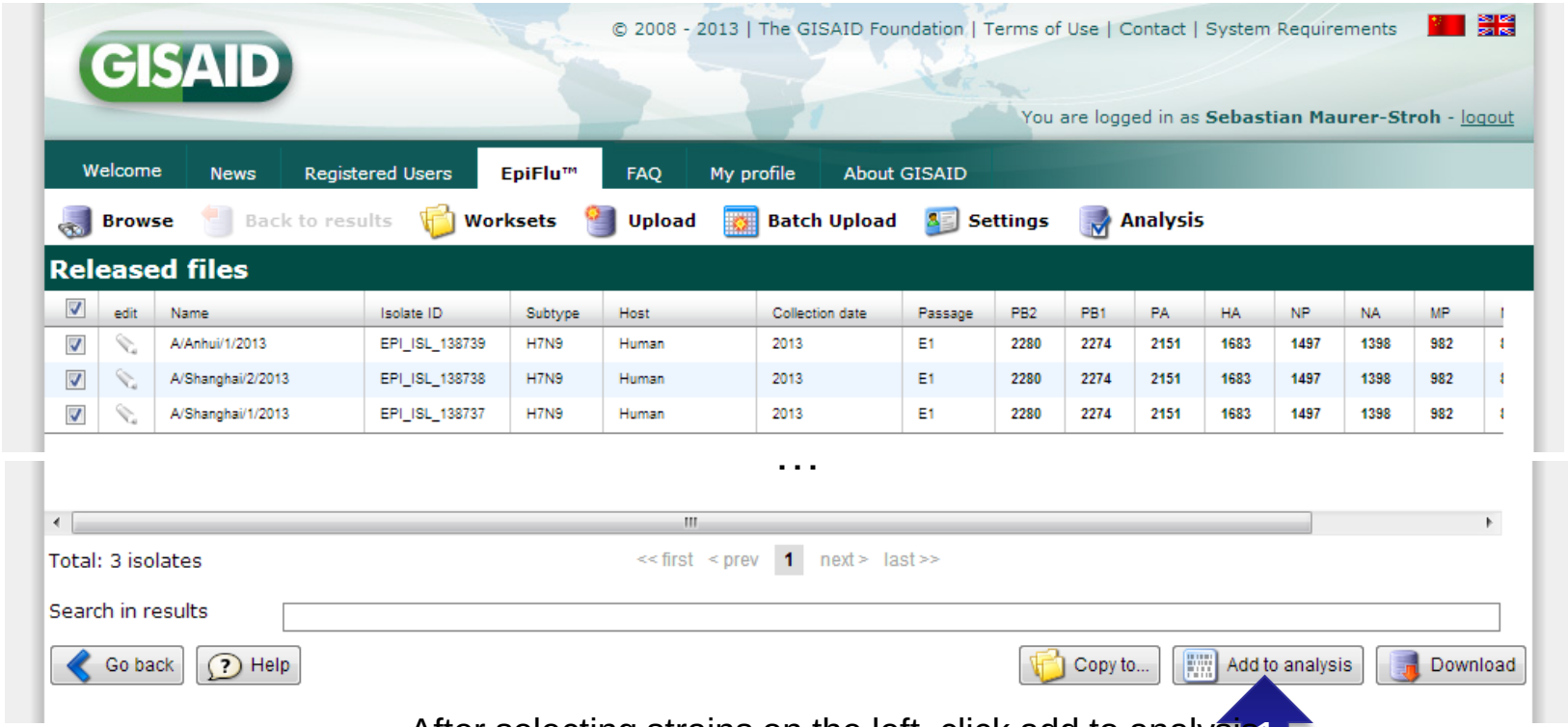
Accessing the FluSurver in GISAID

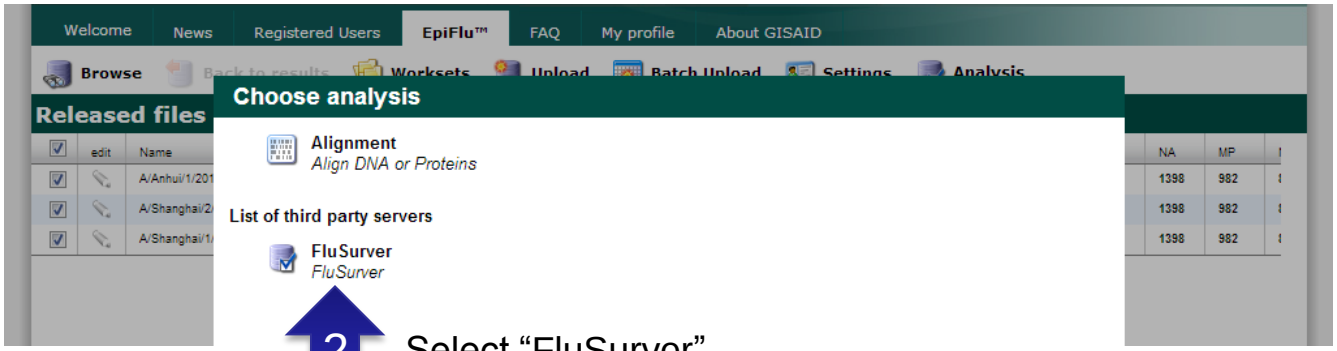
Please send questions and feedback to:
flusurver@gisaid.org

The FluSurver team is located in Singapore and our working day for fast replies may be shifted depending on your local time zone.

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

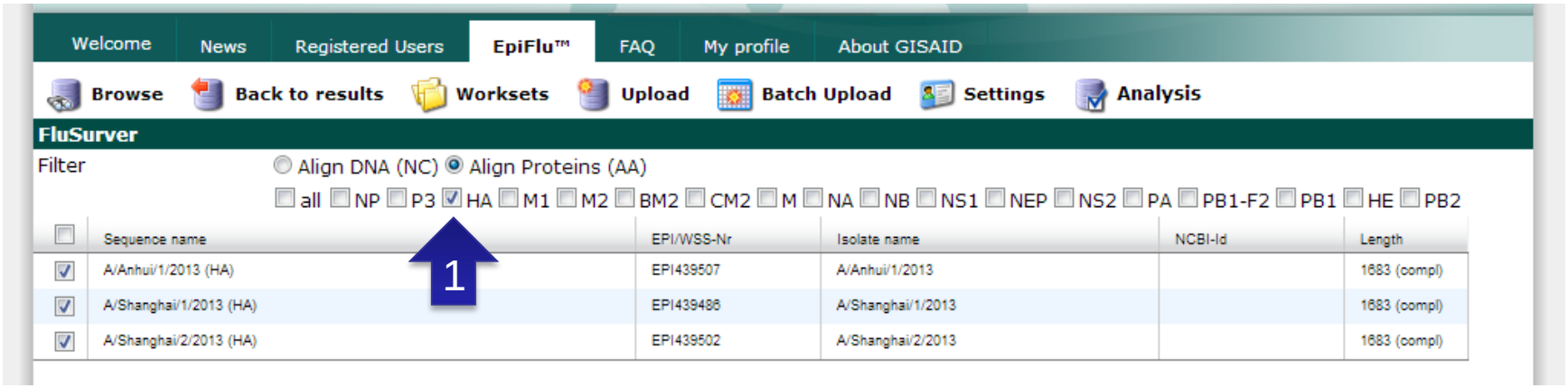


After selecting strains on the left, click add to analysis 1



2 Select “FluSurver”

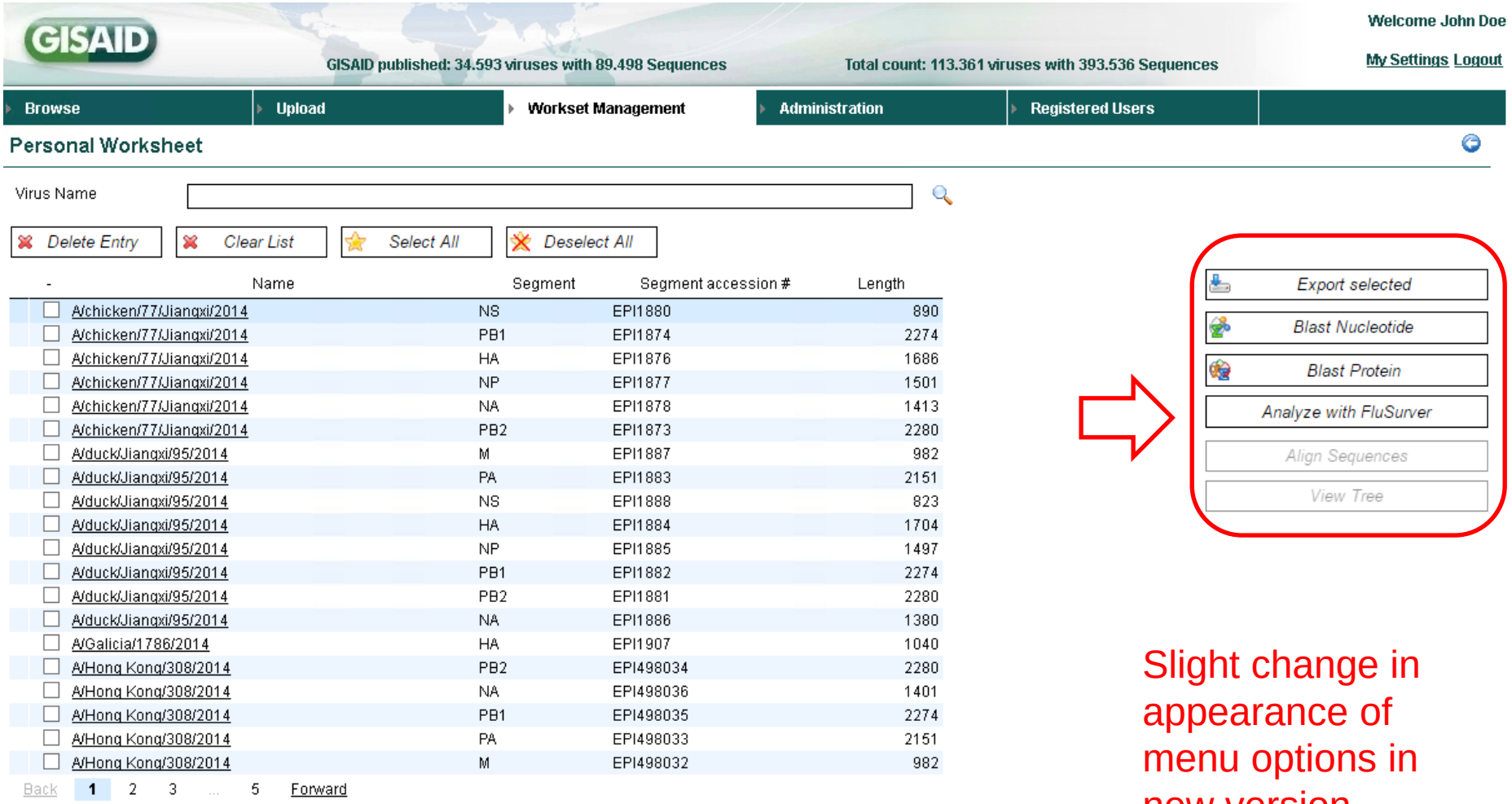
Next steps: Select proteins to analyze[1] , e.g. HA, then click on continue [2], wait for submission form to load and then click “Analyze with FluSurver” [3].



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.

