

LIBRATOR V 1.0 - USER MANUAL

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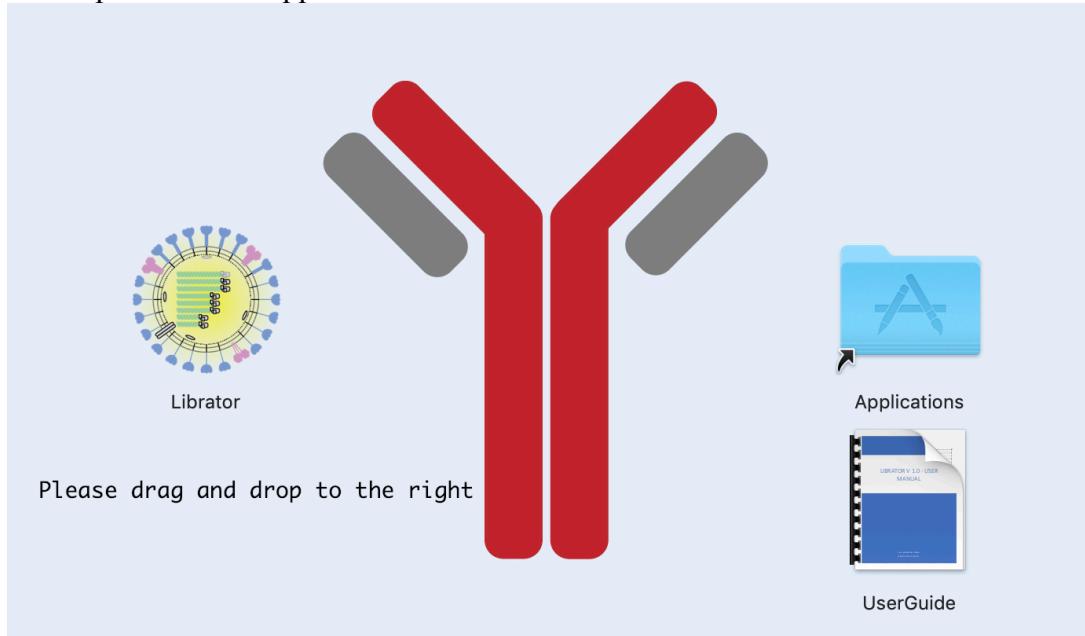
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Installation

Install Librator

Drag and drop liberator to application folder.



Install Dependencies

PyMOL (<https://pymol.org/2/>)

PyMOL for 3D structure visualization of mutated HA protein

Download from official website (license required after 30 days free trial)
or install from anaconda

```
conda install -c schrodinger pymol
```

or install from homebrew (recommended)

```
brew install brewsci/bio/pymol
```

or install from MacPorts

```
sudo port install pymol
```

or install from source (not recommended)

```
python setup.py --osx-frameworks install
```

After you installed PyMOL and setup the correct path, Librator is ready to work. In case some pre-compile tools don't work on some specific MacOS version, you can download and install those missing packages by following our guidelines:

Clustal Omega

We already included pre-compiled clustal omega with Librator. If our version is not working, please download clustal omega from <http://www.clustal.org/omega/> and then setup the correct path in path setting (see basic function->path setting for details).

Muscle

We already included pre-compiled muscle with Librator. If our version is not working, please download muscle from <https://www.drive5.com/muscle/> and then setup the correct path in path setting (see basic function->path setting for details).

Home brew(<https://brew.sh/>)

The Missing Package Manager for macOS (or Linux). You may need the help form homebrew to install some missing packages. Open a terminal and paste the follow commands, then enter

```
/bin/bash -c "$(curl -fsSL  
https://raw.githubusercontent.com/Homebrew/install/master/install.sh)"
```

GhostScript (<https://www.ghostscript.com/>) + Pdf2svg(<https://github.com/dawbarton/pdf2svg>)

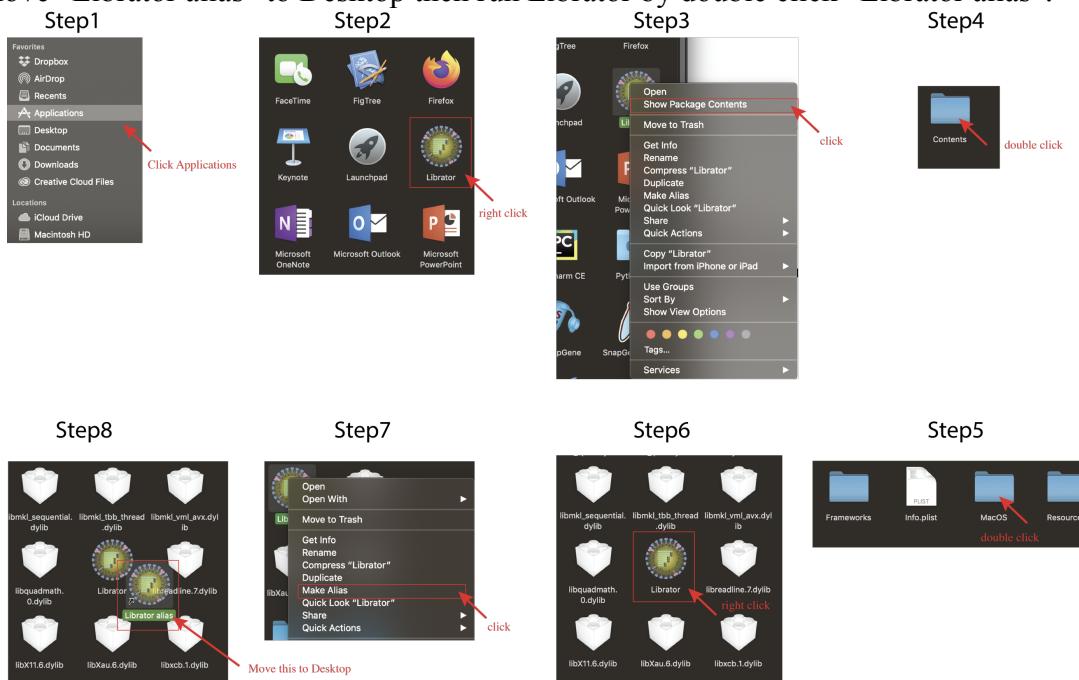
This two libraries are required by embedded sequence logo viewer. Users still can view sequence logo on a popup window without this two libraries.

```
brew install ghostscript  
brew install pdf2svg
```

Run Librator

Users can run Librator directly from Application->Librator (double click).

For faster user experience, users can go to Application folder and right click on Librator icon, then click “Show package contents”, then go to Contents->MacOS, find file “Librator”. Right click “Librator”, choose “make alias”, then a file called “Librator alias” will be generated. Users can move “Librator alias” to Desktop then run Librator by double click “Librator alias”.



User interface

Main Tab (printable)

Sequence Tab (printable)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

Main Sequence Alignment(RTF) Alignment(HTML) SequenceDB Summary Summary(HTML) FragmentDB

Sequence Name: A/California/04/2009 (H1) -S75M(HA1)

Subtype: H1

Click feature to show for sequence:

H3 Numbering H1 Numbering Mutations Donor Regions

Sequence Map: Please scroll to refresh the alignment if the content doesn't change

