

# LIBRATOR V 1.0 - USER MANUAL

Lei Li and Patrick C. Wilson  
WILSON LAB@ UCHICAGO

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## Installation

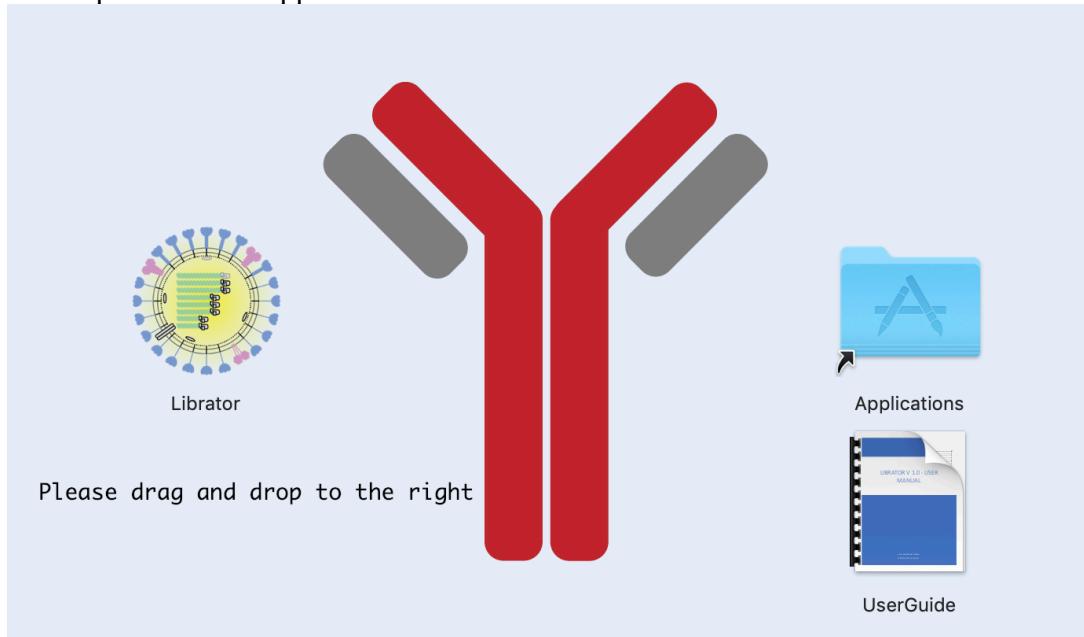
### System requirement

Librator requires MacOS 10.13 or higher version.

Librator was compiled under MacOS 10.14 and has been tested on MacOS 10.14 and MacOS 10.15.

### Install Librator

Drag and drop Librator to application folder.



### Install - essential dependencies

The two aligners are required by most core functions of Librator. We already included pre-compiled **clustal omega** and **muscle** with Librator. In case our versions are not compatible with your MacOS versions, please see below for details.

#### **Clustal Omega**

If our version doesn't work on your computer, 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**

If our version doesn't work on your computer, 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).

### Install - optional dependencies

Here are some optional dependencies. Most of core functions of Librator are still working without those tools.

**PyMOL** (<https://pymol.org/2/>) (highly recommend!)

PyMOL for 3D structure visualization of proteins

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 (see basic function->path setting for details), 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:

**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)"
```

After you installed home brew, you can install the following packages for better user experience.

**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.

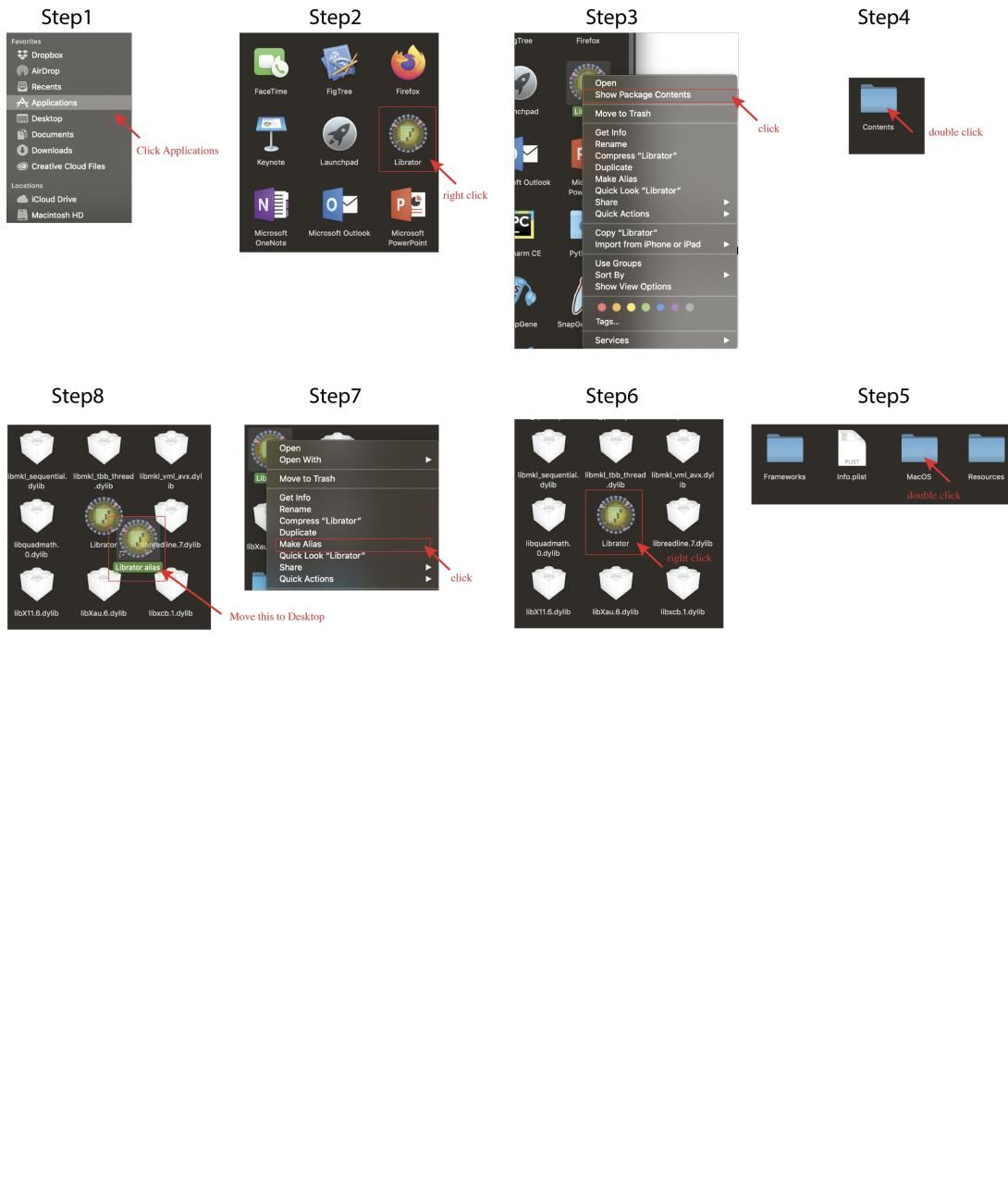
```
brew install ghostscript  
brew install pdf2svg
```

Now you're all set!

Run Librator

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

For better user experience (faster), 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 Settings

Main Sequence Alignment Seq Logo SequenceDB Summary Summary(HTML) FragmentDB

Sequence Name: A/California/04/2009 (H1) -A203V Sequence name

Subtype: H1 Subtype

Click feature to show for sequence: Feature to highlight on the sequence viewer

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) -A203V Sequence viewer

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

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: LRGVAPLHLGKCNIAKGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELRE

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: QLSSVSSFERFEIFPKTSSWPNHDNSKGVTAAACPHAGAKSFYKNLIWLVKKGNSYPKLSK ← Donor region

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: SYINDKGKEVLVWLWGIIHHPSTDQQLSLYQNADTYVFGSSRYSKKPKEIAIRPKVRDQ ← Antigenic sites

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: EGRMNYYWTLVEPGDKITFEATGNLUVPRYAFAMERNAGSGIIISDTPVHDCNTTCQTPK

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: GAIANTSPLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRRGLGAIAGFIEGGWTG

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: MVDCWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKR

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: IENLNKKVDDGFLDIWTYNAELLVLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNG

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: LVLVVSLSLGAISFWMCNSQLQCRCICL

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/California/04/2009 (H1) Gibson Clones

## Alignment Tab (HTML)

## Sequence DB Tab

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Settings

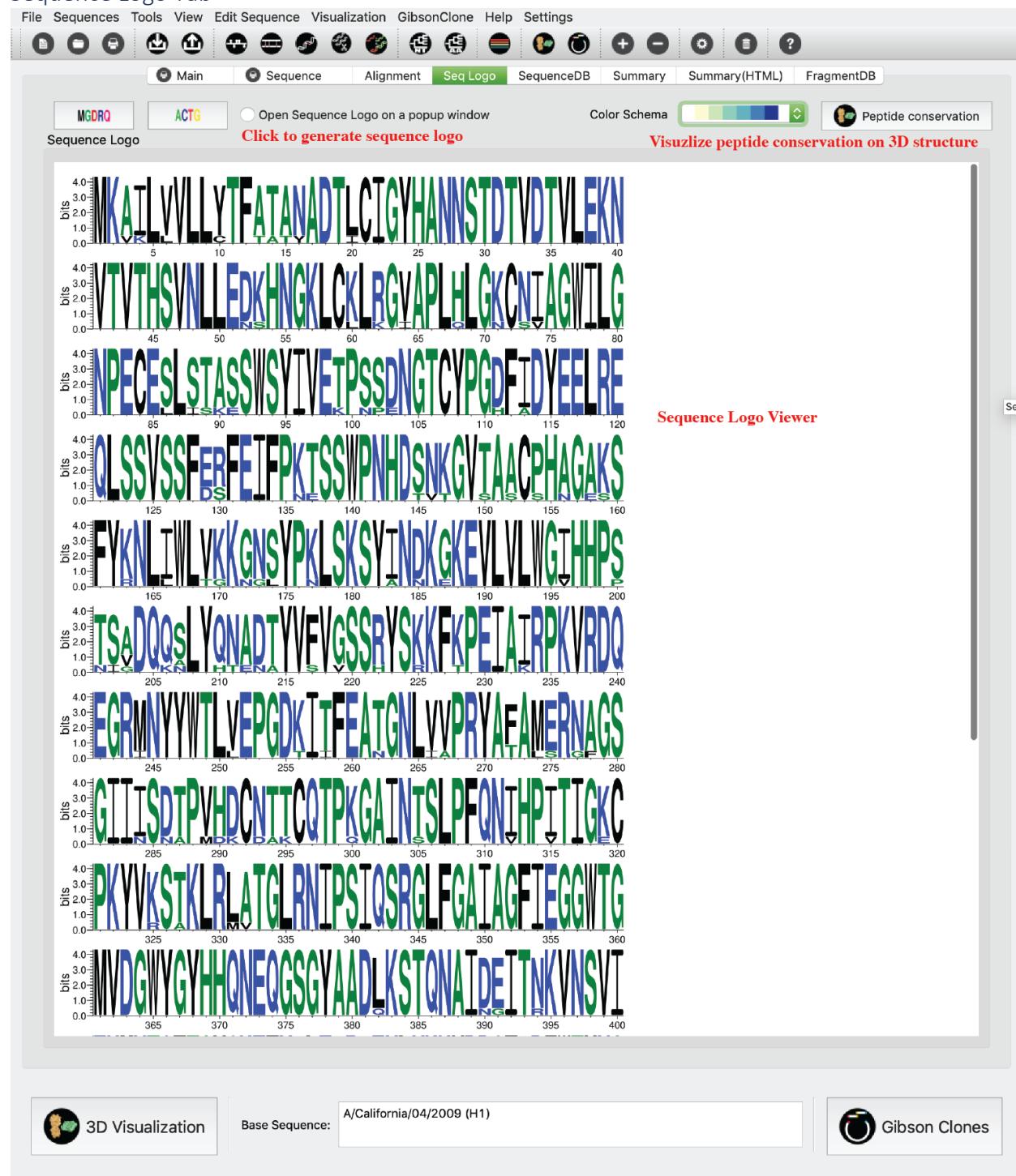
Main Sequence Alignment Seq Logo SequencedB Summary Summary(HTML) FragmentDB

Select entire row Double click on the field to edit records when Edit lock is unlocked Click to enable/disable edit on the table Edit Lock: Locked

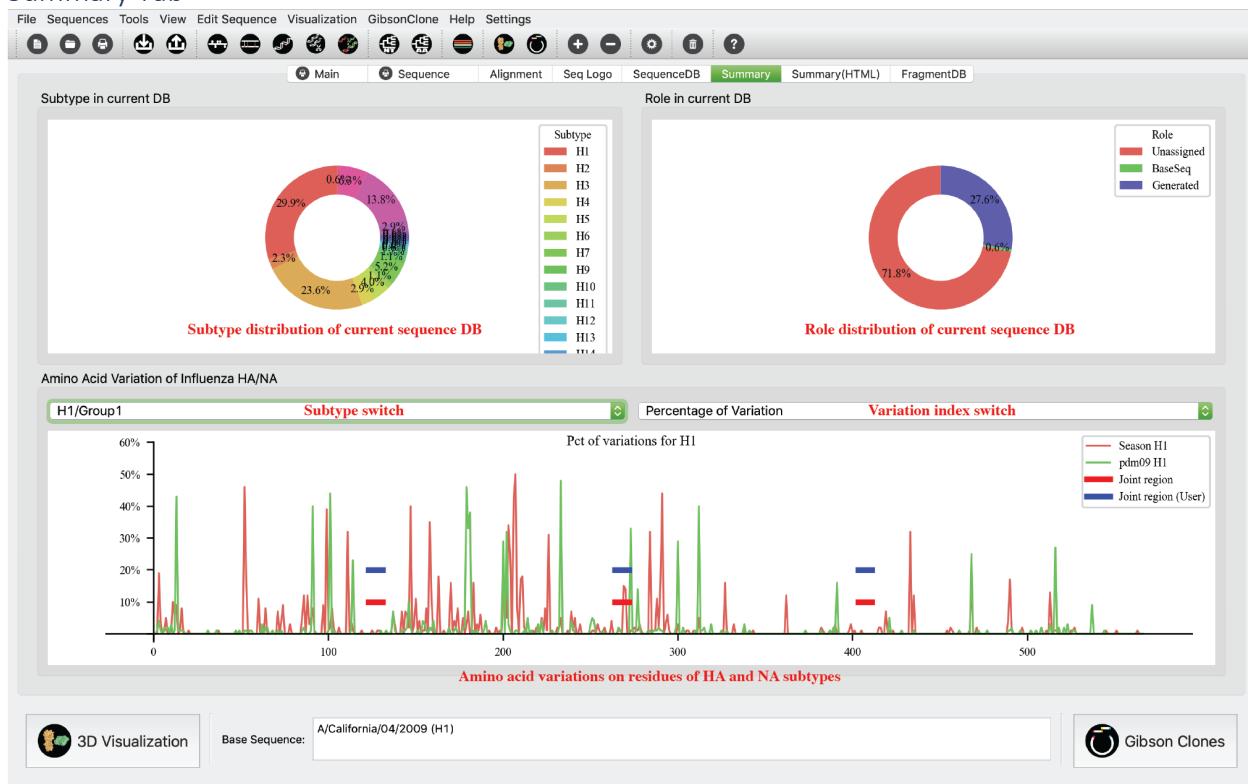
Refresh DB

	SeqName	Sequence	SeqLen	Subtype	Form	VFrom	VTo	Active	Role	Donor	Mutations	ID	Base
1	pH1N1-NA TetraBrachion codon optimized construct	TCCACTCCC...	1524	N1	Full NA	46	5000	False	Unassigned	none	none	0	
2	pH1N1-NA TetraBrachion codon optimized Y406F	TCCACTCCC...	1524	N1	Full NA	46	5000	False	Unassigned	none	none	0	
3	pH1N1-NA TetraBrachion codon optimized Y406F	TCCACTCCC...	1503	N1	Full NA	46	5000	False	Unassigned	none	none	0	
4	pH1N1-NA TetraBrachion codon optimized construct	TCCACTCCC...	1503	N1	Full NA	46	5000	False	Unassigned	none	none	0	
5	WT (1761 bp)	GGAAAAAC...	1761	H1	Full HA	21	5000	True	Unassigned	none	none	0	
6	SARS-CoV 2P triSpike S (R667A)-His isolate CUHK...	TCCACTCCC...	3975	Other	1	5000	False	Unassigned	none	none	0		
7	NC99-test2	ATGAAAGCA...	1702	H1	Full HA	1	5000	True	Unassigned	none	none	0	
8	NC99-test1	ATGAAAGCA...	1705	H1	Full HA	1	5000	True	Unassigned	none	none	0	
9	NC99-test	ATGAAAGCA...	1708	H1	Full HA	1	5000	True	Unassigned	none	none	0	
10	NA construct 6 Gibson Cloning Fragment (1734 bp)	TCCACTCCC...	1734	N1	Full NA	46	5000	False	Unassigned	none	none	0	
11	NA construct 5 Gibson Cloning Fragment (1689 bp)	TCCACTCCC...	1689	N1	Full NA	46	5000	False	Unassigned	none	none	0	
12	NA construct 4 Gibson Cloning Fragment (1530 bp)	TCCACTCCC...	1530	N1	Full NA	46	5000	False	Unassigned	none	none	0	
13	NA construct 3 Gibson Cloning Fragment (1509 bp)	TCCACTCCC...	1509	N1	Full NA	46	5000	False	Unassigned	none	none	0	
14	NA construct 2 Gibson Cloning Fragment (1536 bp)	TCCACTCCC...	1536	N1	Full NA	46	5000	False	Unassigned	none	none	0	