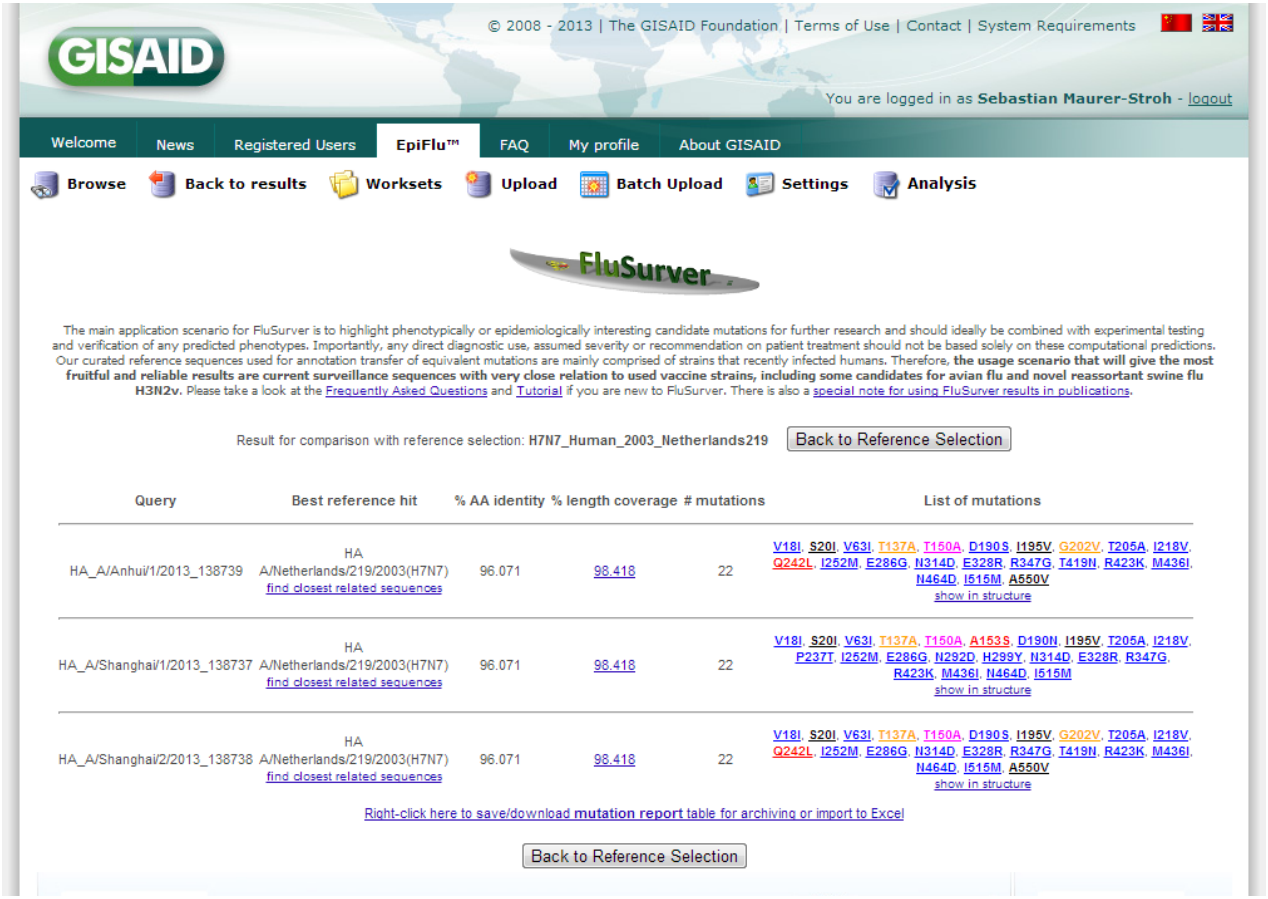
Warning:
In the current EpiFlu 1.0 version, there can be a server time-out if too many sequences are selected, <20 sequences for analysis should work fine.

EpiFlu™ 2.0 – In near Future



-	Name	Segment	Segment accession #	Length
☐	A/chicken/77/Jiangxi/2014	NS	EPI1880	890
☐	A/chicken/77/Jiangxi/2014	PB1	EPI1874	2274
☐	A/chicken/77/Jiangxi/2014	HA	EPI1876	1686
☐	A/chicken/77/Jiangxi/2014	NP	EPI1877	1501
☐	A/chicken/77/Jiangxi/2014	NA	EPI1878	1413
☐	A/chicken/77/Jiangxi/2014	PB2	EPI1873	2280
☐	A/duck/Jiangxi/95/2014	M	EPI1887	982
☐	A/duck/Jiangxi/95/2014	PA	EPI1883	2151
☐	A/duck/Jiangxi/95/2014	NS	EPI1888	823
☐	A/duck/Jiangxi/95/2014	HA	EPI1884	1704
☐	A/duck/Jiangxi/95/2014	NP	EPI1885	1497
☐	A/duck/Jiangxi/95/2014	PB1	EPI1882	2274
☐	A/duck/Jiangxi/95/2014	PB2	EPI1881	2280
☐	A/duck/Jiangxi/95/2014	NA	EPI1886	1380
☐	A/Galicia/1786/2014	HA	EPI1907	1040
☐	A/Hong Kong/308/2014	PB2	EPI498034	2280
☐	A/Hong Kong/308/2014	NA	EPI498036	1401
☐	A/Hong Kong/308/2014	PB1	EPI498035	2274
☐	A/Hong Kong/308/2014	PA	EPI498033	2151
☐	A/Hong Kong/308/2014	M	EPI498032	982

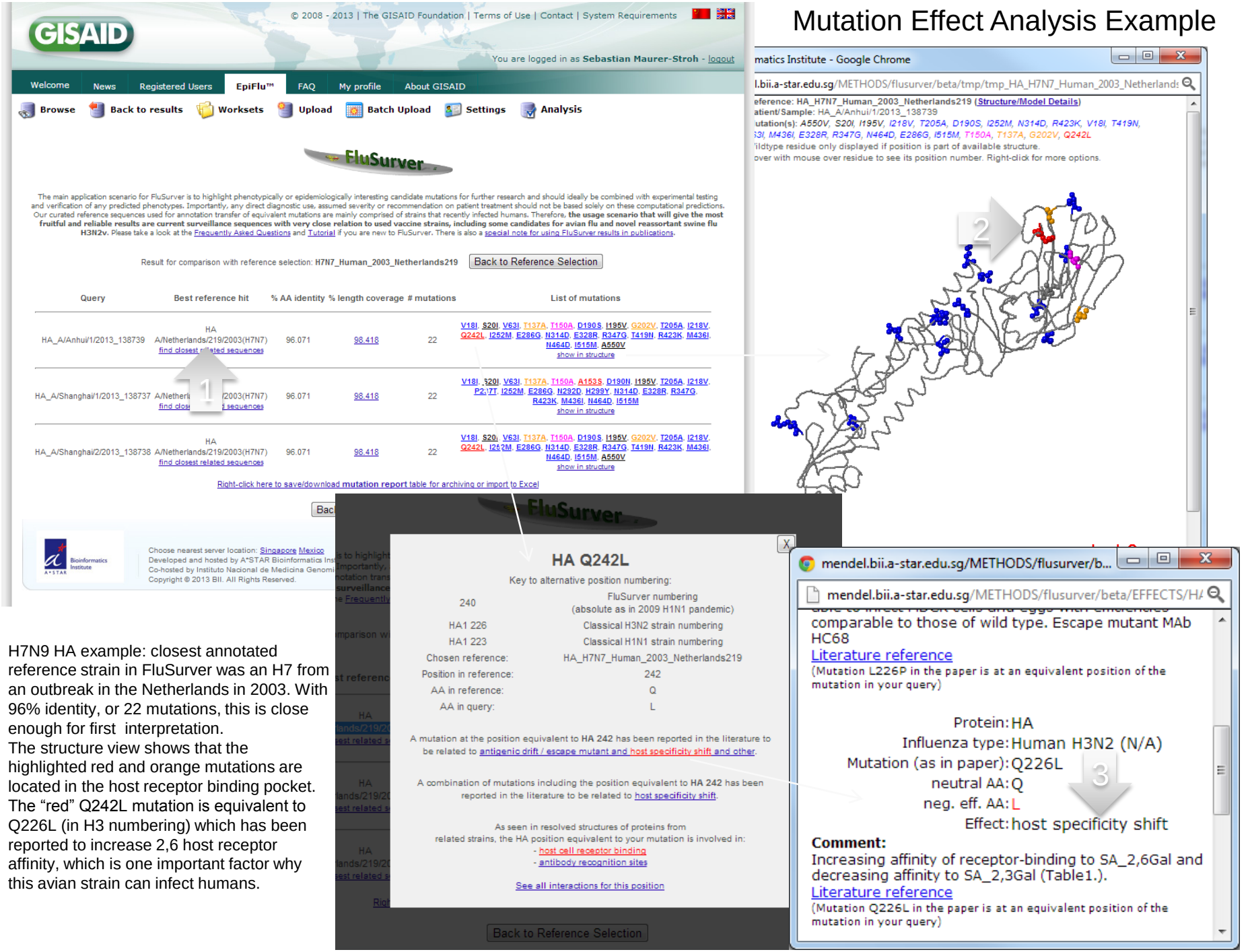
Slight change in appearance of menu options in new version



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

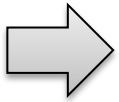
Mutation Effect Analysis Example



1. H7N9 HA example: closest annotated reference strain in FluSurver was an H7 from an outbreak in the Netherlands in 2003. With 96% identity, or 22 mutations, this is close enough for first interpretation.
2. The structure view shows that the highlighted red and orange mutations are located in the host receptor binding pocket.
3. The “red” Q242L mutation is equivalent to Q226L (in H3 numbering) which has been reported to increase 2,6 host receptor affinity, which is one important factor why this avian strain can infect humans.

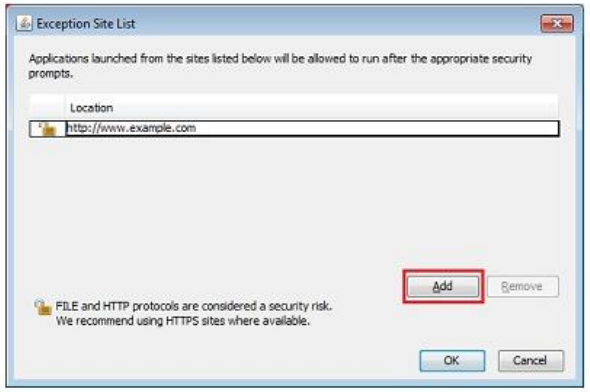
Troubleshooting

- I cannot see the structural view!
  - The structural view uses a free Java program called Jmol which requires:
    - a recent version of the free Java Runtime Environment to be installed (http://java.com/en/download/)
    - accepting the security certificate when prompted.
    - If needed, add “http://mendel.bii.a-star.edu.sg” and “http://flusurver.bii.a-star.edu.sg” to list of trusted sites in the Java configure settings as shown on the right (We have protected the code of the Jmol version used on our server so it cannot be modified by any unauthorized third parties)



Steps to Add URLs to the Exception Site list

- Go to the **Java Control Panel** (On Windows Click Start and then Configure Java)
- Click on the **Security** tab
- Click on the **Edit Site List** button
- Click **Add** in the Exception Site List window

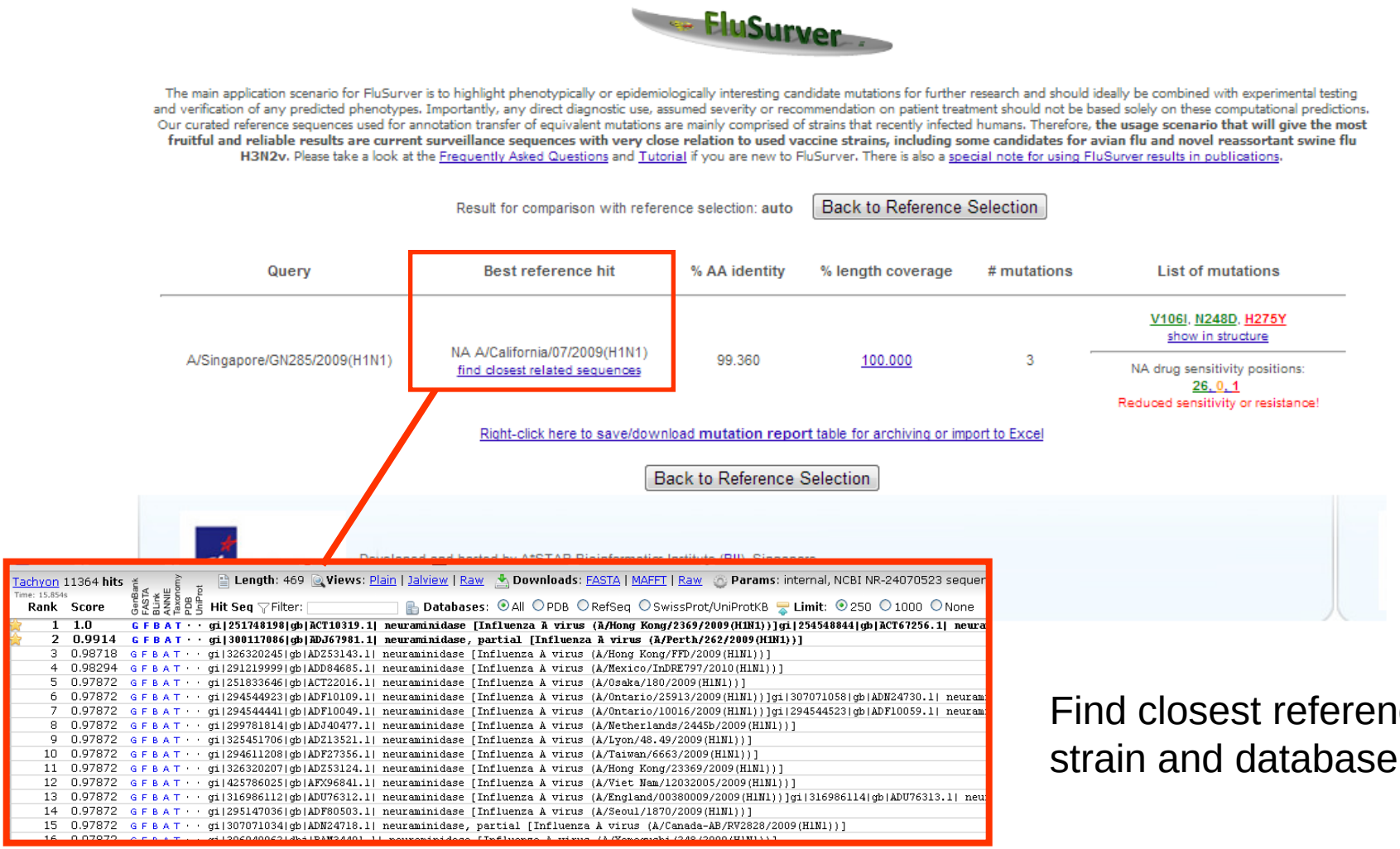


- Click in the empty field under the Location field to enter the URL

Example: http://www.example.com
(URL should begin with **http://** or **https://**)