A/California/04/2009 (H1) -S75M(HA1)

```

Position: ....5....10...15...20...25...30...35...40...45...50...55...6
H1-Numbering: -----10...15...20...25...30...35...40...45...
Sequence: MKAILVVLLYTFTANADTLIGIYHANNSTDVTVDLKEKNVTVTHSVNLLEDKHNGLCK

Position: 0...65...70...75...80...85...90...95...100...105...110...115..1
H1-Numbering: 50...55...60...65...70...75...80...85...90...95...100...105..
Sequence: LRGVAPLHLGKCNIAKGWILGNPECEMLSTASSWSYIVETPSSDNGTCYPGDFIDYEELRE

Position: 20..125..130..135..140..145..150..155..160..165..170..175..1
H1-Numbering: 110..115..120..125..130..135..140..145..150..155..160..165..
Sequence: QLSSVSSFEREIFFGKTSSWPNHDNSNKVTAACPHAGAKSFYKNLIVLVKKGNSYPKLSK

Position: 80..185..190..195..200..205..210..215..220..225..230..235..2
H1-Numbering: 170..175..180..185..190..195..200..205..210..215..220..225..
Sequence: SYINDKGKEVLVLWGIIHHPSTSADQSLYQNADTYVFVGSSRYSKKFPEIAIRPKVRDQ

Position: 40..245..250..255..260..265..270..275..280..285..290..295..3
H1-Numbering: 230..235..240..245..250..255..260..265..270..275..280..285..
Sequence: EGRMNYYWTLVEPGDKITFEATGNLVPRYAFAMERNAGSGIIISDTPVHDCNTTCQTPK

Position: 00..305..310..315..320..325..330..335..340..345..350..355..3
H1-Numbering: 290..295..300..305..310..315..320..325-----1..5...10..15
Sequence: GAIANTSPLFQNIHPITIGKCPKYVKSTKLATGLRNIPSIQSRLGAIAGFIEGGWTG

Position: 60..365..370..375..380..385..390..395..400..405..410..415..4
H1-Numbering: ..20..25..30..35..40..45..50..55..60..65..70..75
Sequence: MVDGWWYGHHQNEQGSGYAADLKSTQNAIDEITNKVNVSIEKMNTQFTAVGKEFNHLEKR

Position: 20..425..430..435..440..445..450..455..460..465..470..475..4
H1-Numbering: ..80..85..90..95..100..105..110..115..120..125..130..13
Sequence: IENLNKKVDDGFLDIWTYNAELLVLENERTLDYHDSNVKNLYEKVRSQLKNNAKEING

Position: 80..485..490..495..500..505..510..515..520..525..530..535..5
H1-Numbering: 5..140..145..150..155..160-----
Sequence: CFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREEIDGVKLESTRIYQILAIYSTVASS

Position: 40..545..550..555..560....
H1-Numbering: -----
Sequence: LVLVVSLGAISFWMCNSGLQCRICI

Sequence elements: HA1 HA2 stop Transmembrane Trimerization-Avitag-H6 Mutations
H1 Antigenic Sites: Ca1 Ca2 Cb Sa Sb Stalk-MN
Position: Donor Region
```

3D Visualization Base Sequence: A/Wyoming/3/2003 (H3) Gibson Clones

Alignment Tab (HTML)

Sequence DB Tab

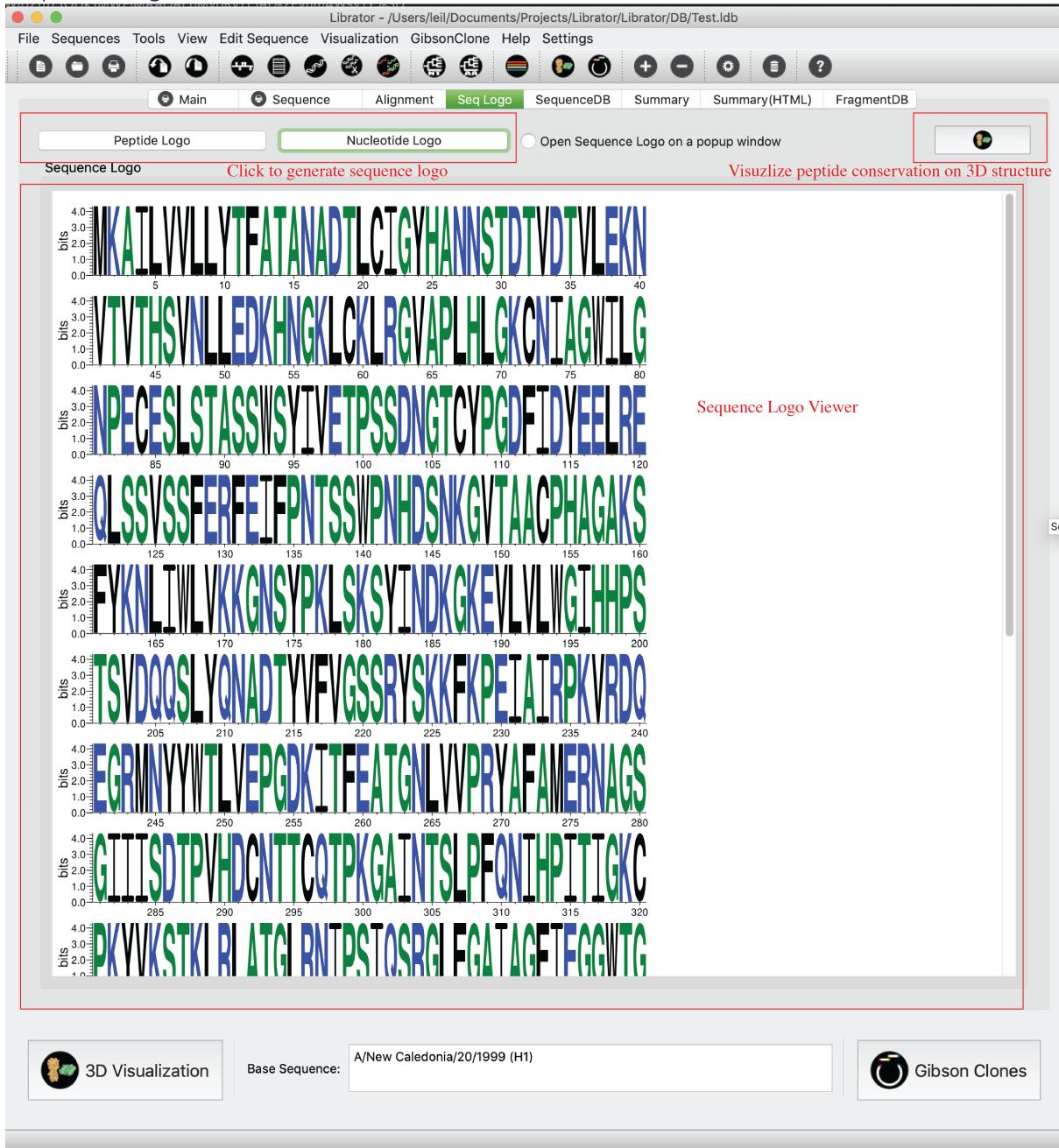
File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

Main Sequence Alignment(RTF) Alignment(HTML) SequenceDB Summary Summary(HTML) FragmentDB

Current Sequence Database Double click on the field to edit records when Edit lock is unlocked Click to enable/disable Edit on the table Edit Lock

	SeqName	Sequence	SeqLen	Subtype	Form	VFrom	VTo	Active	Role	Donor	Mutations	ID	Base
4	B/Malaysia/2506/2004	ATGAAGGCA...	1824	B	Other	1	5000	False	Unassigned	none	none	0	
5	B/Florida/4/2006	ATGAAGGCA...	1755	B	Other	1	5000	False	Unassigned	none	none	0	
6	A/yellow shouldered bat/Guatemala/06/2010 ...	AGCAGAAAG...	1784	H17	Full HA	1	5000	False	Unassigned	none	none	0	
7	A/turkey/Italy/221058/2002 EPI_ISL_2793 A / ...	AGCCAAAAG...	1384	N3	Full NA	1	5000	False	Unassigned	none	none	0	
8	A/turkey/Italy/214845/2002 EPI_ISL_2792 A / ...	AGCCAAAAG...	1384	N3	Full NA	1	5000	False	Unassigned	none	none	0	
9	A/turkey/Indiana/16-001403-1/2016 (H7)	TACAAAAATG...	1705	H7	Full HA	1	5000	False	Unassigned	none	none	0	
10	A/teal/France/2546/2001 EPI_ISL_2888 A / H1N3	GAGATGAAT...	1416	N3	Full NA	1	5000	False	Unassigned	none	none	0	
11	A/swine/Missouri/A01727926/2015 H4	ATGCTATCA...	1713	H4	Full HA	1	5000	False	Unassigned	none	none	0	
12	A/swine/Missouri/4296424/2006 (H2)	TTATTCGTC...	1780	H2	Full HA	1	5000	False	Unassigned	none	none	0	
13	A/swine/MN/02/2011/2008 (H1)	ATGAAAGTA...	1695	H1	Sequence	1	5000	True	Unassigned	none	none	0	
14	A/swine/Jiangsu/40/2011 (H1 avian-swine lineage)	ATGGAAGCA...	1701	H1	Full HA	1	5000	True	Unassigned	none	none	0	
15	A/swine/Hubei/06/2009 H4	ATGCCATAC...	1738	H4	Full HA	1	5000	False	Unassigned	none	none	0	
16	A/swan/Shimane/227/01 EPI_ISL_595 A / H3N9	AAGATGAAT...	1426	N9	Full NA	1	5000	False	Unassigned	none	none	0	
17	A/swan/Shimane/190/2001 EPI_ISL_628 A / H6N9	AAGATGAAT...	1426	N9	Full NA	1	5000	False	Unassigned	none	none	0	
18	A/shoveler/Netherlands/18/1999 (H11)	ATGAAAGAA...	1728	H11	Full HA	1	5000	False	Unassigned	none	none	0	
19	A/shearwater/West Australia/2576/1979 (H15)	AGC AAAAG...	1763	H15	Full HA	1	5000	False	Unassigned	none	none	0	
20	A/rhea/North Carolina/39482/1993 (H7)	AGC AAAAG...	1731	H7	Full HA	1	5000	False	Unassigned	none	none	0	
21	A/pintail/Shimane/324/98 EPI_ISL_498 A / H1N9	AAGATGAAT...	1425	N9	Full NA	1	5000	False	Unassigned	none	none	0	
22	A/mallard/Sweden/81/2002 (H6)	AGC AAAAG...	1742	H6	Full HA	1	5000	False	Unassigned	none	none	0	
23	A/mallard/Netherlands/5/1999 (H2)	AGC AAAAG...	1771	H2	Full HA	1	5000	False	Unassigned	none	none	0	
24	A/mallard/Netherlands/12/2000 (H7)	AGC AAAAG...	1732	H7	Full HA	1	5000	False	Unassigned	none	none	0	
25	A/mallard/Interior Alaska/7MP0167/2007 (H12)	ATGGAAAAAA...	1712	H12	Full HA	1	5000	False	Unassigned	none	none	0	
26	A/mallard/Interior Alaska/10BM01929/2010 (H10)	GGTACAAT...	1703	H10	Full HA	8	5000	False	Unassigned	none	none	0	
27	A/mallard/Curlew/7R3/19R2 (H14)	AGC AAAAG...	1740	H14	Full HA	1	5000	False	Unassigned	none	none	0	