15	NA Construct 1 Gibson Cloning Fragment (1509 bp)	TCCACTCCC...	1509	N1	Full NA	46	5000	False	Unassigned	none	none	0	
16	Mutation (1761 bp)	GGAAAAAC...	1761	H1	Full HA	3	5000	True	Unassigned	none	K142N,A20...	0	WT (1761 bp)
17	B/Victoria/2/1987	ATGAAGGCA...	1824	B	Full HA	1	5000	False	Unassigned	none	none	0	
18	B/Phuket/3073/2013	ATTTCTAA...	1853	B	Full HA	1	5000	False	Unassigned	none	none	0	
19	B/Massachusetts/2/2012	ATGAAGGCA...	1755	B	Full HA	1	5000	False	Unassigned	none	none	0	
20	B/Malaysia/2506/2004	AAATGAAGG...	1824	B	Full HA	3	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 Settings

Main Sequence Alignment Seq Logo 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 Click to connect to DB Connect

Content of fragment DB

	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 A / ...	TCDRNWQGPNRPV QIDPVAMTHTSQYICSPVLT...	ACATGCAGAGACAATTGGCAGGGCCCAAATAGA...	Yes
2	N9-F3-0004	NA	3	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A / ...	TCDRNWQGSNRPV QIDPVAMTHTSQYICSPVLT...	ACATGAGAGATAATTGCAAGGCTCAAATAGAC...	No
3	N9-F3-0003	NA	3	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / H3N9	TCDRNWQGSNRPV QIDPVAMTHTSQYICSPVLT...	ACATGCAGAGATAATTGCAAGGCTCAAATAGAC...	No
4	N9-F3-0002	NA	3	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 A / ...	TCDRNWQGSNRPV QINPTMMTHTSQYICSPVLT...	ACGTGTAGAGACAATTGGCAAGGCTCGAATAGA...	No
5	N9-F3-0001	NA	3	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 A / ...	TCDRNWQGSNRPV QIDPTMMTHTSQYICSPVLT...	ACGTGTAGAGACAATTGGCAAGGCTCGAATAGA...	No
6	N9-F2-0006	NA	2	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / H1N9	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATC...	No
7	N9-F2-0005	NA	2	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 A / ...	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATC...	No
8	N9-F2-0004	NA	2	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A / ...	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATC...	No
9	N9-F2-0003	NA	2	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / H3N9	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATC...	No
10	N9-F2-0002	NA	2	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 A / ...	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATAA...	No
11	N9-F2-0001	NA	2	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 A / ...	RFYALSQGTTIRGKHSNGTIHRSQYRALISWPLS...	AGGTTCTATGCTCTCAGCCAAGGGACAACATAA...	No
12	N9-F1-0007	NA	1	N9	0007	A/duck/Siberia/700/1996 EPI_ISL_618 A / ...	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	No
13	N9-F1-0006	NA	1	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / H1N9	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	No
14	N9-F1-0005	NA	1	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 A / ...	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	TCCACTCCCAGGTCCAAC TGCACCTCGTTCTA...	No

Edit "in stock" status here

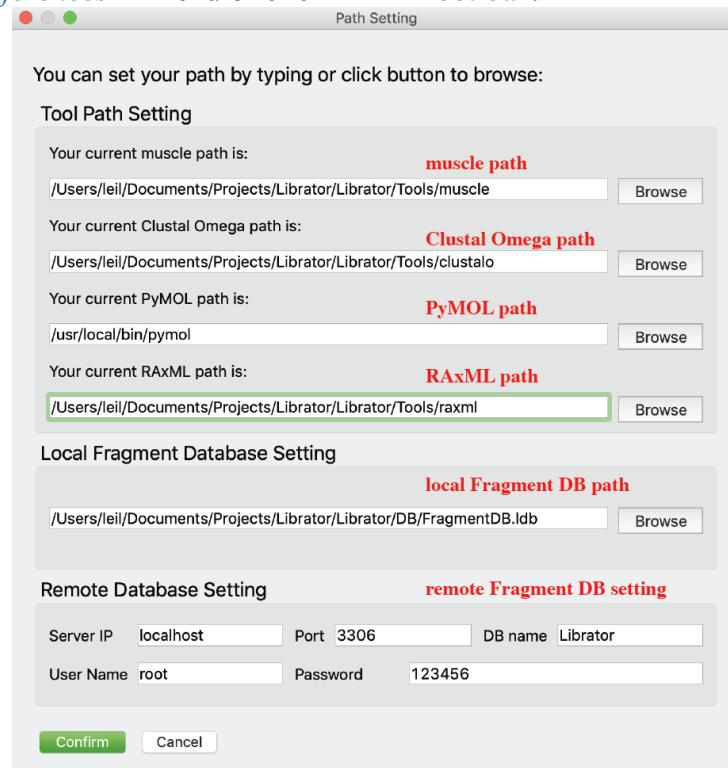
3D Visualization Base Sequence: A/California/04/2009 (H1) Gibson Clones

## 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*.

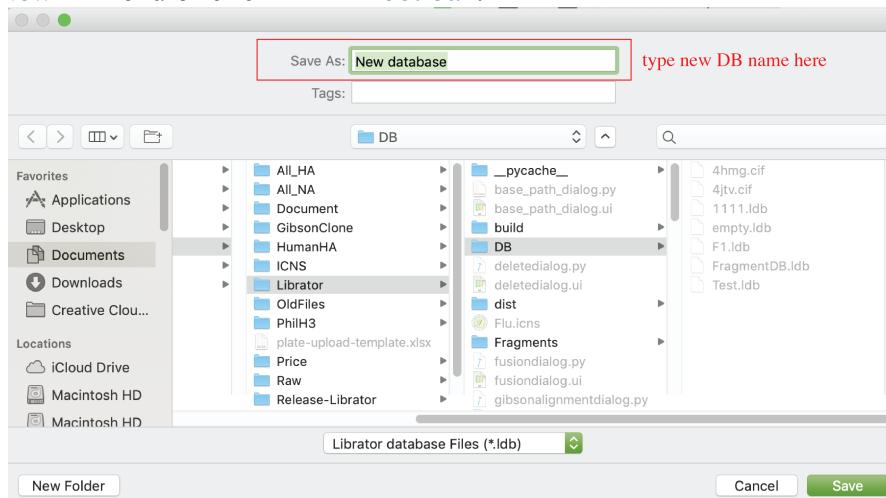


You can type path or click Browse button to choose the correct path.

### Create new sequence database

Users can create new sequence database.

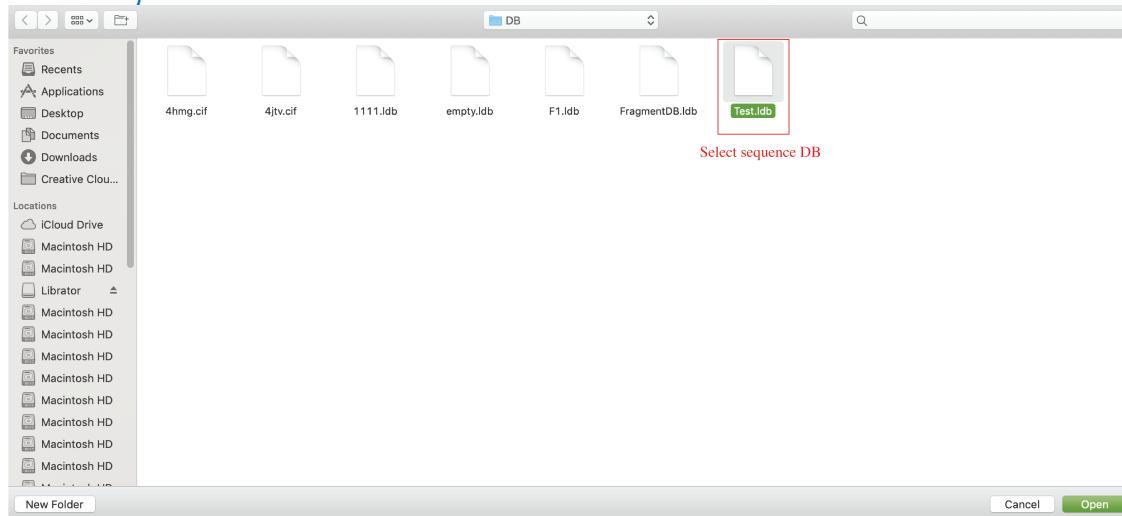
Click *File-> New* in menu or click  in *Tool bar*.



Open existing sequence database

Users can create new sequence database.

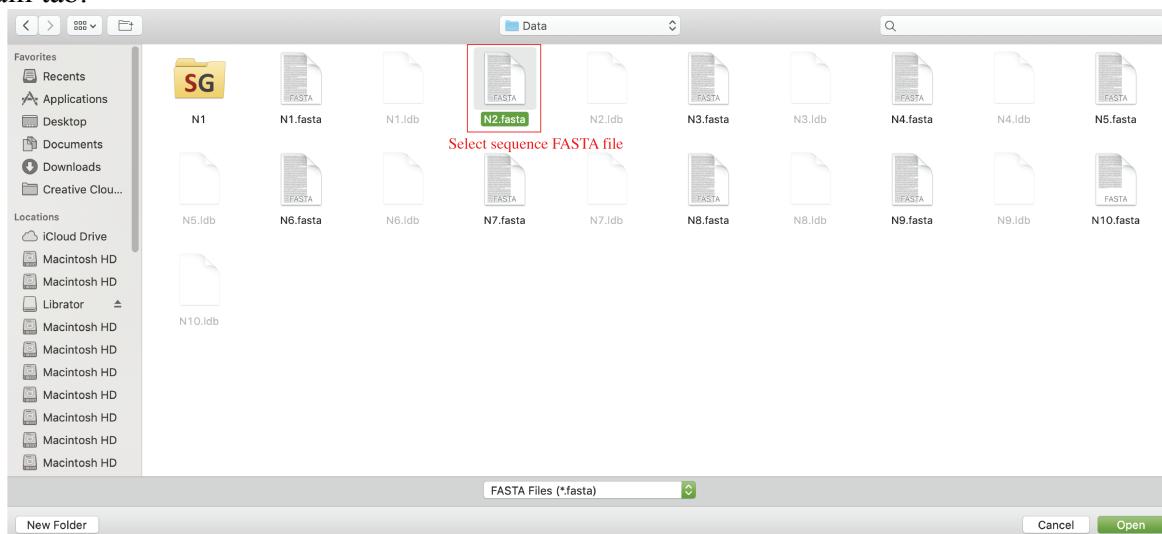
Click *File-> Open* in menu or click  in *Tool bar*.



### Import sequences

After load an existing sequence database, users can import sequence into current database. The input files should be in FASTA format. Please also try to make your sequence name concise and easy to recognize. Try not to include space in your sequence name.

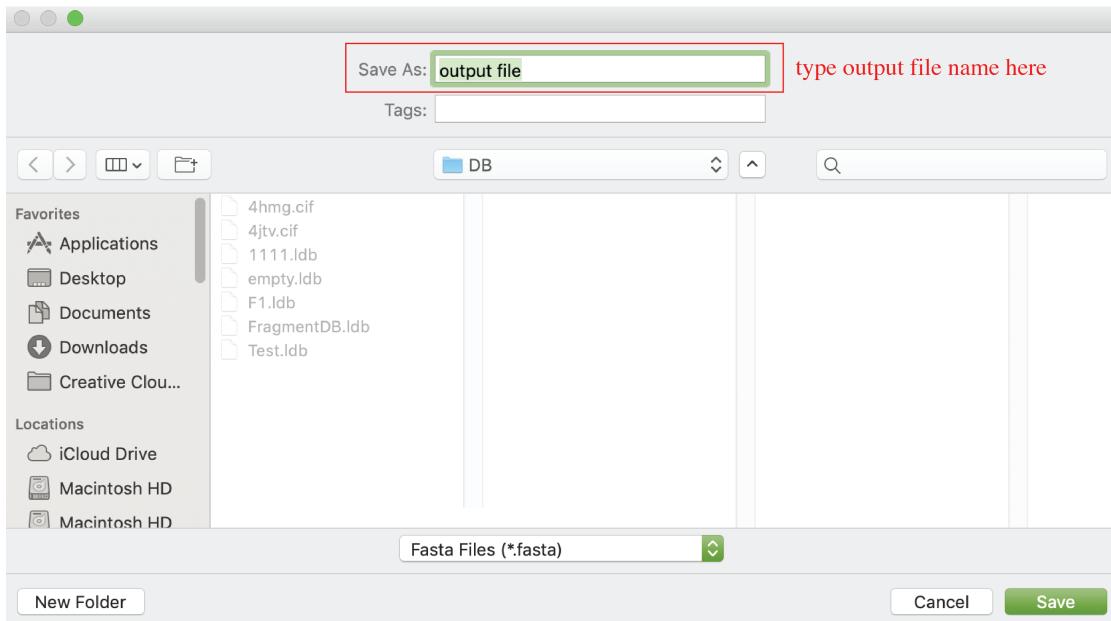
Click *Sequences-> Import* in menu or click  in *Tool bar* or click “import sequences” button in Main tab.



### 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*.

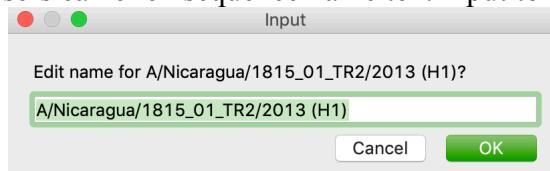


## Functions - advanced functions:

Edit sequence information (on Main tab)