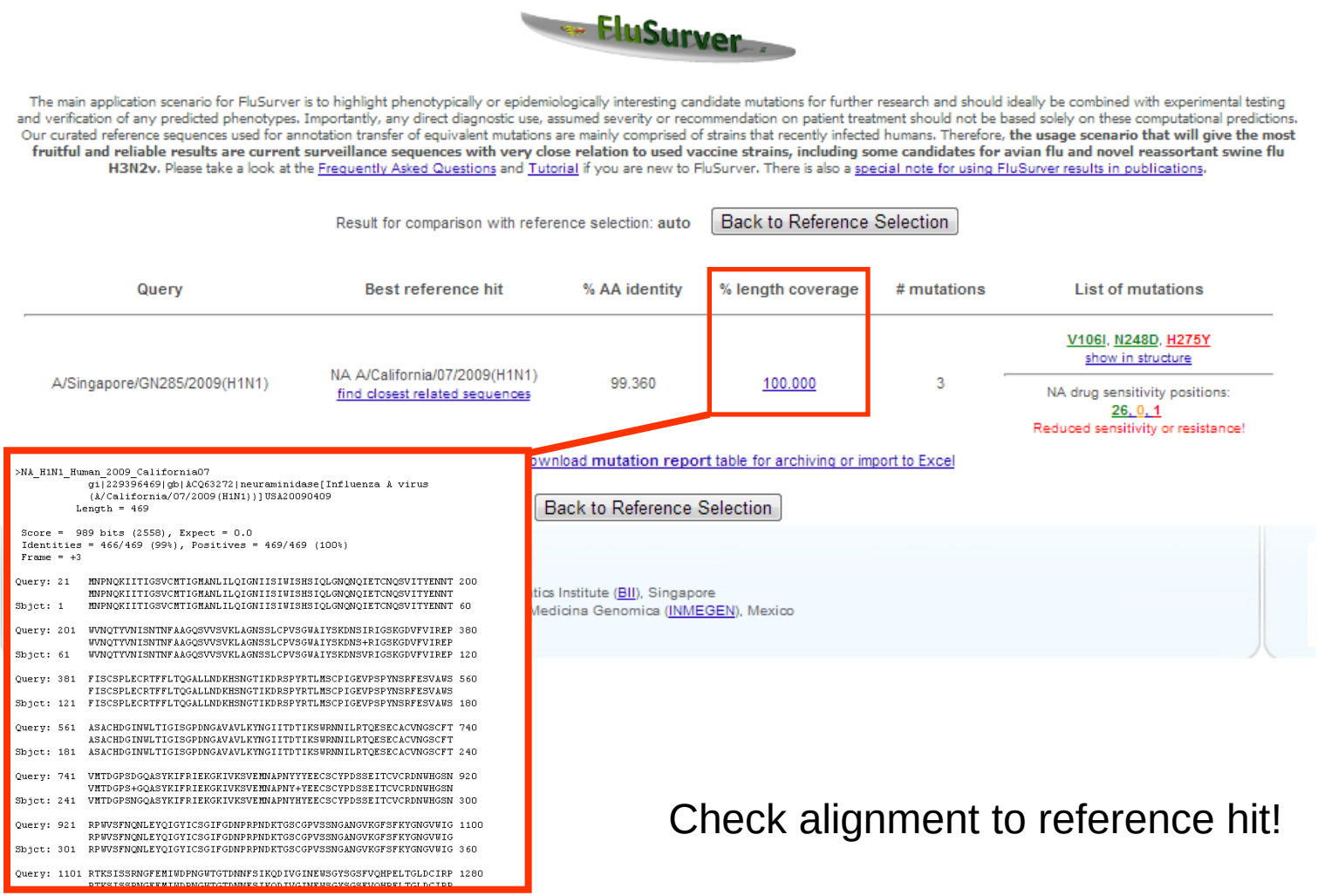
- Answers to more frequently asked questions: http://flusurver.bii.a-star.edu.sg/help/faq.html
- If there are any access problems via GISAID and urgent analysis is needed, you can also use our general site (http://flusurver.bii.a-star.edu.sg/) but this version does not include statistics of GISAID sequences!

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



Find closest reference strain and database hits!

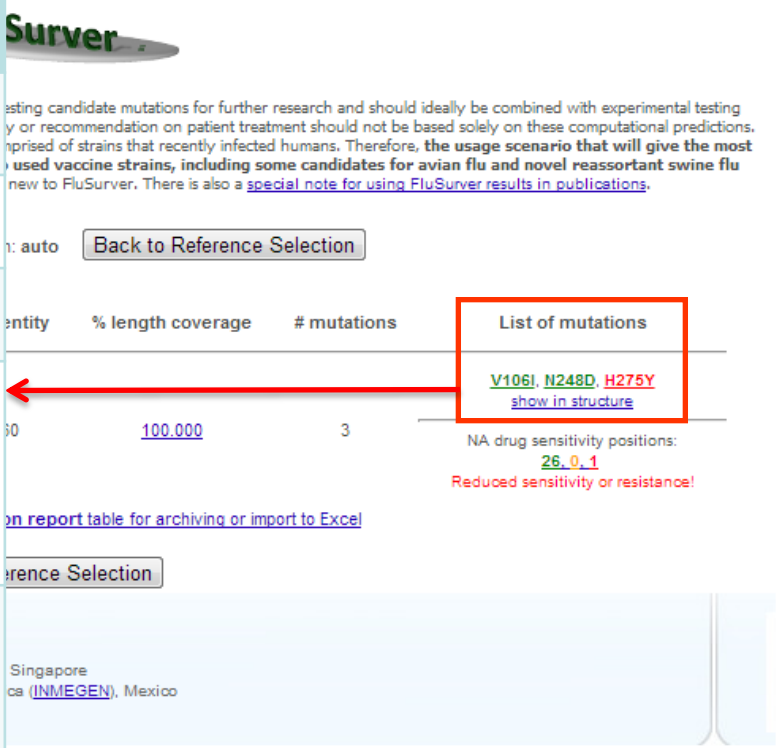
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



Check alignment to reference hit!

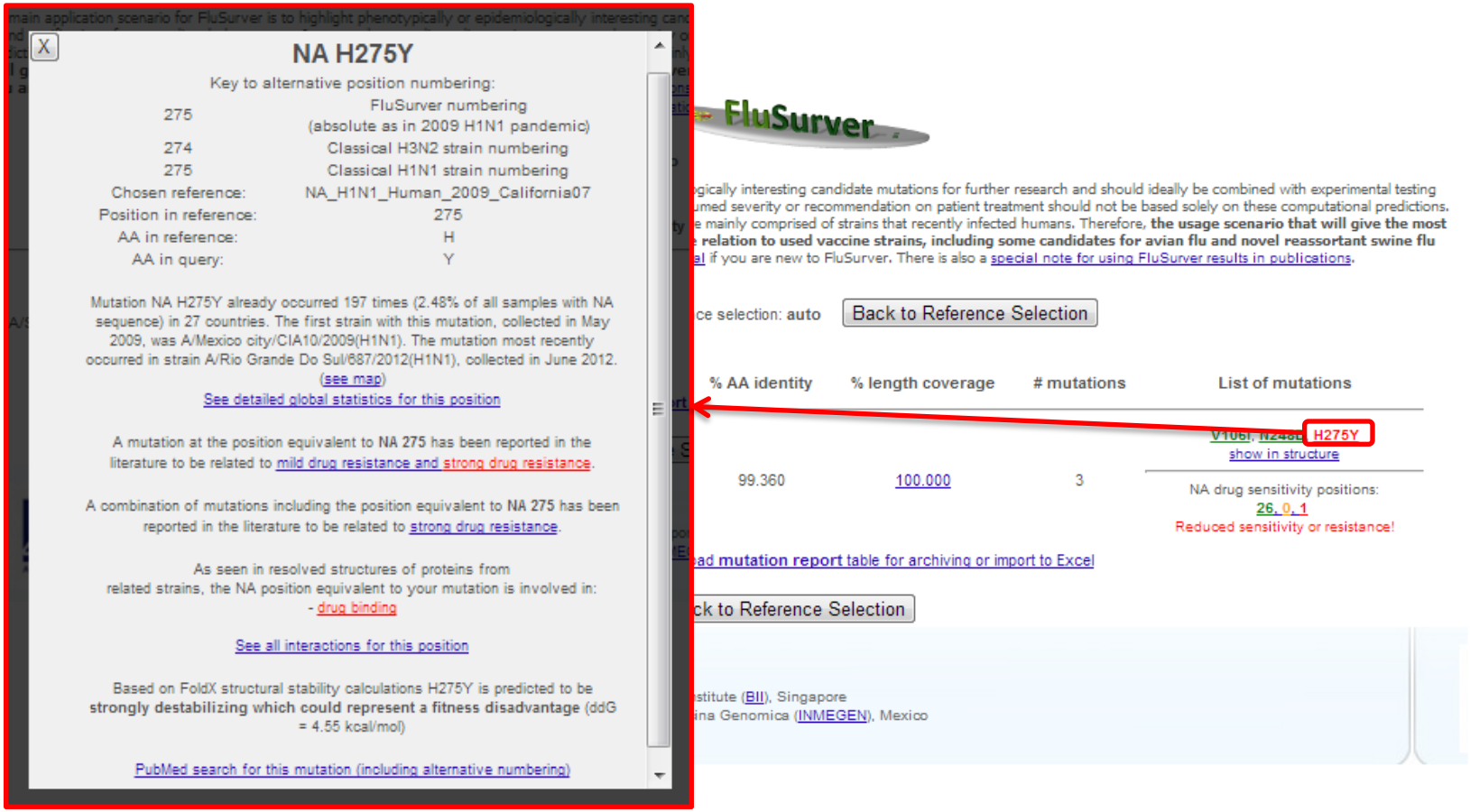
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

Color	Interest level	Remarks
Black	0 (least significant)	No known effects
Green	0	Common
Blue	1	At site of interaction
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.



