

# User manual

## User interface

### 1) Main Tab (printable)

**Main Tab (printable)**

The Main Tab provides a comprehensive interface for managing and analyzing sequence data. Key features include:

- Menu Bar:** File, Sequences, Tools, View, Edit Sequence, Visualization, GibsonClone, Help, Setting.
- Tool Bar:** Includes icons for opening files, saving, zooming, and other common operations.
- Tab Switch:** Allows switching between Main, Sequence, Alignment(RTF), Alignment(HTML), SequenceDB, Summary, Summary(HTML), and FragmentDB tabs.
- Sequence Editing Functions:** Options for copying selected sequences into another DB or a new DB, generating sequences, and selecting report types.
- Recent Files:** Shows recently opened files, such as /Users/leil/Documents/Projects/Librator/Librator/DB/Test.lldb.
- Available Sequences:** Tree view showing All Sequences, grouped by Subtype, Role, or Form. Active sequences are highlighted in red.
- Active Sequences:** List of currently active sequences, including A/Brisbane/02/2018|EPI\_ISL\_330190|2018-01-04|H1|1312566 and others.
- Import/Export:** Buttons for Import Sequences, Import sequences, Delete Records, and Delete records.
- Donor Regions:** Settings for Role (Generated), Form (Full HA), and Subtype (H1).
- Mutations:** Information for the E290K mutation, including residue position and count from the first AA of the original sequence.
- DNA Sequence:** Position 0 of 1711 total nucleotides.
- Amino Acid Sequence:** Position 1 of 572 total residues.
- Search:** Motif search function.
- Annotations:** Detailed annotations for the sequence, including reading frames and sequence switches.
- Visualizations:** Links to 3D Visualization and Gibson Clones.

## 2) Sequence Tab (printable)

File Sequences Tools View Edit Sequence Visualization GibsonClone Help Setting

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

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

Click feature to show for sequence:  
H3 Numbering      H1 Numbering      Mutations      Donor Regions

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

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

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

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: LRGVAPLHLGKCNIAKGWLGNPECEMLSTASSWSYIVETPSSDNGTCYPGDFIDYEELRE

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: QLSSVSSFERFEIIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLVKKGNNSYPKLSK

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: SYINDKGKEVLWLWGIHHPSTSADQQSLYQNADTYVEFGSSRSKKPKPEIAIRPKVRDQ

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: EGRMNYYWTLVEPGDKITFEATGNLVPVRYAFAMERNAGSGIIISDTPVHDCTTCQTPK

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 LPFQNIHPITIGKCPVKSTKLRLATGLRNIPSIQSRGLFGAIAGFIEGGWTG

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: MVDGWYGHQQNEQGSQYAIDLKSTQNAIDEITNKVNNSVIEKMNTQFTAVGKEFNHLEKR

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: IENLNK KVDDGFLDIW TYNAEL LVLENERTLDYHDSNVKNLYEKVRSQLNNNAKEIGNG

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

Position: 40....545....550....555....560....  
H1-Numbering: -----  
Sequence: LVLVVS LG AISFW MCSNGSLQCRCI

