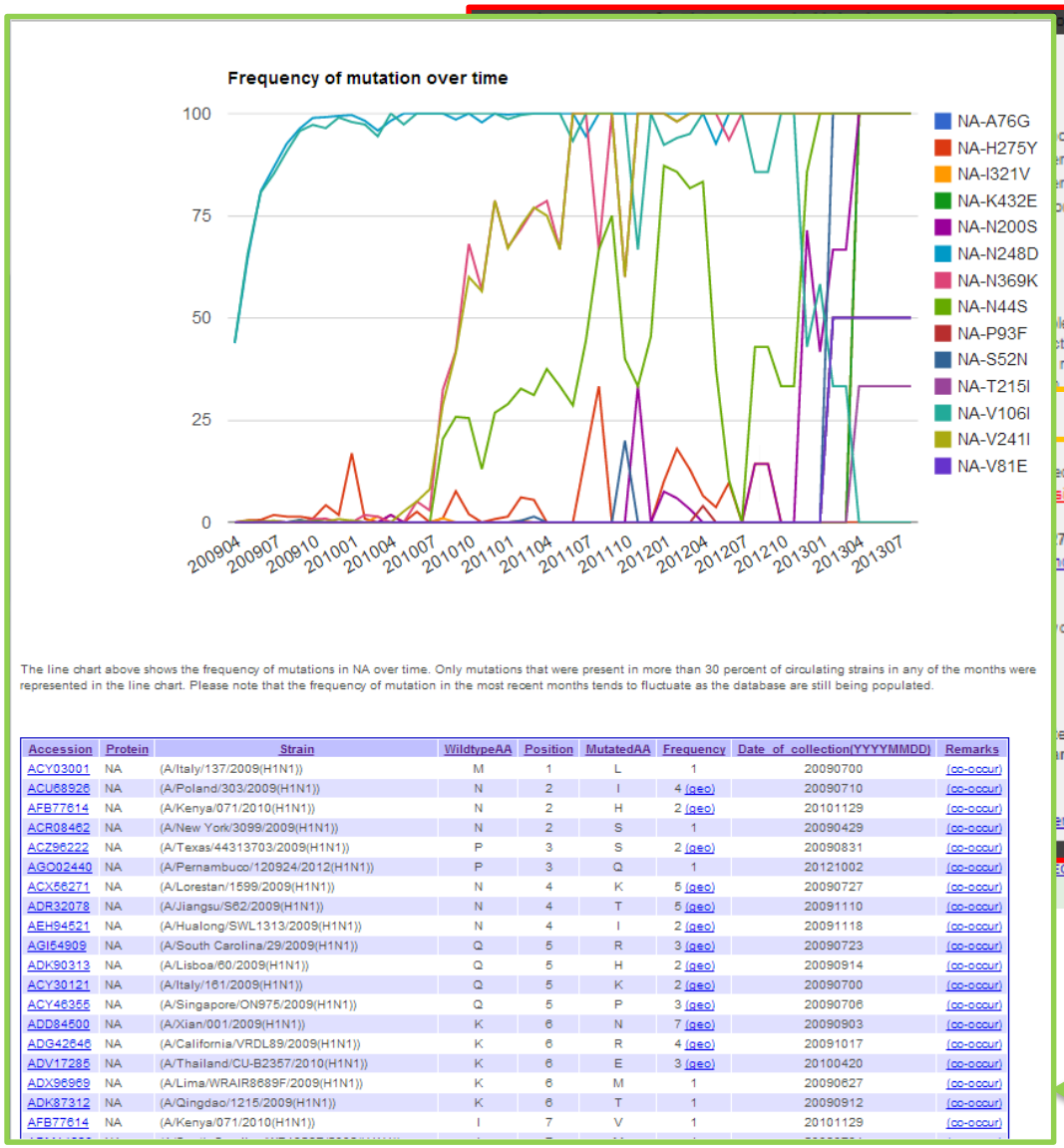
Check for geographic occurrence pattern!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



Check if there are other mutations at same position!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



The line chart above shows the frequency of mutations in NA over time. Only mutations that were present in more than 30 percent of circulating strains in any of the months were represented in the line chart. Please note that the frequency of mutation in the most recent months tends to fluctuate as the database are still being populated.

Accession	Protein	Strain	WildtypeAA	Position	MutatedAA	Frequency	Date_of_collection(YYYYMMDD)	Remarks
ACY03001	NA	(A/Italy/137/2009(H1N1))	M	1	L	1	20090700	(co-occur)
ACU88926	NA	(A/Poland/303/2009(H1N1))	N	2	I	4 (geo)	20090710	(co-occur)
AFB77614	NA	(A/Kenya/071/2010(H1N1))	N	2	H	2 (geo)	20101129	(co-occur)
ACR08462	NA	(A/New York/3099/2009(H1N1))	N	2	S	1	20090429	(co-occur)
ACZ96222	NA	(A/Texas/44313703/2009(H1N1))	P	3	S	2 (geo)	20090831	(co-occur)
AGO02440	NA	(A/Pernambuco/120924/2012(H1N1))	P	3	Q	1	20121002	(co-occur)
ACX56271	NA	(A/Lorestan/1599/2009(H1N1))	N	4	K	5 (geo)	20090727	(co-occur)
ADR32078	NA	(A/Jiangsu/S62/2009(H1N1))	N	4	T	5 (geo)	20091110	(co-occur)
AEH94521	NA	(A/Hualong/SWL1313/2009(H1N1))	N	4	I	2 (geo)	20091118	(co-occur)
AGI54909	NA	(A/South Carolina/29/2009(H1N1))	Q	5	R	3 (geo)	20090723	(co-occur)
ADK90313	NA	(A/Lisboa/60/2009(H1N1))	Q	5	H	2 (geo)	20090914	(co-occur)
ACY30121	NA	(A/Italy/161/2009(H1N1))	Q	5	K	2 (geo)	20090700	(co-occur)
ACY46355	NA	(A/Singapore/ON975/2009(H1N1))	Q	5	P	3 (geo)	20090706	(co-occur)
ADD84600	NA	(A/Xian/001/2009(H1N1))	K	6	N	7 (geo)	20090903	(co-occur)
ADG42646	NA	(A/California/VRDL89/2009(H1N1))	K	6	R	4 (geo)	20091017	(co-occur)
ADV17285	NA	(A/Thailand/CU-B2357/2010(H1N1))	K	6	E	3 (geo)	20100420	(co-occur)
ADX96969	NA	(A/Lima/WRAIR8689F/2009(H1N1))	K	6	M	1	20090627	(co-occur)
ADK87312	NA	(A/Qingdao/1215/2009(H1N1))	K	6	T	1	20090912	(co-occur)
AFB77614	NA	(A/Kenya/071/2010(H1N1))	I	7	V	1	20101129	(co-occur)

Mutation statistics for NA at position 275

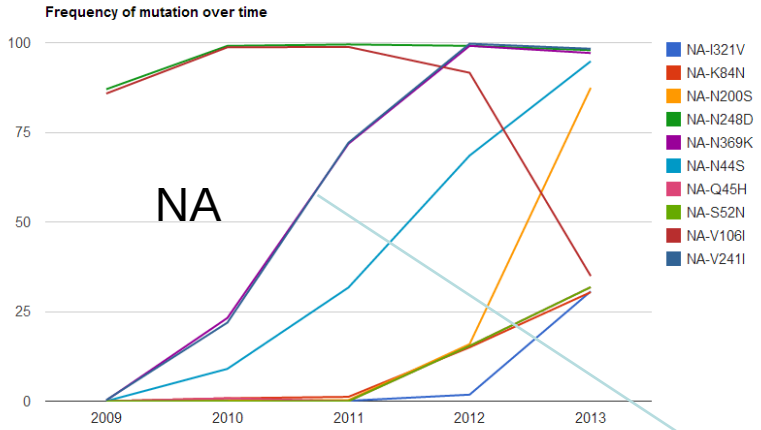
AA	# Occ.	%	Geo Distribution	Co-occurrences
X	8	0.10		
Y	197	2.48	(geo)	(co-occur)
-	13	0.16		
H	7740	97.26	reference aa	reference aa
ALL	7958	100.00		