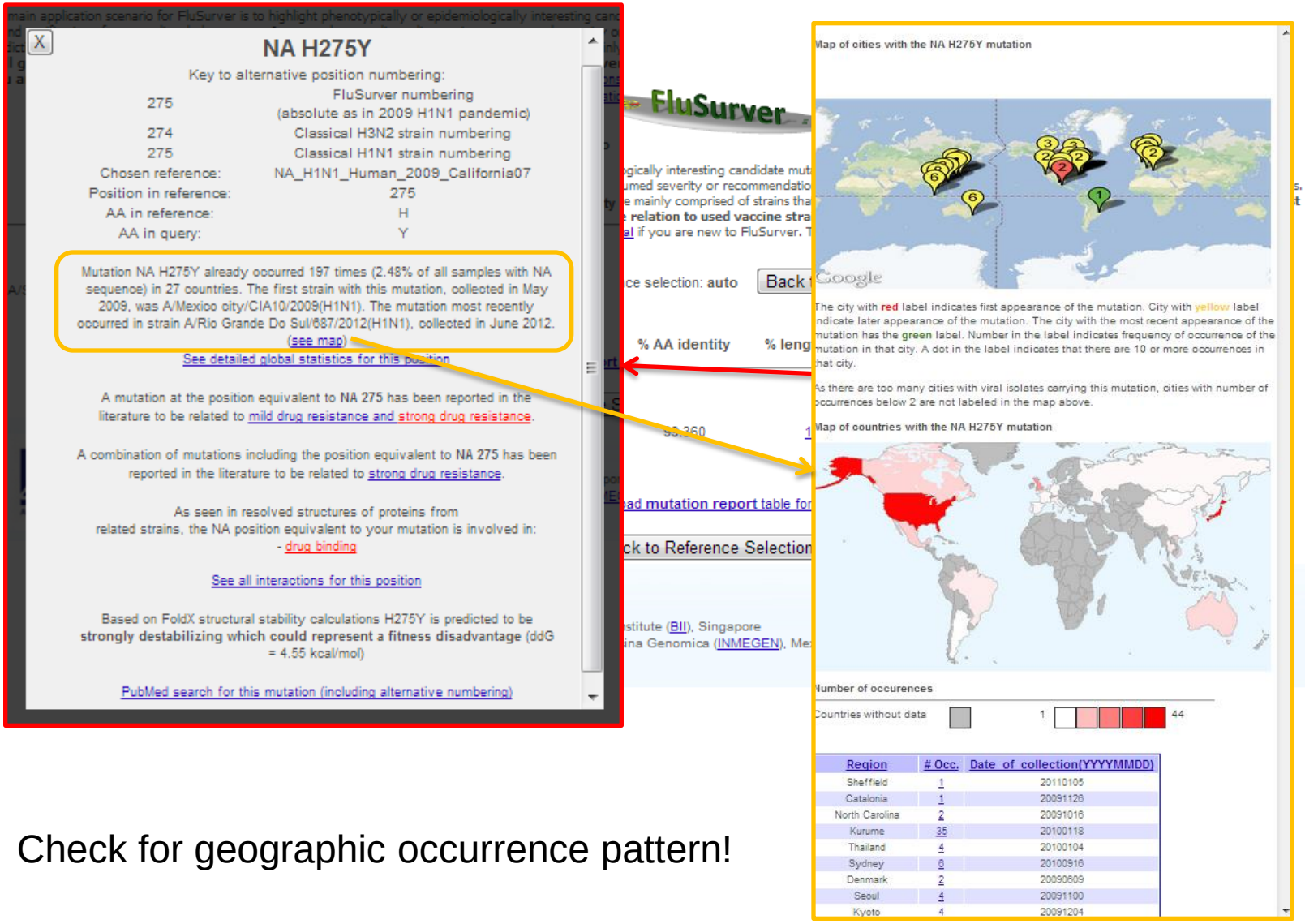
Check list of mutations!

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



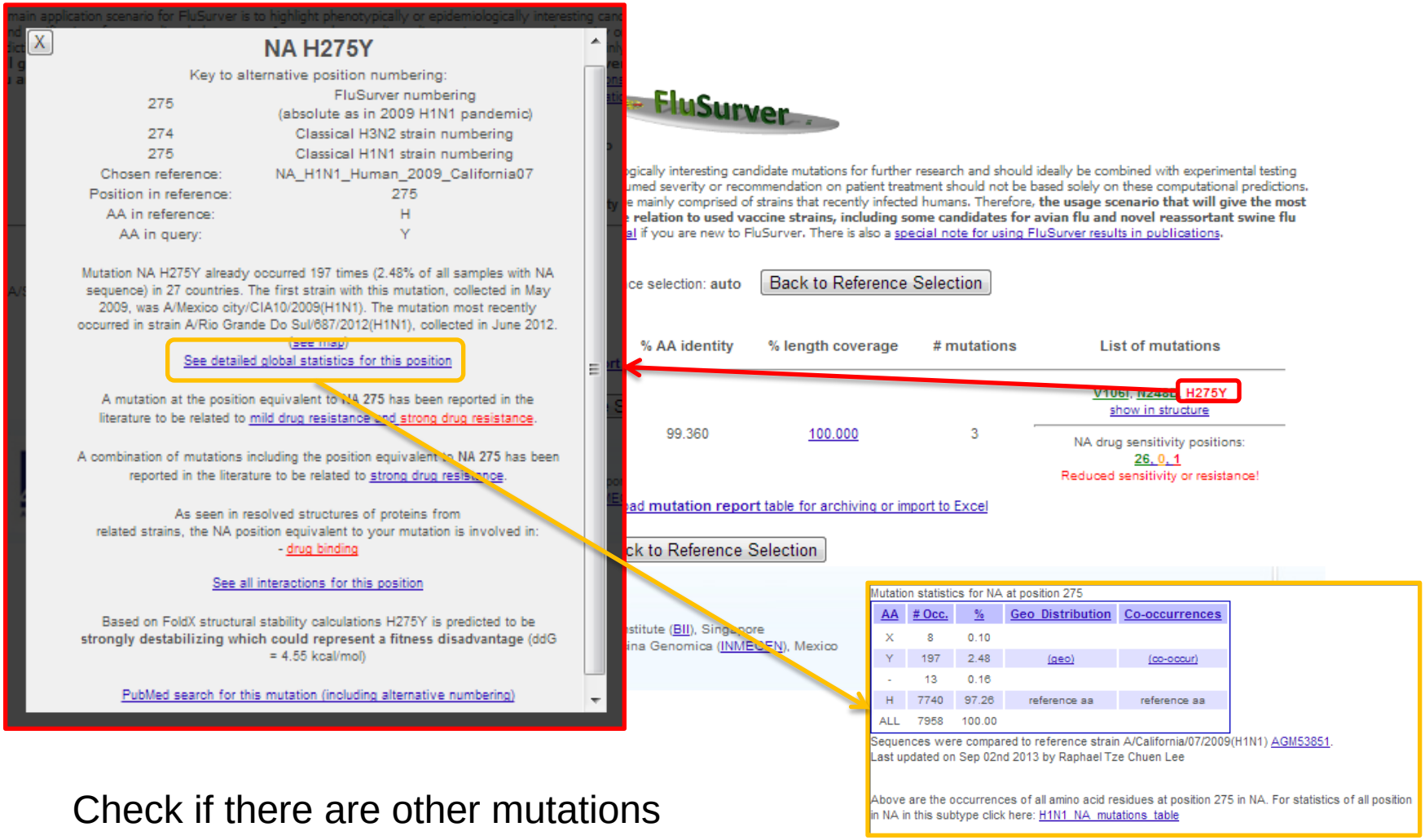
Click on mutation of interest for details!

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



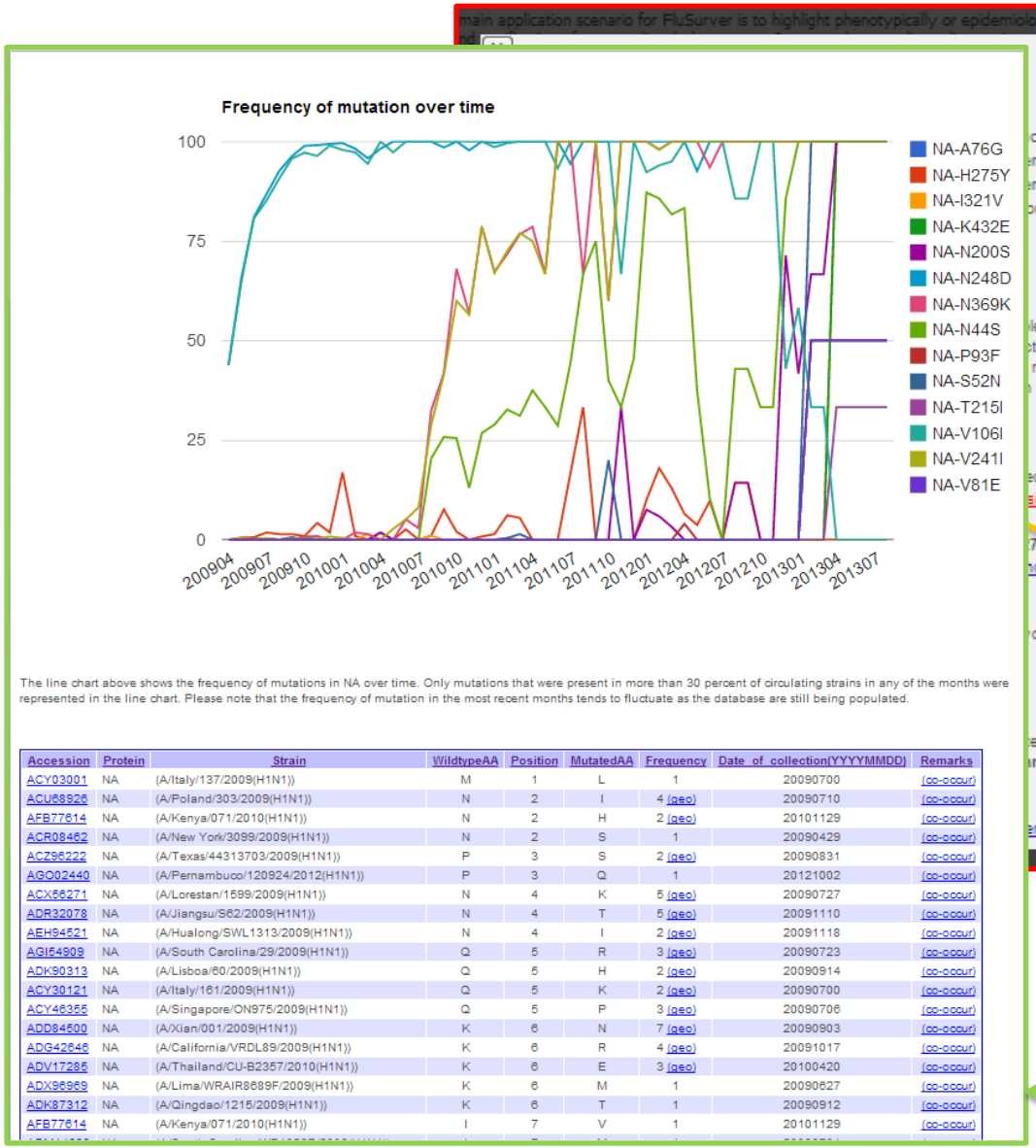
Check for geographic occurrence pattern!

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

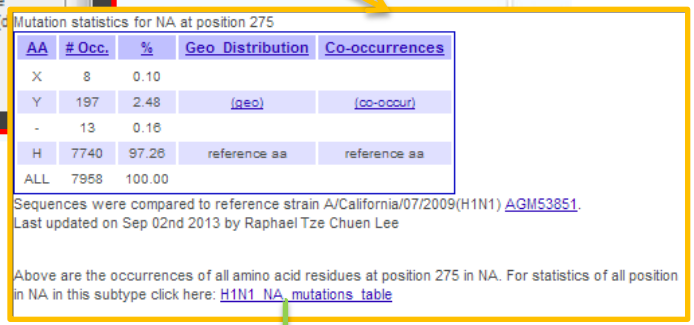


Check if there are other mutations at same position!