Sequence Logo Tab



Summary Tab



Summary Tab (HTML)



Fragment DB Tab

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

Main Sequence Alignment(RTF) Alignment(HTML) SequenceDB Summary Summary(HTML) FragmentDB

Setup Database information

Local DB (SQLite) Remote DB (MySQL)

Server IP: localhost Port: 3306 DB name: Librator
User Name: root Password: 123456

Connect to Local or remote Fragment DB Connect

DB content Content of fragment DB Edit "in stock" status here

	Name	Segment	Fragment	Subtype	ID	Template	AA seq	NT seq	In stock
1	N9-F3-0005	NA	3	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 ...	TCDRNWQGPNRPVIQIDPVAMTHTSQYICSP...	ACATGCAGAGACAATTGGCAGGGCCCAAATA...	Yes
2	N9-F3-0004	NA	3	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A / ...	TCDRNWQGSNRPVIIQIDPVAMTHTSQYICSP...	ACATGCAGAGATAATTGGCAAGGCTCAAATA...	No
3	N9-F3-0003	NA	3	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	TCDRNWQGSNRPVIIQIDSVAMTHTSQYICSP...	ACATGCAGAGATAATTGGCAAGGCTCAAATA...	No
4	N9-F3-0002	NA	3	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	TCDRNWQGSNRPVIIQINPTMMTHTSQYICSP...	ACGTGTAGAGACAATTGGCAAGGCTGAATA...	No
5	N9-F3-0001	NA	3	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	TCDRNWQGSNRPVIIQIDPTMMTHTSQYICSP...	ACGTGTAGGGACAATTGGCAAGGCTGAATA...	No
6	N9-F2-0006	NA	2	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
7	N9-F2-0005	NA	2	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
8	N9-F2-0004	NA	2	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A / ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
9	N9-F2-0003	NA	2	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGTCAGGGAACACAA...	No
10	N9-F2-0002	NA	2	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGATTCTATGCTCTCAGCCAAGGGACAACAA...	No
11	N9-F2-0001	NA	2	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	RFYALSQGTTIRGKHSNGTIHDRSQYRALISW...	AGATTCTATGCTCTCAGCCAAGGGACAACAA...	No
12	N9-F1-0007	NA	1	N9	0007	A/duck/Siberia/700/1996 EPI_ISL_618 A / ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
13	N9-F1-0006	NA	1	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
14	N9-F1-0005	NA	1	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
15	N9-F1-0004	NA	1	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A / ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
16	N9-F1-0003	NA	1	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
17	N9-F1-0002	NA	1	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No
18	N9-F1-0001	NA	1	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	TCCACTCCCAGGTCCAACTGCACCTCGGTT...	No

3D Visualization Base Sequence: A/Wyoming/3/2003 (H3) Gibson Clones

Functions:

Basic functions:

Path setting

Users can set paths for all required tools and databases.

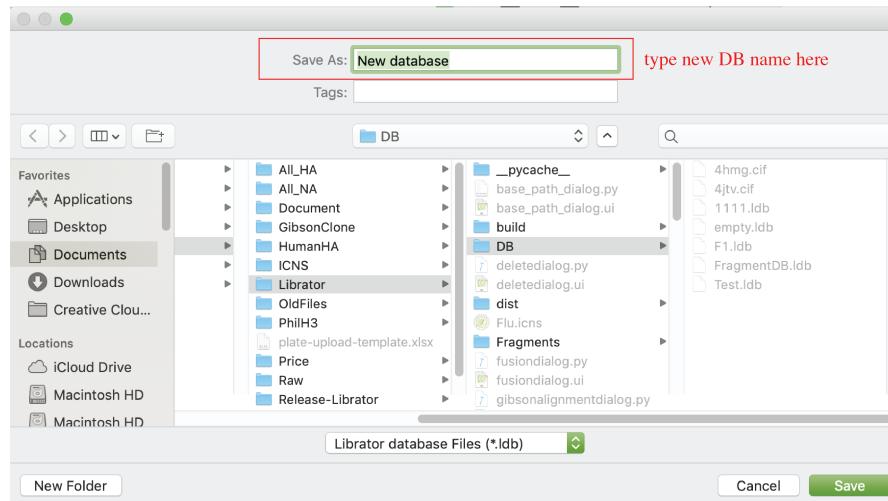
Click *Setting-> Preferences* in menu or click  in *Tool bar*.



Create new sequence database

Users can create new sequence database.

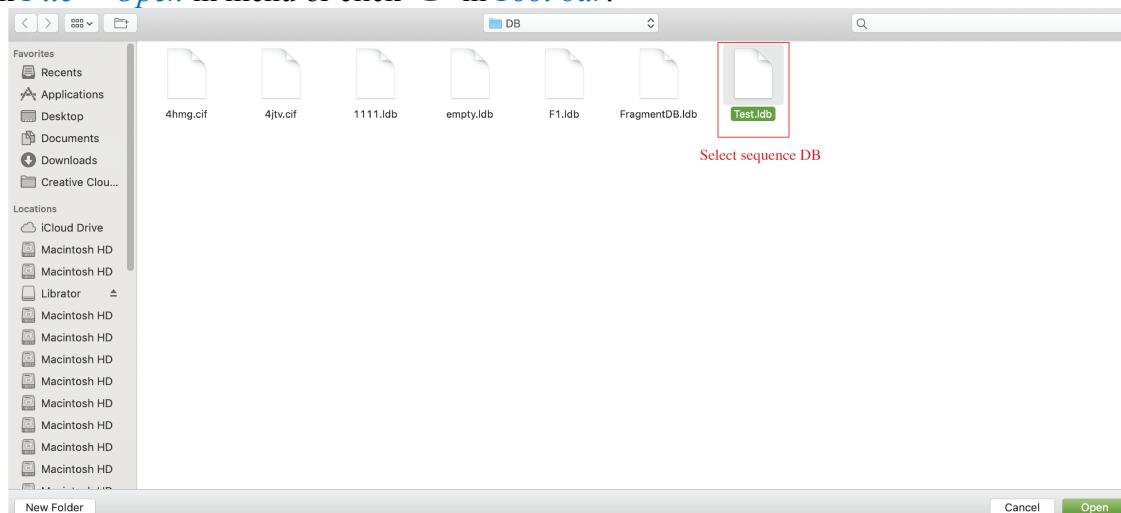
Click *File-> New* in menu or click  in *Tool bar*.



Open existing sequence database

Users can create new sequence database.

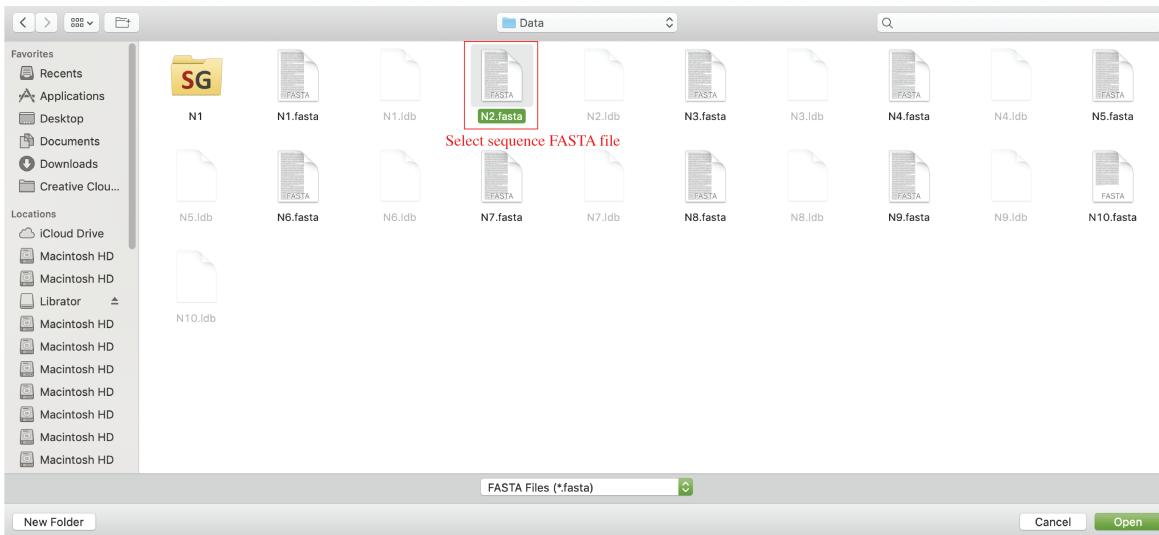
Click *File-> Open* in menu or click  in *Tool bar*.



