

LIBRATOR V 1.0 - USER MANUAL

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User interface

Main Tab (printable)

Sequence Tab (printable)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

Main Sequence Alignment(RTF) Alignment(HML) SequenceDB Summary Summary(HML) 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: MKAILVVLLYTFATANADTLCIGYHANNSTDVTDTVLEKNVTVTHSVNLLEDKHNGKLCK

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: LRGVAPLHLGKCNIAAGWILGNPECEMLSTASSWSYIVETPSSDNGTCYPGDFIDYEELRE

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: QLSSVSSFERFEIFPKTSSWPNHDNSNKVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSK

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: SYINDKGKEVLWLWGIHHPSSTSADQQSLYQNAADTYVFVGSSRYSKKFPEIAIRPKVRDQ

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: EGRMNYYWTLVEPGDKITFEATGNLVPYAFAMERNAGSGIIISDTPVHDCNTTCQTPK

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: GAIANTS LPFQNIHPI TIGKCPK VVKST KRLAT GLRN I P S I Q SRGL F GAI AGFIEGGWTG

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: MVDGWYHQQNEQGSGYAIDLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKR

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: IENLNKKVDDGFLDIW TYNAELL VLLENERTLDYHDSNVKNLYEKVRSQLNNAAKEIGNG

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: LVLVSLGAISFWMCSNGSLQCRCI

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/220158/2002 EPI_ISL_2793 A / ...	AGCAAAAG...	1384	N3	Full NA	1	5000	False	Unassigned	none	none	0	
8	A/turkey/Italy/214845/2002 EPI_ISL_2792 A / ...	AGCAGAAAG...	1384	N3	Full NA	1	5000	False	Unassigned	none	none	0	
9	A/turkey/Indiana/16-001403-1/2016 (H7)	TACAAATG...	1705	H7	Full HA	1	5000	False	Unassigned	none	none	0	
10	A/teal/France/2546/2001 EPI_ISL_2888 A / H1N2	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)	TTATTCTGC...	1780	H2	Full HA	1	5000	False	Unassigned	none	none	0	
13	A/swine/MN/02011/2008 (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	ATGCTATCA...	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)	ATGAAGAAA...	1728	H11	Full HA	1	5000	False	Unassigned	none	none	0	
19	A/shearwater/West Australia/2576/1979 (H15)	AGCAGAAAG...	1763	H15	Full HA	1	5000	False	Unassigned	none	none	0	
20	A/rhea/North Carolina/39482/1993 (H7)	AGCAGAAAG...	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)	AGCAGAAAG...	1742	H6	Full HA	1	5000	False	Unassigned	none	none	0	
23	A/mallard/Netherlands/5/1999 (H2)	AGCAGAAAG...	1771	H2	Full HA	1	5000	False	Unassigned	none	none	0	
24	A/mallard/Netherlands/12/2000 (H7)	AGCAGAAAG...	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)	GGTCACAT...	1703	H10	Full HA	8	5000	False	Unassigned	none	none	0	
27	A/mallard/Sri Lanka/263/1982 (H14)	AGCAGAAAG...	1749	H14	Full HA	1	5000	False	Unassigned	none	none	0	

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

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 ...	TCDRNWQGPNRVIIQIDPVAMTHTSQYICSP...	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 / ...	TCDRNWQGSNRPVIIQIDSVMTHTSQYICSP...	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...	AGGTTCTATGCTCTCAGTCAAGGGACAACAA...	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.