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

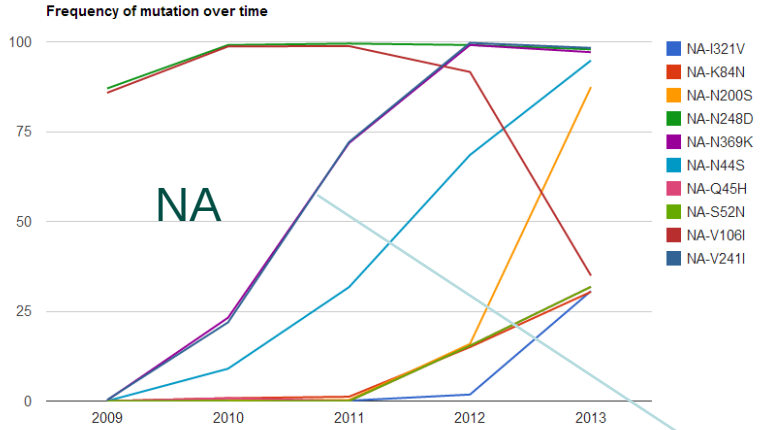


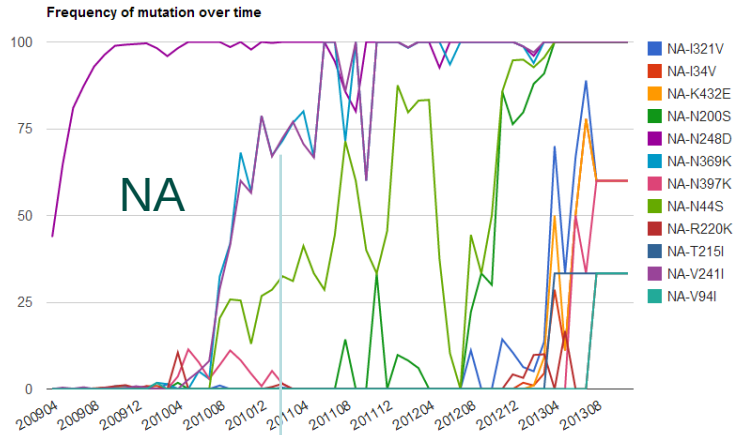
Accession	Protein	Strain	WildtypeAA	Position	MutatedAA	Frequency	Date_of_collection(YYYYMMDD)	Remarks
ACY03001	NA	(A/Italy/137/2009(H1N1))	M	1	L	1	20090700	(co-occur)
ACU88926	NA	(A/Poland/303/2009(H1N1))	N	2	I	4 (geo)	20090710	(co-occur)
AFB77614	NA	(A/Kenya/071/2010(H1N1))	N	2	H	2 (geo)	20101129	(co-occur)
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)

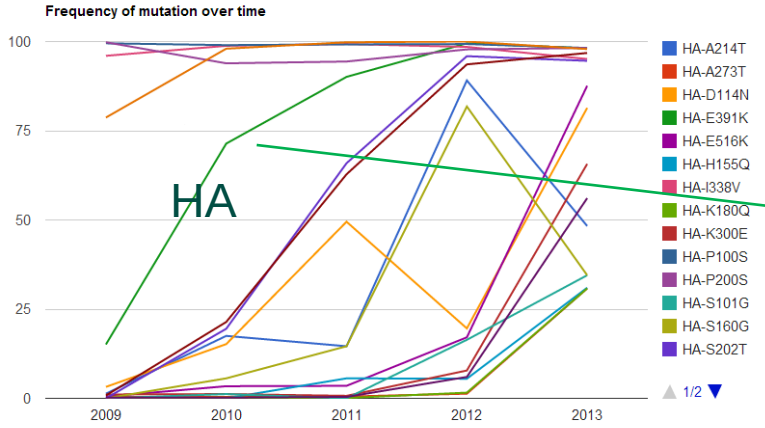


Check for temporal occurrence patterns!

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.

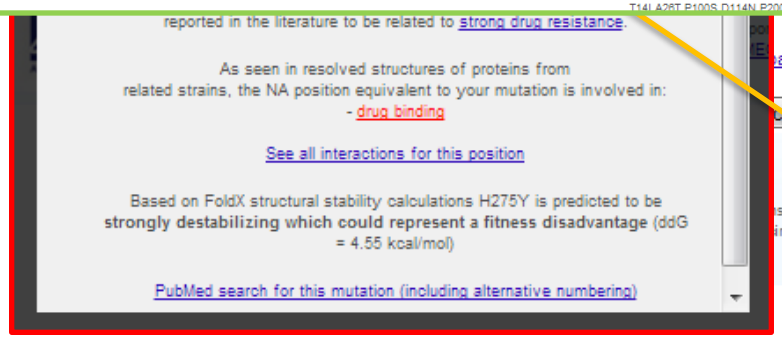
Change in pH-dependency of fusion

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

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 N158S	-	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
						T14I A26T P100S D114N P200S S220T R222K I233V							



Reduced sensitivity or resistance!

ad mutation report table for archiving or import to Excel

k to Reference Selection

stitute (BII), Singapore
ina Genomica (INMEGEN), Mexico

Mutation statistics for NA at position 275

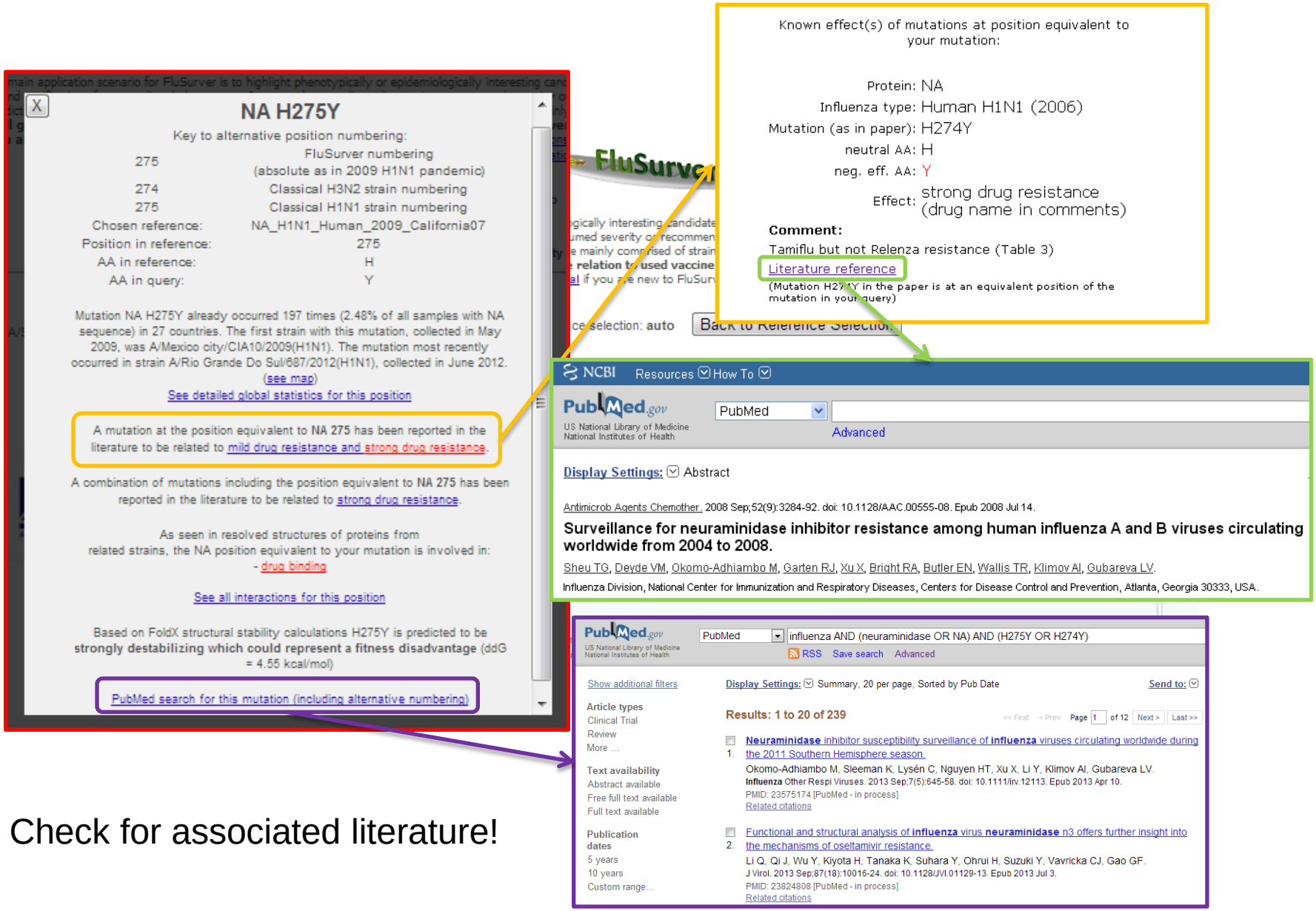
AA	# Occ.	%	Geo_Distribution	Co-occurrences
X	8	0.10		
Y	197	2.48	(geo)	(co-occur)
-	13	0.16		
H	7740	97.26	reference aa	reference aa
ALL	7958	100.00		

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

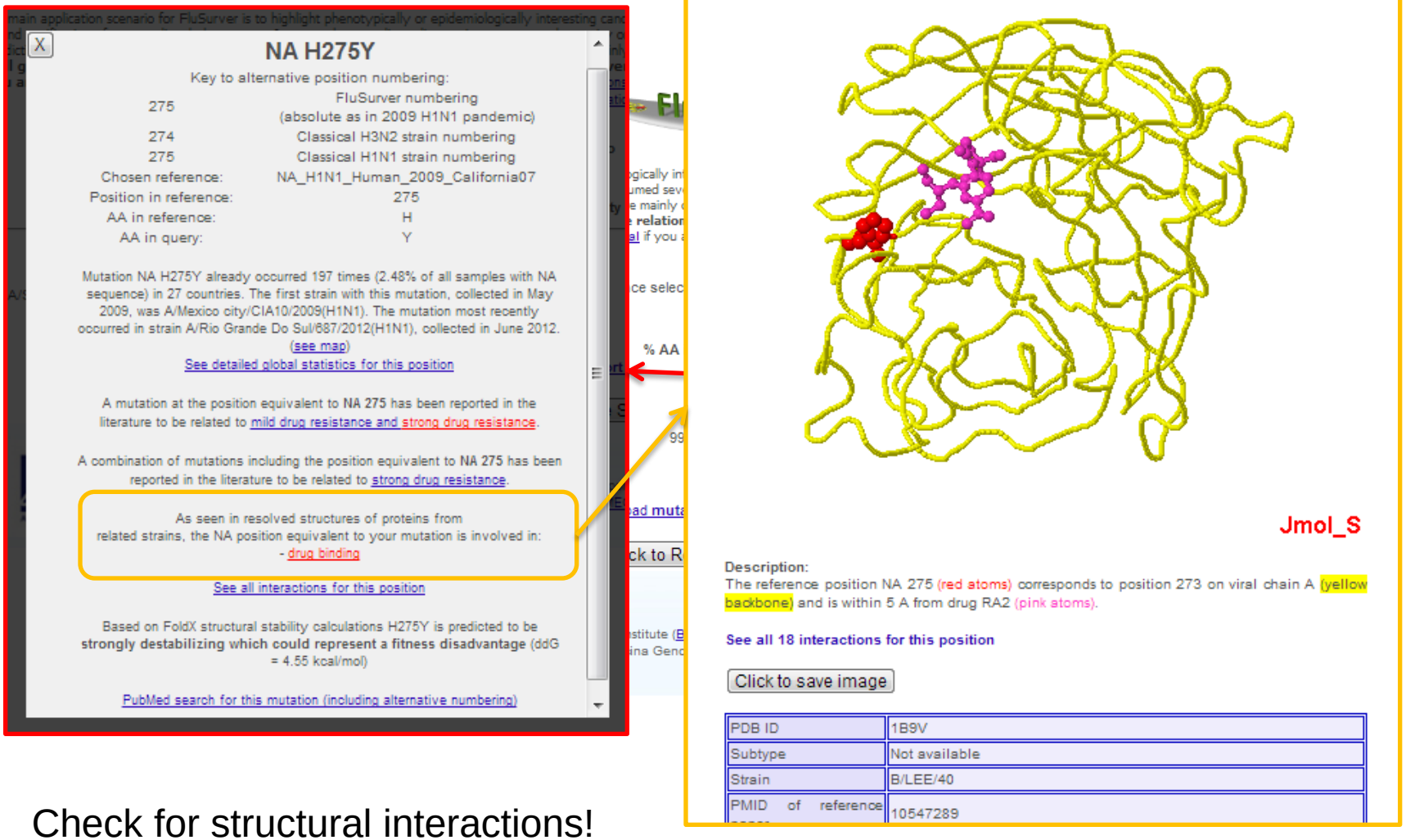
Check for co-occurring mutations!