Import sequences

After load an existing sequence database, users can import sequence into current database. The input files should be in FASTA format.

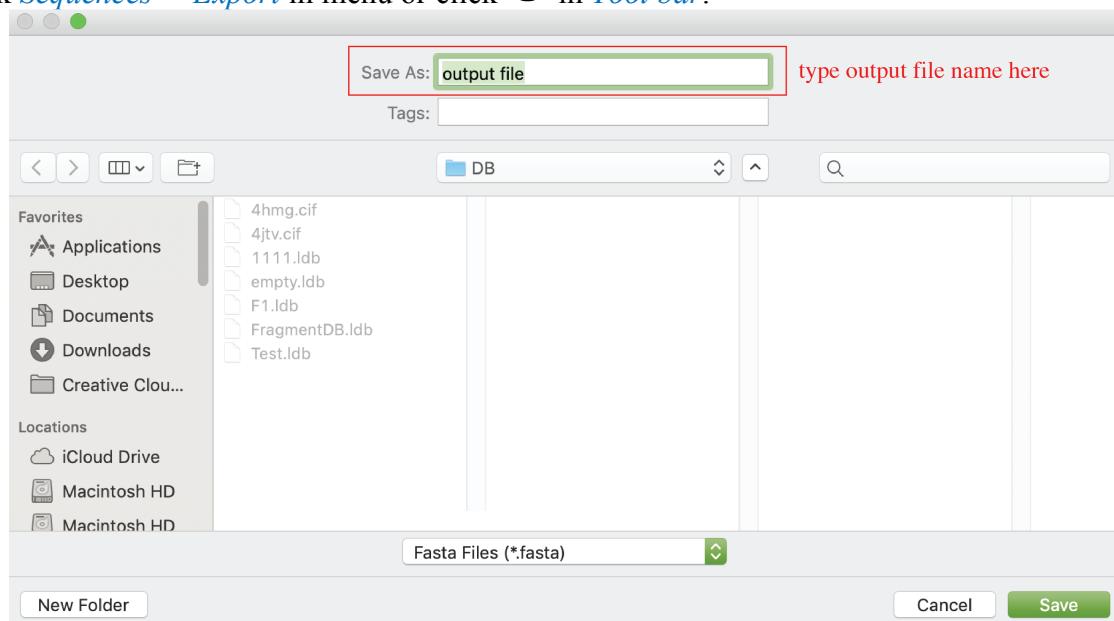
Click *Sequences-> Import* in menu or click  in *Tool bar* or click “import sequences” button in Main tab.



Export sequences

After load an existing sequence database, users can import sequence into current database. Selected sequences will be exported to a FASTA file.

Click *Sequences-> Export* in menu or click  in *Tool bar*.

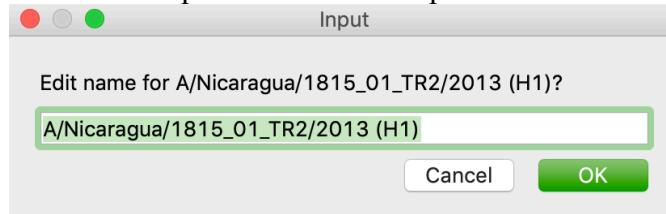


Advanced functions:

Edit information of sequences (on Main tab)

Users can edit information (**sequence name, Role, Form, Subtype, NT sequence, reading frame, donor regions**) of sequences on main tab.

For **Sequence name**, users can click sequence name text input to edit it.



For **Role, Form, and Subtype**, users can edit by the left panel. Users can select multiple sequences in active sequence panel to do bulk update.

The screenshot shows the main application window. On the left, there's a tree view under 'Available sequences' with categories like 'All Sequences', 'Base Sequence', 'B', and 'H1'. Under 'Base Sequence', several sequences are listed with checkboxes. On the right, a list of 'Active sequences' is shown, also with checkboxes. A red box highlights the 'Edit here' section at the bottom left, which contains dropdown menus for 'Role' (set to 'Unassigned'), 'Form' (set to 'Full HA'), and 'Subtype' (set to 'H1'). Below this, there are sections for 'Donor regions' (set to 'none') and 'Mutations' (set to 'none').

For **reading frame start and end**, users can use the “Coding region” inputs.

For **NT sequences**, users can click “edit sequence” button to edit sequences.

Update Sequence

Sequence Name:	sequence name		
<input type="text" value="A/Denver/1/1957 (H1)"/>			
<input type="button" value="Search"/> atg	<input type="text" value="search motif"/>	Coding Region From: <input type="text" value="20"/> To: <input type="text" value="5000"/>	reading frame setting
Sequence: current coding region highlighted in gray <pre>GAAAATAAACACAACCAAAATGAAAGCAAAACTACTGATCCTGTATGTGCACTTCCGCTACAGATGCAGA CACAATATGTATAGGCTTACCATGCAAAACATTCAACCGACACTGTTGACACACTCTGAGAAGAATGTGAC AGTGACACACTCTGTAACATTGGTCTAGGAAACCCAGAATGCGAATCATTGCTCTCC CACTACAATTGGGAACGTAACTCGAACAGCCACAATGGAAATTATGAGATTTAAAGGAAAGGCC AATAGATCATGGTCTTACATTGCAAAACACCAAAACTCTGAGAATGGACATGCTACCCAGGGATTTGCC GACTATGAGGAAGGGAGGAGCAATTGAGCTCAGTATCATTCATTGAGAGATTGAAATATTCCCAGGAA AGATCATGGCCAACCACACAACCAAGGAGGAGTGACGGCAGCATGGCCCCATGCGAGGAAAGCAGTTTT ACAAAAAATTGGTCTGGCTGACGGAGGCAAATGGCTCATACCCAAATCTGAGCAGGTCTATGTAACAA CAAGAGAAAAGAAGTCTTGCTATGGGAGTTCATACCCGCTAATAGAGGAACAAAGGGCACTCTA TCGGAAAGATAATGCTATGCTCTGAGTCTCAATTATAACAGGAGATTACCCAGAAATAGCAAA AGGCCAAAGTAAGAGATCAATCAGGAGAATGAACTACTGGACTTTGCTAGAACCCGGAGACACAATA ATATTTGAGGCAACTGAAATCTAATAGCACCATGGTATGCTTCGCACTGAGTAGAGGCCCTGGATCAGGG</pre>			
Translated Amino Acid sequence: (Using current reading frame) <pre>MKAKLILLCALSATDADTCIGYHANNSTDVTVDLKNVTVTHSVNLLEDSHNGKLCRLKGKAPLQLGN CNIAGWVLGNPECESLLSNRWSYIAETPSENNGTYPDFADYEELREQLSSVSSFERFEIFPKERSWP NHTTRGVTAACPARKSSFYKNLVWLTEANGSYPNLRSYVNNQKEVFLVWGVHHP SNIEEQRALYRKDNAYVS VVSNNYNRRTPEIAKRPKVRDQSGRMNYYWTLEPGDTIIFEA TGNLIAPWYAFALSRGP GSGIITSNAPLDEC DTKCQTPQGAI NSSLPFQNIHPVTIGECPKYVRSTKLRMVTGLRNIPSVQSRGLFGAIA GFIEGGWTGMMDGWYGH HQNEQGSGYAADQKSTQNAINGITKVNSVIEKMNTQFTAVGKEFNKLEKRMENLNKKVDDGFM DIWTYNAELLLENERTLDFHDSNVKNLYEKVKNQLRNNAKELGNGCFEFYHKCDNECMESVNGTYD PYSEESKLNREKIDGVKLES MGYRILAIYSTVASSLVLVSLGAISFWMCNSNGSLQCRCI*DQNFRNIRK~</pre>			
<input type="button" value="Confirm"/>		<input type="button" value="Cancel"/>	

Note: coding region and NT sequence editing is disabled for sequences with mutation information.

