

LIBRATOR V 1.0 - USER MANUAL

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User interface

Main Tab (printable)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

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

Tool bar

Open recent DB

Recent files: /Users/leil/Documents/Projects/Librator/Librator/DB/Test.lldb

Copy selected sequences into another DB

Sequence editing functions

Generate Sequences: Select report type:

Available sequences: All sequences in the tree can be grouped by Subtype, Role or Form

All sequences in current DB

Group by: SubType

Active sequences: A/Brasilia/02/2018|EPI_ISL_330190|2018-01-04|H1|1312566

Highlight sequences by Role

Highlight: Unassigned

All sequence in current DB

Import Sequences Import sequences

Delete Records Delete records

Clear active sequence panel

Reset active list

A/New Caledonia/20/1999 (H1)-E290K(Cocktail)

Current sequence

Donor regions: I100-200

Mutations: E290K

Donor region information

Mutation information information

Open a popup window show all three Reading Frames

Open a popup window to edit sequence

Coding region: Users can adjust coding region for selected sequence. This function is disabled for sequences with mutations

Coding Region: From: 1 To: 5000

Show Reading Frames

DNA Sequence: position = 0 of 1711 total nucleotides

Nucleotide sequence

Search Motif search function

Search target sequence switch

Amino Acid Sequence: position = 1 of 572 total residues

Amino Acid sequence

Design GibsonClones fragments for selected sequences

open PyMOL to visualize selected sequence

3D Visualization

Base Sequence: A/New Caledonia/20/1999 (H1)

Base sequence of current DB. It's unique.

Gibson Clones

SMYAKLILVLCFTTATYADTCIGYHANNSTDVTLEKNVTVTHSVNLLEDHSNKGKLLKGIAPLQLGNCSVAGWILGNPECLLISKEWSVSYIETPNPENGTCYPGYFADYEELREQLSVSSFSERFEIPKESWPNHTVTGVSVASCNSHNKS SFYRNLLWLTLGKNGLYPNLSKSYNNKEKEVLVWGVHIPPNGVSYSSRFTPEAKPRVKVRDQEGRINRYVWTLEPGDTIIEANGLNJAPWFALSRSRGFSGIITSNAPMDKCDKCTQPGAINSSLPFQNVHPVTI GECPKYVRSALKRMVTGLRNIPSQSRGLFGIAIGFIEGGWTGMVDGWYGHNEQNGSGYIAODKSTONAINGITNKVNSVIEKMNTQFTAVGKEFNKLERRMENLNKKVDDGFLDIWVNAELLVLLENERLTDFHDSNVKNLYEVKSQLKN NAEIGNGCFFYHKCNNECMESVKNGTYDYPKYSSEESKLNRKEDGVKLEMGSVYQILAYSTVASSLVLVSLGAISFWMCNSGSLQCRIC*DNF*

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: 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: 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: LVLVSLGAISFWMCSNGSLQCRICI

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 RTF Tab (printable)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

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

Muscle Alignment: Set font size: Font Size (Ctrl+, Ctrl-): 13

VGenes multiple alignment using Clustal Omega.

Consensus:

```

      - - - M K A K L L V L L C A F A A T N
      -----ATGAAGGCAAACACTACTGGCTCTGTTATGTGCATTGAGCTACAGAT 60
      I V Y T T A
      K A T K I V Y T T A
      aaagcaacaaa-----t-g-a-t-c-a-a-----a-cg-a- 60
      E R
      -----g-----t-----a----- 60
      V T T Y
      -----a-----ca-----a----- 60
      L A D
      -----c-----L S D 60
      I L S D
      I L S D
      -----a-----c----- 60

```

Alignment viewer

Consensus:

```

      A D T I C I G Y H A N N S T D T V D T V
      GCAGACACAATATGTATAAGGCTTACCATGCGAACATTCAACCGACACTGTTGACACAGTA 120
      L
      L
      -----t-----t-----a-----a----- 120
      -----t-----t-----a-----a----- 120
      -----t-----c----- 120
      -----c-----c----- 120
      -----c-----c----- 120
      -----c-----c----- 120
      -----a----- 120
      -----a----- 120
      -----c----- 120

```

Consensus:

```

      L E K N V T V T H S V N L L E D S H N G
      CTCGAAAAGAAATGTGACAGTGACACACTCTGTTAACCTGCTCGAAGACAGCCACACGGG 180
      K
      -----a-----a-----t-----a-----ag-----t----- 180
      -----a-----a-----t-----a-----ag-----t----- 180
      -----c----- 180
      -----c-----t-----g-----t-----t-----a 180
      -----g----- 180
      -----g-----a-----a-----t--- 180
      -----a-----a----- 180