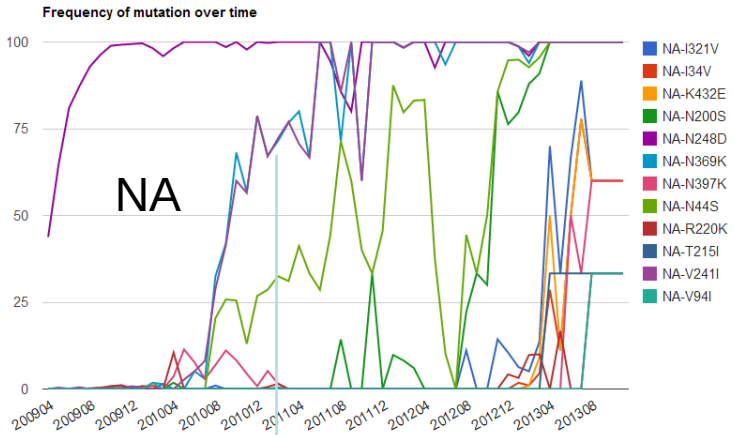
Sequences were compared to reference strain A/California/07/2009(H1N1) AGM53851.
Last updated on Sep 02nd 2013 by Raphael Tze Chuen Lee

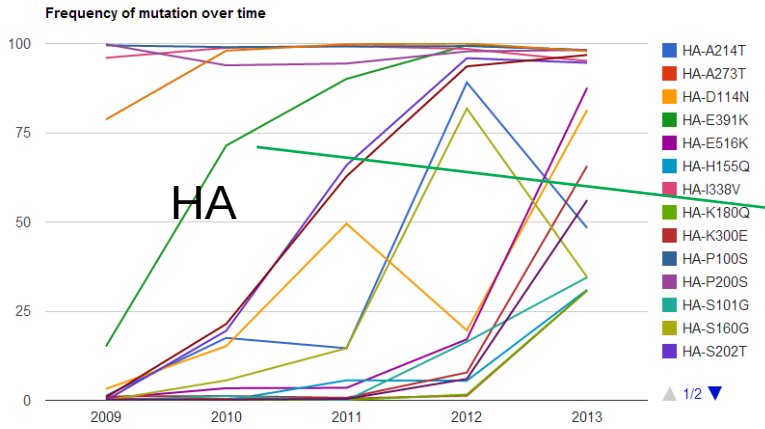
Above are the occurrences of all amino acid residues at position 275 in NA. For statistics of all position in NA in this subtype click here: H1N1_NA_mutations_table

Check for temporal mutation frequency changes!

Mutation temporal 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

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

Country	Strain	PB2	PB1	PB1-F2	PA	HA	NP	NA	M1	M2	NS1	NS2	Date_of_collection(YYYYMMDD)
Taiwan	(A/Taiwan/7336/2009(H1N1))	-	-	-	-	A26T P100S P200S S220T I338V E391K	-	V106I N248D H275Y	-	-	-	-	20091105
Japan	(A/Kurume/R8/2010(H1N1))	-	-	-	-	-	-	V53A V80M S82P V106I N248D H275Y Y282H	-	-	-	-	20100118
South Korea	(A/Daejeon/1871/2009(H1N1))	-	-	-	-	K39R N73S P100S S145P G172E P200S S220T I338V	-	A86T V106I I117M N248D H275Y	-		-	-	20091215
United Kingdom	(A/England/94840152/2009(H1N1))	-	-	-	-	P100S P200S S220T I338V	-	V106I N248D H275Y E462K	-	-	-	-	20091119
Japan	(A/Kurume/N6/2010(H1N1))	-	-	-	-	-	-	V80M S82P V106I N248D H275Y	-	-	-	-	20100118
United Kingdom	(A/England/00380015/2009(H1N1))	-	-	-	-	P100S P200S S220T I338V	-	V106I N248D H275Y E462K	-	-	-	-	20091117
USA	(A/California/21/2012(H1N1))	-	-	-	-	S86T P100S K136N S160G P200S S202T A214T S220T D239G N277D I338V F347L E391K S468N V537A	-	A20V G41R N44S V106I V241I N248D H275Y N369K	V80I	S13N	-	-	20120220
Viet Nam	(A/Viet Nam/835/2009(H1N1))			-	P224S	P100S P200S S220T I338V	V100I	V106I N248D H275Y			L115F I123V		20090727
Mexico	(A/Mexico/InDRE3354/2012(H1N1))	-	-	-	-	S86T P100S S160G P200S S202T A214T S220T N277D I338V E391K S468N V537A	-	G41R N44S S95I V106I V241I N248D H275Y N369K	-	-	-	-	20120208
Japan	(A/Kurume/N1/2010(H1N1))	-	-	-	-	-	-	V80M S82P V106I N248D H275Y	-	-	-	-	20100118
USA	(A/Bethesda/NIH106-D14/2009(H1N1))	R591Q	K736G	-	V14I P224S K716Q	A15T P100S P200S S220T I338V E391K F432L	V100I V270I V444I	V106I N248D H275Y			I123V		20091105
Japan	(A/Kurume/L19/2010(H1N1))	-	-	-	-	-	-	V80M S82P V106I N248D H275Y	-	-	-	-	20100118
China	(A/Haishu/SWL110/2010(H1N1))	-	-	-	-	P100S S179N P200S S220T I338V	-	V106I N248D H275Y	-	-	-	-	20100104
Germany	(A/Munich/INS541/2011(H1N1))	R299K V344M I354L N456S	V645I	-	P224S N321K I330V M548I	P100S D114N P200S S202T S220T I338V E391K S468N	V100I	V106I V241I N248D K260R H275Y I321V N369K	V80I		I123V		20110218
Canada	(A/Canada-AB/RV2828/2009(H1N1))		M92V N156S	-	P224S	P100S P200S S220T T258I I338V		V106I N248D H275Y V394I					20090804
USA	(A/Texas/33/2012(H1N1))	-	-	-	-	S86T P100S S160G P200S S202T A214T S220T N277D I338V E391K S468N V537A	-	G41R N44S V106I L127W V241I N248D H275Y N369K	V80I	S13N	-	-	20120312
USA	(A/Texas/48/2012(H1N1))	-	-	-	-	S86T P100S S160G P200S S202T A214T S220T N277D I338V E391K S468N V537A	-	G41R N44S S95N V106I V241I N248D H275Y N369K	V80I	S13N	-	-	20120316
United Kingdom	(A/England/00380020/2009(H1N1))	-	-	-	-	P100S P200S S220T I338V	-	V106I N248D H275Y E462K	-	-	-	-	20091120
USA	(A/North Carolina/59/2009(H1N1))	-	-	-	-	P100S V169I P200S S220T P288Q I312V I338V	-	V106I V234I N248D H275Y		P25T	-	-	20091107
Spain	(A/Catalonia/NS7362/2009(H1N1))	-	-	-	-	P100S S179N P200S S220T T249A I338V G411D	-	V106I N248D H275Y	-	-	-	-	20091126

A mutation at the position equivalent to **NA 275** has been reported in the literature to be related to mild drug resistance and strong drug resistance.