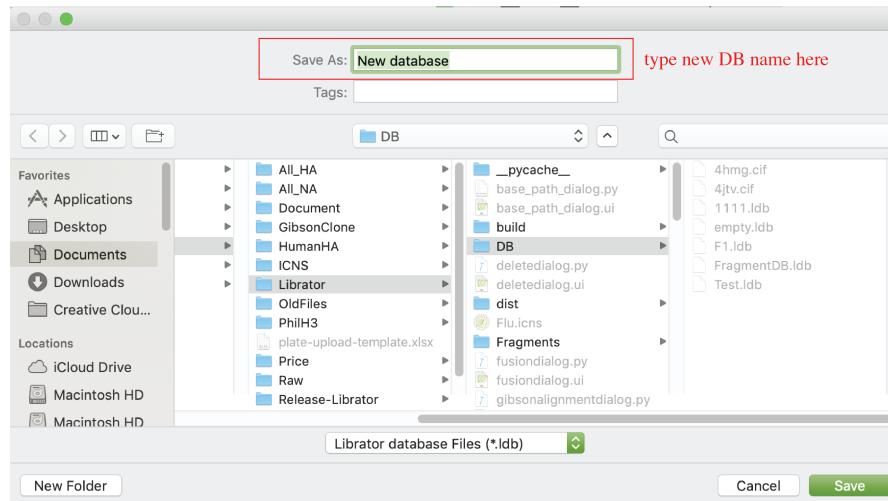
Click *Setting-> Preferences* in menu or click  in *Tool bar*.



Create new sequence database

Users can create new sequence database.

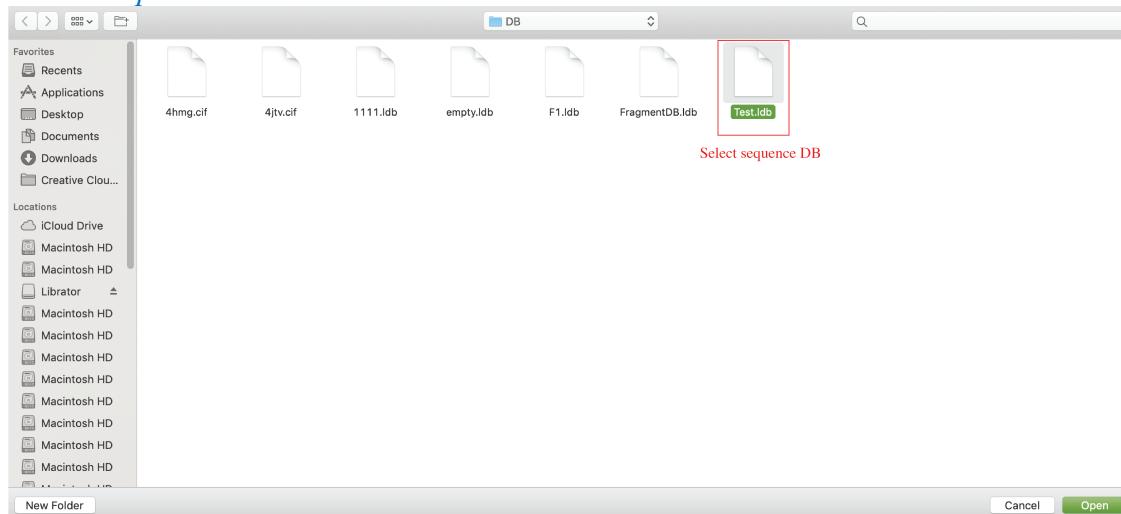
Click *File-> New* in menu or click  in *Tool bar*.



Open existing sequence database

Users can create new sequence database.