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

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

### 3) Alignment RTF Tab (printable)

Main		Sequence	Alignment(RTF)	Alignment(HTML)	SequenceDB	Summary	Summary(HTML)	FragmentDB							
Muscle Alignment:		Set font size		Font Size (Ctrl+, Ctrl-):		13 C									
VGenes multiple alignment using Clustal Omega.															
Consensus:															
A/California/04/2009 (H1) -S75M(HA1)	-	M K A K L L V L L C A F A A T N	-----ATGAAGC AAA ACTCTGGTCTGTATGCAATTGCAGCTACAGAT	I V Y T T A				60							
A/Nicaragua/1815_01_TR2/2013 (H1)	K A T K	t-g-a-t-c-a-a-----a-cg-a-	t-g-a-t-c-a-a-----a-cg-a-	Y T T A				60							
A/South Carolina/1/1918 (H1)	aaagcaaaaaa-	t-g-a-t-c-a-a-----a-cg-a-	t-g-a-t-c-a-a-----a-cg-a-					60							
A/Solomon Islands/3/2006 (H1)	E R			T T Y				60							
A/PR/8/1934 (H1)	V			ca-a-----L A D				60							
A/Denver/1/1957 (H1)	a-t			I L S D				60							
A/Fort Monmouth/1/1947 (H1)				I L S D				60							
				c-t-----c-----t-----				60							
Consensus:															
A/California/04/2009 (H1) -S75M(HA1)	L	A D T I C I G Y H A N N S T D T V D T V	GCAGACACAATATGTATAAGGCTACCATGCGAACATTCAACCGACACTGTTGACACAGTA					120							
A/Nicaragua/1815_01_TR2/2013 (H1)	L	t-----t-----a-----a-----a-----a-----	t-----t-----a-----a-----a-----a-----					120							
A/South Carolina/1/1918 (H1)				t-----c-----				120							
A/Solomon Islands/3/2006 (H1)				c-----c-----				120							
A/PR/8/1934 (H1)								120							
A/Denver/1/1957 (H1)				a-----a-----				120							
A/Fort Monmouth/1/1947 (H1)				c-----c-----				120							
								120							
Consensus:															
A/California/04/2009 (H1) -S75M(HA1)		L E K N V T V T H S V N L L E D S H N G	CTCGAAAAGAATGTGACAGTGACACACTCTGTTAACCTGTCGAAGCACCCACAAACGGG					180							
A/Nicaragua/1815_01_TR2/2013 (H1)		--a-----a-----a-----t-----a-----ag-----t-----	--a-----a-----a-----t-----a-----ag-----t-----	K				180							
A/South Carolina/1/1918 (H1)				--a-----a-----a-----t-----a-----ag-----t-----	K			180							
A/Solomon Islands/3/2006 (H1)				c-----a-----a-----a-----a-----a-----a-----				180							
A/PR/8/1934 (H1)				t-----g-----c-----t-----g-----at-----t-----a				180							
A/Denver/1/1957 (H1)				g-----a-----a-----a-----a-----a-----a-----				180							
A/Fort Monmouth/1/1947 (H1)				g-----a-----a-----a-----a-----a-----a-----				180							
								180							
Consensus:															
A/California/04/2009 (H1) -S75M(HA1)	R V H	K L C K L K G I A P L Q L G K C N I A G	AAACTATGCAGATAAAAGGAATAGCCCCACTACAATTGGGGAAATGTAACATTGCCGG					240							
A/Nicaragua/1815_01_TR2/2013 (H1)	R V H	a-c-----g-----gg-----t-----g-----t-----t-----c-----	a-c-----g-----gg-----t-----g-----t-----t-----c-----					240							
A/South Carolina/1/1918 (H1)	L	t-----a-----t-----t-----c-----	t-----a-----t-----t-----c-----	N S V				240							
A/Solomon Islands/3/2006 (H1)	R	t-----tct-----t-----c-----g-----g-----	t-----tct-----t-----c-----g-----g-----					240							
A/PR/8/1934 (H1)	R K N	t-----t-----c-----	t-----t-----c-----					240							
A/Denver/1/1957 (H1)	R	t-----a-----a-----c-----	t-----a-----a-----c-----					240							
A/Fort Monmouth/1/1947 (H1)		t-----	t-----					240							
								240							
Consensus:															
A/California/04/2009 (H1) -S75M(HA1)	M S	W I L G N P E C E S L L T A S S W S Y I	TGGATCTTGGGAAACCCAGAACATGCGAATCACTGCTCTCAGCGAGATCATGGTCTACATT					300							
A/Nicaragua/1815_01_TR2/2013 (H1)	S	c-----t-----g-----t-----at-----ctc-----a-----a-----c-----	c-----t-----g-----t-----at-----ctc-----a-----a-----c-----					300							
A/South Carolina/1/1918 (H1)	L D L	c-----t-----g-----t-----at-----ctc-----a-----a-----t-----	c-----t-----g-----t-----at-----ctc-----a-----a-----t-----					300							
A/Solomon Islands/3/2006 (H1)	L D P	t-----t-----a-----t-----a-----c-----	t-----t-----a-----t-----a-----c-----	I S R E				300							
A/PR/8/1934 (H1)	V P V R	c-----t-----cc-----t-----tc-----	c-----t-----cc-----t-----tc-----	P V R				300							
	V S N R	- - - - -	- - - - -	S N R				300							