A combination of mutations including the position equivalent to **NA 275** has been reported in the literature to be related to strong drug resistance.

As seen in resolved structures of proteins from related strains, the NA position equivalent to your mutation is involved in:
- drug binding

See all interactions for this position

Based on FoldX structural stability calculations H275Y is predicted to be **strongly destabilizing which could represent a fitness disadvantage** (ddG = 4.55 kcal/mol)

PubMed search for this mutation (including alternative numbering)

I34V, L40I, N44S, V116I, N189S, N200S, V241I, N248D, G249R, R257K, H275Y, I321V, N369K, N386S, I389R, K432E
show in structure

100.000 16

NA drug sensitivity position
32, 1, 1
Reduced sensitivity or resistance!

mutation report table for archiving or impo

to Reference Selection

Bioinformatics Institute (BII), Singapore
cional de Medicina Genomica (INMEGEN), Me
served.

Mutation statistics for NA at position 275

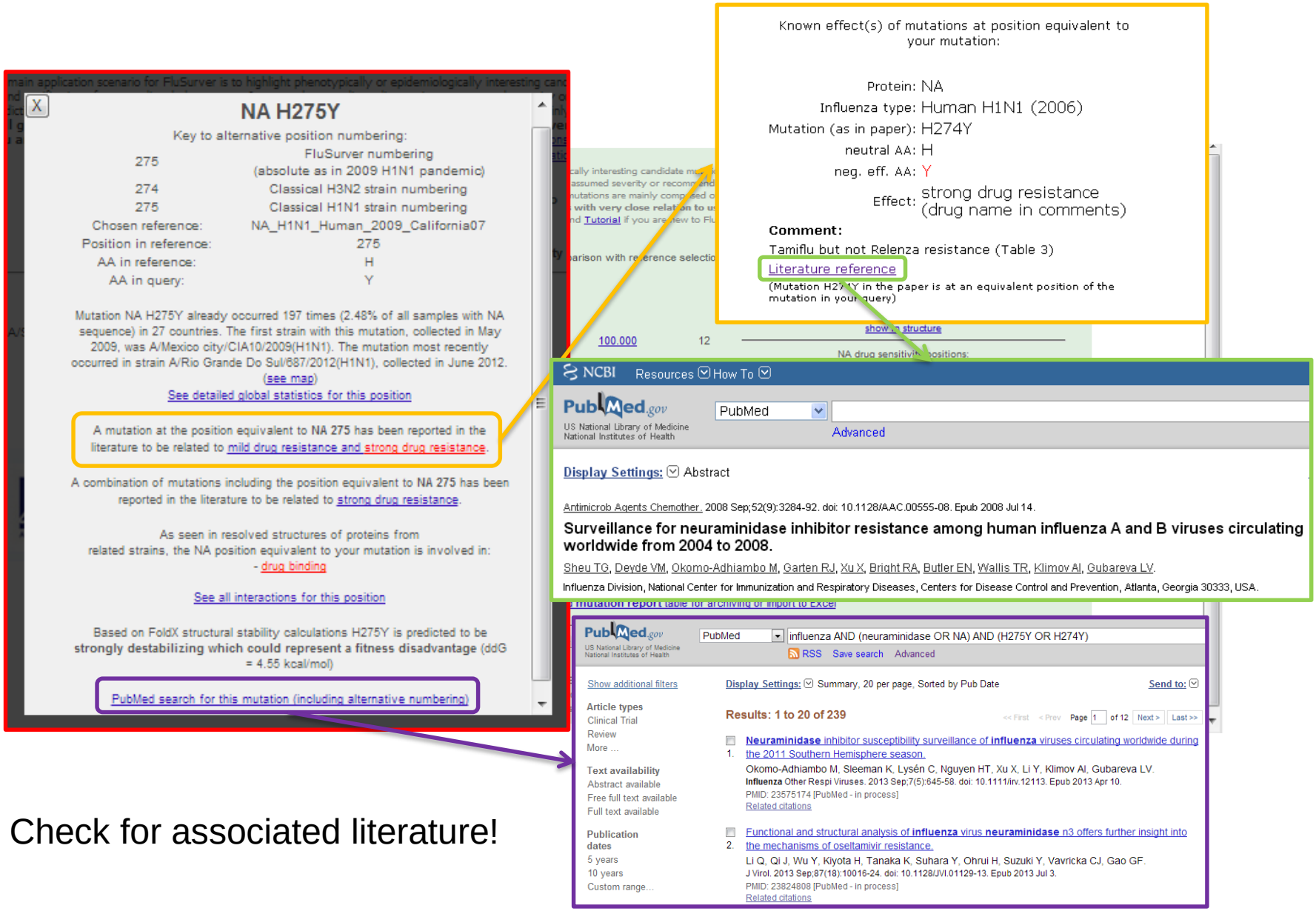
AA	# Occ.	%	Geo_Distribution	Co-occurrences
X	8	0.10		
Y	197	2.48	(geo)	(co-occur)
-	13	0.16		
H	7740	97.26	reference aa	reference aa
ALL	7958	100.00		

Sequences were compared to reference strain A/California/07/2009(H1N1) AGM53851.
Last updated on Sep 02nd 2013 by Raphael Tze Chuen Lee

Above are the occurrences of all amino acid residues at position 275 in NA. For statistics of all position in NA in this subtype click here: H1N1_NA_mutations_table

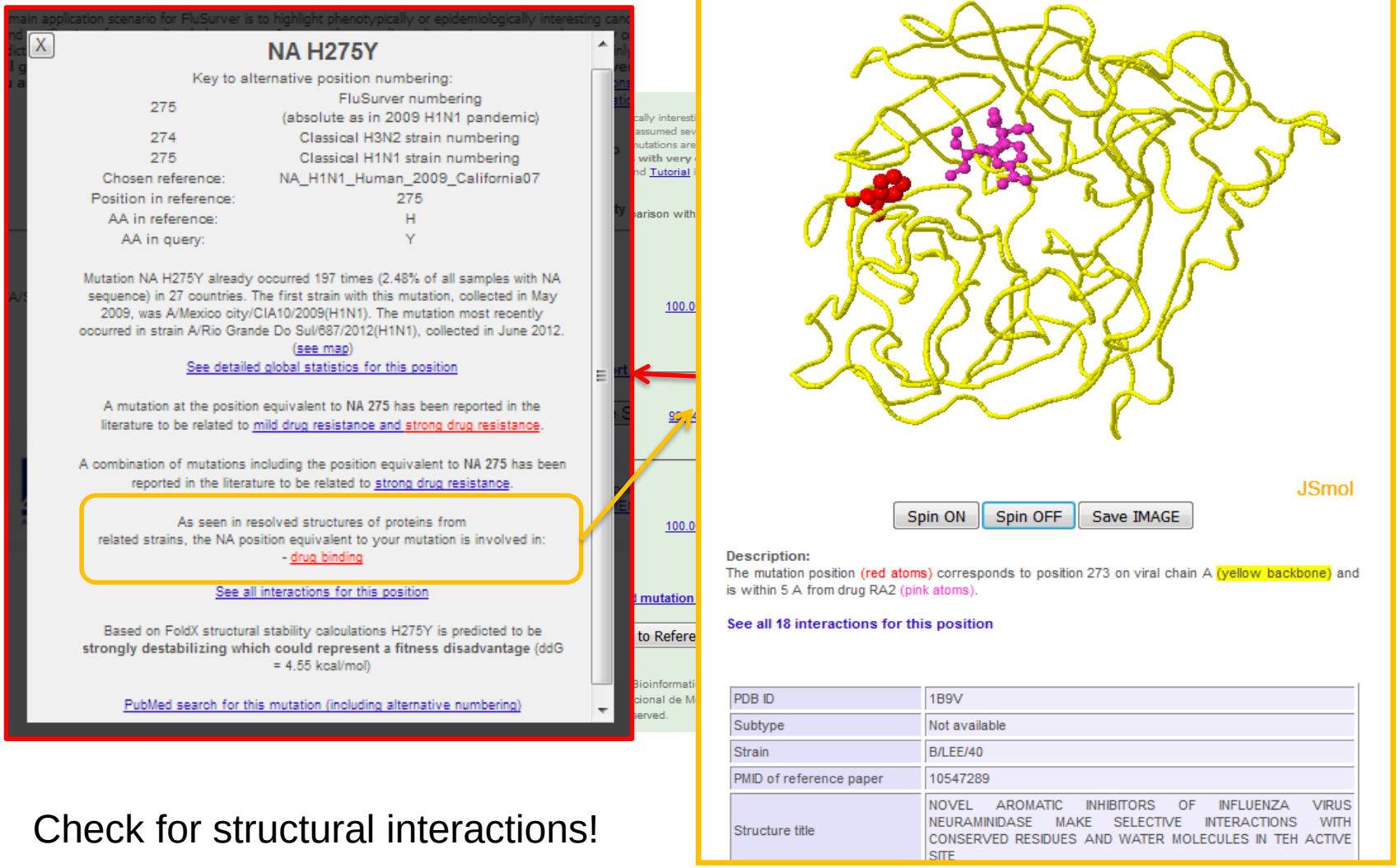
Check for co-occurring mutations!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