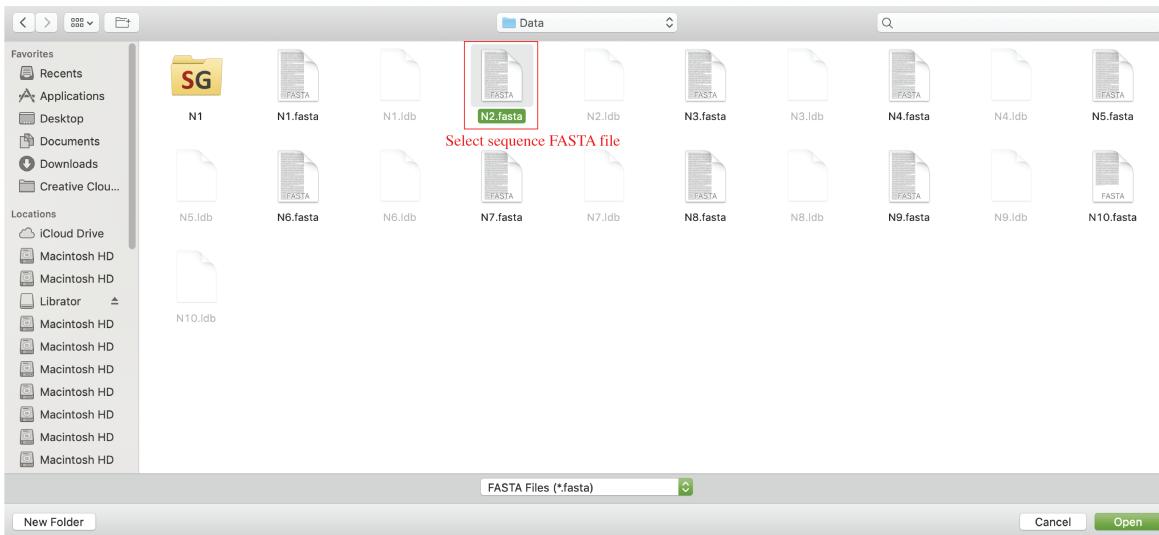
Click *File-> Open* in menu or click  in *Tool bar*.



Import sequences

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

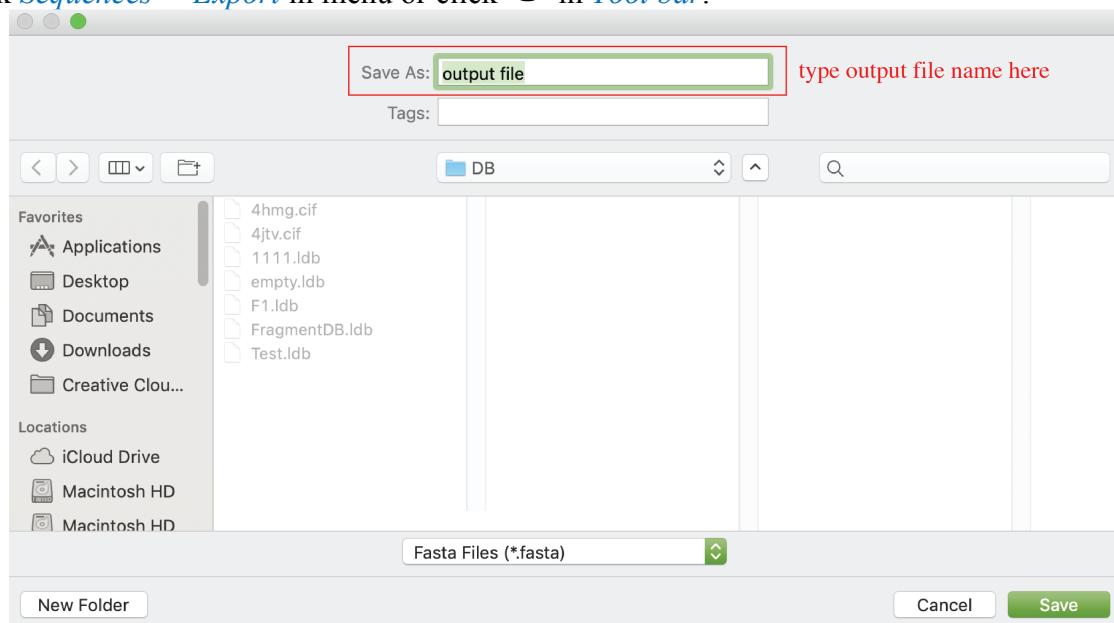
Click *Sequences-> Import* in menu or click  in *Tool bar* or click “import sequences” button in Main tab.