#### 4) Alignment Tab (HTML)

Multiple Sequence Alignment Alignment viewer

**Options:** AA NT HI H3 Alignment display options

**Display Mode:** Original: 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: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35  
H1 numbering: . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50  
H3 numbering: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50  
Position NT: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50 . . . . . 55 . . . . . 60 . . . . . 65 . . . . . 70 . . . . . 75 . . . . . 80 . . . . . 85 . . . . . 90 . . . . . 95 . . . . . 100 . . . . . 105  
A/Brisbane/59/2007 (H1) - . . V K L L V L L C T F T A T Y A H N N S T D T V D T  
A/Brisbane/59/2007 (H1) - . . G T A A A A C T A C T G G T C T G T T A T G C A C A T T A C A G C T A C A T A G C A G A C A A C A T A T G C T A C C A C T G C A C G C A C T G T T G A C A C A  
A/California/04/2009 (H1) - M K A I L V L V L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/California/04/2009 (H1) - M K A I L V V L L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/California/04/2009 (H1) - M K A I L V L V L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/Denver/1/1957 (H1) - M K A K L L G L T I L C A L S A T D A D T I C I G Y H A N N S T D T V D T  
A/Denver/1/1957 (H1) - A T G A A A G C A A A A C T A C T G A T C C T G T T A T G T G C A T T T C C G C T A C A G A T G C A G A C A A C A T A T G T A T A G G G T C C A T G C G A A C A A T T C A C C G A C A C T G T T G A C A C A  
  
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105  
H3 numbering: HAI 10

Position information of current residue: Position AA: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35  
H1 numbering: . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50  
H3 numbering: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50  
Position NT: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40 . . . . . 45 . . . . . 50 . . . . . 55 . . . . . 60 . . . . . 65 . . . . . 70 . . . . . 75 . . . . . 80 . . . . . 85 . . . . . 90 . . . . . 95 . . . . . 100 . . . . . 105  
A/Brisbane/59/2007 (H1) - . . V K L L V L L C T F T A T Y A H N N S T D T V D T  
A/Brisbane/59/2007 (H1) - . . G T A A A C T A C T G G T C T G T T A T G C A C A T T A C A G C T A C A T A G C A G A C A A C A T A T G C T A C C A C T G C A C G C A C T G T T G A C A C A  
A/California/04/2009 (H1) - M K A I L V L V L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/California/04/2009 (H1) - M K A I L V V L L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/California/04/2009 (H1) - M K A I L V L V L Y T F A T A T A N A A D T L C I G Y H A N N S T D T V D T  
A/California/04/2009 (H1) - A T G A A G G C A A T C A T G A T G T T C T G C T A T A C T A C T T G C A C C G C A A A T G C A G A C A C A T T A T G C T A T A G G G T T A T C G G C A A C A T T C A C A G A C A C T G T A G A C A C A  
A/Denver/1/1957 (H1) - M K A K L L G L T I L C A L S A T D A D T I C I G Y H A N N S T D T V D T  
A/Denver/1/1957 (H1) - A T G A A A G C A A A A C T A C T G A T C C T G T T A T G T G C A T T T C C G C T A C A G A T G C A G A C A A C A T A T G T A T A G G G T C C A T G C G A A C A A T T C A C C G A C A C T G T T G A C A C A  
  