For **donor regions** information, users can click “Donor region” input to update information.

HA numbering

Users can access H1/H3 numbering of selected sequence.

Click [Tools-> HA Numbering](#) in menu or just click “Sequence” tab.

Please see “Sequence” Tab for interface and details.

Identify mutations

Users can identify mutations between any two sequences.

Click [Tools-> Identify Mutation](#) in menu or just click  in [Tool bar](#) to open the function window. Then select one sequence as template, determine the sequence that user want to identify mutation from as target sequence. Then the alignment will show in the HTML viewer and all mutations will be listed in the bottom text box. Users can save mutation/template information to the target sequence by clicking “Confirm” button.

Identify mutations

Template Sequence WT (1761 bp) Target Sequence Mutation (1761 bp)

Sequence Alignment

Options:

AA NT H1 H3

H1 highlight region:	Ca1	Ca2	Cb	Sa	Sb	Stalk-MN
H3 highlight region:	A	B	C	D	E	Stalk-MN

Position AA:
H1 numbering
H3 numbering
Position NT:
WT (1761 bp)
Mutation (1761 bp)

Alignment Viewer

Mutations between current template and target sequences:
K142N,A209V,V273L

Mutation information

Confirm Cancel

Multiple sequence alignment

Users can align multiple sequences together and check the results in a graphical viewer.

Click [Tools-> Multiple Alignment\(HTML\)](#) in menu or just click  in [Tool bar](#) for an alignment viewer in a popup window or click Alignment(HTML) tab for an integrated alignment viewer. There are two display modes: original sequence mode and template mode. In template mode, users can choose any sequence (including consensus sequence) as template to only highlight sequence difference.

Options: AA NT H1 H3

Display Mode: Original: Template: Alignment display options A

Legend:
H1 highlight region:

Ca1	Ca2	Cb	Sa	Sb	Stalk-MN
A	B	C	D	E	Stalk-MN

H3 highlight region:

Ca1	Ca2	Cb	Sa	Sb	Stalk-MN
A	B	C	D	E	Stalk-MN

Legend

Position AA:
H1 numbering
H3 numbering
Position NT:
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/59/2007 (H1)
A/Brisbane/59/2007 (H1)
A/California/04/2009 (H1)
A/California/04/2009 (H1)-
A/Denver/1/1957 (H1)
A/Fort Monmouth/1/1947
A/Fort Monmouth/1/1947

Position AA:
H1 numbering
H3 numbering
Position NT:
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/59/2007 (H1)
A/Brisbane/59/2007 (H1)
A/California/04/2009 (H1)
A/California/04/2009 (H1)-
A/Denver/1/1957 (H1)
A/Denver/1/1957 (H1)
A/Fort Monmouth/1/1947
A/Fort Monmouth/1/1947

Position AA:
H1 numbering
H3 numbering
Template NT:
Position NT:
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/02/2018IEP1_ISL
A/Brisbane/59/2007 (H1)
A/Brisbane/59/2007 (H1)
A/California/04/2009 (H1)
A/California/04/2009 (H1)-
A/Denver/1/1957 (H1)
A/Denver/1/1957 (H1)
A/Fort Monmouth/1/1947
A/Fort Monmouth/1/1947

Generate phylogenetic tree (ML tree)

Users can generate phylogenetic tree (Maximum Likelihood tree) for selected sequences. Nucleotide and Amino Acid trees are available.

For Nucleotide tree:

Click *Tools-> Generate Maximum Likelihood Tree (nucleotide)* in menu or just click  in *Tool bar*.

For Amino acid tree:

Click *Tools-> Generate Maximum Likelihood Tree (Amino Acid)* in menu or just click  in Tool bar.

Alignment Reviewer

Review alignment of your selected sequences:

Sequence: 35 to 35 (1 bases) selected of 581 BP select AA(NT) to see their position

A/Nicaragua/1815_01_TR2/2013_H1
A/California/04/2009_H1
A/California/04/2009_H1_S75M_HA1
A/South_Carolina/1/1918_H1
A/Brisbane/59/2007_H1
A/Solomon_Islands/3/2006_H1
A/PR/8/1934_H1
A/Denver/1/1957_H1
A/Fort_Monmouth/1/1947_H1

Click sequence name to highlight name and alignment by gray background

Sequence Name Sequence Alignment

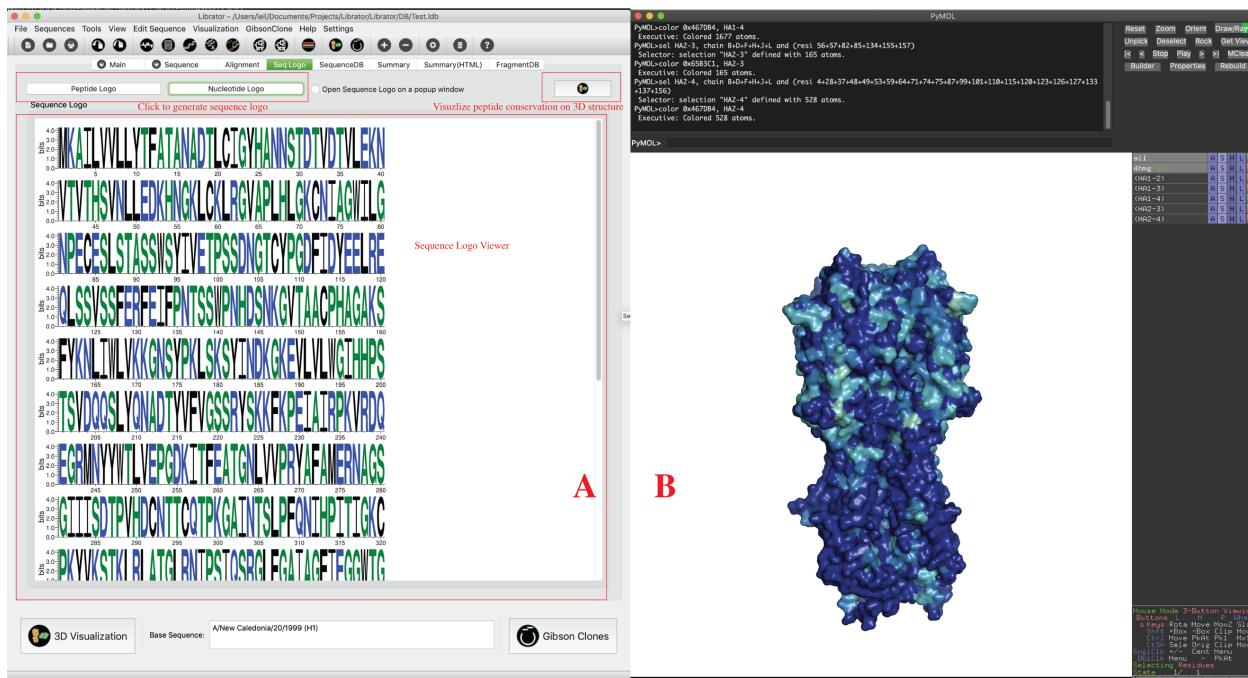
Determine region:

Start:(0-5000) 5 End:(0-5000) 570 Sequence region to generate tree Generate Tree Cancel

Generate sequence Logo (sequence conservation)

Users can generate sequence logo for multiple selected sequences.

Users should select multiple sequence in main page, then click “Seq logo” tab. On “Seq logo” tab, users can click *Peptide logo* or *Nucleotide logo* to generate sequences logos (Panel A). Users also can check the checkbox called “open Sequence logo on a popup window” to view the sequence logo on a popup window and then can be saved for further use. Users also can click the button on the right top corner to visualize peptide conservations on a 3D structure using PyMOL (panel B).



Mutate sequence

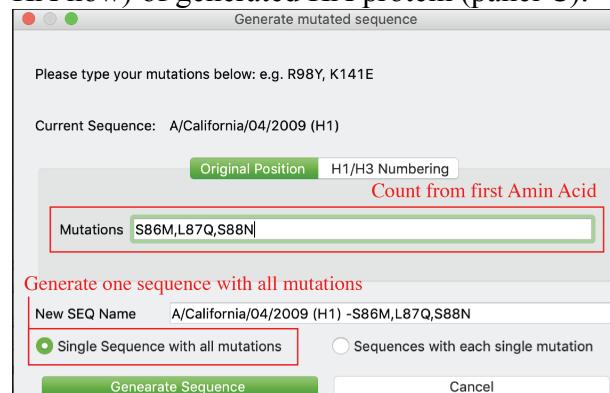
Users can generate new sequences with mutations based on any existing sequence.

Click *Edit Sequence-> Mutation* in menu or just click  in *Tool bar*.