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*.

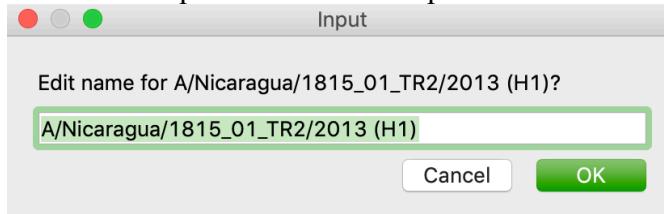


Advanced functions:

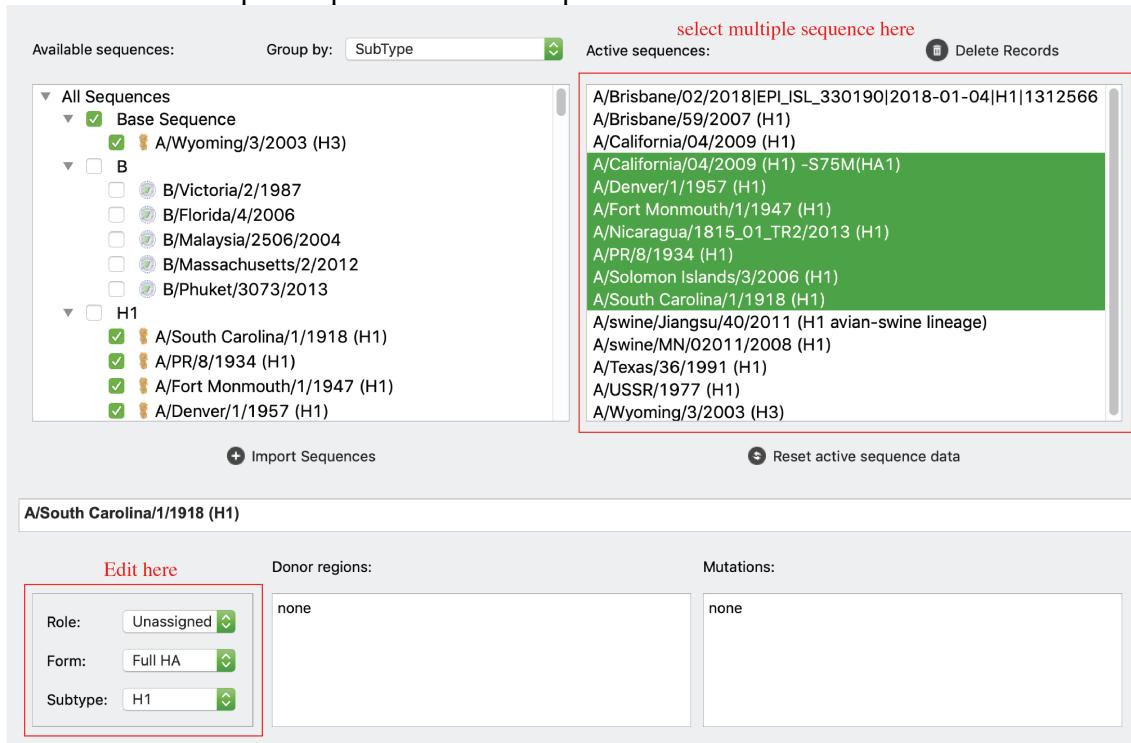
Edit information of sequences (on Main tab)