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105  
Codon indicator

## 5) 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	ATGAAGGC...	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 ...	AGCAGAAG...	1784	H17	Full HA	1	5000	False	Unassigned	none	none	0	
7	A/turkey/Italy/22015/2002/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)	TTATTCGTC...	1780	H2	Full HA	1	5000	False	Unassigned	none	none	0	
13	A/swine/MN/0211/2008 (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)	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	AAGATGAAT...	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)	ATGGAAAAA...	1712	H12	Full HA	1	5000	False	Unassigned	none	none	0	
26	A/mallard/Interior Alaska/10BM01929/2010 (H10)	GGTCACAAT...	1703	H10	Full HA	8	5000	False	Unassigned	none	none	0	
27	A/mallard/Guinea/263/1982 (H14)	AGC^AAAAG	1749	H14	Full HA	1	5000	False	Unassigned	none	none	0	

## 6) Summary Tab



## 7) Summary Tab (HTML)



## 8) 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

Load 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 ...	TCRDNWQGPNRVIIQIDPVAMHTSQYICSP...	ACATGCAGAGACAATTGGCAGGGCCCAAATA...	Yes
2	N9-F3-0004	NA	3	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A /...	TCRDNWQGSNRPVIQIDPVAMHTSQYICSP...	ACATGCAGAGATAATTGCAAGGCTCAAATA...	No
3	N9-F3-0003	NA	3	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	TCRDNWQGSNRPVIQIDSVMTHTSQYICSP...	ACATGCAGAGATAATTGCAAGGCTCAAATA...	No
4	N9-F3-0002	NA	3	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	TCRDNWQGSNRPVIQINPTMMHTSQYICSP...	ACGTGTAGAGACAATTGGCAAGGCTCGAATA...	No
5	N9-F3-0001	NA	3	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	TCRDNWQGSNRPVIQIDPTMMHTSQYICSP...	ACGTGTAGGGACAATTGCAAGGCTCGAATA...	No
6	N9-F2-0006	NA	2	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / ...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
7	N9-F2-0005	NA	2	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 ...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
8	N9-F2-0004	NA	2	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A /...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGCCAAGGGACAACAA...	No
9	N9-F2-0003	NA	2	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGGTTCTATGCTCTCAGTCAAGGGACAACAA...	No
10	N9-F2-0002	NA	2	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGATTCTATGCTCTCAGCCAAGGAACAACAA...	No
11	N9-F2-0001	NA	2	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	RFYALSQTTIRGKHSNGTIHDRSQYRALISW...	AGATTCTATGCTCTCAGCCAAGGAACAACAA...	No
12	N9-F1-0007	NA	1	N9	0007	A/duck/Siberia/700/1996 EPI_ISL_618 A / ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
13	N9-F1-0006	NA	1	N9	0006	A/pintail/Shimane/324/98 EPI_ISL_498 A / ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
14	N9-F1-0005	NA	1	N9	0005	A/duck/Hokkaido/W245/2004 EPI_ISL_645 ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
15	N9-F1-0004	NA	1	N9	0004	A/swan/Shimane/190/2001 EPI_ISL_628 A /...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
16	N9-F1-0003	NA	1	N9	0003	A/swan/Shimane/227/01 EPI_ISL_595 A / ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
17	N9-F1-0002	NA	1	N9	0002	A/duck/Hong Kong/562/1979 EPI_ISL_617 ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No
18	N9-F1-0001	NA	1	N9	0001	A/duck/Hong Kong/278/1978 EPI_ISL_619 ...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	TCCACTCCCAGGTCCAAC TGCACCTCGGTT...	No

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

## Functions:

### Basic functions:

#### 1) Path setting

Users can set paths for all required tools and databases.