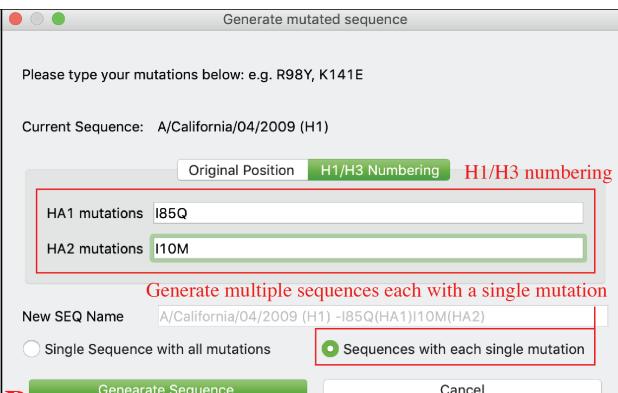
As shown below, users can generate mutations on a template sequence using either original position (count from first amino acid, panel A) or H1/H3 numbering (H1 numbering for group1 virus and H3 numbering for group2 virus, panel B). H1/H3 numbering function is only enabled for HA sequences of FLU A.

Users are allowed to input multiple mutations at the same time, and are allowed to decide from two options: 1) generate one sequence with all mutations; 2) generate multiple sequences, each of them with a single mutation.

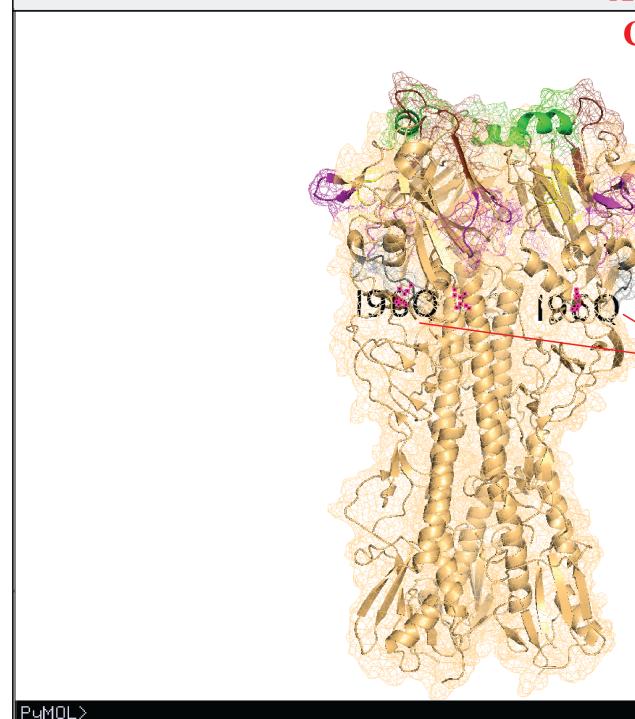
After the mutated sequence was generated, users can visualize the 3D structure (only enabled for HA now) of generated HA protein (panel C).



A



B



C

	A	S	H	L	C
all	A	S	H	L	C
4jtv 1/1	A	S	H	L	C
(ABS-Ca1)	A	S	H	L	C
(ABS-Ca2)	A	S	H	L	C
(ABS-Cb)	A	S	H	L	C
(ABS-Sa)	A	S	H	L	C
(ABS-Sb)	A	S	H	L	C
(haimutation)	A	S	H	L	C

Mouse Mode 3-Button Viewing
 Buttons L M R Wheel
 & Keys Rota Move MovZ Slab
 Shft +Box -Box Clip MovS
 Ctrl Move PkAt Pk1 MvSz
 CtSh Sele Orig Clip MovZ
 SnglClk +/- Cent Menu
 DblClk Menu - PkAt
 Selecting Residues
 State 1 1

|<< << □ >> >> □| F

Compare sequences and generate screening mutations

Users can compare sequences and generate consensus sequences (Base biased) or new sequences with screening mutations (Cocktail).

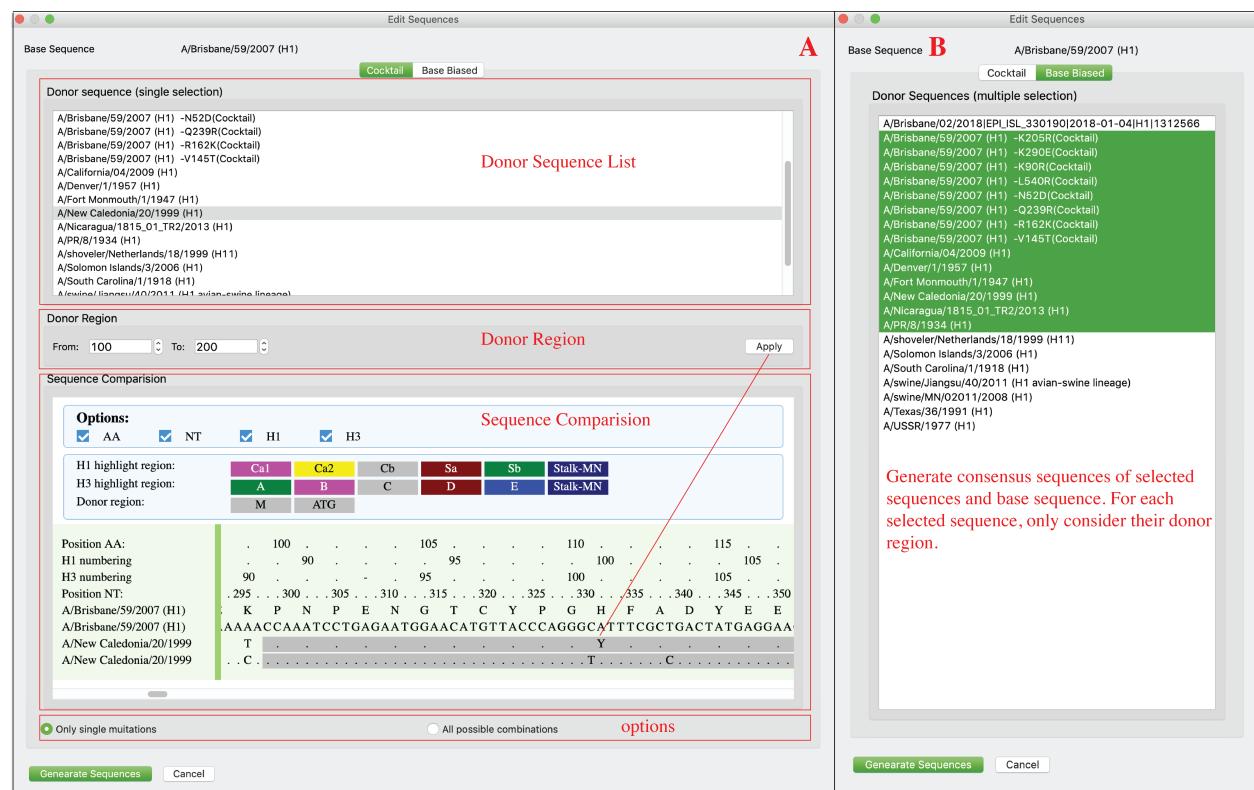
Click *Edit Sequence-> Editing* in menu or just click  in *Tool bar*.

Cocktail mode (A):

Compare base sequence to another sequence, identify all mutations between two sequences on donor region of donor sequence (user can setup donor region on this interface), then generate new sequences with those mutations using base sequence as template. Users can choose to only generate sequences with single mutations or all combinations of mutations.

Base biased mode (B):

Generate consensus sequences of base sequence and selected sequences. For each donor sequence, only their donor region will be considered (full sequence will be considered when donor region is none).



Epitope transplant across different subtypes

Users can transplant epitopes/regions from sequences of different subtypes.

Regions being removed from base sequence and that being inserted from donor sequence are not necessary to have same length.

Users can add multiple replacements on one sequence (click ‘Add’). Those replacements could be from different sequences. Users can review the replacement design in the bottom window. Users can double click replacement to delete it. Users also can click ‘Clear’ button to rest current design.

Click *Edit Sequence-> Fusion* in menu or just click  in *Tool bar*. This window has two layouts: high resolution layout and low resolution layout. Liberator will automatically choose the best fit based on current display resolution. Users also can click *Edit Sequence-> Fusion (high resolution)* or *Edit Sequence-> Fusion (low resolution)* to determine the layouts they prefer (As shown below).