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 software's main window. On the left, a tree view labeled 'Available sequences' shows categories like 'All Sequences', 'Base Sequence', 'B', and 'H1', each with a list of specific sequences. On the right, a list of 'Active sequences' is shown, with many entries highlighted in green. At the bottom, there are three main editing sections: 'Edit here' (with dropdown menus for Role, Form, and Subtype), '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 CACAAATATGATAGGCTTACCATGCAACAAATTCAACCGACACTGTTGACACACTCTGAGAAGAATGTGAC AGTGACACACTCTGAACACTCGAAGACAGCCACAATGGAAATTATGCAGATTAAGGAAAGGCC CACTACAATTGGGAACGTAACTTGGCGGATGGTCTTAGGAAACCCAGAATGCGAATCATTGCTCTCC AATAGATCATGGTCTTACATTGCAAGAACACCCAAACTCTGAGAATGGACATGCTACCCAGGGATTCGCG GACTATGAGGAAGGGAGGAGCAATTGAGCTCAGTATCATCATTGAGAGATTGAAATATTCCCAGGAA AGATCATGGCCAACCACACAACCAAGAGGAGTGACGGCAGCAGTGGGGGGATGCGAGGAAAGCAGTTTT ACAAAAAATTGGTCTGGCTGACGGAGGCAAATGGCTCATACCCAAATCTGAGCAGGTCTATGTGAAACAT CAAGAGAAAGAAGTCTTGCTATGGGAGTTCATACCCGCTAACATAGAGGAACAAAGGGCACTCTA TCGGAAAGATAATGCTATGCTCTGAGTCTCAATTATAACAGGAGATTACCCAGAAATAGCAAA AGGCCAAAGTAAGAGATCAATCAGGAGAATGAACTACTGGACTTTGCTAGAACCCGGAGACACAATA ATATTTGAGGCAACTGAAATCTAATAGCACCATGGTATGCTTCGCACTGAGTAGAGGCCCTGGATCAGGG</pre>			
Translated Amino Acid sequence: (Using current reading frame) <pre>MKAKLILLCALSATDADTCIGYHANNSTDVTVDLKNVTVTHSVNLLEDSHNGKLCRLKGKAPLQLGN WVLGNPECESLLSNRWSYIAETPSENCGTYPDFADYEELREQLSSVSSFERFEIFPKERSWP NHTTRGVTAACPHARKSSFYKNLVWLTEANGSYPNLRSYVNNQKEVFLVWGVHHP SNIEEQRALYRKDNAYVS VSSNNYNR FTPEIAKRPKVRDQSGRMNYYWTLEPGDTIIFEA TGNLIAPWYAFALSRGP GSGIITSNAPLDEC DTKCQTPQGAI NSSLPFQNIHPV TIGEC PKYVRSTKLRM VTLNIP VSQSRGLFG AIGFIEGGWTG MMDGWYGH HQNEQGSG YAADQKSTQNA INGIT NKVNSV IEKMNT QFTAVG KEFN KLEM LNKK VDDGF MDI WTYNA ELL LEN RTL DFHDSNV KVNLYEK VKNQLRN NAEL NGC FEFY HKCD NECM EV KNGTYD P KYSE ES KLN REK IDGV KLES MG Y Y RILAI Y STV ASS L V L L G A I F W M C S N G L Q C R I C I *DQN F R N R K~</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.

A RTF alignment page is also available, users can click Alignment(RTF) tab. This tab is printable. Please see “Alignment (RTF)” Tab for interface and details.

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	KATKMKAIIVVVLLYT
A/California/04/2009_H1	FATANADTLCIGYHANNST
A/California/04/2009_H1_S75M_HA1	DTVDTVLEKNVTVTHSVNLLLEDKHNGKLCKLRGVAPLHLGKCNIA
A/South_Carolina/1/1918_H1	GWLGNPECESLSTASSWSYI
A/Brisbane/59/2007_H1	VETSSDNGTCYI
A/Solomon_Islands/3/2006_H1	---
A/PR/8/1934_H1	MKAIVLVLLYT
A/Denver/1/1957_H1	FATANADTLCIGYHANNST
A/Fort_Monmouth/1/1947_H1	DTVDTVLEKNVTVTHSVNLLLEDKHNGKLCKLRGVAPLHLGKCNIA

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

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).