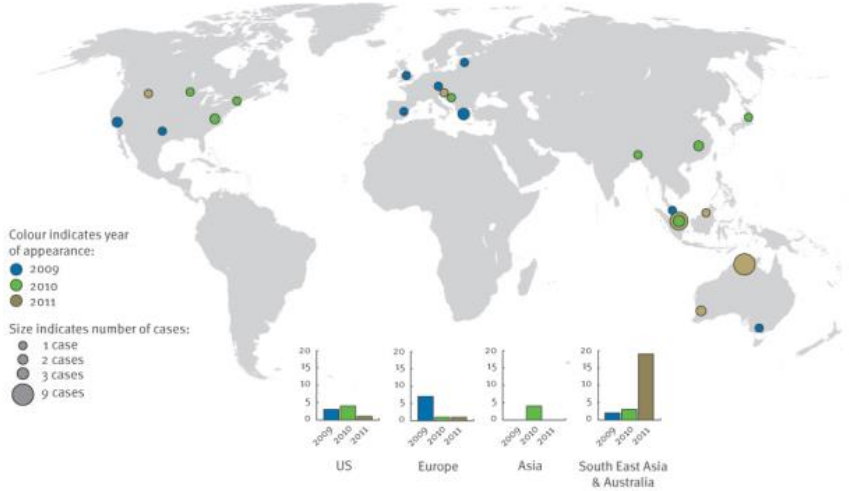
Check for associated literature!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

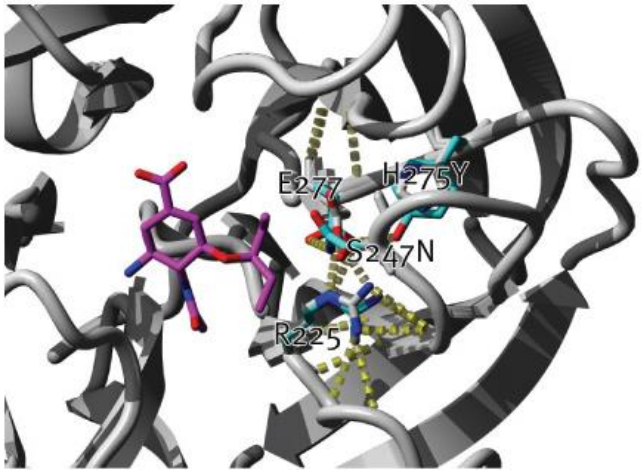


Check for structural interactions!

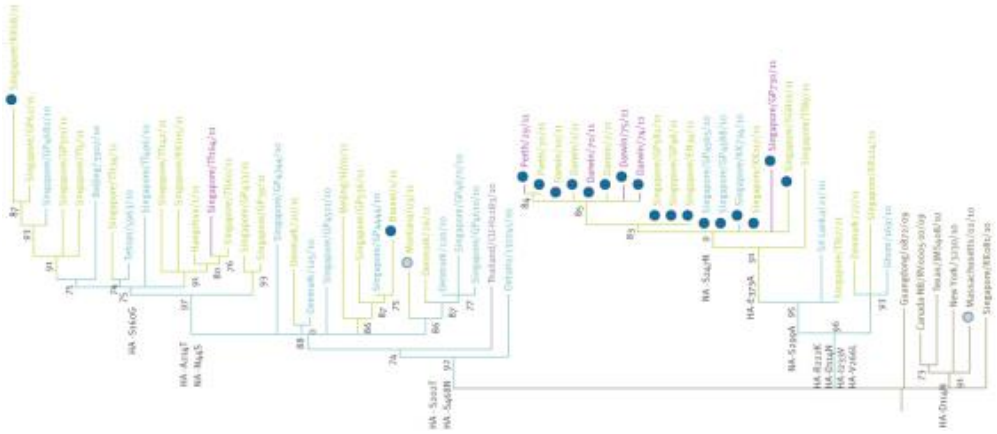
Example of new drug sensitivity altering mutation NA S247N discovered with the help of FluSurver