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

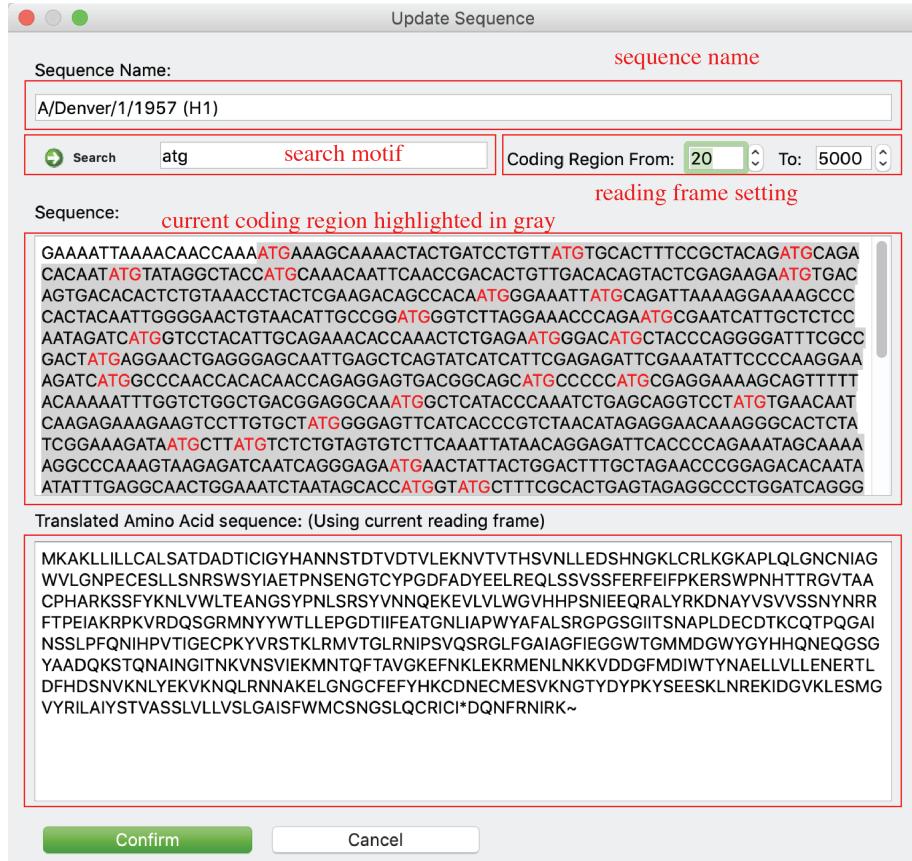
1. For **Sequence name**, users can click sequence name text input to edit it.



2. 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 'Available sequences' tree on the left with categories like All Sequences, Base Sequence, B, and H1. The 'Active sequences' list on the right contains a large number of entries, many of which are highlighted with a red border. The 'Edit here' section at the bottom left, which includes dropdown menus for Role (Unassigned), Form (Full HA), and Subtype (H1), is also highlighted with a red border.

3. For **reading frame** start and end, users can use the “Coding region” inputs. When users change coding region setting, the amino acid sequences will be updated accordingly.
4. For **NT sequences**, users can click “edit sequence” button to edit sequences in the following pop-up window.



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 system

Users are allowed to access H1/H3 numbering of selected sequence. Two ways:

- Please select your sequence on active sequence panel and then click *Tools-> HA Numbering* in menu, or click on *Tool bar*, or just click “Sequence” tab. Please see “Sequence” Tab for interface and details.

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

A/Brisbane/59/2007 (H1)

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

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: LKGIAPLQLGNCSVAGWILGNPECELLISKESWSYIIVEKPNPENGTCYPGHFADYEELRE

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....1  
Sequence: QLSSVSSFERFEIFPKESSWPNTVTGVSASCNHGESSFYRNLLWLTGKNGLYPNLSKS

Position: 80....185....190....195....200....205....210....215....220....225....230....235....2  
H1-Numbering: 70....175....180....185....190....195....200....205....210....215....220....225....2  
Sequence: YANNKEKEVLVLWGVHPPNIDQKALYHTENAYSVVSSHYSRKFTPEIAKRPKVRDQE

Position: 40....245....250....255....260....265....270....275....280....285....290....295....3  
H1-Numbering: 30....235....240....245....250....255....260....265....270....275....280....285....2  
Sequence: GRINYYWTLLEPGDTIIFEANGNLIAPRYAFALSRGFGSGIINSNAPMDKCDAKCQTPQG

- 2) Please select your sequence on active sequence panel and then click “Alignment” tab or



**Options:**  
 AA     NT     H1     H3

**Display Mode:**  
 Original:     Template:

**Legend:**  
 H1 highlight region: Ca1 (pink), Ca2 (yellow), Cb (grey), Sa (red), Sb (green), Stalk-MN (blue), Lateral Patch (black)  
 H3 highlight region: A (green), B (pink), C (grey), D (red), E (blue), Stalk-MN (blue), Lateral Patch (black)

Position AA:	.	.	.	20	.	.	.	25	.	.	.	30	.	.	.	35	.			
H1 numbering	-	.	.	10	.	15	.	20	.	25	.	30	.	.	.	35	.			
H3 numbering	10	.	15	.	20	.	25	.	30	.	35	.	40	.	45	.	50	.		
Position NT:	50	..	55	..	60	..	65	..	70	..	75	..	80	..	85	..	90	..		
A/Brisbane/59/2007 (H1)	A	D	T	I	C	I	G	Y	H	A	N	N	S	T	D	T	V	D	T	V
	CAGACACAATATGTATAGGCTACCATGCTAACAACTCGACCGACACTGTTGACACAGTAC																			

### Identify mutations

Users are allowed to identify mutations between any two sequences. If you have a template sequence and a mutated sequence (e.g. escape mutants) and want to quickly know the mutated residues, you can use this function to identify all mutations and annotate mutation information on your mutated sequence.

Click **Tools->Identify Mutation** in menu or just click on **Tool bar** to open the function window. Then select one sequence as template, determine the mutated sequence as target sequence. Then the alignment will be displayed 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 on [Tool bar](#) for an alignment viewer in a popup window or click Alignment (HTML) tab for an integrated and alignment viewer. There are two display modes: original sequence mode (panel A) and template mode (panel B). In template mode, users can choose any sequence (including consensus sequence) as template to only highlight sequence differences.

**Options:**

 AA    NT    H1    H3