```

Consensus:

```

      K L C K L K G I A P L Q L G K C N I A G
      AACTATGCAGATTAAAGGAATAGCCCCACTACAATTGGGAAATGTAACATGCCGGA 240
      R V H
      -----a-----g-----gg-----t-----t-----t-----t-----c 240
      -----a-----g-----gg-----t-----t-----t-----t-----c 240
      -----t-----a-----t-----t-----c----- 240
      -----L-----N-----S-----V 240
      -----t-----tct-----t-----t-----c-----g-----g----- 240
      -----R----- 240
      -----R-----K-----N----- 240
      -----R----- 240
      -----t----- 240

```

Consensus:

```

      W I L G N P E C E S L L T A S S W S Y I
      TGGATCTTGGGAAACCCAGAAATGCGAATCACTGCTCTCAGCGAGATCATGGTCTACATT 300
      M S
      -----c-----t-----g-----t-----atg-----ctc-----a-----a-----c----- 300
      -----c-----t-----g-----t-----ctc-----a-----a-----t----- 300
      -----c-----g-----t-----t-----a-----c----- 300
      -----L-----D-----L-----I-----S-----R-----E 300
      -----a-----t-----a-----c-----g-----ga----- 300
      -----L-----D-----P-----P-----V-----R----- 300
      -----V-----cc-----tc-----t-----S-----N-----R----- 300
      ----- 300

```

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

Alignment Tab (HTML)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

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

Multiple Sequence Alignment Alignment viewer

Options: AA NT H1 H3 Alignment display options

Display Mode: Original Template: Alignment display Mode

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

Position information of current residue

Position AA:
H1 numbering
H3 numbering
Position NT:
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/California/04/2009 (H1)-
A/California/04/2009 (H1)-
A/California/04/2009 (H1)-
A/California/04/2009 (H1)-
A/Denver/I/1957 (H1)
A/Denver/I/1957 (H1)

A/Hong Kong/1/1968 (H3)

Gibson Clones

Sequence DB Tab

Current Sequence Database															Double click on the field to edit records when Edit lock is unlocked		Click to enable/disable Edit on the table	
	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/22015B/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 / ...	AGCAAAAG...	1384	N3	Full NA	1	5000	False	Unassigned	none	none	0						
9	A/turkey/Indiana/16-001403-1/2016 (H7)	TACAAAATG...	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)	TTATTTCGTC...	1780	H2	Full HA	1	5000	False	Unassigned	none	none	0						
13	A/swine/MN/0211/2008 (H1)	ATGAAAGTGA...	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)	AGCAAAAG...	1763	H15	Full HA	1	5000	False	Unassigned	none	none	0						
20	A/rhea/North Carolina/39482/1993 (H7)	AGCAAAAG...	1731	H7	Full HA	1	5000	False	Unassigned	none	none	0						
21	A/pintail/Shimane/324/98[EPI_ISL_498]A / H1N9	AGAGATGAAT...	1425	N9	Full NA	1	5000	False	Unassigned	none	none	0						
22	A/mallard/Sweden/81/2002 (H6)	AGCAAAAG...	1742	H6	Full HA	1	5000	False	Unassigned	none	none	0						
23	A/mallard/Netherlands/5/1999 (H2)	AGCAAAAG...	1771	H2	Full HA	1	5000	False	Unassigned	none	none	0						
24	A/mallard/Netherlands/12/2000 (H7)	AGCAAAAG...	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/10BMO1929/2010 (H10)	GGTCACAAAT...	1703	H10	Full HA	8	5000	False	Unassigned	none	none	0						
27	A/mallard/Interior Alaska/263/1982 (H14)	AGCAAAAG...	1749	H14	Full HA	1	5000	False	Unassigned	none	none	0						

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

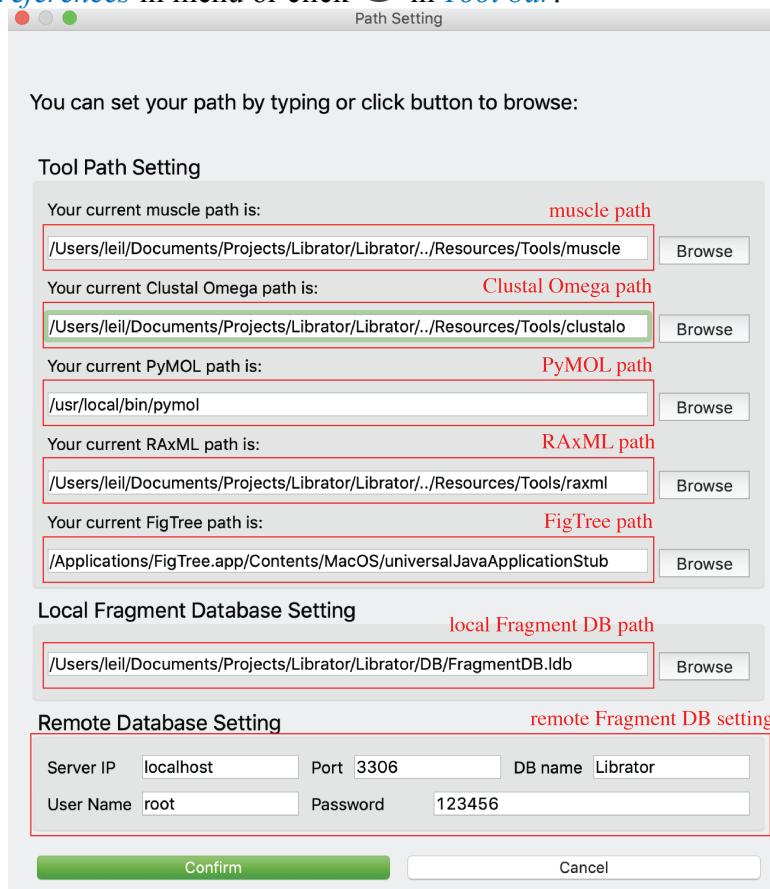
Functions:

Basic functions:

Path setting

Users can set paths for all required tools and databases.

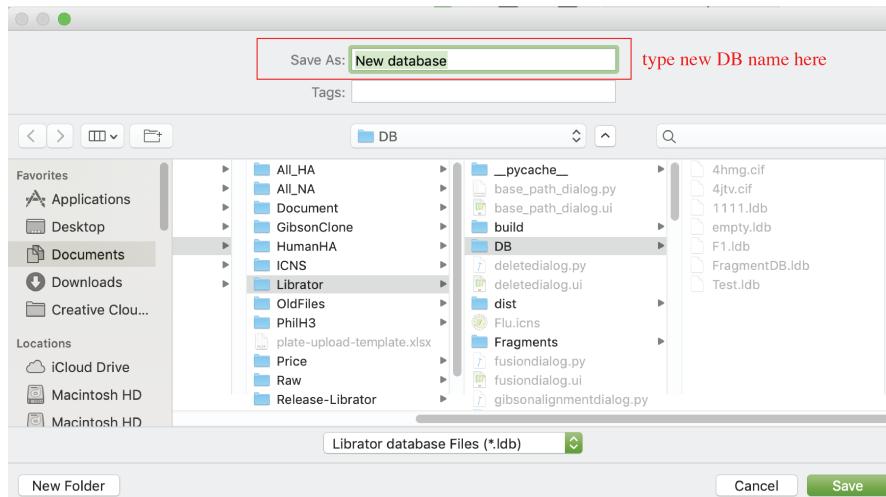
Click *Setting-> Preferences* in menu or click  in *Tool bar*.



Create new sequence database

Users can create new sequence database.

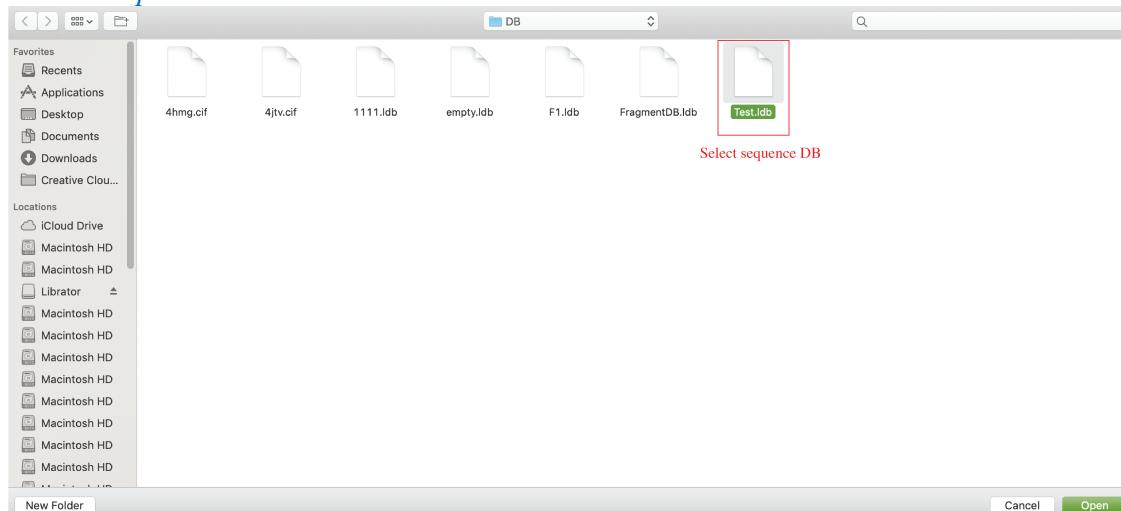
Click *File-> New* in menu or click  in *Tool bar*.



Open existing sequence database

Users can create new sequence database.