Global occurrence of new variant



Structural context of mutation



Phylogenetic context of new variant

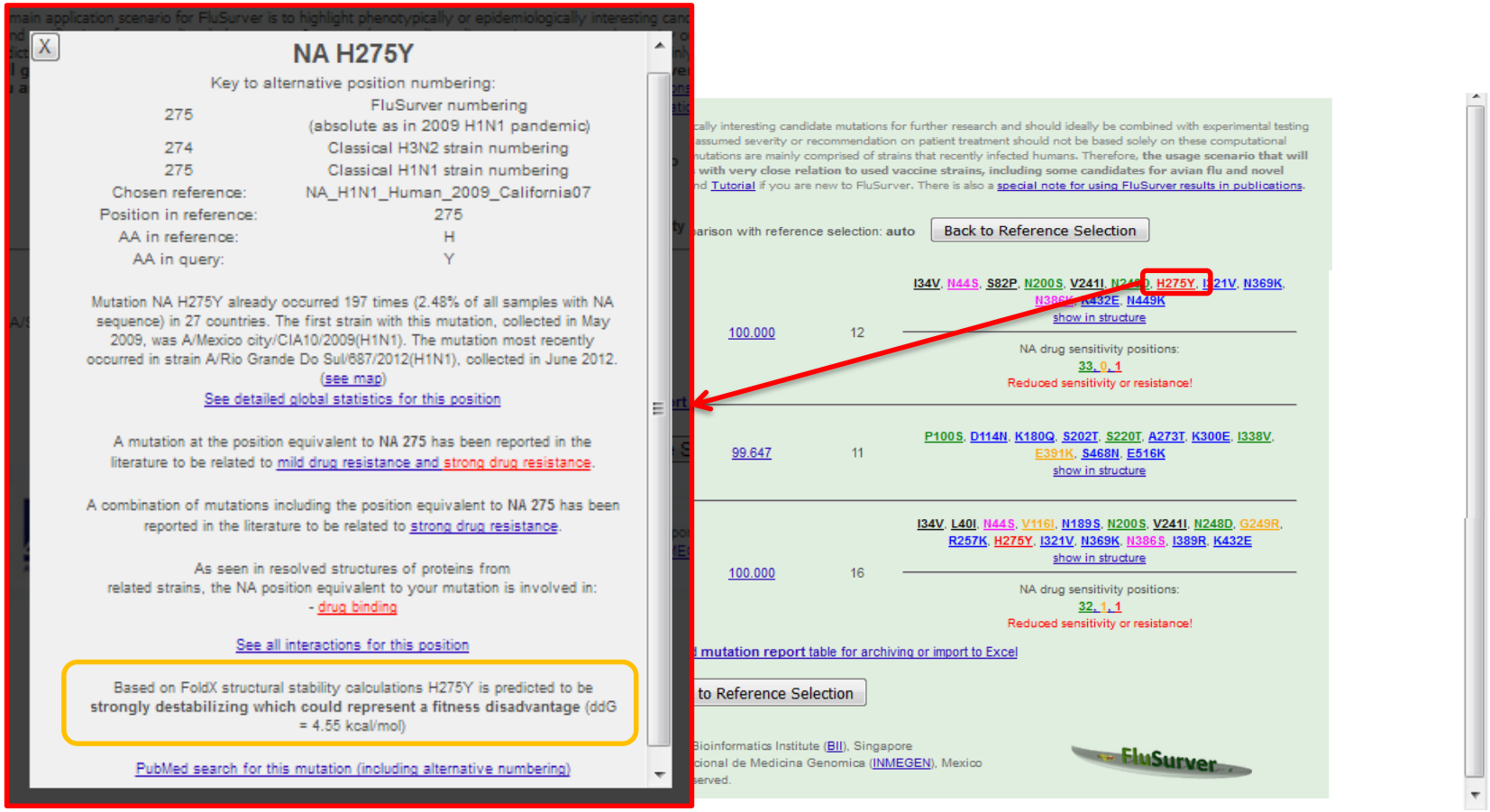
Found circulating in 10% of samples in Singapore and 30% of samples in Northern Australia in early 2011.

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.*

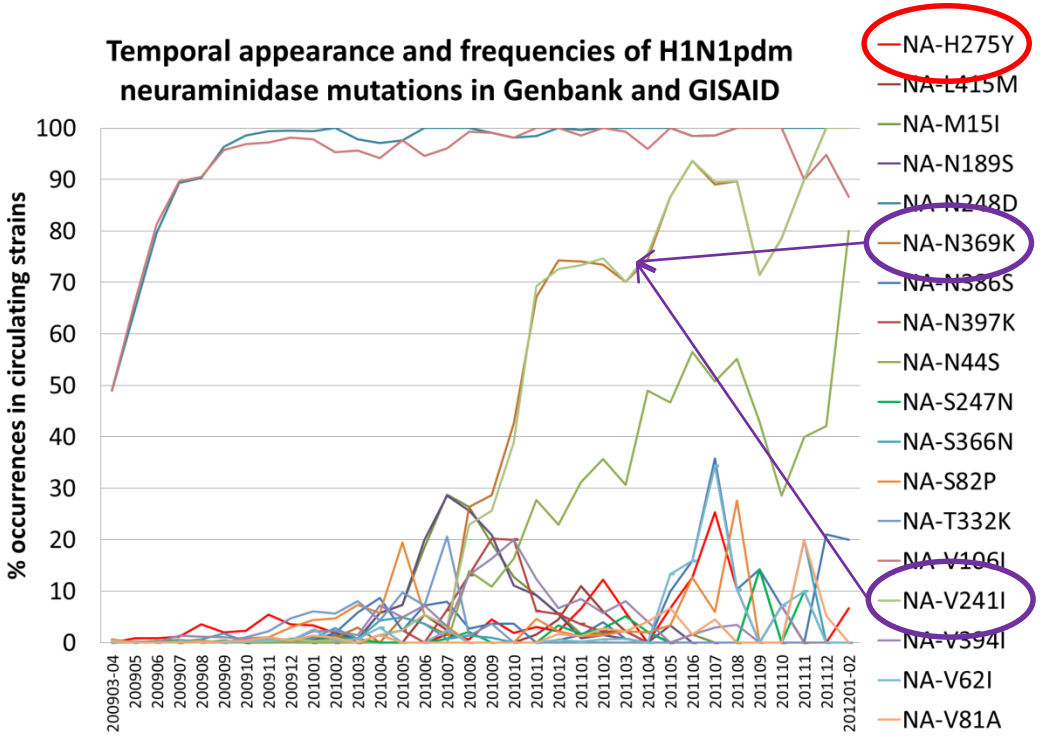
Euro Surveill. 2011;16(23):pii=19884.

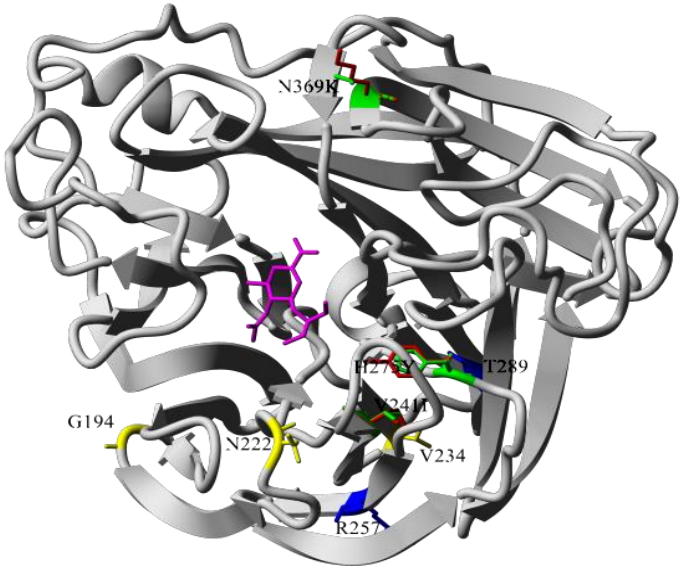
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

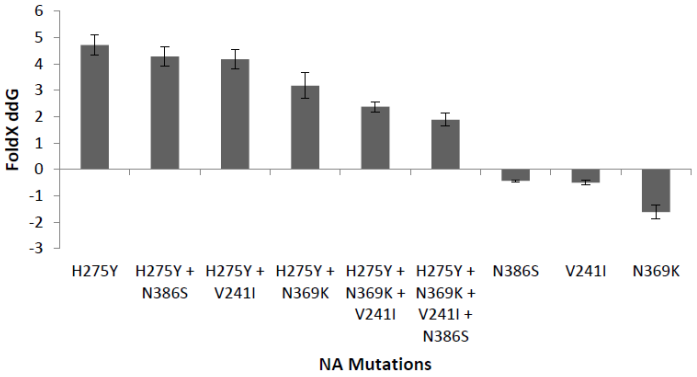


Check for stability
or passage effect
(if available)!