Sequence Fusion across subtypes

High Resolution Layout

Selected donor sequence:	Base Sequence: A/Hong Kong/1/1968 (H3)		Base sequence	Donor Sequence: A/Brisbane/59/2007 (H1)		Selected donor sequence															
<p>A/Brisbane/02/2018/EPL_ISL_330190[20] A/Brisbane/59/2007 (H1) A/California/04/2009 (H1) A/California/04/2009 (H1)-10M(HA2) A/California/04/2009 (H1)-I86Q(HA1) A/California/04/2009 (H1)-S75M(HAI) A/Denver/1957 (H1) A/Fort Monmouth/1947 (H1) A/Icaraguan/185.1.01/TR2/2013 (H1) A/PR/8/1934 (H1) A/Solomon Islands/3/2006 (H1) A/South Carolina/1/1918 (H1) A/swine/Jiangsu/40/2011 (H1 avian swin) A/swine/MN/2021/2008 (H1) A/Texas/36/1991 (H1) A/US/SSR/1977 (H1)</p>																					
<p>Position: 1...6....11..16....21....26....31....36....41....46....51....56.... H3-Numbering: -----1.....5.....10.....15.....20.....25.....30.....35.....40..... H1-Numbering: 1.....5.....10.....15.....20.....25.....30.....35.....40..... Sequence: MKTTIALSYIFCLALGQDLPGNNDNSTATLCLGHAVPNGLTVKTTIDDDTEVTVNAELVQSSSTGKICNNPHRILGDIDCTLIDALLGDPF Position: 61....66....71....76....81....86....91....96....101....106....111....116.... H3-Numbering: 45....50....55....60....65....70....75....80....85....90....95....100.... H1-Numbering: 0....45....50....55....60....65....70....75....80....85....90....95....100.... Sequence: BSBTGCKNNPHRILGDIDCTLIDALLGDPFHCVDQNFTWDLFVERSRAFSNCYCPDV Position: 121....126....131....136....141....146....151....156....161....166....171....176.... H3-Numbering: 105....110....115....120....125....130....135....140....145....150....155....160.... H1-Numbering: 105....115....120....125....130....135....140....145....150....155....160.... Sequence: YASLRSLIVASQGLEEFTTEGFITWVQNGGSNACKRGPSGCFSSRLNLTSGSSTYVL Position: 181....186....191....196....201....206....216....221....226....231....236.... H3-Numbering: 165....170....175....180....185....190....195....200....205....210....215....220.... H1-Numbering: 1....10....15....180....185....190....195....200....205....210....215....220.... Sequence: NVTMPNNNFQDKLYIVHWHFPTNQQTSLVQASGRVTVSTRRSQQTTPINQISGRPW Position: 241....246....251....256....261....266....271....276....281....286....291....296.... H3-Numbering: 20....23....23....24....24....25....250....255....260....265....270....275....280.... H1-Numbering: 230....235....240....245....250....255....260....265....270....275....280....285.... Sequence: GLSSRISIYIWTVKPGFVULVINSNGLIAPIRGYFKMRGKGSSTMSRDAFDICSECIPE Position: 301....306....311....316....321....326....331....336....341....346....351....356.... </p>																					
						Position: 61....66....71....76....81....86....91....96....101....106....111....116.... H3-Numbering: 45....50....55....60....65....70....75....80....85....90....95....100.... H1-Numbering: 0....45....50....55....60....65....70....75....80....85....90....95....100.... Sequence: VKLVLVLLCTTATYATADTCIGHANNSTTGTDTVLEKNTVYTRVNLLENLSNRXLLK Position: 61....66....71....76....81....86....91....96....101....106....111....116.... H3-Numbering: 45....50....55....60....65....70....75....80....85....90....95....100.... H1-Numbering: 0....45....50....55....60....65....70....75....80....85....90....95....100.... Sequence: PFTATYQDQGDSVAGWILNGPECLLJSKESWSGYIVEKPNPENGTCPYGHFRADYELERQL Position: 121....126....131....136....141....146....151....156....161....166....171....176.... H3-Numbering: 110....118....120....125....130....135....140....145....150....155....160....165.... H1-Numbering: 0....115....120....125....130....135....140....145....150....155....160....165.... Sequence: SSVSFERFEFLPKFESNNPWFNTVGEASCSCHGESSFTYRLWLJCGRNLYPLNSRSVA Position: 181....186....191....196....201....206....211....216....221....226....231....236.... H3-Numbering: 170....175....180....185....190....195....200....205....210....215....220.... H1-Numbering: 1....15....180....185....190....195....200....205....210....215....220.... Sequence: NNEKEEVILVLUHGWHVHPNIIQGARLYHTENAYAVSVSSHSRKTFPEIAKRPVKRDQQR Position: 241....246....251....256....261....266....271....276....281....286....291....296.... H3-Numbering: 20....23....23....24....24....25....250....255....260....265....270....275....280....285.... H1-Numbering: 235....240....245....250....255....260....265....270....275....280....285....290.... Sequence: INYWYTLLEPFGDITFFANGNUIAPIRAYPAFSLRGFGSSGIIINSNAMDFCDAKCPQGQAI Position: 301....306....311....316....321....326....331....336....341....346....351....356.... 															
						Region on base sequence: 60 ⌂ to 70 ⌂ Replaced region on base sequence: 60 ⌂ to 70 ⌂															
						Region on donor sequence: 60 ⌂ to 70 ⌂ Donor region on donor sequence: 60 ⌂ to 70 ⌂															
						Region on base sequence to be replaced: 60 ⌂ to 70 ⌂ Region on donor sequence to be inserted: 60 ⌂ to 70 ⌂															
<p>Active sequence list</p> <p>A/Hong Kong/1/1968 (H3)</p> <p>Current Base sequence:</p> <p>Base sequence name</p> <p>Current product</p> <p>Legend:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> H1 numbering <input checked="" type="checkbox"/> H3 numbering <table border="1" style="margin-top: 10px; border-collapse: collapse;"> <tr> <td style="width: 15%;">Ca1</td> <td style="width: 15%;">Ca2</td> <td style="width: 15%;">Cb</td> <td style="width: 15%;">Sa</td> <td style="width: 15%;">Sb</td> <td style="width: 15%;">Stalk-MN</td> <td style="width: 15%;">Stalk-MN</td> </tr> <tr> <td style="text-align: center;">A</td> <td style="text-align: center;">B</td> <td style="text-align: center;">C</td> <td style="text-align: center;">D</td> <td style="text-align: center;">E</td> <td colspan="2"></td> </tr> </table> <p>Position AA: H1 numbering H3 numbering Original Seq:</p> <p>MKT I I A L S Y I F C L A L G Q D L P G N D N S T A T L C L G H A V P N G L T V K T I T D D O T E V T N A E L V Q S S T G K I C N N P H R I L G D I D C T L I D A L L G D P F</p> <p style="text-align: center;">FTATYADTCI TVDTVLEKVNVT KLCLLKGIAPL</p> <p style="text-align: center;">Double click to delete</p> <p>Insert information</p>						Ca1	Ca2	Cb	Sa	Sb	Stalk-MN	Stalk-MN	A	B	C	D	E			<input type="button" value="Add"/> <input type="button" value="Clear"/>	
Ca1	Ca2	Cb	Sa	Sb	Stalk-MN	Stalk-MN															
A	B	C	D	E																	

Sequence Fusion across subtypes

Low Resolution Layout

Select donor sequence:

A/Wisconsin/67/2006 (H3)

Base Sequence: A/Wisconsin/67/2006 (H3) **Base sequence**

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: -----1...5...10...15...20...25...30...35...40...4
H1-Numbering: -----1...5...10...15...20...25...30...35...40...4
Sequence: M K T I I A L S Y I C L V F A Q K L P G N D N S T A T L C L G H H A V P N G T I V K T I T N D Q I E V T N A T E L V Q
Position: 61...66...71...76...81...86...91...96...101...106...111...116...
H3-Numbering: 45...50...55...60...65...70...75...80...85...90...95...100...
H1-Numbering: 0...45...55...60...65...70...75...80...85...90...95...100...
Sequence: S S T G C I C D S H Q I L D G E R T I L D A L L O P C D F C O N K R N D L E V E R S K A Y S K Y P V D P D
Position: 121...126...131...136...141...146...151...156...161...166...171...176...
H3-Numbering: 105...110...115...120...125...130...135...140...145...150...155...160...
H1-Numbering: 105...115...120...125...130...135...140...145...150...155...160...
Sequence: Y A S L R L V I V A S G T Y L E P N D E S P W T G T Q M G T S S C K R R Q N N S F F S R L N W I T H L R K R I P A L
Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: -----1...5...10...15...20...25...30...35...40...4
H1-Numbering: -----1...5...10...15...20...25...30...35...40...4
Sequence: K A T K M K A I L V L I V T F A T A N A D T C I G Y W H T D F D V D T V E L K R V T V H S V N L E D C H K G
Position: 61...66...71...76...81...86...91...96...101...106...111...116...
H3-Numbering: 50...55...60...65...70...75...80...85...90...95...100...10
H1-Numbering: 5...50...55...60...65...70...75...80...85...90...95...100...1
Sequence: K L I C K I G V A D I H L G K O N I A G W I G N E C E S L S T A S S W S Y I V E T S S D N G T C Y P S D F I N Y E
Position: 121...126...131...136...141...146...151...156...161...166...171...176...
H3-Numbering: 5...10...15...20...25...30...35...40...45...50...55...60...
H1-Numbering: 05...10...15...20...25...30...35...40...45...50...55...60...
Sequence: E L R Q Q I S V S J F I R F E I F P K T S W N F H D D N K G V T A C P H A G A K S P Y T K N I W M L V R K G N S I P