Alignment display options

A

**Display Mode:**

 Original:    Template: A/Fort Monmouth/1/1947 (H1)

Alignment display Mode

**Legend:**

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

Position AA:  
H1 numbering  
H3 numbering  
Position NT:  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/59/2007 (H1)  
A/Brisbane/59/2007 (H1)  
A/California/04/2009 (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 AA:  
Position NT:  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/59/2007 (H1)  
A/California/04/2009 (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

**Options:**

 AA    NT    H1    H3

Alignment display options

B

Alignment display Mode

**Legend:**

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

Position AA:  
H1 numbering  
H3 numbering  
Template NT:  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/02/2018/EPI\_ISL\_...  
A/Brisbane/59/2007 (H1)  
A/California/04/2009 (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 are allowed to 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 on *Tool bar*.

For Amino acid tree:

Click *Tools-> Generate Maximum Likelihood Tree (Amino Acid)* in menu or just click on *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\_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

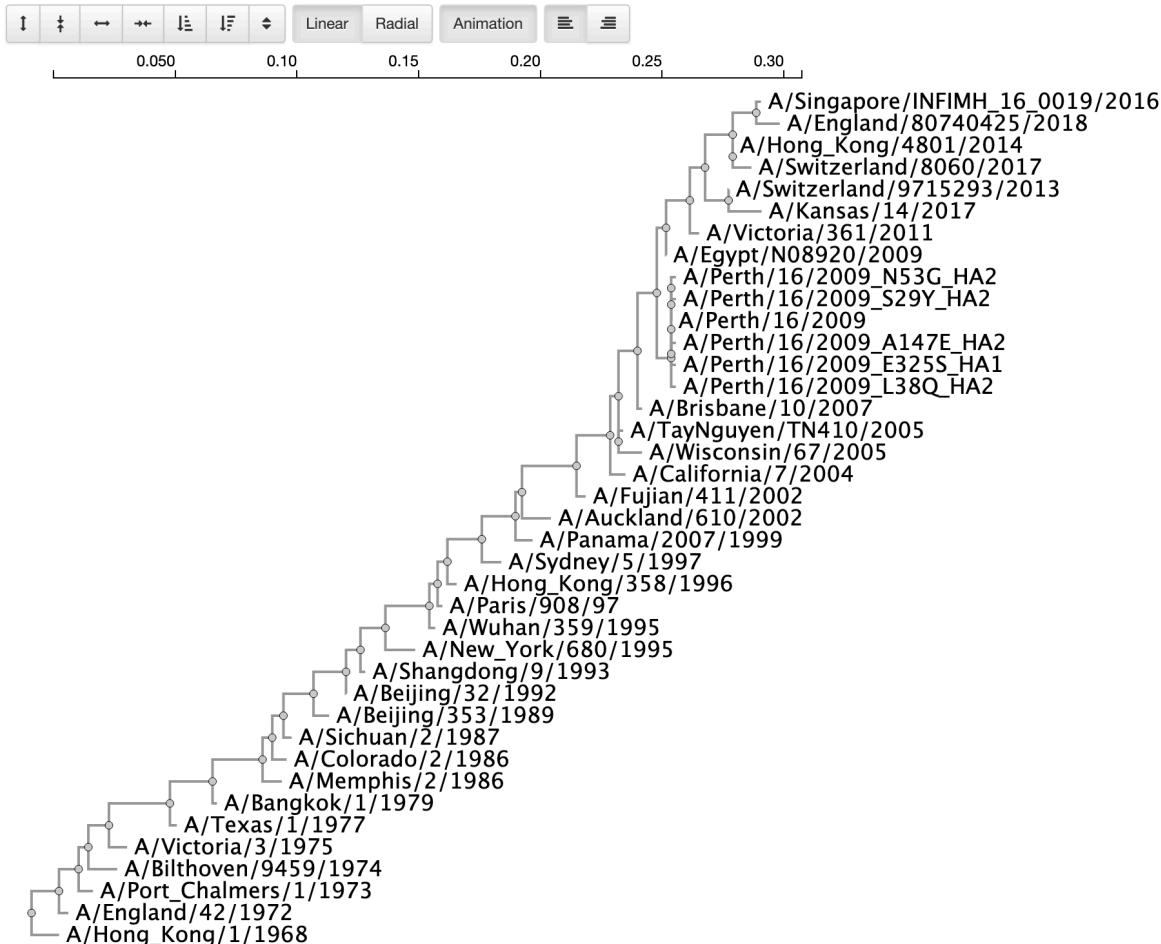
Click sequence name to highlight name and alignment by gray background

Sequence Name Sequence Alignment

Determine region:

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

Users are allowed to review alignments before building the tree. Users can determine a specific region of the alignment to avoid interference from sporadic insertion/deletion (as shown in the figure, selected regions were highlighted by red).

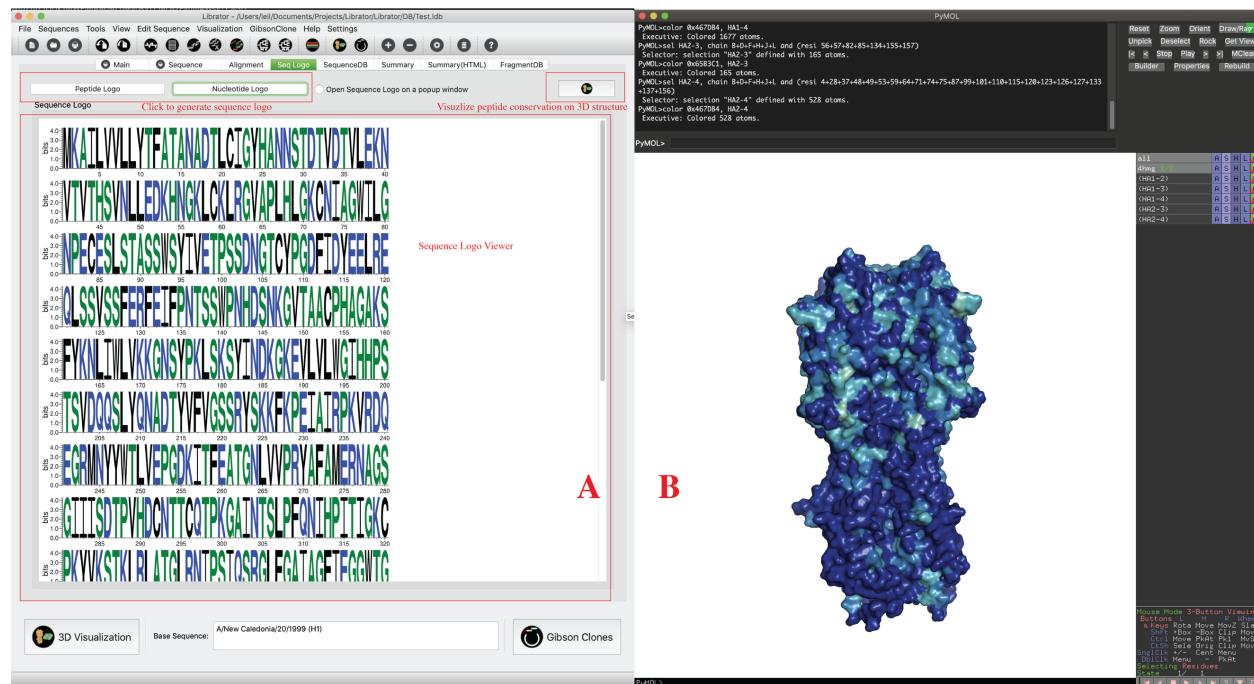


## Generate sequence Logo (peptide/nucleotide conservation)

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 as an EPS file (vector graph) for further use.

Users also can click the button on the right top corner to visualize peptide conservations for selected sequences on a 3D structure using PyMOL (panel B).



## Mutate sequence

Users can generated 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 then 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**

PyMOL> \_

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 on *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. This function could be helpful for designing chimeric HA protein.

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**

Select donor sequence:

- A/Brisbane/02/2018/EPI\_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) -18Q(HA1)
- A/California/04/2009 (H1) -S75M(HA1)
- A/Denver/1/1957 (H1)
- A/Fort Monmouth/1947 (H1)
- A/Iceland/1815\_01/TR2/2013 (H1)
- APR/0/1934 (H1)
- A/Solomon Islands/3/2006 (H1)
- A/South Carolina/1918 (H1)
- A/swine/Jiangsu/40/2011 (H1 avian-swine)
- A/swine/MN/0201/2008 (H1)
- A/Texas/36/1991 (H1)
- A/USSR/1977 (H1)

Active sequence list

Base Sequence: A/Hong Kong/1/1968 (H3)

Base sequence

H3-Numbering: Position: 1...6...11...16...21...26...31...36...41...46...51...56...  
H1-Numbering: ...1...5...10...15...20...25...30...35...40...45...50...  
Sequence: M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

H3-Numbering: Position: 61...66...71...76...81...86...91...96...101...106...111...116...  
H1-Numbering: ...10...15...20...25...30...35...40...45...50...  
Sequence: S S T G K T C H I R I L L D C T I D A L L G D P H C D V F Q N T W D I L V E R S K A F S N C Y Y D P V

H3-Numbering: Position: 121...126...131...136...141...146...151...156...161...166...171...176...  
H1-Numbering: ...105...110...115...120...125...130...135...140...145...150...155...160...  
Sequence: Y A S L R S I V A S S G T L E F I T Q N G S N A C K S P G S G F F S R L N L W T K S G S T P V

H3-Numbering: Position: 181...186...191...196...201...206...211...216...221...226...231...236...  
H1-Numbering: ...165...170...175...180...185...190...195...200...205...210...215...220...  