Example of frequency rise associated with stability changes leading to permissive mutations



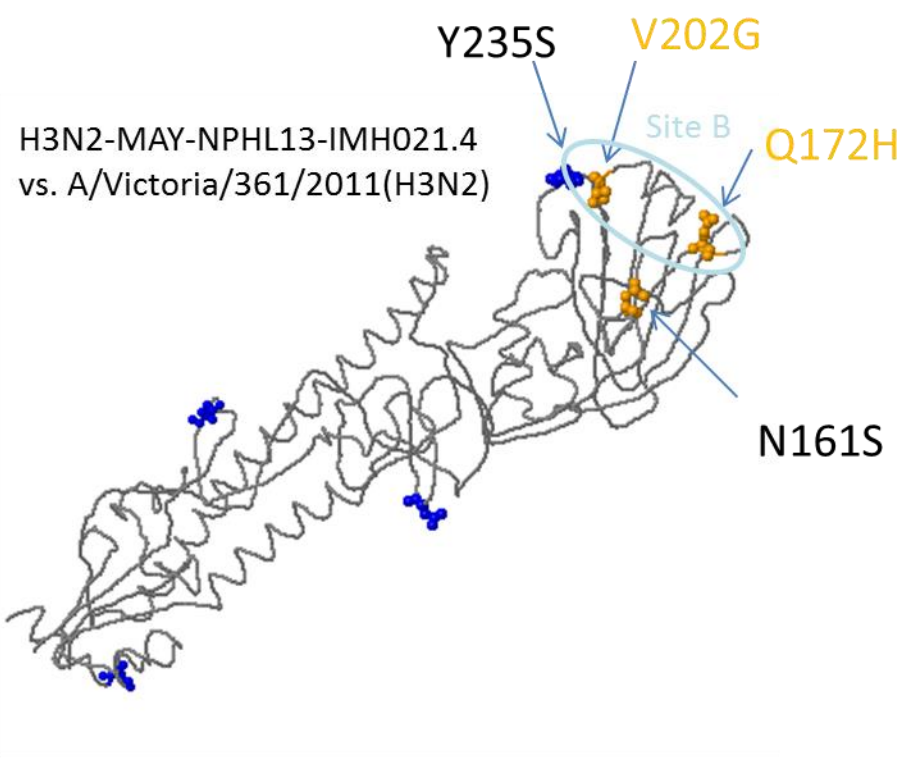


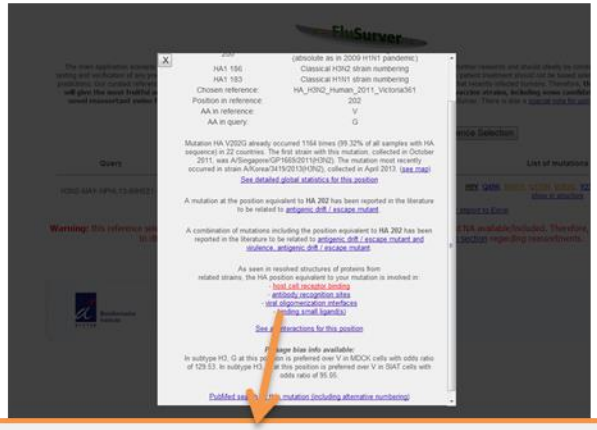


FoldX predicts increase in structural stability for mutations that were increasing in frequency and were fixed in Newcastle strains.

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 RC, 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.

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

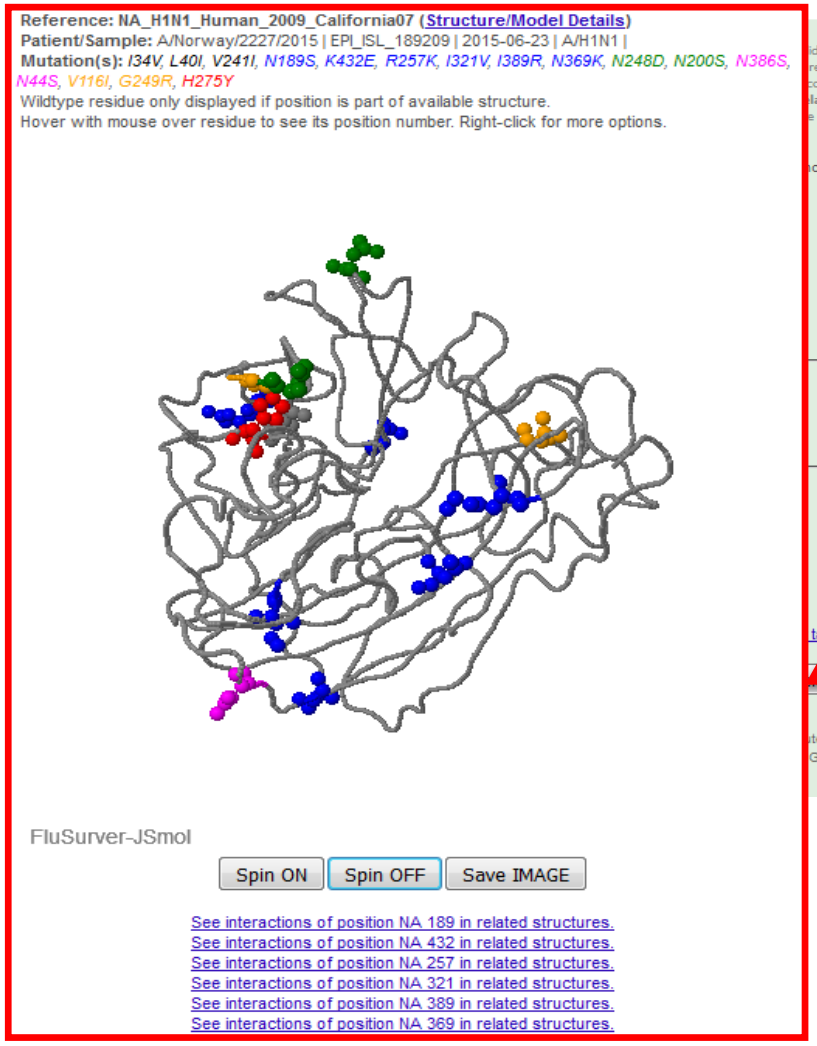
See all interactions for this position

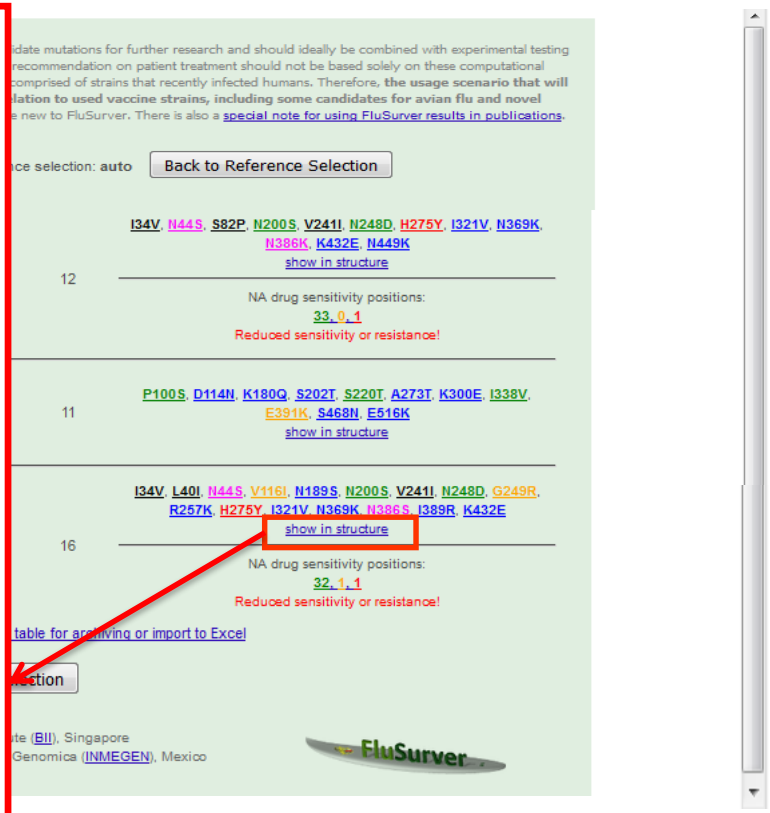
Passage bias info available:
In subtype H3, H at this position is preferred over Q in SIAT cells with odds ratio of 67.59.