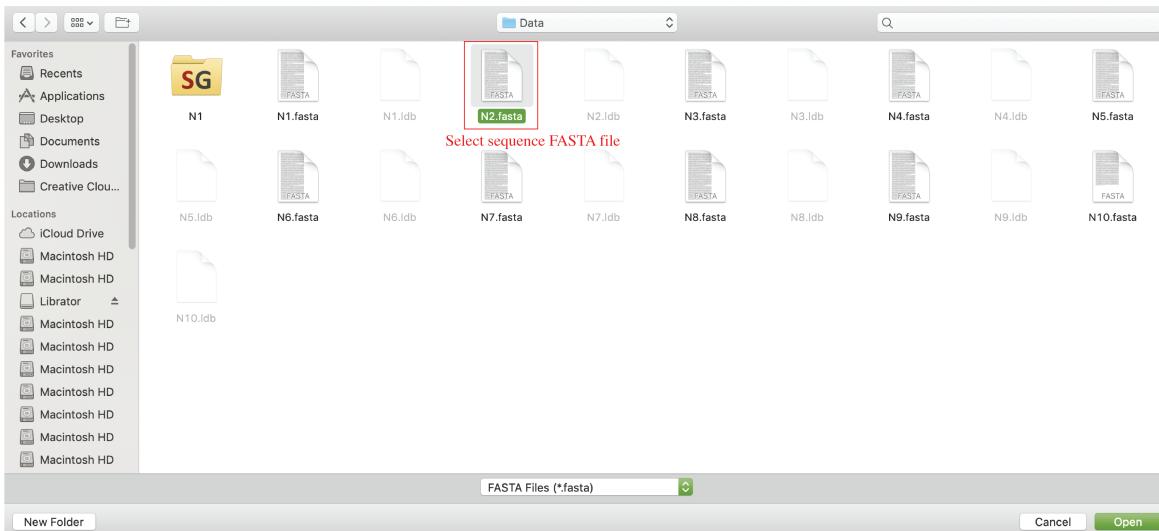
Click *File-> Open* in menu or click in *Tool bar*.



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.

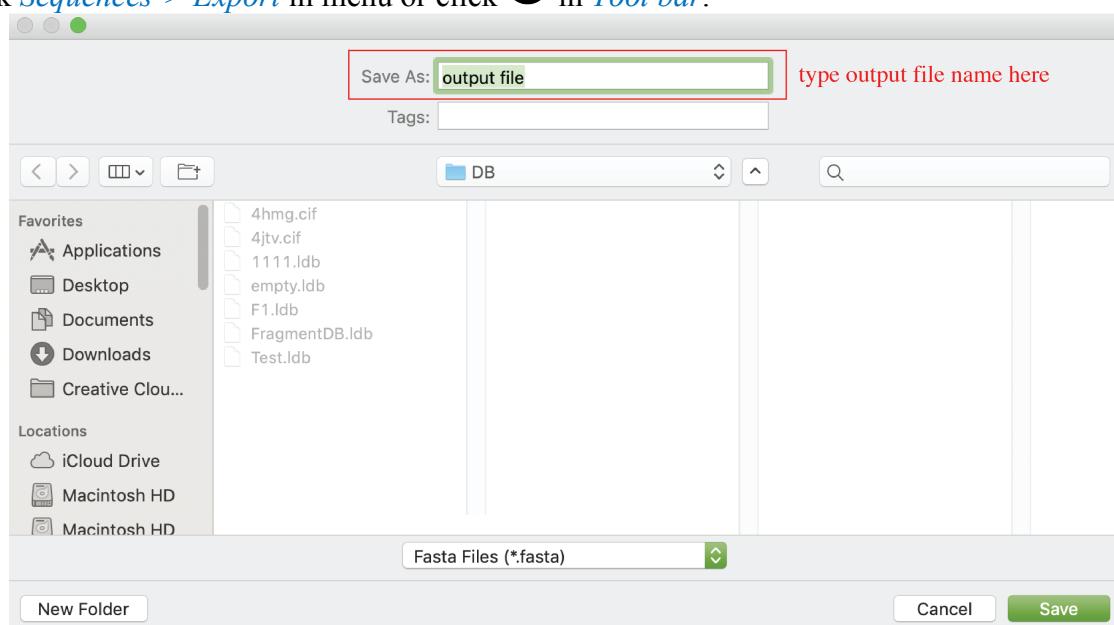


Export sequences

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*.

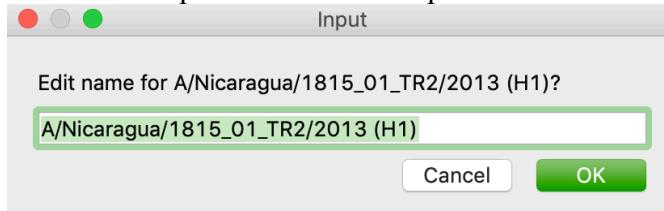


Advanced functions:

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.