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



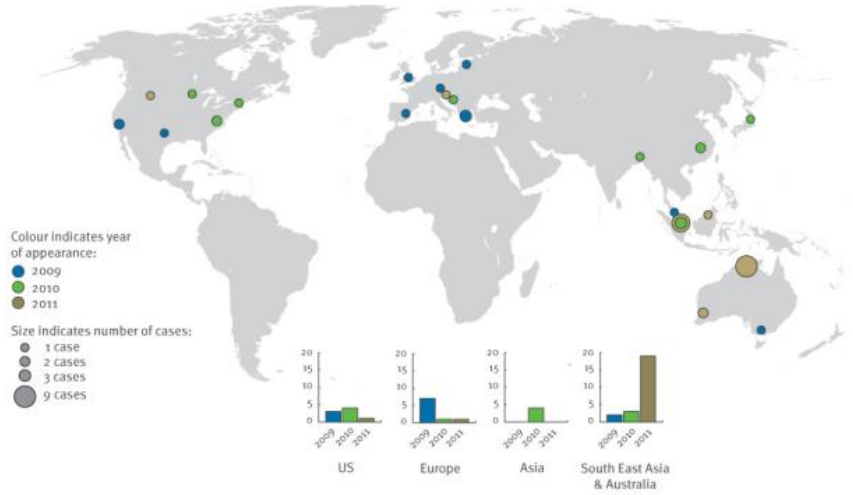
Check for associated literature!

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

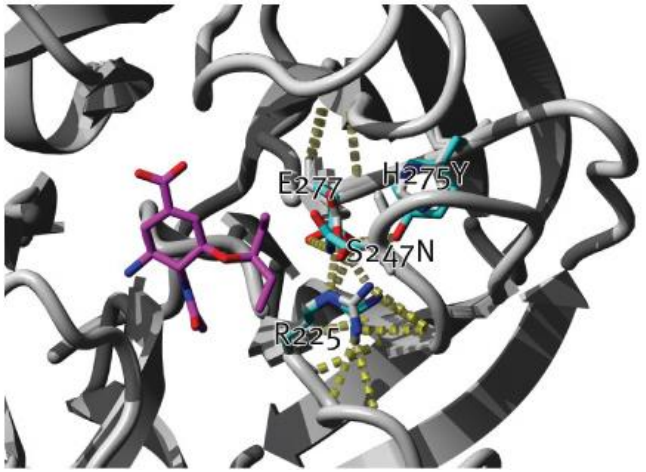


Check for structural interactions!

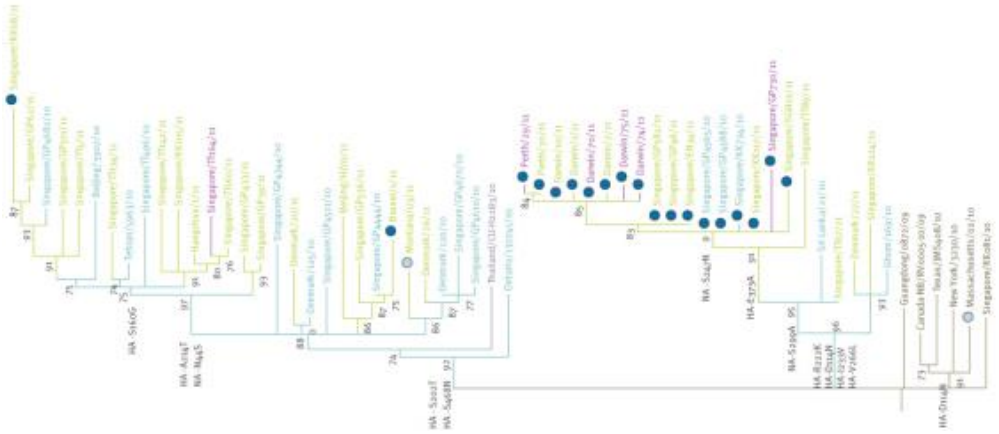
New drug sensitivity altering mutation NA S247N



Global occurrence of new variant



Structural context of mutation



Phylogenetic context of new variant

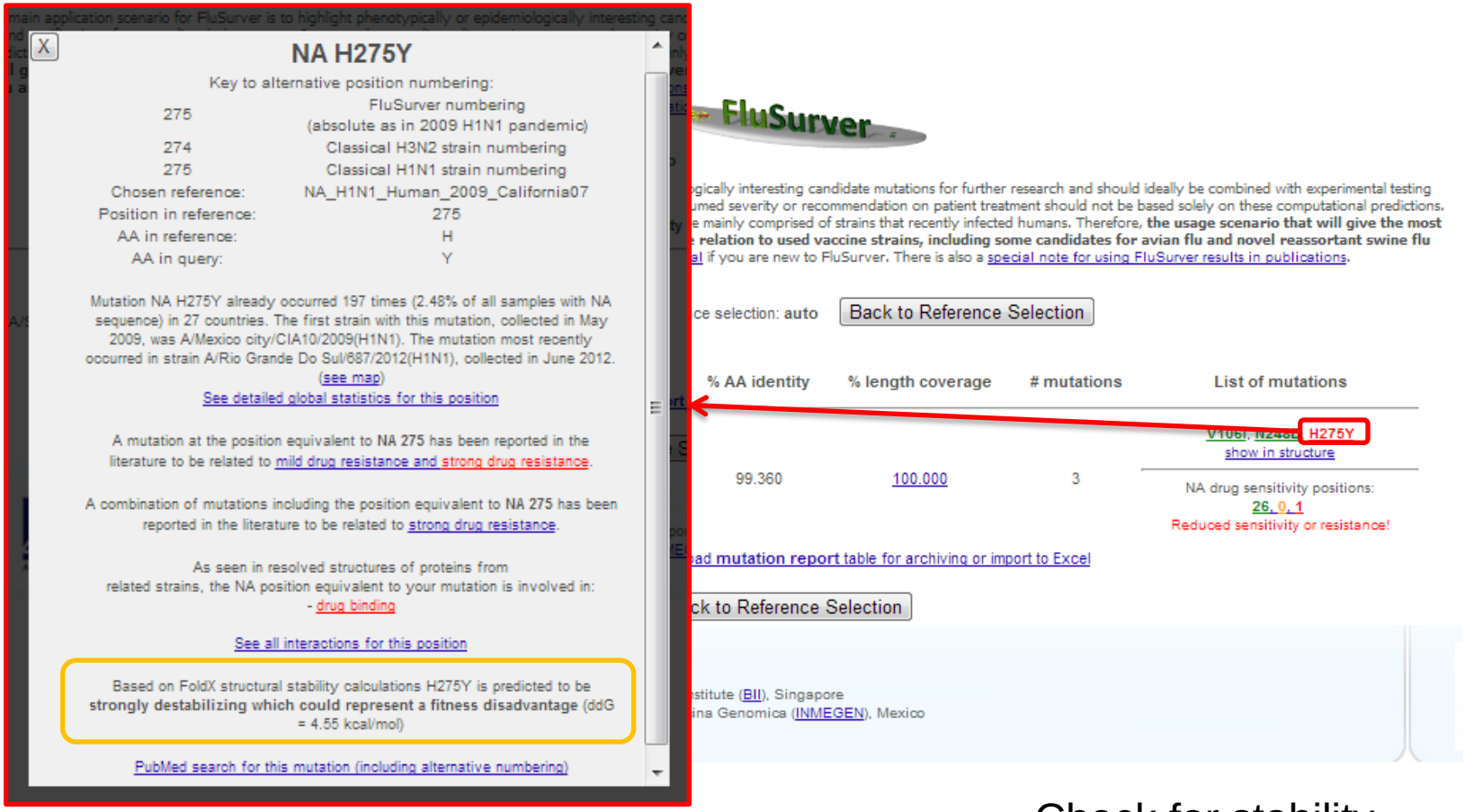
Found circulating in 10% of samples in Singapore and 30% of samples in Northern Australia in early 2011.

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.*

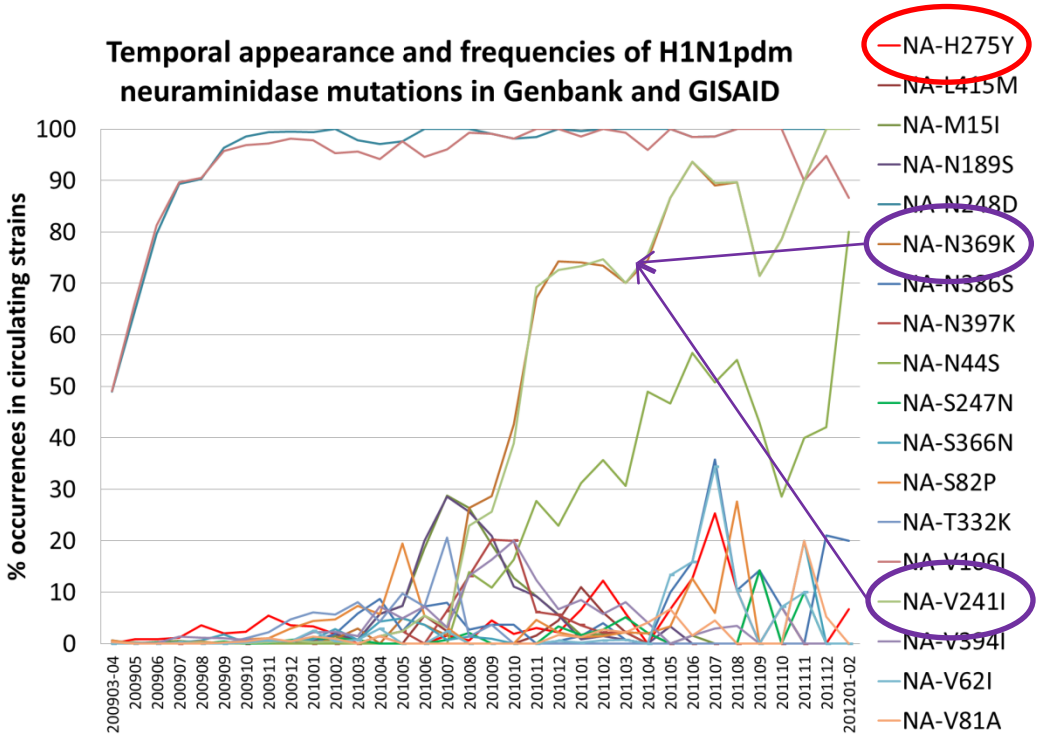
Euro Surveill. 2011;16(23):pii=19884.

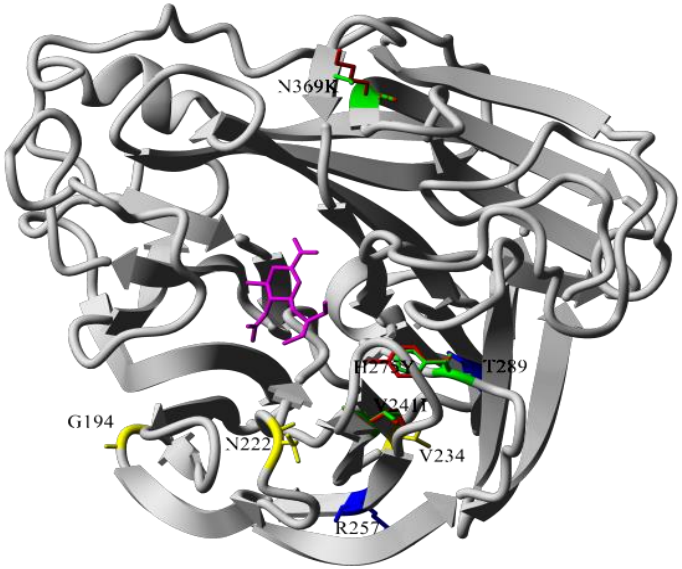
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier

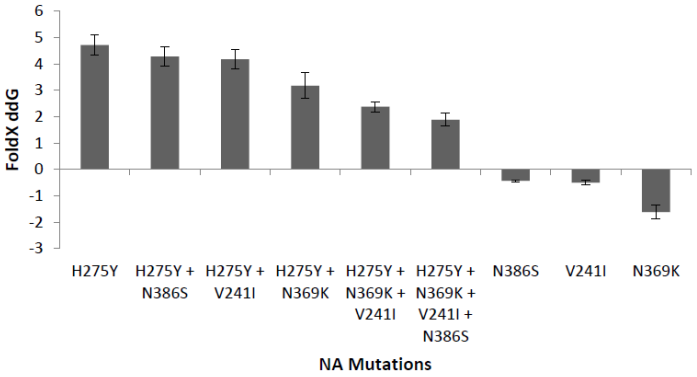


Check for stability or passage effect (if available)!