Sequence: N V T M P N N D N F U K L Y I N G W H H H P S T N E C T S L Y Q A S G R V T V S T R S Q Q T I I P N I G S R P W

H3-Numbering: Position: 241...246...251...256...261...266...271...276...281...286...291...296...  
H1-Numbering: ...205...210...215...220...225...230...235...240...245...250...255...260...265...270...275...280...285...  
Sequence: G L S R R I S Y W T I V K P G D V L V I N S N G N L I A P R G Y K M R T G K S I M B S D A P I D T C I S E C T P

Position: 301..306..311..316..321..326..331..336..341..346..351..356..

Donor Sequence: A/Brisbane/59/2007 (H1)

Selected donor sequence

H3-Numbering: Position: 1...6...11...16...21...26...31...36...41...46...51...56...  
H1-Numbering: ...1...5...10...15...20...25...30...35...40...45...50...  
Sequence: V K L L V L C L T T F A T Y A D T I C G H A N N S T D T V D L E R N V T T H S V N L E N S H N G K C L L R

H3-Numbering: Position: 61...66...71...76...81...86...91...96...101...106...111...116...  
H1-Numbering: ...10...15...20...25...30...35...40...45...50...  
Sequence: G T A P I T Q I G S V A G W I L N F C E C L I S E K S W Y I V E K P N E G T C Y P H F H A D Y E E L R Q L

H3-Numbering: Position: 121...126...131...136...141...146...151...156...161...166...171...176...  
H1-Numbering: ...0...115...120...125...130...135...140...145...150...155...160...165...  
Sequence: S S V S F S P E R F I F K E S N F N H T V T G V S A C S I N G E S F Y R N L L I T G N G L Y F N L S K S Y A

H3-Numbering: Position: 181...186...186...191...196...201...206...211...216...221...226...231...236...  
H1-Numbering: ...170...175...180...185...190...195...200...205...210...215...220...225...  
Sequence: N H K E K E V I A P F E R F I E R K F T E I A R K P R K V R V D Q E R

H3-Numbering: Position: 241...246...251...256...261...266...271...276...281...286...291...296...  
H1-Numbering: ...203...208...213...218...223...228...233...238...243...248...253...  
Sequence: I N Y Y T W L E P G D T I I F E R A N G N L I A P R A F A L S R G F G S G J I N S N A P M D C D A K C Q T P Q G A I

Position: 301..306..311..316..321..326..331..336..341..346..351..356..

Current Base sequence:

A/Hong Kong/1/1968 (H3)

Base sequence name

Base sequence

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

Cal-A	Cal-B	Cb-C	Sa-D	Sb-E	Stalk-MN
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Position AA: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

H1 numbering: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

H3 numbering: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

Original Seq: M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

FTATYADTICL TVTDVLEKNTV KLCLLKGIAPL

Double click to delete

Insertion information

Confirm Cancel

**Sequence Fusion across subtypes**

**Low Resolution Layout**

Select donor sequence:

- A/Brisbane/02/2018/EPI\_ISL\_330190/201
- A/Brisbane/59/2007 (H1)
- A/California/04/2009 (H1)
- A/Alabama/1918 (H1)
- A/Fort Monmouth/1947 (H1)
- A/New Caledonia/20/1999 (H1)
- A/Caraguata/1815\_01/TR2/2013 (H1)
- APR/0/1934 (H1)
- A/Solomon Islands/3/2006 (H1)
- A/South Carolina/1918 (H1)
- A/swine/Jiangsu/40/2011 (H1 avian-swine)
- A/swine/MN/0201/2008 (H1)
- A/Texas/36/1991 (H1)
- A/USSR/1977 (H1)

Active sequence list

Base Sequence: A/Wisconsin/6/2005 (H3)

Base sequence

H3-Numbering: Position: 1...6...11...16...21...26...31...36...41...46...51...56...  
H1-Numbering: ...1...5...10...15...20...25...30...35...40...45...  
Sequence: M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

H3-Numbering: Position: 61...66...71...76...81...86...91...96...101...106...111...116...  
H1-Numbering: ...10...15...20...25...30...35...40...45...50...  
Sequence: S S T G K T C H I R I L L D C T I D A L L G D P H C D V F Q N T W D I L V E R S K A F S N C Y Y D P V

H3-Numbering: Position: 121...126...131...136...141...146...151...156...161...166...171...176...  
H1-Numbering: ...105...110...115...120...125...130...135...140...145...150...155...160...  