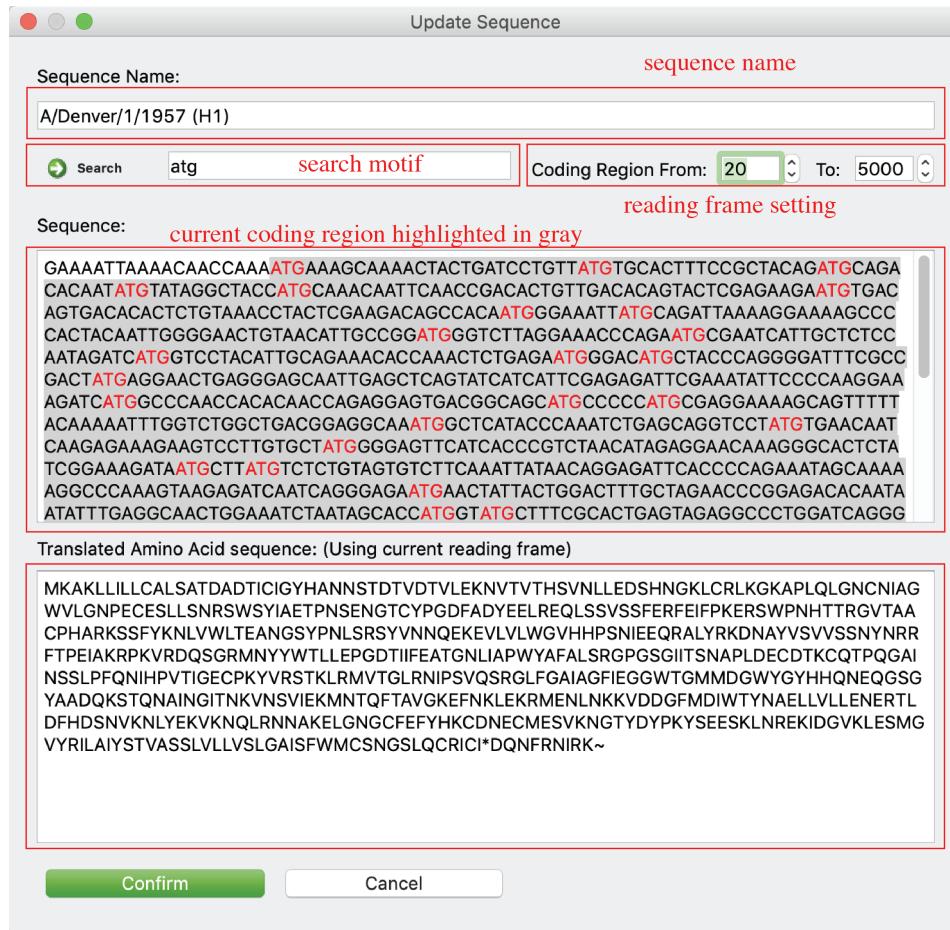


For **Role, Form, and Subtype**, users can edit by the left panel. Users can select multiple sequences in active sequence panel to do bulk update.

The screenshot shows the application's main window. On the left, a tree view lists 'Available sequences' under categories like 'All Sequences', 'Base Sequence', 'B', and 'H1'. Several sequences are selected, indicated by green checkmarks. On the right, a list of 'Active sequences' is shown, also with green checkmarks. A red box highlights the 'Edit here' section at the bottom left, which contains dropdown menus for 'Role' (Unassigned), 'Form' (Full HA), and 'Subtype' (H1). Other sections include 'Donor regions' (none) and 'Mutations' (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.



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.

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 and 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	KATKMKAIIVVLLYTFATANADTLCIGYHANNSTDVTDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIETVETSSSDNGTCYI
A/California/04/2009_H1	---MKAIIVVLLYTFATANADTLCIGYHANNSTDVTDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIETVETPSSDNGTCYI
A/California/04/2009_H1_S75M_HA1	---MKAIIVVLLYTFATANADTLCIGYHANNSTDVTDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAAGWILGNPECEMLSTASSWSYIETVETPSSDNGTCYI
A/South_Carolina/1/1918_H1	---MEARLLVLLCAFAATNAADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLEDKHNGKLCKLKGIAPLQLGKCNIAAGWILGNPEC DLLTASSWSYIETVETNSSENGTCYI
A/Brisbane/59/2007_H1	---VKLLVLLCCTFTATYADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLEN SHNGKLCLLKGIAPLQLGNCSVAGWILGNPECELLISKESWSYIIVEKPNPENGTCYI
A/Solomon_Islands/3/2006_H1	---MKVKLLVLLCCTFTATYADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLED SHNGKLCLLKGIAPLQLGNCSVAGWILGNPECELLISRESWSYIIVEKPNPENGTCYI
A/PR/8/1934_H1	-----CALAADADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLED SHNGKLCLLKGIAPLQLGKCNIAAGWILGNPEC DLLPVRSWSYIETVTPNSENGICYI
A/Denver/1/1957_H1	-----MKAKL LLLCALSATDADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLED SHNGKLCLLKKGAPLQLGNCNIAAGWVLGNPECESLLSNRSWSYIAETPNSENGTCYI
A/Fort_Monmouth/1/1947_H1	-----MKAKL LLLCALSATDADTICIGYHANNSTDVTDTVLEKNVTVTHSVNLLED SHNGKLCLLKGIAPLQLGKCNIAAGWILGNPECESLLSKRSWSYIAETPNSENGTCYI

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).