Frequency rise points to role of permissive mutations



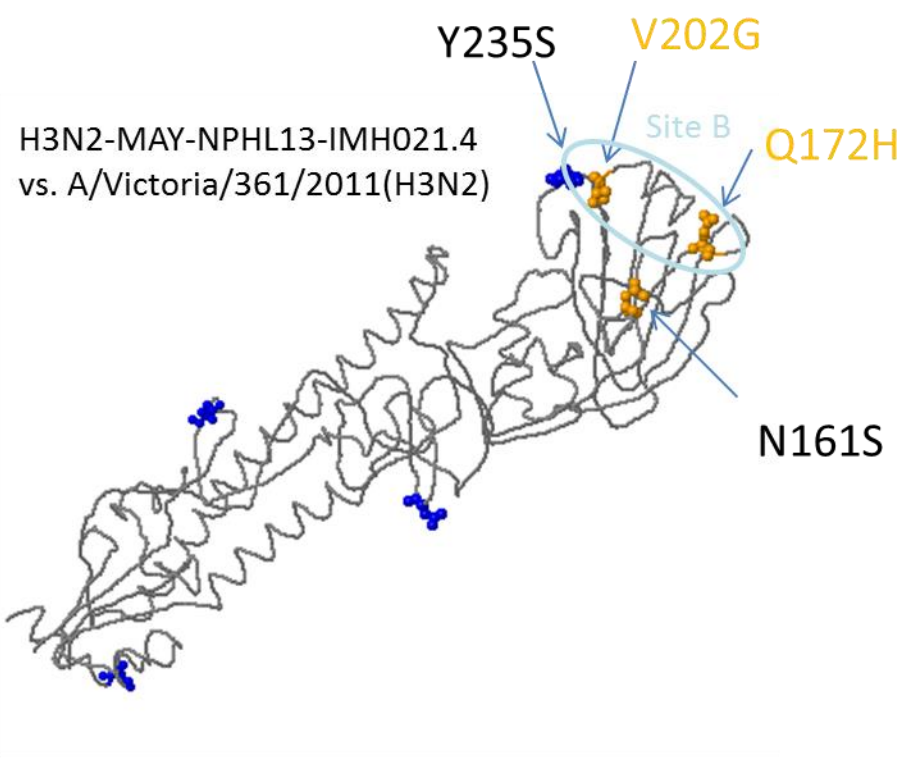


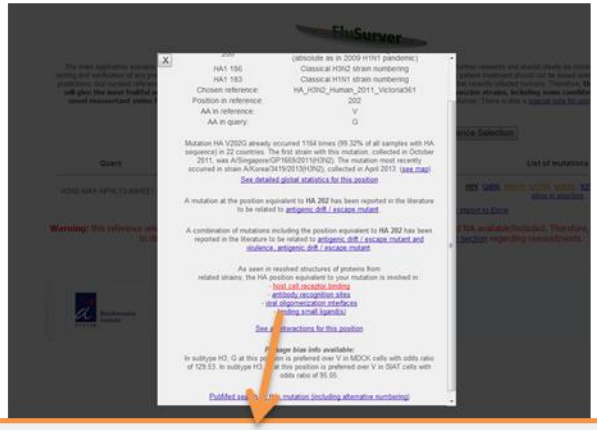


FoldX predicts increase in structural stability for mutations that were increasing in frequency and were fixed in Newcastle strains.

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 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.

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

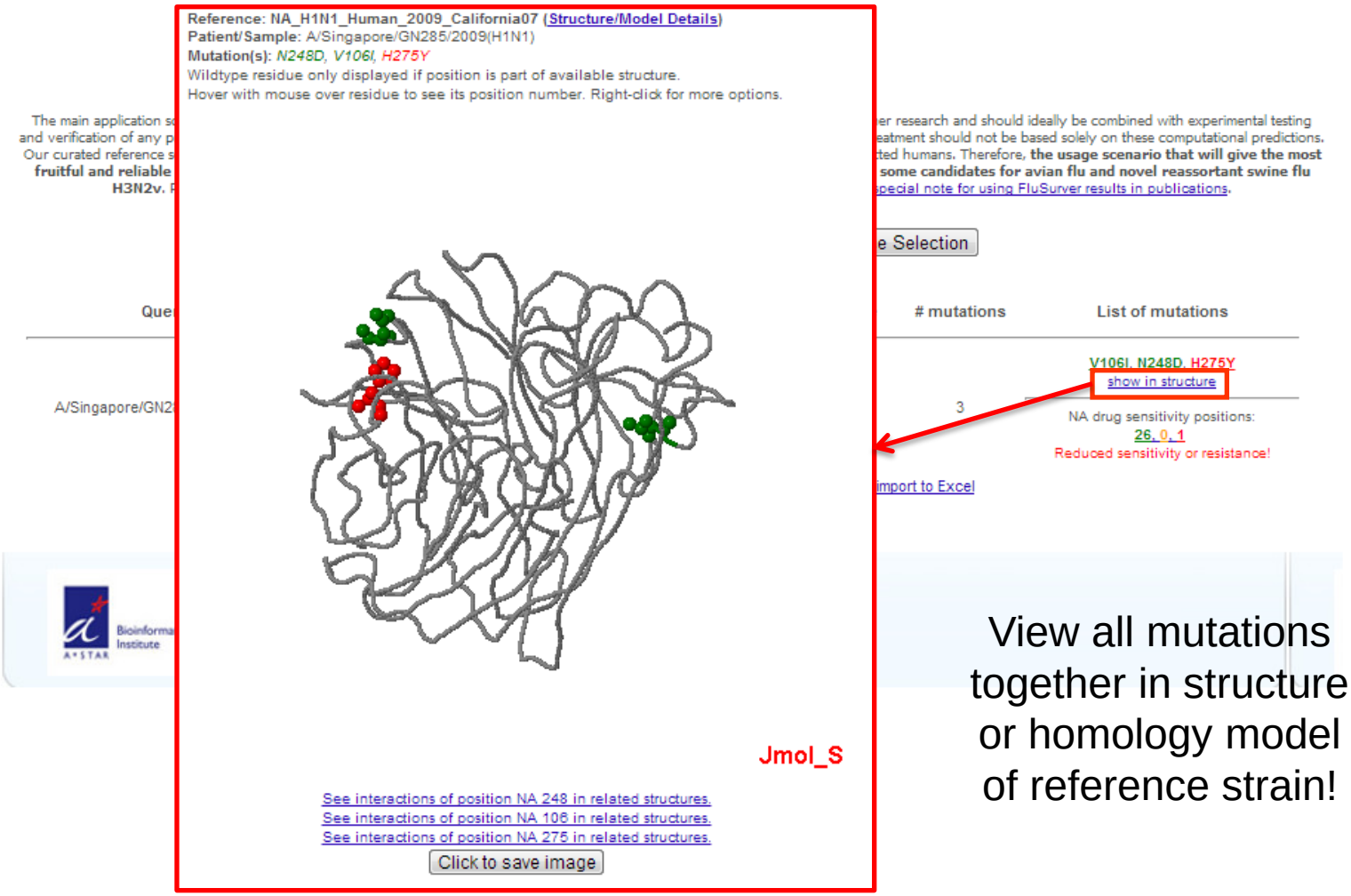
See all interactions for this position

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)

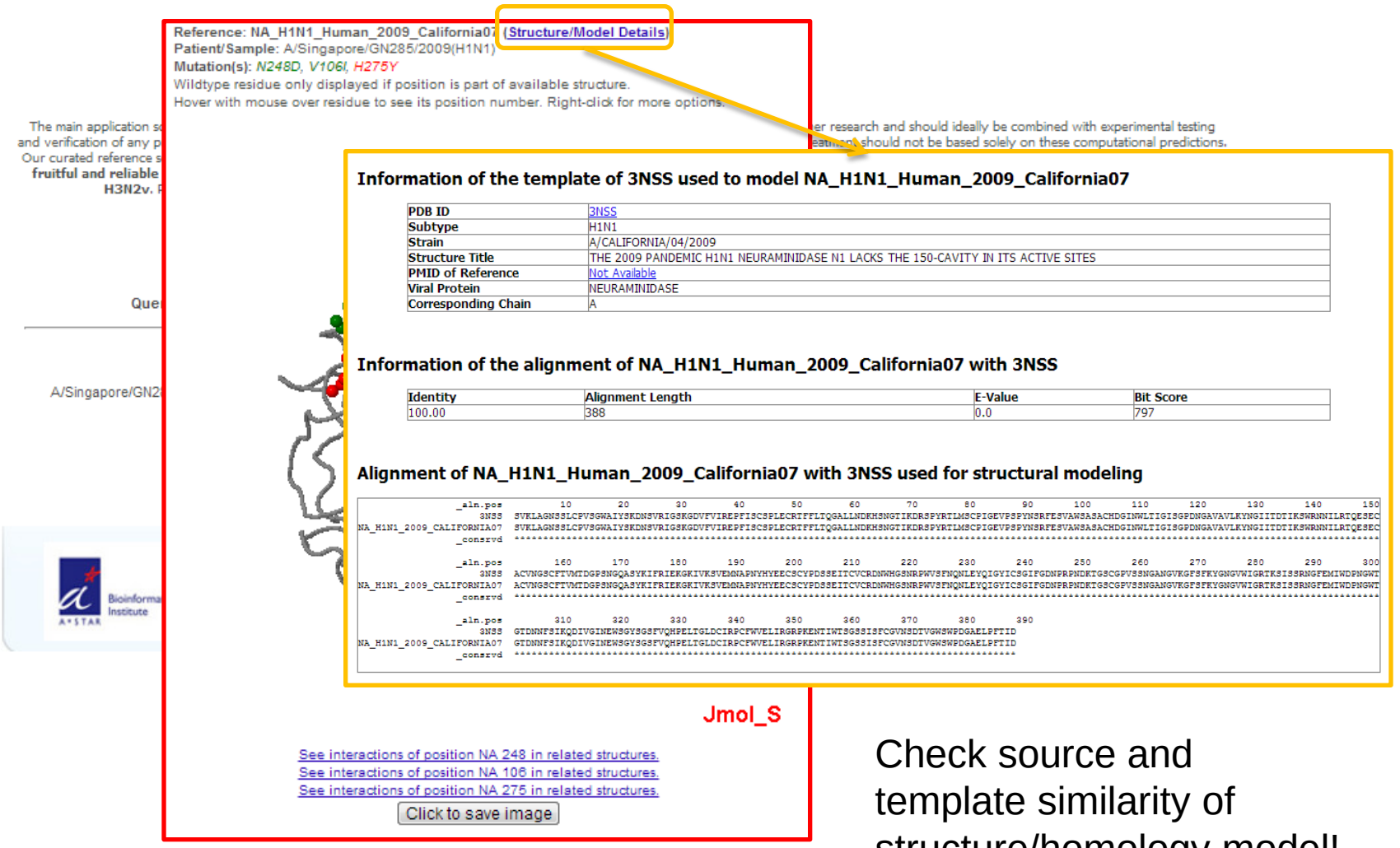
GISAID ID	Submitter	Passage	Mutations relative to Victoria/361
EPI_ISL_128750	WHO CC Melbourne via NPHL	**MDCK0, MDCK1**	H9Y, Q49R, N161S, **Q172H, V202G, Y235S**, N294K
EPI_ISL_135838	US CDC via WHO CC Melbourne	**E4/E1**	H9Y, Q49R, N161S, N294K

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



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

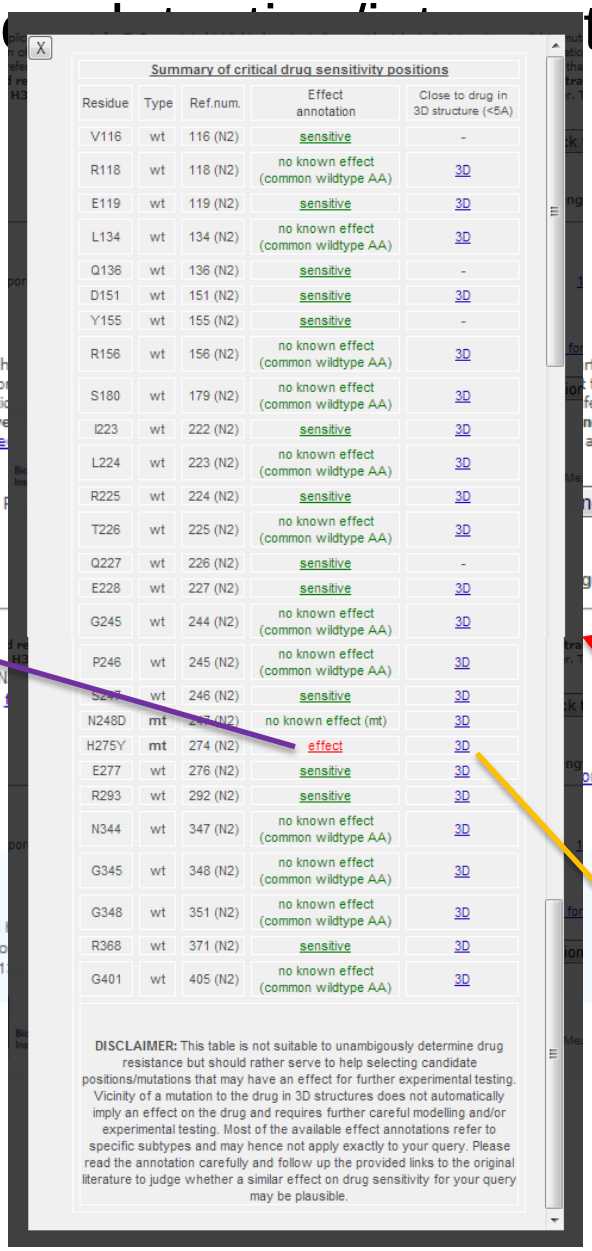
FluSurver – an online tool to make sequence analysis and mutation detection/interpretation easier



Check source and template similarity of structure/homology model!