Active sequence list

Donor Sequence: A/Nicaragua/1815_01_IR2/2013 (H1) **Selected donor sequence**

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: -----1...5...10...15...20...25...30...35...40...4
H1-Numbering: -----1...5...10...15...20...25...30...35...40...4
Sequence: K A T K M K A I L V L I V T F A T A N A D T C I G Y W H T D F D V D T V E L K R V T V H S V N L E D C H K G
Position: 121...126...131...136...141...146...151...156...161...166...171...176...
H3-Numbering: 105...110...115...120...125...130...135...140...145...150...155...160...
H1-Numbering: 105...115...120...125...130...135...140...145...150...155...160...
Sequence: E L R Q Q I S V S J F I R F E I F P K T S W N F H D D N K G V T A C P H A G A K S P Y T K N I W M L V R K G N S I P

Current Base sequence: A/Wisconsin/67/2005 (H3) **Base sequence name**

Replaced region on base sequence: Donor region on donor sequence: **Add** **Clear**

Region on base sequence to be replaced: **Region on donor sequence to be inserted:**

Current product

Legend:

- H1 numbering
- H3 numbering
- Ca1
- Ca2
- Cb
- Sa
- Sb
- Stalk-MN
- Stalk-MN

Position AA: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . .
H1 numbering: -----1...5...10...15...20...25...30...35...40...45...50...
H3 numbering: -----1...5...10...15...20...25...30...35...40...45...50...
Original Seq: M K T I I A L S Y I C L V F A Q K L P G N D N S T A T L C L G H H A V P N G T I V K T I T N D Q I E V T N A T E L V Q

Double click to delete

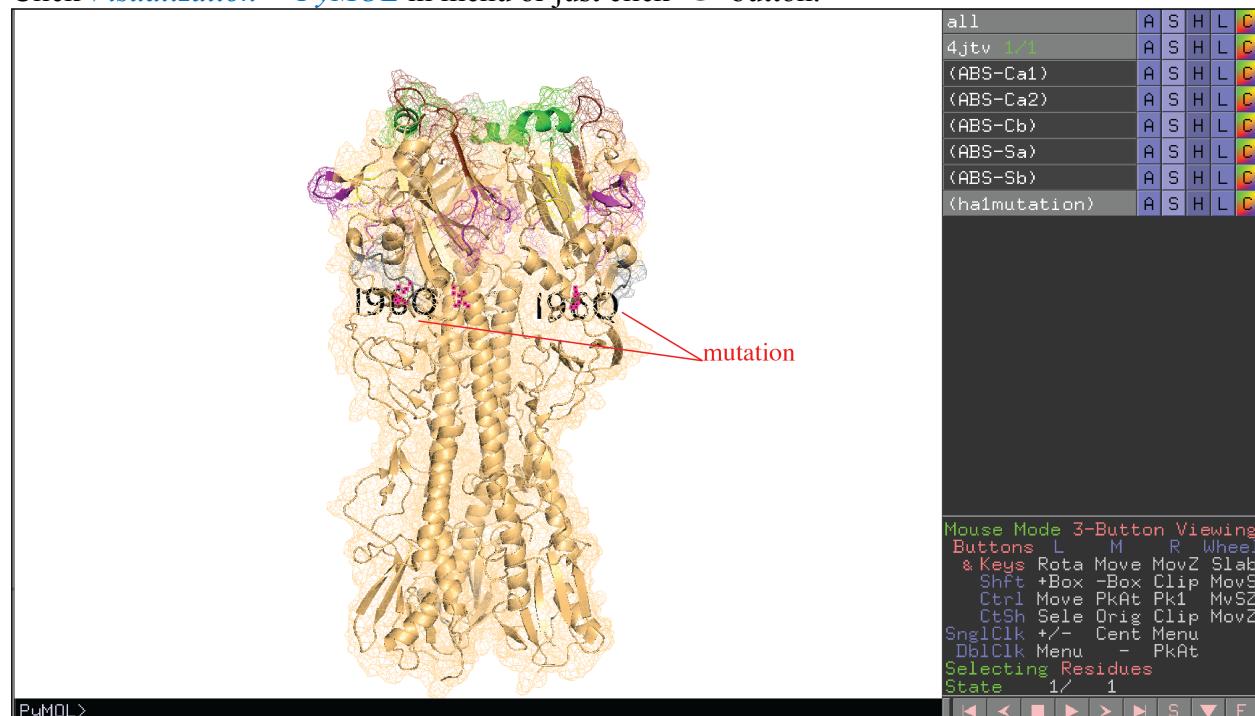
Insertion information

Confirm **Cancel**

3D visualization via PyMOL

Users can see 3D structure of selected sequence via PyMOL (only for HA).

Click *Visualization-> PyMOL* in menu or just click  button.



Generate Gibson Clone fragments

Users can generate Gibson Clone Fragments.

Click *GibsonClone*-> *GibsonClone* in *menu* or just click  button on Main tab.

Note: *GibsonClone* only works for sequences within same subtype!

Users can select sequences, determine fragment DB, output path, and joint region plan (panel A), after click “Generate Fragments” button, a dialog will pop up for users to review and confirm (panel B). On panel B, users can review the generated Fragments (both amino acid sequences and nucleotide sequences), joint regions that shared by neighbor fragments are highlighted. Joint region that connect fragment 1 and 4 to the vector not displayed for AA sequences but can be found in NT sequences. ‘-’ in AA alignments indicate incomplete sequences or deletions and will be deleted in NT fragments. Users can click “confirm” button to generate fragments after they confirmed current fragments. After a few second, a notice will be popped up with summarized results (panel C).

Welcome to Gibson Clone Fragment Design page!

Select All

	Name	Subtype
1	A/South Carolina/1/1918 (H1)	H1
2	A/PR/8/1934 (H1)	H1
3	A/Fort Monmouth/1/1947 (H1)	H1
4	A/Denver/1/1957 (H1)	H1
5	A/USSR/1977 (H1)	H1
6	A/Texas/36/1991 (H1)	H1
7	A/Solomon Islands/3/2006 (H1)	H1
8	A/Brisbane/59/2007 (H1)	H1

Sequence candidates

Vector Connector Sequences

Joint region for upstream end (Gibson cloning into the vector):

Joint region for 3' end (instead of transmembrane region):

Fragments Database:

Server IP: localhost Port: 3306 DB name: Librator
User Name: root Password: 123456

Gibson clone fragments files output path:

Subtype: H1/Group1 H3/Group2 NA

Choose joint region plan: Default User Defined

Joint Region Design

Fragment 1: 1 to 131 Fragment 2: 123 to 272
 Fragment 3: 264 to 411 Fragment 4: 403 to 518

Output path:

The files were generated under: /Users/eil/Documents/Projects/Librator/Resources/Temp

Existing fragments used: H3-F4-0001, H3-F4-0002, H3-F4-0003 Existing fragments

New fragments generated: H3-F1-0003, H3-F1-0005, H3-F1-0011, H3-F1-0012, H3-F1-0013, H3-F1-0014, H3-F1-0015, H3-F2-0007, H3-F2-0008, H3-F2-0014, H3-F2-0015, H3-F2-0016, H3-F2-0017, H3-F2-0018, H3-F3-0002, H3-F3-0003, H3-F3-0009, H3-F3-0010, H3-F3-0011, H3-F3-0012, H3-F3-0013, H3-F4-0008, H3-F4-0009, H3-F4-0010

New fragments need to order: H3-F1-0014, H3-F2-0014, H3-F2-0015, H3-F2-0016, H3-F2-0017, H3-F2-0018, H3-F3-0002, H3-F3-0003, H3-F3-0009, H3-F3-0010, H3-F3-0011, H3-F3-0012, H3-F3-0013, H3-F4-0008, H3-F4-0009, H3-F4-0010

Please review Gibson Fragments Design:

Selected sequences: " will be highlighted

Alignment of Fragments

" in sequences will be removed before generating Fragments

Switch between AA and NT

Amino Acid Nucleotide

Contact

For comments and feedbacks please E-mail Lei Li(leil@uchicago.edu) or Patrick C. Wilson(wilsonp@uchicago.edu)