Sequence: Y A S L R S I V A S S G T L E F I T Q N G S N A C K S P G S G F F S R L N L W T K S G S T P V

H3-Numbering: Position: 181...186...191...196...201...206...211...216...221...226...231...236...  
H1-Numbering: ...165...170...175...180...185...190...195...200...205...210...215...220...  
Sequence: N V T M P N N D N F U K L Y I N G W H H H P S T N E C T S L Y Q A S G R V T V S T R S Q Q T I I P N I G S R P W

H3-Numbering: Position: 241...246...251...256...261...266...271...276...281...286...291...296...  
H1-Numbering: ...205...210...215...220...225...230...235...240...245...250...255...260...265...270...275...280...285...  
Sequence: G L S R R I S Y W T I V K P G D V L V I N S N G N L I A P R G Y K M R T G K S I M B S D A P I D T C I S E C T P

Position: 301..306..311..316..321..326..331..336..341..346..351..356..

Donor Sequence: A/Nicaragua/1815\_01/TR2/2013 (H1)

Selected donor sequence

H3-Numbering: Position: 1...6...11...16...21...26...31...36...41...46...51...56...  
H1-Numbering: ...1...5...10...15...20...25...30...35...40...45...  
Sequence: R A T K Y M A D V I L V L Y P A T A N A D T C I G H A N N S T D T V D L E R N V T T H S V N L E N S H N G K C L L R

H3-Numbering: Position: 61...66...71...76...81...86...91...96...101...106...111...116...  
H1-Numbering: ...10...15...20...25...30...35...40...45...50...  
Sequence: S S T G K T C H I R I L L D C T I D A L L G D P H C D V F Q N T W D I L V E R S K A F S N C Y Y D P V

H3-Numbering: Position: 121...126...131...136...141...146...151...156...161...166...171...176...  
H1-Numbering: ...105...110...115...120...125...130...135...140...145...150...155...160...  
Sequence: Y A S L R S I V A S S G T L E F I T Q N G S N A C K S P G S G F F S R L N L W T K S G S T P V

H3-Numbering: Position: 181...186...186...191...196...201...206...211...216...221...226...231...236...  
H1-Numbering: ...170...175...180...185...190...195...200...205...210...215...220...225...  
Sequence: I N Y Y T W L E P G D T I I F E R A N G N L I A P R A F A L S R G F G S G J I N S N A P M D C D A K C Q T P Q G A I

H3-Numbering: Position: 241...246...251...256...261...266...271...276...281...286...291...296...  
H1-Numbering: ...203...208...213...218...223...228...233...238...243...248...253...  
Sequence: N H K E K E V I A P F E R F I E R K F T E I A R K P R K V R V D Q E R

Position: 301..306..311..316..321..326..331..336..341..346..351..356..

Current Base sequence:

A/Wisconsin/6/2005 (H3)

Base sequence name

Base sequence

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

Cal-A	Cal-B	Cb-C	Sa-D	Sb-E	Stalk-MN
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Position AA: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

H1 numbering: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

H3 numbering: . . . 5 . . . 10 . . . 15 . . . 20 . . . 25 . . . 30 . . . 35 . . . 40 . . . 45 . . . 50 . . . 55 . . . 60 . . . 65 . . . 70 . . . 75 . . . 80 . . . 85 . . . 90

Original Seq: M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

M K T 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 H A V P N G T I V K T I T D D Q I E V T N A T E L V Q

FTVLLYFTATAN ANNSTDITVDTV

Double click to delete

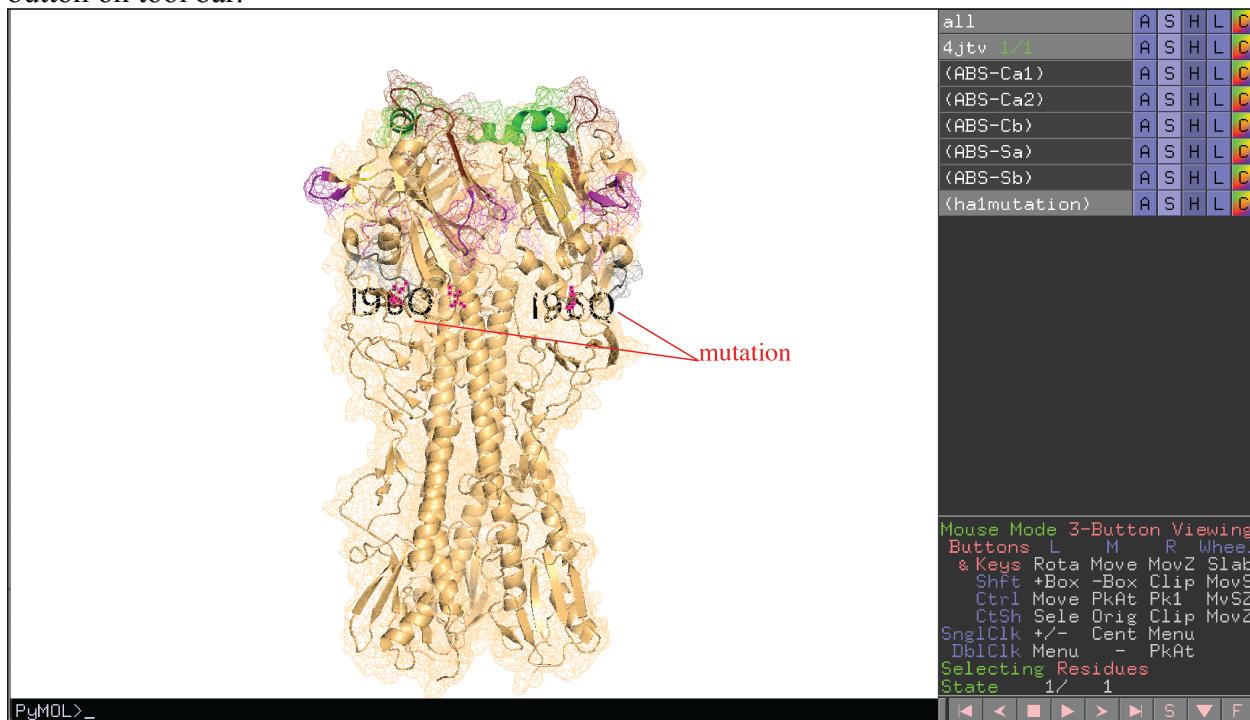
Insertion information

Confirm Cancel

### 3D visualization via PyMOL

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

Please select any sequence in active panel and then click *Visualization-> PyMOL* in menu or  button on tool bar.



Generate Standardized Gibson Clone fragments for HA/NA in batches

Users can generate standardized Gibson Clone Fragments for HA/NA sequences in batches. Those fragments are reusable due to they are following the same design.

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 region that connect fragment 1 and 4 to the vector not displayed for AA sequences but will be automatically added to 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):  **joint region to vector**

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

Joint Region Design:  Default  User Defined