The screenshot shows two side-by-side PyMOL windows. Both windows have the title "Generate mutated sequence".

Left Window (A):

- Text: "Please type your mutations below: e.g. R98Y, K141E"
- Text: "Current Sequence: A/California/04/2009 (H1)"
- Buttons: "Original Position", "H1/H3 Numbering", "Count from first Amin Acid". The "Count from first Amin Acid" button is highlighted.
- Text input: "Mutations S86M,L87Q,S88N" (highlighted with a red box).
- Section: "Generate one sequence with all mutations"
 - New SEQ Name: A/California/04/2009 (H1) -S86M,L87Q,S88N
 - Radio buttons: "Single Sequence with all mutations" (selected), "Sequences with each single mutation".
- Buttons: "Generate Sequence", "Cancel".

Right Window (B):

- Text: "Please type your mutations below: e.g. R98Y, K141E"
- Text: "Current Sequence: A/California/04/2009 (H1)"
- Buttons: "Original Position", "H1/H3 Numbering", "H1/H3 numbering". The "H1/H3 numbering" button is highlighted.
- Text input: "HA1 mutations I85Q" and "HA2 mutations I10M" (both highlighted with red boxes).
- Section: "Generate multiple sequences each with a single mutation"
 - New SEQ Name: A/California/04/2009 (H1) -I85Q(HA1)I10M(HA2)
 - Radio buttons: "Single Sequence with all mutations", "Sequences with each single mutation" (selected).
- Buttons: "Generate Sequence", "Cancel".

Bottom Panel (C):

- 3D Protein Structure: A yellow ribbon model of a protein with two residues labeled "I98Q" and "I98Q" highlighted in magenta.
- Text: "mutation" pointing to the second residue.
- Table: A grid showing amino acid preferences at various positions. The columns are labeled "all", "4jtv 1/1", and sequence names. The rows are labeled with positions and names like "(ABS-Ca1)", "(ABS-Ca2)", etc. The last row is labeled "(haimutation)".
- Text: "PyMOL>_"
- Text: "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 Drig Clip MovZ SnglClk +/- Cent Menu DblClk Menu - PkAt Selecting Residues State 1/ 1" (PyMOL menu bar).

Compare sequences and generate screening mutations

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

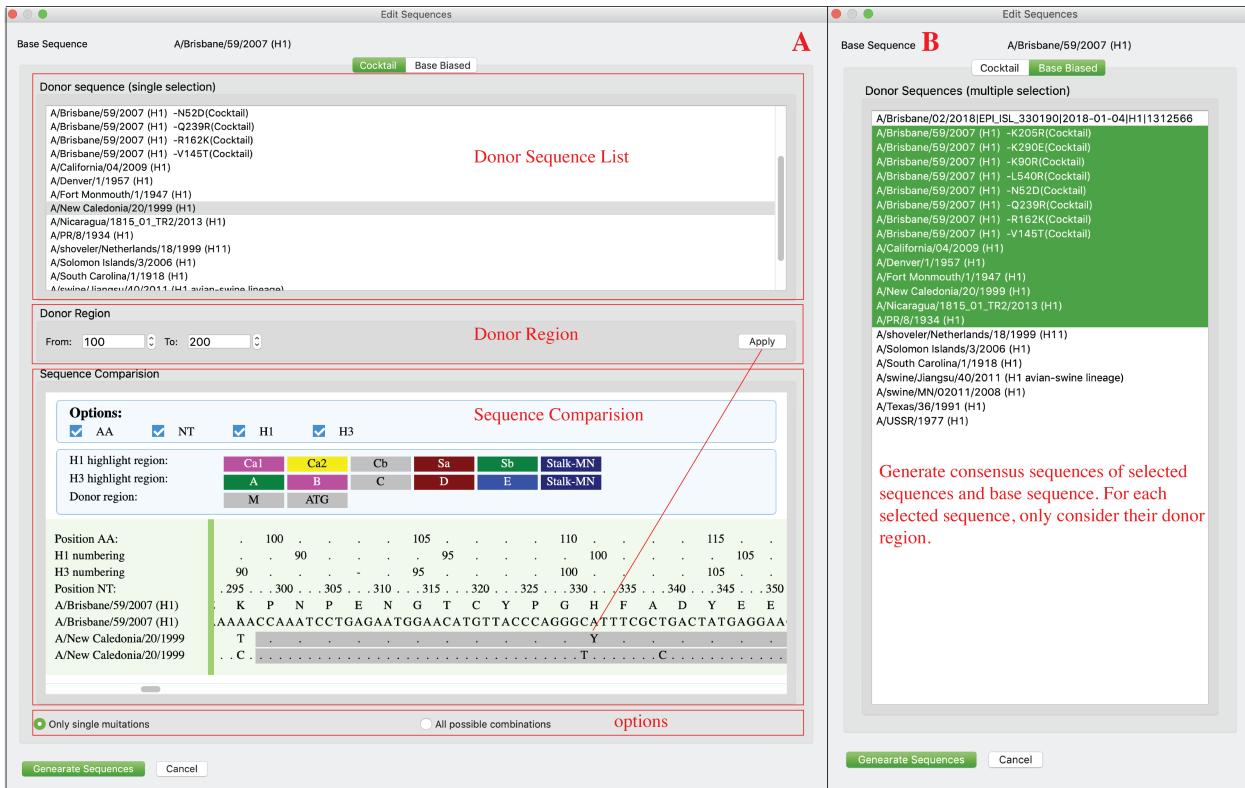
Click *Edit Sequence-> Editing* in menu or just click in *Tool bar*.