FluSurver – an online tool to make sequence analysis and mutati tation easier

Check drug summary table!



Summary of critical drug sensitivity positions

Residue	Type	Ref.num.	Effect annotation	Close to drug in 3D structure (<5A)
V116	wt	116 (N2)	sensitive	-
R118	wt	118 (N2)	no known effect (common wildtype AA)	3D
E119	wt	119 (N2)	sensitive	3D
L134	wt	134 (N2)	no known effect (common wildtype AA)	3D
Q136	wt	136 (N2)	sensitive	-
D151	wt	151 (N2)	sensitive	3D
Y155	wt	155 (N2)	sensitive	-
R156	wt	156 (N2)	no known effect (common wildtype AA)	3D
S180	wt	179 (N2)	no known effect (common wildtype AA)	3D
I223	wt	222 (N2)	sensitive	3D
L224	wt	223 (N2)	no known effect (common wildtype AA)	3D
R225	wt	224 (N2)	sensitive	3D
T226	wt	225 (N2)	no known effect (common wildtype AA)	3D
Q227	wt	226 (N2)	sensitive	-
E228	wt	227 (N2)	sensitive	3D
G245	wt	244 (N2)	no known effect (common wildtype AA)	3D
P246	wt	245 (N2)	no known effect (common wildtype AA)	3D
S247	wt	246 (N2)	sensitive	3D
N248D	mt	247 (N2)	no known effect (mt)	3D
H275Y	mt	274 (N2)	effect	3D
E277	wt	276 (N2)	sensitive	3D
R293	wt	292 (N2)	sensitive	3D
N344	wt	347 (N2)	no known effect (common wildtype AA)	3D
G345	wt	348 (N2)	no known effect (common wildtype AA)	3D
G348	wt	351 (N2)	no known effect (common wildtype AA)	3D
R368	wt	371 (N2)	sensitive	3D
G401	wt	405 (N2)	no known effect (common wildtype AA)	3D

DISCLAIMER: This table is not suitable to unambigously determine drug resistance but should rather serve to help selecting candidate positions/mutations that may have an effect for further experimental testing. Vicinity of a mutation to the drug in 3D structures does not automatically imply an effect on the drug and requires further careful modelling and/or experimental testing. Most of the available effect annotations refer to specific subtypes and may hence not apply exactly to your query. Please read the annotation carefully and follow up the provided links to the original literature to judge whether a similar effect on drug sensitivity for your query may be plausible.

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

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 drug sensitivity positions:
26, 0, 1
Reduced sensitivity or resistance!

# mutations	List of mutations
3	V106I, N248D, H275Y show in structure

Jmol_S

Also useful for analysis of other segments!

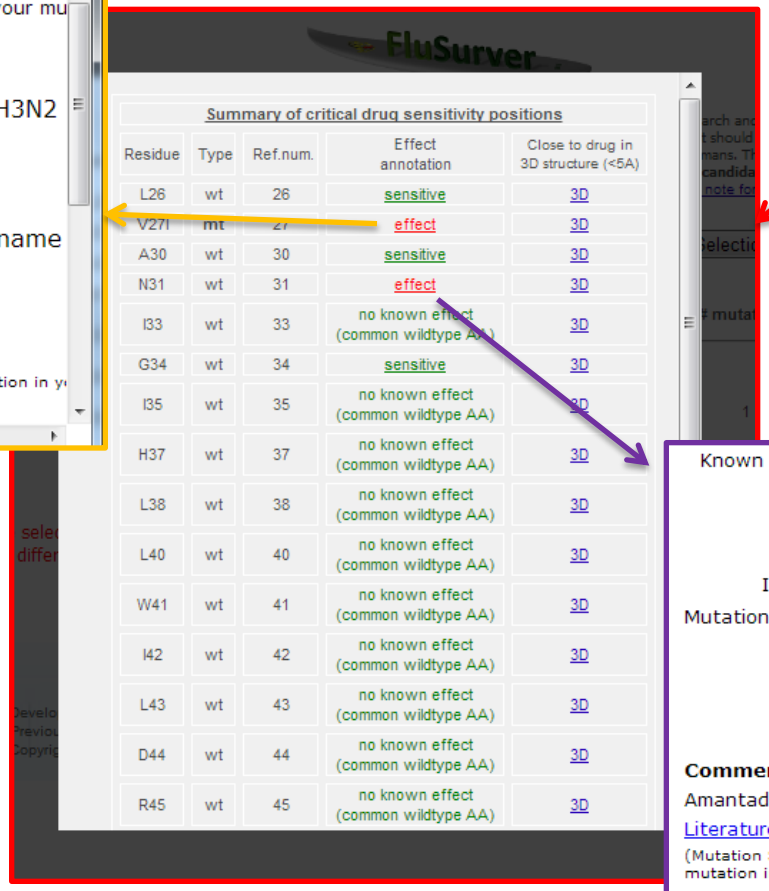
Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
RIVM4541200051|H3 clade 3B|N2|	M2 A/Wisconsin/67/2005(H3N2) find closest related sequences	98.780	84.536	1	V27I show in structure

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)



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)

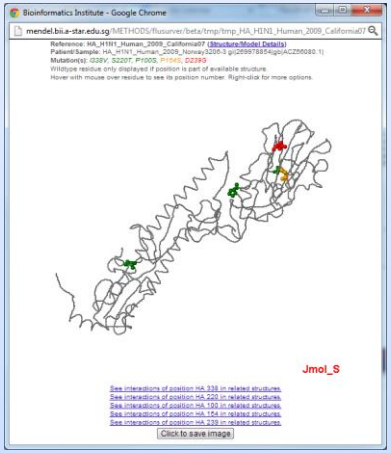
If WT residues in reference strains are associated with resistance it will be shown in drug summary table!

Summary of FluSurver features 2014

Bioinformatics Institute A*STAR

GISAID

Structure

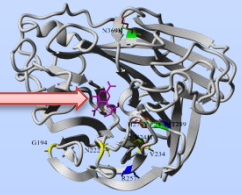


Map mutations to structure

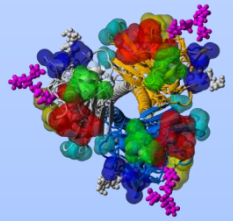
250+ reference homology models

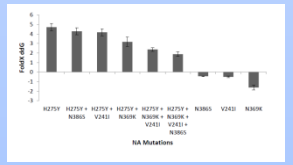
1568	self/oligomerization
975	other small ligand
268	antibody
188	host protein
182	antigen-presenting MHC molecule
132	other viral protein
46	drug
45	nucleic acids
13	host cell receptor
3417	**total interactions for 2062 positions**

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

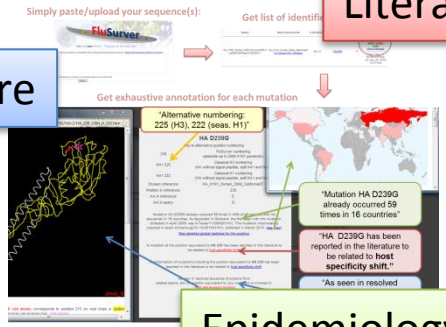
Passage bias (egg/cell adaptation) for ~1300 mutations

Literature-curated mutation effect database

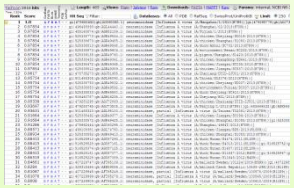
~250 entries

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

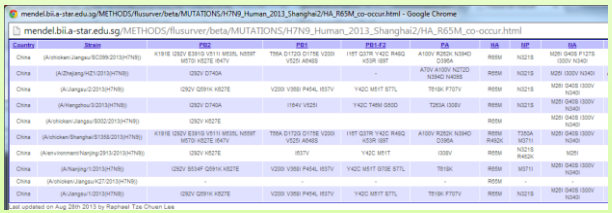
Epidemiology



Closest DB hits



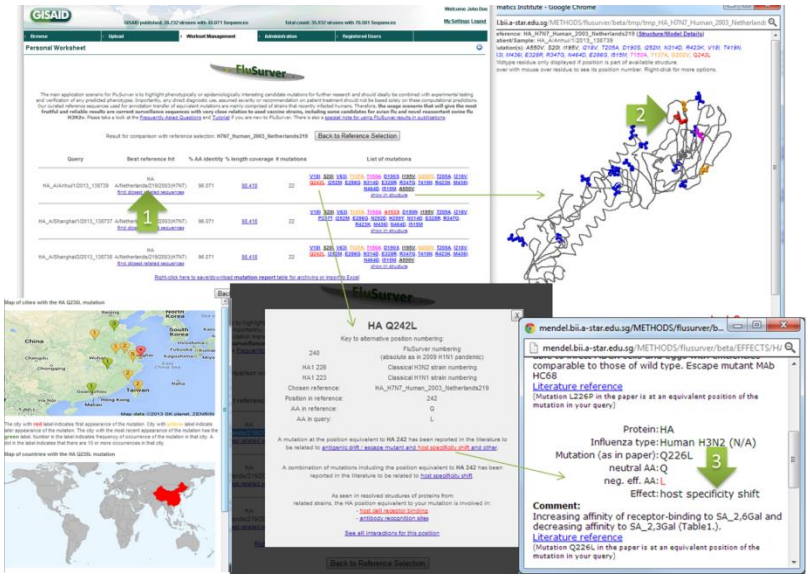
Temporal pattern



Genomic co-occurrence

Regional & global occurrence

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.

FluSurver Acknowledgements

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

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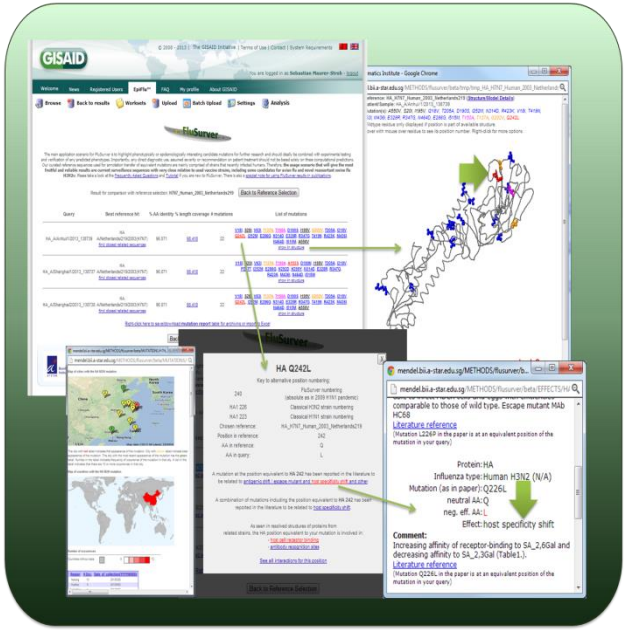
The FluSurver would be nothing without the valuable feedback and interaction with the influenza research and surveillance community, including especially and in chronological order:

- Genome Institute of Singapore (GIS), Singapore
- INMEGEN Mexico City, Mexico
- 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)



Contact: flusurver@gisaid.org

Fishing for Flu Mutations since 2009!

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