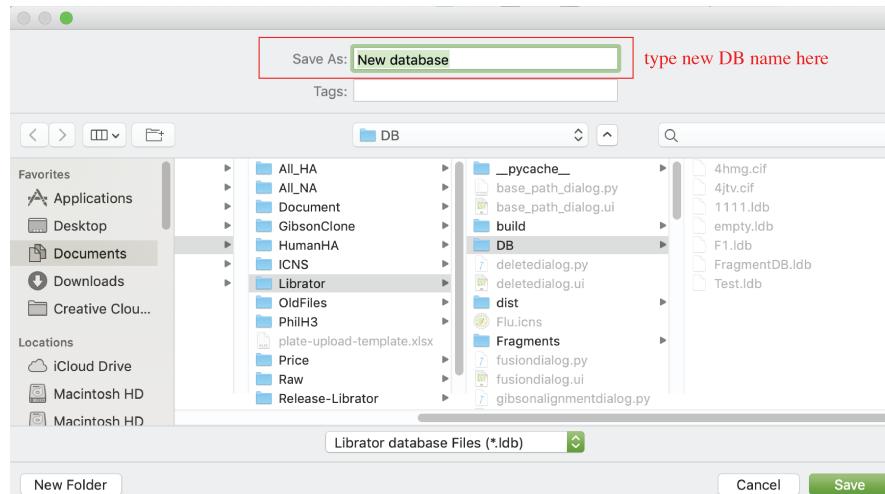
Click *Setting-> Preferences* in menu or click  in *Tool bar*.



#### 2) Create new sequence database

Users can create new sequence database.

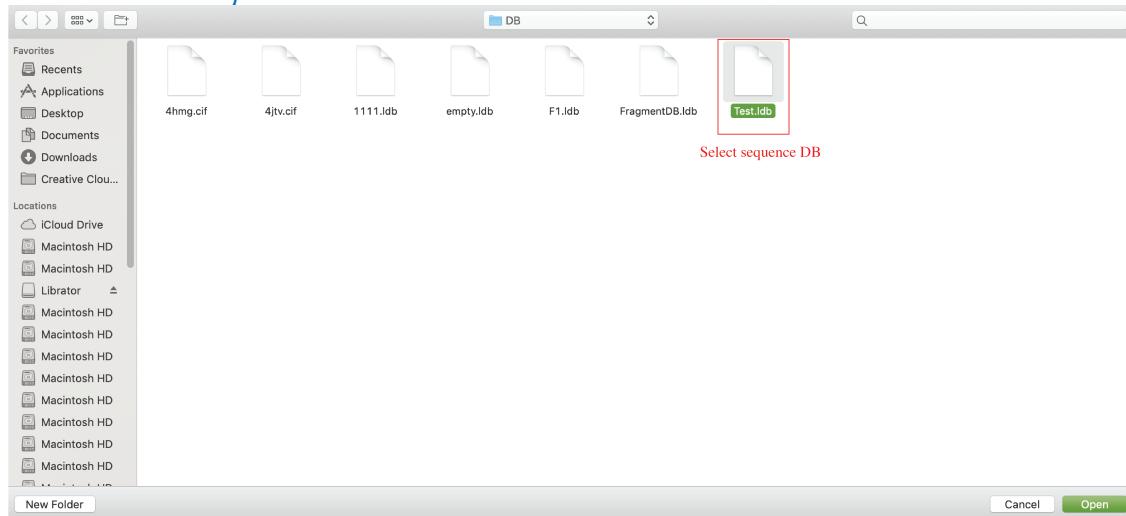
Click *File-> New* in menu or click  in *Tool bar*.



### 3) Open existing sequence database

Users can create new sequence database.