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

Select donor sequence:

- A/Brisbane/02/2018/EPI_ISL_330190/20
- A/Brisbane/59/2007 (H3)
- A/California/04/2009 (H1)
- A/California/04/2009 (H1) -10M(HA2)
- A/California/04/2009 (H1) -185Q(HA1)
- A/California/04/2009 (H1) -S75M(HA1)
- A/Denver/1/1957 (H1)
- A/Fort Monmouth/1947 (H1)
- A/Icaragua/1815_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-swine)
- A/swine/MN/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

```

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: -----1...5...10...15...21...25...30...35...40...45...
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
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: S S T G K T C H I N R H 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 I
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 S I V A S S G T L E F I T E G S Q A C K S P G G F S R L N L W L T K S G S T Y P V I
Position: 181...186...191...196...201...206...211...216...221...226...231...236...
H3-Numbering: 165...170...175...180...185...190...195...200...205...210...215...220...
H1-Numbering: 170...175...180...185...190...195...200...205...210...215...220...
Sequence: N V T M P N N D N D N F U K L Y I N G W 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 V
Position: 241...246...251...256...261...266...271...276...281...286...291...296...
H3-Numbering: 235...230...235...240...245...250...255...260...265...270...275...280...285...
H1-Numbering: 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

```

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: 1...5...10...15...21...25...30...35...40...45...50...
H1-Numbering: 10...15...20...25...30...35...40...45...50...55...
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 N V T T H S V N L E N S H N G K C L L R
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: G T A P I Q O G S V A G W I L G N E C C E 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
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: 0...115...120...125...130...135...140...145...150...155...160...
Sequence: S S V S F S P E R F I F K E S N F N P H I C Q A L Y H E T A N V Y V V S S S Y S R K F T F E I A R P K V R V D Q E G R
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: 175...180...185...190...195...200...205...210...215...220...
Sequence: S S V S F S P E R F I F K E S N F N P H I C Q A L Y H E T A N V Y V V S S S Y S R K F T F E I A R P K V R V D Q E G R
Position: 241...246...251...256...261...266...271...276...281...286...291...296...
H3-Numbering: 230...235...240...245...250...255...260...265...270...275...280...285...
H1-Numbering: 235...240...245...250...255...260...265...270...275...280...285...
Sequence: I N Y Y T W I L L E P G D T I I F E A R G A L I A P R Y A F A L S R G R F G S G J I N S N A P M D C D A 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	Ca2	Cb	Sa	Sb	Stalk-MN
A	B	C	D	E	Stalk-MN

Position AA:

H1 numbering

H3 numbering

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

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_330190/201
- A/Brisbane/59/2007 (H3)
- A/California/04/2009 (H1)
- A/Alabama/1999 (H1)
- A/Fort Monmouth/1947 (H1)
- A/New Caledonia/20/1999 (H1)
- A/Icaragua/1815_01_TR2/2013 (H1)
- A/PR/8/1934 (H1)
- A/Solomon Islands/3/2006 (H1)
- A/Alaska/04/1999 (H1)
- A/South Carolina/1/1918 (H1)
- A/swine/Jiangsu/40/2011 (H1 avian-swine)
- A/swine/MN/2011/2008 (H1)
- A/Texas/36/1991 (H1)
- A/USSR/1977 (H1)

Active sequence list

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

Base sequence

```

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: -----1...5...10...15...21...25...30...35...40...45...
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
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: S S T G K T C H I N R H 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 I
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 S I V A S S G T L E F I T E G S Q A C K S P G G F S R L N L W L T K S G S T Y P V I
Position: 181...186...191...196...201...206...211...216...221...226...231...236...
H3-Numbering: 165...170...175...180...185...190...195...200...205...210...215...220...
H1-Numbering: 170...175...180...185...190...195...200...205...210...215...220...
Sequence: N V T M P N N D N D N F U K L Y I N G W 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 V
Position: 241...246...251...256...261...266...271...276...281...286...291...296...
H3-Numbering: 235...230...235...240...245...250...255...260...265...270...275...280...285...
H1-Numbering: 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