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

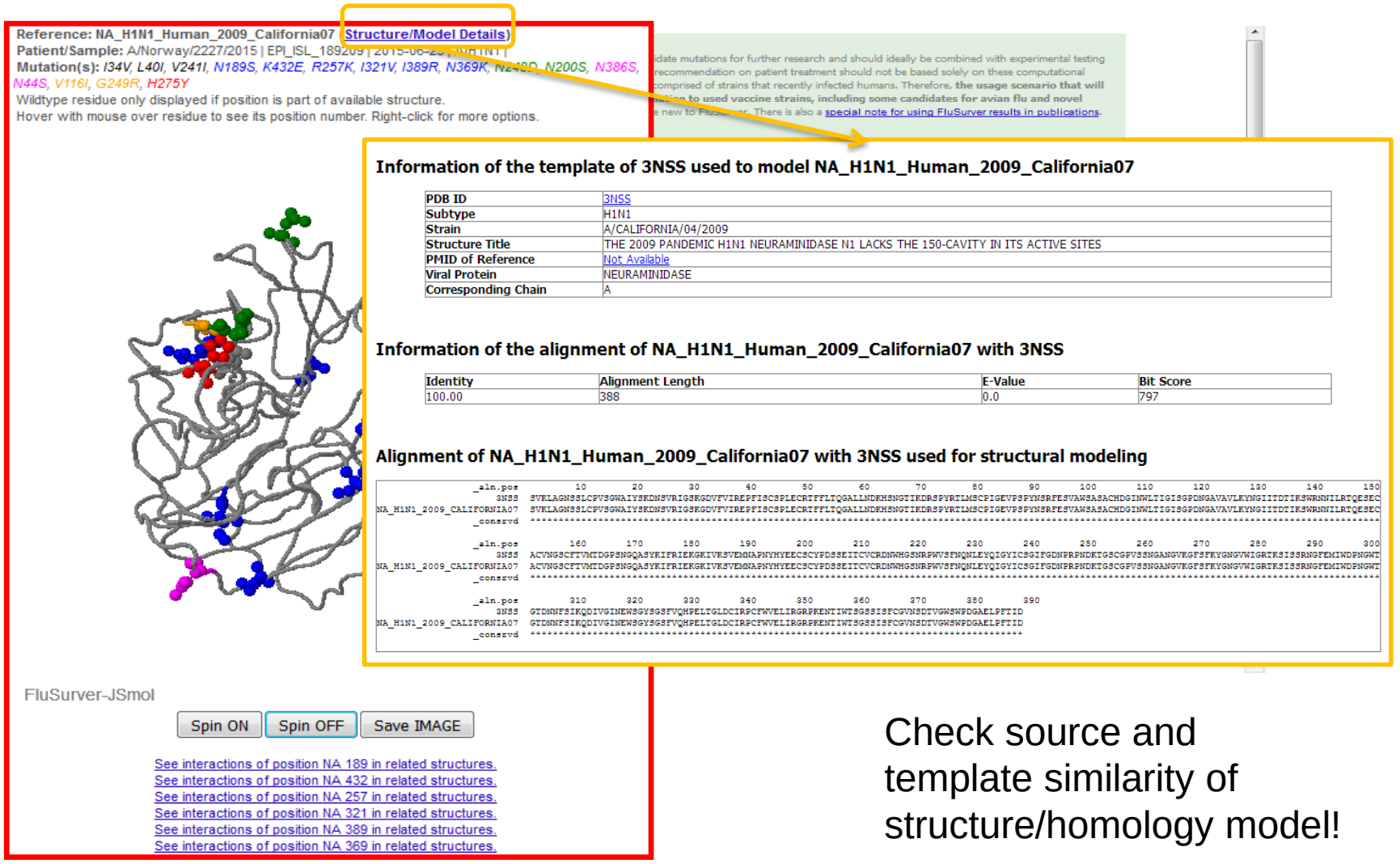
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier





View all mutations together in structure or homology model of reference strain!

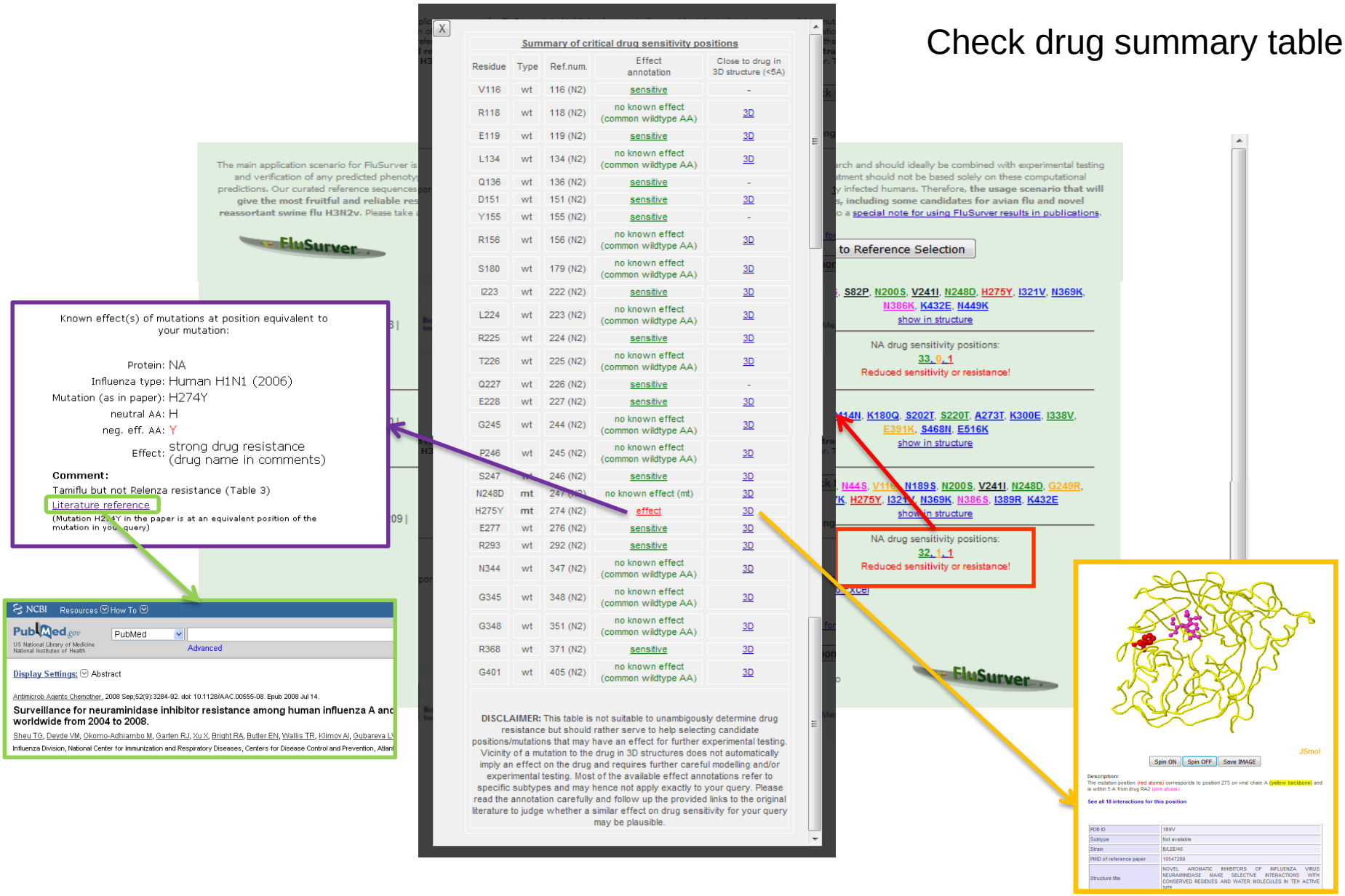
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



Check source and template similarity of structure/homology model!

FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

Check drug summary table!



Also useful for analysis of other segments!

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
RIVM4541200051|H3 clade 3B|N2|	M2 A/Wisconsin/67/2005(H3N2) find closest related sequences	98.780	84.536	1	V27I show in structure

M2 drug sensitivity positions: 16, 0, 2
Reduced sensitivity or resistance!

Known effect(s) of mutations at position equivalent to your mu

Protein: M2
Influenza type: Duck (live poultry market) H3N2
Mutation (as in paper): V27I
neutral AA: V
neg. eff. AA: I
Effect: mild drug resistance (drug name comments)

Comment:
conferred Amantadine resistance (Table 1).
Literature reference
(Mutation V27I in the paper is at an equivalent position of the mutation in y query)

FluSurver

Summary of critical drug sensitivity positions

Residue	Type	Ref.num.	Effect annotation	Close to drug in 3D structure (<5A)
L26	wt	26	sensitive	3D
V27I	mt	27	effect	3D
A30	wt	30	sensitive	3D
N31	wt	31	effect	3D
I33	wt	33	no known effect (common wildtype AA)	3D
G34	wt	34	sensitive	3D
I35	wt	35	no known effect (common wildtype AA)	3D
H37	wt	37	no known effect (common wildtype AA)	3D
L38	wt	38	no known effect (common wildtype AA)	3D
L40	wt	40	no known effect (common wildtype AA)	3D
W41	wt	41	no known effect (common wildtype AA)	3D
I42	wt	42	no known effect (common wildtype AA)	3D
L43	wt	43	no known effect (common wildtype AA)	3D
D44	wt	44	no known effect (common wildtype AA)	3D
R45	wt	45	no known effect (common wildtype AA)	3D

Known effect(s) of mutations at position equivalent to your mutation:

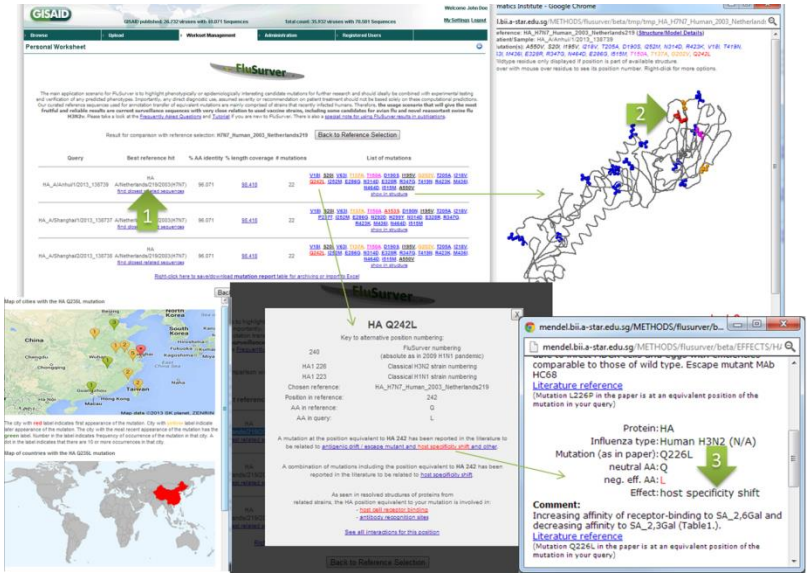
Protein: M2
Influenza type: Human H1N1 (2007)
Mutation (as in paper): S31N
neutral AA: S
neg. eff. AA: N
Effect: strong drug resistance (drug name in comments)

Comment:
Amantadine resistance (Table)
Literature reference
(Mutation S31N in the paper is at an equivalent position of the mutation in your query)

If WT residues in reference strains are associated with resistance it will be shown in drug summary table!

Bioinformatics Institute
A*STAR

Analysis – FluSurver for Mutation Interpretation



Important disclaimer:

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

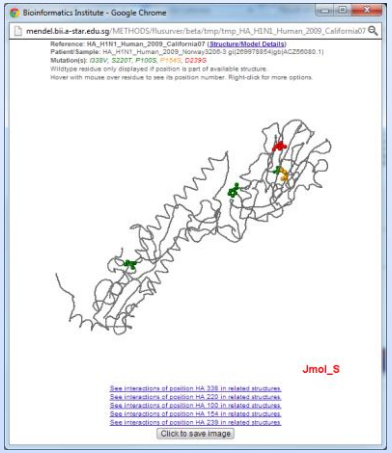
While we have placed great emphasis on avoiding false positive alerts and provide tutorials, one still needs to read the associated papers and interpret the provided evidence carefully to judge any effect realistically.

Summary of FluSurver annotations

Bioinformatics Institute A*STAR

GISAID

Structure

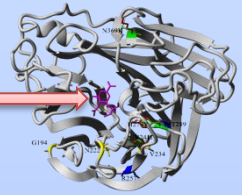


Map mutations to structure

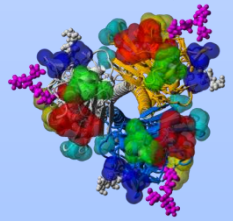
300+ reference homology models

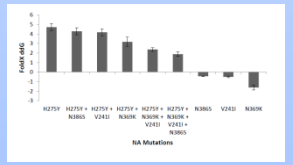
1568	self/oligomerization
975	other small ligand
268	antibody
188	host protein
182	antigen-presenting MHC molecule
132	other viral protein
46	drug
45	nucleic acids
13	host cell receptor
3417	**total interactions for 2062 positions**

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

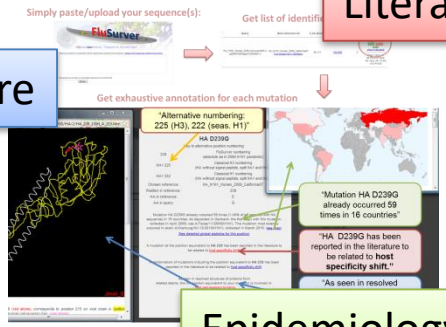
Updated

Literature-curated mutation effect database

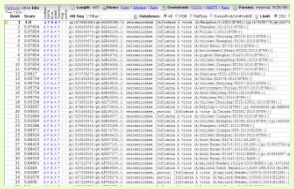
~400 entries

mild drug resistance	30
strong drug resistance	40
virulence	106
antigenic drift / escape mutant	84
host specificity shift	136
other	23

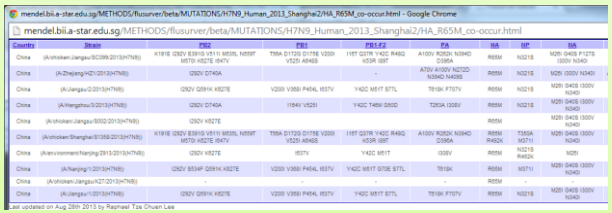
Epidemiology



Closest DB hits



Temporal pattern



Genomic co-occurrence

Regional & global occurrence