AB

C

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

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
 CtrSh Sele Drag Clip MovZ
 SnglClk +/- Cent Menu
 DblClk Menu - PkAt
 Selecting Residues
 State 1/ 1

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 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).

A

Base Sequence A/Brisbane/59/2007 (H1)

Donor sequence (single selection)

- A/Brisbane/59/2007 (H1) -N52D(Cocktail)
- A/Brisbane/59/2007 (H1) -Q239R(Cocktail)
- A/Brisbane/59/2007 (H1) -R162K(Cocktail)
- A/Brisbane/59/2007 (H1) -V145T(Cocktail)
- A/California/04/2009 (H1)
- A/Denver/1/1957 (H1)
- A/Fort Monmouth/1/1947 (H1)
- A/New Caledonia/20/1999 (H1)
- A/Nicaragua/1815_01_TR2/2013 (H1)
- A/PR/8/1934 (H1)
- A/shoveler/Netherlands/18/1999 (H1)
- A/Solomon Islands/3/2006 (H1)
- A/South Carolina/1/1918 (H1)
- A/swine/Jiangsu/40/2011 (H1 avian-swine lineage)

Donor Region

From: 100 To: 200

Donor Region

Apply

Sequence Comparision

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
Donor region:	M	ATG				

Position AA:
H1 numbering 105 110 115 . .
H3 numbering 95 100 105 . .
Position NT:
A/Brisbane/59/2007 (H1) 295 . . . 300 . . . 305 . . . 310 . . . 315 . . . 320 . . . 325 . . . 330 . . . 335 . . . 340 . . . 345 . . . 350
A/Brisbane/59/2007 (H1) K P N P E N G T C Y P G H F A D Y E E
A/Brisbane/59/2007 (H1) AAAACCAAT CCTGAGGAATGGAACATGTTACCCAGGGCATTCGCTGACTATGAGGAA
A/New Caledonia/20/1999 T
A/New Caledonia/20/1999 . . C

 Only single mutations All possible combinations options

Generate Sequences Cancel

B

Base Sequence B A/Brisbane/59/2007 (H1)

Donor Sequences (multiple selection)

- A/Brisbane/59/2007 (H1) -K205R(Cocktail)
- A/Brisbane/59/2007 (H1) -K290E(Cocktail)
- A/Brisbane/59/2007 (H1) -K90R(Cocktail)
- A/Brisbane/59/2007 (H1) -L840R(Cocktail)
- A/Brisbane/59/2007 (H1) -N52D(Cocktail)
- A/Brisbane/59/2007 (H1) -Q239R(Cocktail)
- A/Brisbane/59/2007 (H1) -R162K(Cocktail)
- A/Brisbane/59/2007 (H1) -V145T(Cocktail)
- A/California/04/2009 (H1)
- A/Denver/1/1957 (H1)
- A/Fort Monmouth/1/1947 (H1)
- A/New Caledonia/20/1999 (H1)
- A/Nicaragua/1815_01_TR2/2013 (H1)
- A/PR/8/1934 (H1)
- A/shoveler/Netherlands/18/1999 (H1)
- A/Solomon Islands/3/2006 (H1)
- A/South Carolina/1/1918 (H1)
- A/swine/Jiangsu/40/2011 (H1 avian-swine lineage)
- A/swine/MN02011/2008 (H1)
- A/Texas/38/1991 (H1)
- A/USSR/1977 (H1)

Generate consensus sequences of selected sequences and base sequence. For each selected sequence, only consider their donor region.

Generate Sequences Cancel

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

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) -I85Q(HAI)
- A/California/04/2009 (H1) -S75M(HAI)
- 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/02/2011/2008 (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...
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...81...86...91...96...101...106...111...116...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 M 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: ...1...5...10...15...20...25...30...35...40...
Sequence: I 2 1 . . . 1 2 6 . . . 1 3 1 . . . 1 3 6 . . . 1 4 1 . . . 1 4 6 . . . 1 5 1 . . . 1 5 6 . . . 1 6 1 . . . 1 6 6 . . . 1 7 1 . . . 1 7 6 . . .