```

Position: 1...6...11...16...21...26...31...36...41...46...51...56...
H3-Numbering: 1...5...10...15...21...25...30...35...40...45...
H1-Numbering: 10...15...20...25...30...35...40...45...50...55...
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 N V T T H S V N L E N S H N G K C L L R
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: G T A P I Q O G S V A G W I L G N E C C E 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
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: S S V S F S P E R F I F K E S N F N P H I C Q A L Y H E T A N V Y V V S S S Y S R K F T F E I A R P K V R V D Q E G R
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: 175...180...185...190...195...200...205...210...215...220...
Sequence: I N Y Y T W I L L E P G D T I I F E A R G A L I A P R Y A F A L S R G R F G S G J I N S N A P M D C D A C Q T P Q G A I
Position: 241...246...251...256...261...266...271...276...281...286...291...296...
H3-Numbering: 235...240...245...250...255...260...265...270...275...280...285...
H1-Numbering: 235...240...245...250...255...260...265...270...275...280...285...
Sequence: E L R Q I S S V S V S F S P E R F I F K P T S S W N H D S N K G V T A C P H A G A K S F Y K N D I W L V R K G N S Y P
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	Ca2	Cb	Sa	Sb	Stalk-MN
A	B	C	D	E	Stalk-MN

Position AA:

H1 numbering

H3 numbering

Original Seq:

M K T I I A L S Y I F C L V F 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

VVLLYTFATAN| ANNSTDITVDTV |

Double click to delete

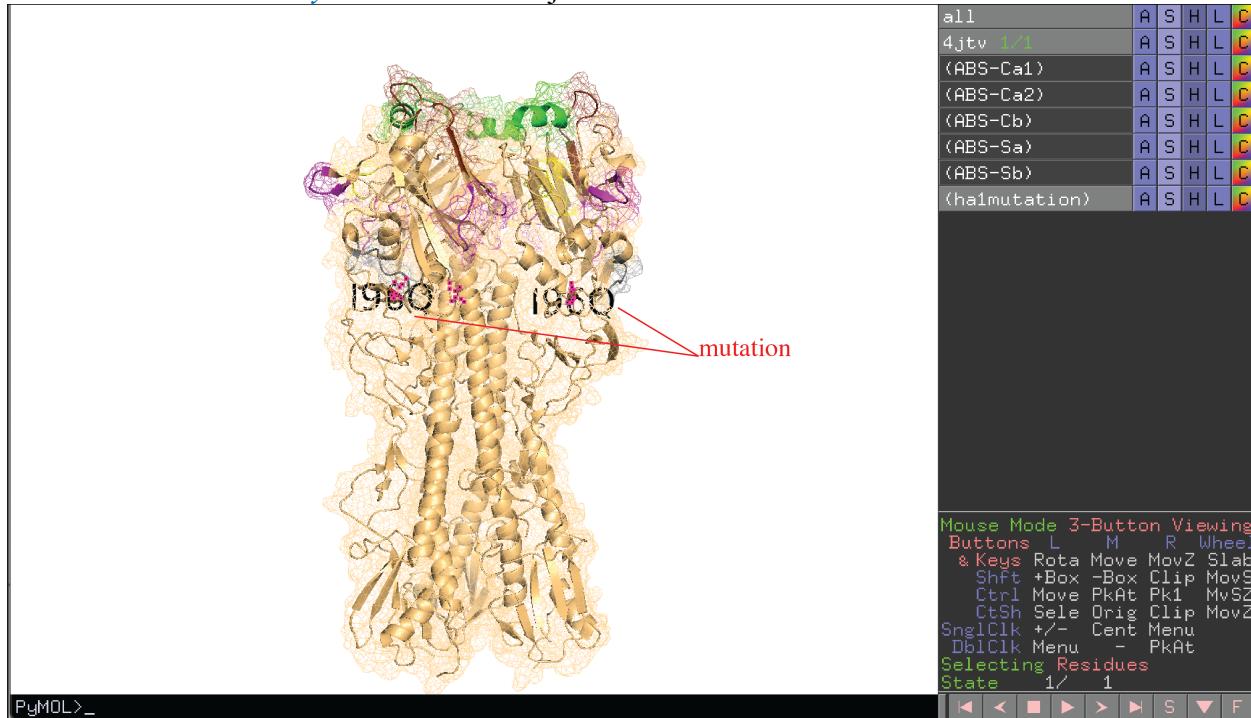
Insertion information

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): 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

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:

- 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)

Selected sequences: Amino Acid Nucleotide
 " " in sequences will be removed before generating Fragments

Alignment of Fragments

joint region shared by fragments will be highlighted

Confirm

Contact

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