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

**Gibson Clone Fragments Preview**

Options:  AA  NT

Position AA:	Sequence
A/England/80740425/2018	. . . 90 . . . 95 . . . 100 . . . 105 . . . 110 . . . 115 . . . 120 . . . 125 . . . 130 . . . 135 . . . 140 . . . 145 . . . 150 . . . 155 . . . 160 . . . 165 . . .
A/Auckland/610/2002	GDPQCDGFQNKKWDLFVERSRAYSNCYPYDVPDYASLRSVLVASS
A/Memphis/2/1986	GDPHCDGFQNKEWDLFVERSKAFNCYPYDVPDYASLRSVLVASS
A/New York/680/1995	GDPHCDGFQNKEWDLFVERSKAYNSNCYPYDVPDYASLRSVLVASS
A/England/80740425/2018	SLRSLVASSGTLERFKNESFNWAGVTQNGKSSACIRGSSSSFFSRLNW
A/Auckland/610/2002	SLRSLVASSGTLERFNNEFSNWTGVAQNGTSSACKRRSDKSFFSRLNW
A/Memphis/2/1986	SLRSLVASSGTLERFINEGFNWTGTVQSGGSYACKRGSVNSFFSRLNW
A/New York/680/1995	SLRSLVASSGTLERFTNENFNWWTGVAQDGKSYACKRGSVNSFFSRLNW
A/England/80740425/2018	
A/Auckland/610/2002	
A/Memphis/2/1986	
A/New York/680/1995	

**Alignment of Fragments**

Selected sequences

**Output path**

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

Existing fragments used: H3-F1-0001 H3-F4-0002 H3-F4-0003 Existing fragments

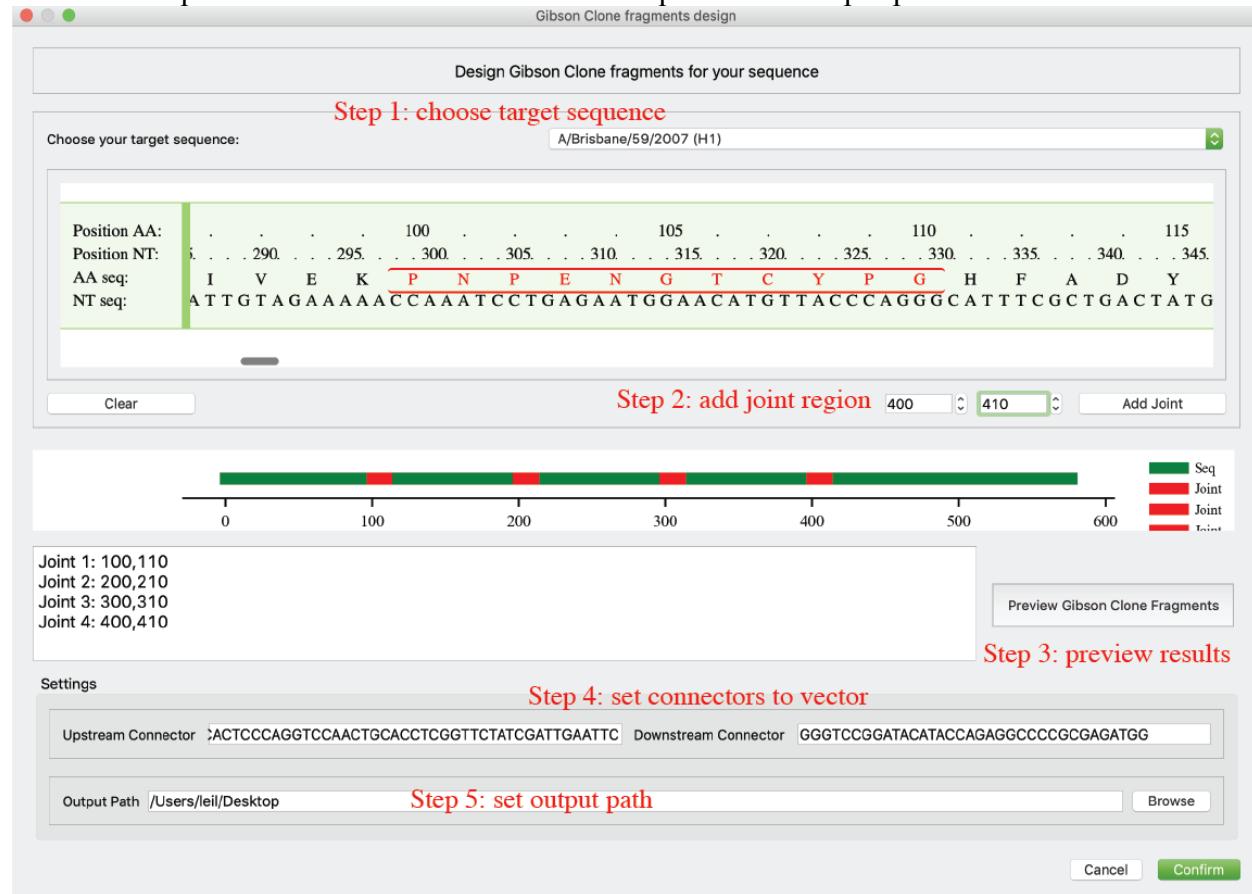
New fragments generated: H3-F1-0003 H3-F1-0005 H3-F1-0011 H3-F1-0012 H3-F1-0013 H3-F1-0014 H3-F1-0015 H3-F2-0007 H3-F2-0008 H3-F2-0014 H3-F2-0015 H3-F2-0016 H3-F2-0017 H3-F2-0018 H3-F3-0002 H3-F3-0003 H3-F3-0009 H3-F3-0010 H3-F3-0011 H3-F3-0012 H3-F3-0013 H3-F4-0008 H3-F4-0009 H3-F4-0010

New fragments need to order

Generate Gibson Clone fragments for any sequence

Beside HA/NA sequences, Librator also allows users to split any of their sequences into fragments. Users can use this function for some frontier and novel research, e.g. COVID-19.

Users can click [GibsonClone-> GibsonClone for single sequence](#) in [menu](#). Users can view their sequence and add at most 12 joint regions (where to split the sequence) to their sequence. Then users can preview the fragment products under current design. After that, users can setup upstream connector sequence and downstream connector sequence and output path.



Identify possible key residues from two groups of sequences

Librator allows users to identify possible key residues from two groups of sequences. This function could be helpful in the case of identifying key residues that potentially responsible for antigenic change or antibody binding.

Users can click [Tools-> Identify key residues](#) in [menu](#). Users can double click sequence names on the left panel to add them into positive group or negative group to the right. Users can click the corresponding arrow to change the destination (green arrow indicates current destination). Users also can double click sequences on the right panel to remove them from current panel. After that, users can click 'Identify possible key residues' button to run analysis. The importance of residues are quantified and ranked by numerical score, which indicates the difference of amino acid composition between two groups. All residues will be listed in a table and be sorted by the score (inverse order). Multiple sequence alignment will also be popped up for users to investigate the details.

The screenshot displays two windows of the Multiple Sequence Alignment Viewer. The left window shows 'Test Positive sequences' and 'Test negative sequences' with various sequence entries. The right window shows 'Options' (AA, NT, H1, H3 selected), 'Display Mode' (Original, Template, Consensus Sequence), 'Legend' (H1 highlight region, H3 highlight region), and a sequence alignment table with columns for Position AA, H1 numbering, H3 numbering, Template AA, and various patch regions (Ca1, Ca2, Cb, Sa, Sh, Stalk-MN, Lateral Patch). The alignment table includes rows for Positive/Australia/2009, Positive/Australia/2009, Positive/Australia/2009, Positive/Australia/2009, Positive/Australia/2009, Positive/Australia/2009, Negative/A/New, Negative/A/New, Negative/A/New, Negative/A/New, Negative/A/New, and Negative/A/New.

## Contact

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