H3-Numbering: Position: 105...110...115...120...125...130...135...140...145...150...155...160...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
Sequence: A 1 0 5 . . . 1 1 0 . . . 1 1 5 . . . 1 2 0 . . . 1 2 5 . . . 1 3 0 . . . 1 3 5 . . . 1 4 0 . . . 1 4 5 . . . 1 5 0 . . . 1 5 5 . . . 1 6 0 . . .

H3-Numbering: Position: 181...186...191...196...201...206...211...216...221...226...231...236...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 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: ...1...5...10...15...20...25...30...35...40...
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 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...
Sequence: V K L L V L C L T T F A T Y A D T I C G H A N S T D T V D L E R K 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...81...86...91...96...101...106...111...116...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
Sequence: G T A P T J O N S E V A G N I L G N F C E C L I S E K S W Y I V E K P N F 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: ...1...5...10...15...20...25...30...35...40...
Sequence: S S V S F S P E R F I F K E S E N W 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...191...196...201...206...211...216...221...226...231...236...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
Sequence: N N K E K E V I A P F E R F I E R K F T E I A R K P 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: ...1...5...10...15...20...25...30...35...40...
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 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
60 0 to 70 60 0 to 70 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 . . .

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

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

FTATYADTIC| 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_330190/2011
- 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/02/2011/2008 (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...
Sequence: A B R I S B A N E / 0 2 / 2 0 1 8 / E P I _ I S L _ 3 3 0 1 9 0 / 2 0 1 1

H3-Numbering: Position: 105...110...115...120...125...130...135...140...145...150...155...160...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 M 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: ...1...5...10...15...20...25...30...35...40...
Sequence: I 2 1 . . . 1 2 6 . . . 1 3 1 . . . 1 3 6 . . . 1 4 1 . . . 1 4 6 . . . 1 5 1 . . . 1 5 6 . . . 1 6 1 . . . 1 6 6 . . . 1 7 1 . . . 1 7 6 . . .

H3-Numbering: Position: 181...186...191...196...201...206...211...216...221...226...231...236...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 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: ...1...5...10...15...20...25...30...35...40...
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 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...
Sequence: A W I S C O N S I N / 6 / 2 0 0 5

H3-Numbering: Position: 61...66...71...81...86...91...96...101...106...111...116...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 M 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: ...1...5...10...15...20...25...30...35...40...
Sequence: I 2 1 . . . 1 2 6 . . . 1 3 1 . . . 1 3 6 . . . 1 4 1 . . . 1 4 6 . . . 1 5 1 . . . 1 5 6 . . . 1 6 1 . . . 1 6 6 . . . 1 7 1 . . . 1 7 6 . . .

H3-Numbering: Position: 181...186...191...196...201...206...211...216...221...226...231...236...
H1-Numbering: ...1...5...10...15...20...25...30...35...40...
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 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: ...1...5...10...15...20...25...30...35...40...
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 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/Wisconsin/6/2005 (H3)

Base sequence name

Base sequence

Replaced region on base sequence: Donor region on donor sequence: Add
0 0 to 0 0 0 to 0 0 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 . . .

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

Original Seq: M K T I I A L S Y I F C L V A Q K L F 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 V A Q K L F 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