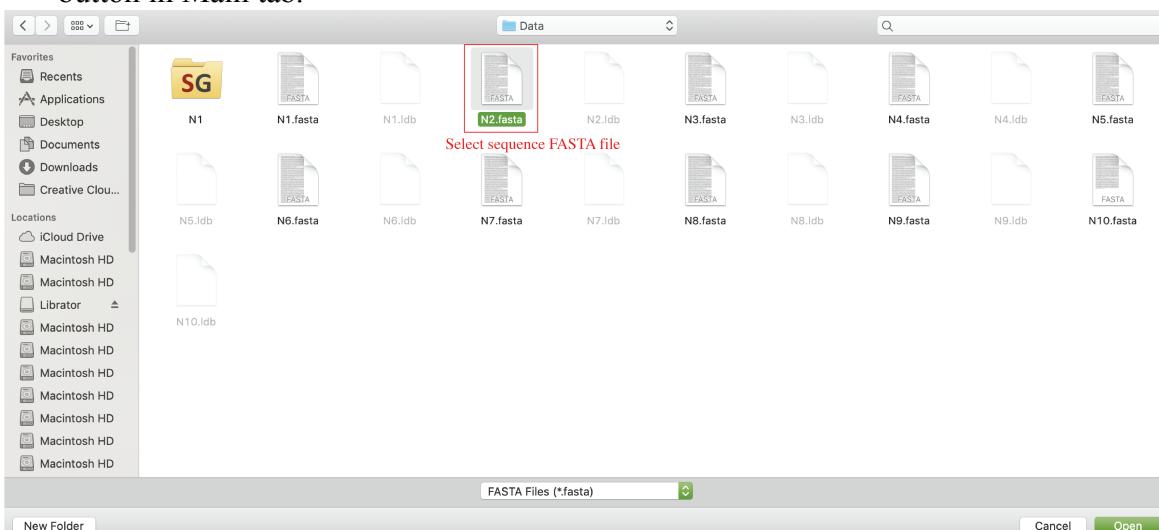
Click *File-> Open* in menu or click  in *Tool bar*.



### 4) Import sequences

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

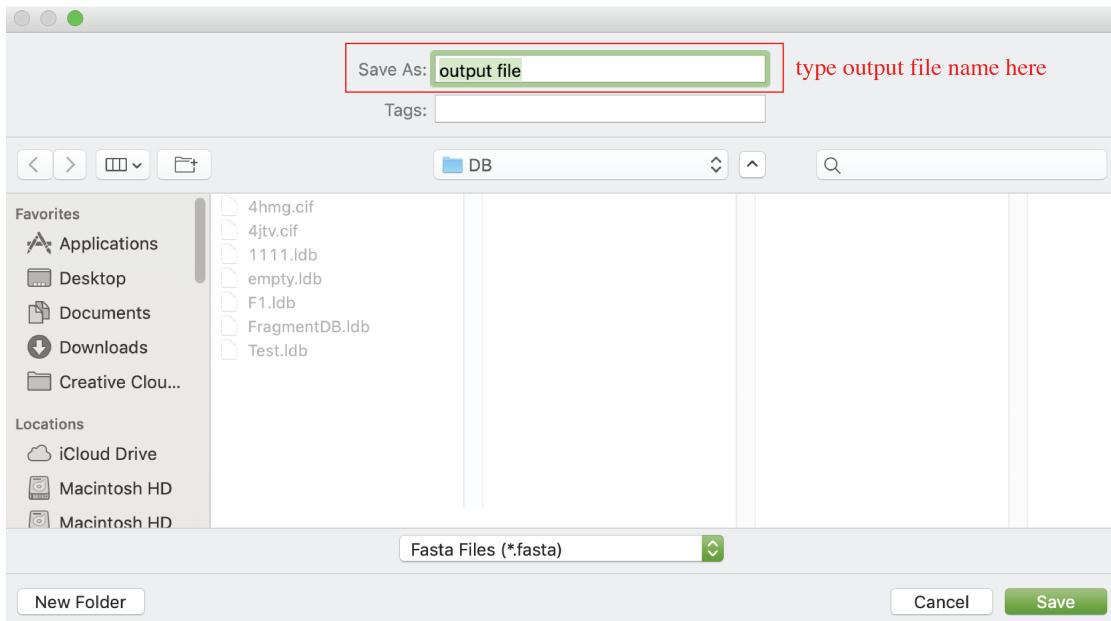
Click *Sequences-> Import* in menu or click  in *Tool bar* or click “import sequences” button in Main tab.