TVVLLYTFATAN | ANNSTDITVDTV |

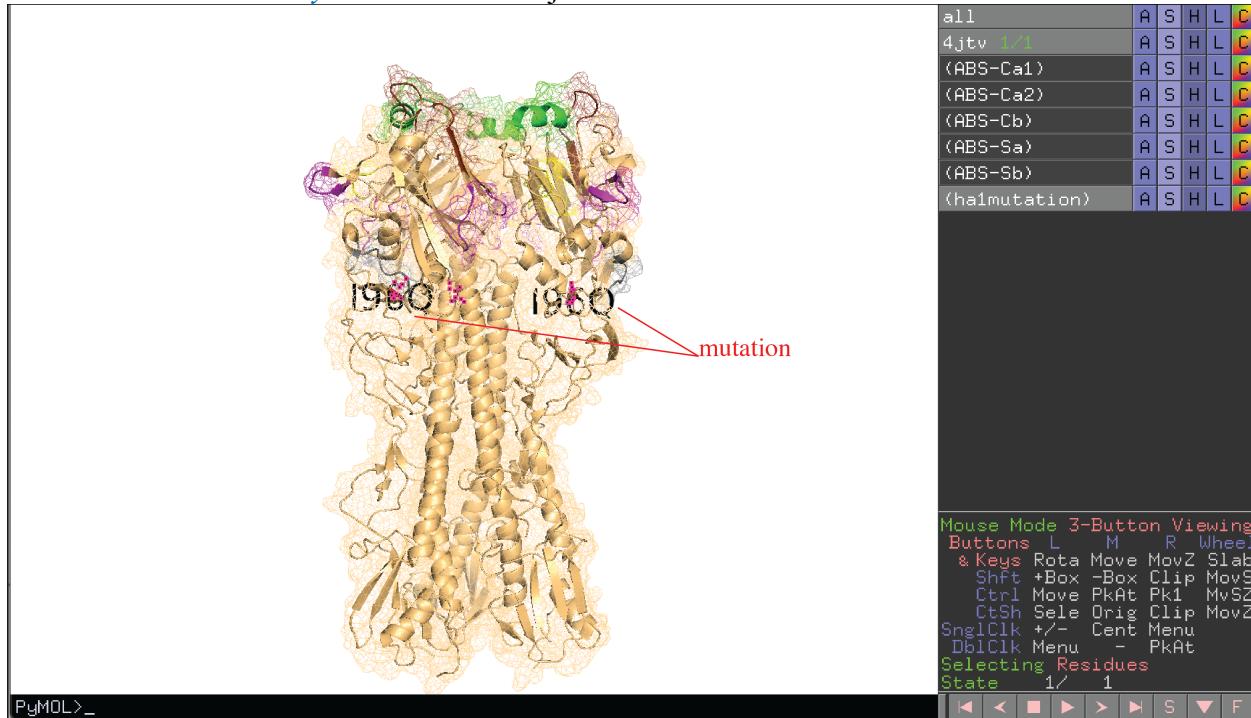
Double click to delete

Insertion information

Confirm Cancel

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

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: A/Indiana/10/2011 (H3v), A/Hong Kong/1/1966 (H3), A/Harbor seal/Massachusetts/1/2011, A/Philippines/2/1982 (H3), A/Panama/2007/1999 (H3), A/Perth/16/2009 (H3), A/Victoria/36/2011 (H3)

Alignment of Fragments

Fragment 1: MKTIIIAFSCILCLIAQAKLPGSDNSMATICLGHNVAPNGTLVKITDDQIEVTNATELVQSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFVERSTAYNCYPYDVPYA
 Fragment 2: MKTIIIALSYIPLCALQDLPQNDNSTATLCLGHNVAPNGTLVKITDDQIEVTNATELVQSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFVERSKAFNSCYDVPYA
 Fragment 3: MKTIIIALSYIPLSCPFCLAPCONSENNNTATLCLGHNVAPNGTLVKITDDQIEVTNATELVQSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFVERSKAFNSCYDVPYA
 Fragment 4: MKTIIIALSYIPLSYMLCVAQNLPGNDNSTATLCLGHNVAPNGTLVKITDDQIEVTNATELVQSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFVERSKAFNSCYDVPYA
 joint region shared by fragments will be highlighted

“*” will be highlighted

“*” in sequences will be removed before generating Fragments

Switch between AA and NT

Amino Acid Nucleotide

Confirm Cancel

Contact

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