### 5) 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*.

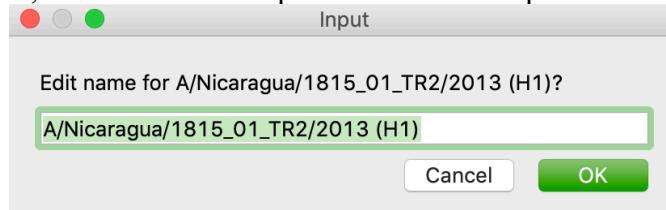


Advanced functions:

6) Edit information of sequences (on Main tab)

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

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

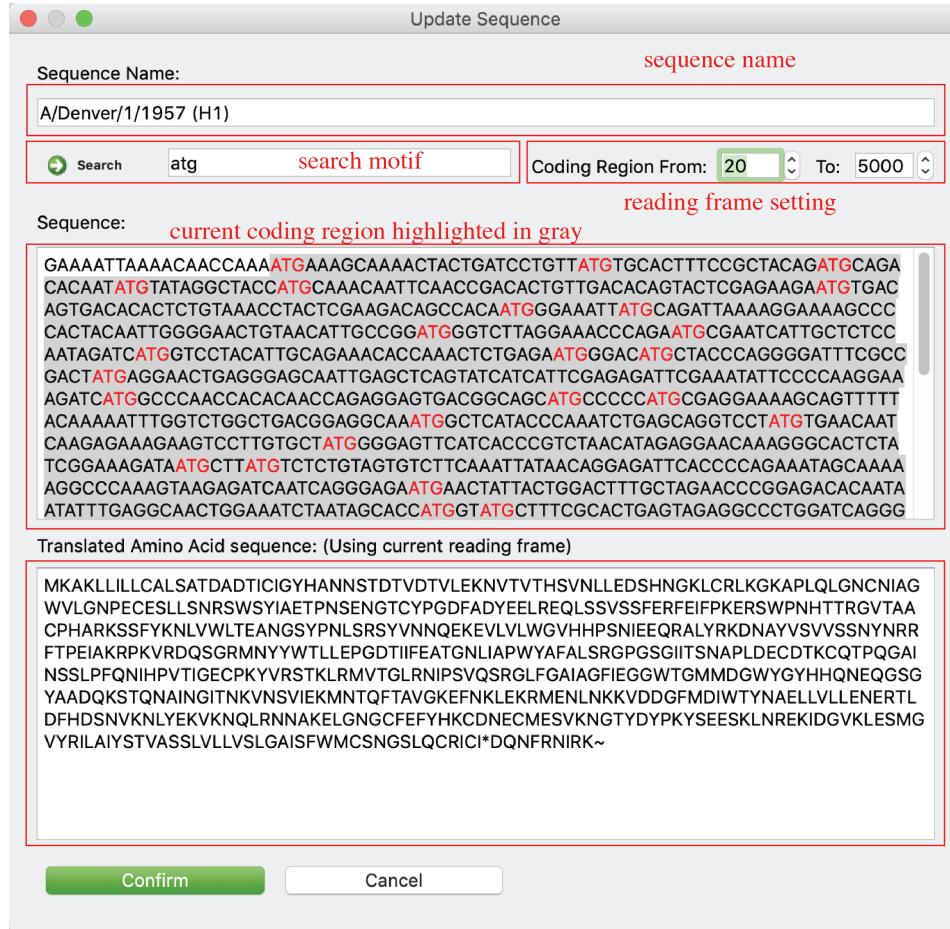


For **Role**, **Form**, and **Subtype**, users can edit by the left panel. Users can select multiple sequences in active sequence panel to do bulk update.

The screenshot shows the main user interface for managing sequences. 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's an 'Edit here' section with dropdown menus for 'Role' (Unassigned), 'Form' (Full HA), and 'Subtype' (H1). Buttons for 'Import Sequences' and 'Reset active sequence data' are also present.

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.

#### 7) 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.

#### 8) 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.

**Options:**  AA  NT  H1  H3

**Alignment display options** A

**Display Mode:**  Original:  Template: Alignment display Mode

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

Position AA: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 H1 numbering . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 H3 numbering . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 Position NT: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/02/2018/EPI\_ISL\_ . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/02/2018/EPI\_ISL\_ . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/59/2007 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/59/2007 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Denver/1/1957 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Denver/1/1957 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/For Monmouth/1/1947 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/For Monmouth/1/1947 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40

**Options:**  AA  NT  H1  H3

**Alignment display options** B

**Display Mode:**  Original:  Template: A/For Monmouth/1/1947 (H1) Alignment display Mode

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

Position AA: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 H1 numbering . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 H3 numbering . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 Position NT: . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/02/2018/EPI\_ISL\_ . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/02/2018/EPI\_ISL\_ . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/59/2007 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Brisbane/59/2007 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/California/04/2009 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Denver/1/1957 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/Denver/1/1957 (H1) . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/For Monmouth/1/1947 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40  
 A/For Monmouth/1/1947 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 . . . . . 30 . . . . . 35 . . . . . 40

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.

## 9) 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	KATKMKAIILVVLLYTFTANADATLICIGYHANNSTDVTLEKNVTVTHSVNLLLEDKHNGKLCKLRLGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIVETSSSDNGTCYI
A/California/04/2009_H1	---MKAIILVVLLYTFTANADATLICIGYHANNSTDVTLEKNVTVTHSVNLLLEDKHNGKLCKLRLGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIVETPSSDNGTCYI
A/California/04/2009_H1_S75M_HA1	---MKAIILVVLLYTFTANADATLICIGYHANNSTDVTLEKNVTVTHSVNLLLEDKHNGKLCKLRLGVAPLHLGKCNIAAGWILGNPECESLSTASSWSYIVETPSSDNGTCYI
A/South_Carolina/1/1918_H1	---MEARLILVLLCAFAATNAADTICIGYHANNSTDVTLEKNVTVTHSVNLLLEDKHNGKLCKLRLGVAPLHLGKCNIAAGWILGNPECEMLSTASSWSYIVETPSSDNGTCYI
A/Brisbane/59/2007_H1	---VKLLVLCLCTFTATYADTICIGYHANNSTDVTLEKNVTVTHSVNLLLENSHNGKLCLLKGIAPLQLGKCNIAAGWILGNPECDLLITASSWSYIVETNSSENGTCYI
A/Solomon_Islands/3/2006_H1	---MKVKLLVLCLCTFTATYADTICIGYHANNSTDVTLEKNVTVTHSVNLLLEDSHNGKLCLLKGIAPLQLGKCNIAAGWILGNPECELLISRESWSYIVEKPNPENGTCYI
A/PR/8/1934_H1	-----CALAAADADTICIGYHANNSTDVTLEKNVTVTHSVNLLLEDSHNGKLCLLKGIAPLQLGKCNIAAGWILGNPECDLLPVRSWSYIVETPNSENGICYI
A/Denver/1/1957_H1	-----MKAKLILLLCALSATDADTICIGYHANNSTDVTLEKNVTVTHSVNLLLEDSHNGKLCLLKGIAPLQLGKCNIAAGWVLGNPECESLLSNRSWSYIAETPNSENGTCYI
A/Fort_Monmouth/1/1947_H1	-----MKAKLILLLCALSATDADTICIGYHANNSTDVTLEKNVTVTHSVNLLLEDSHNGKLCLLKGIAPLQLGKCNIAAGWILGNPECESLLSKRSWSYIAETPNSENGTCYI

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

## 10) Mutate sequence

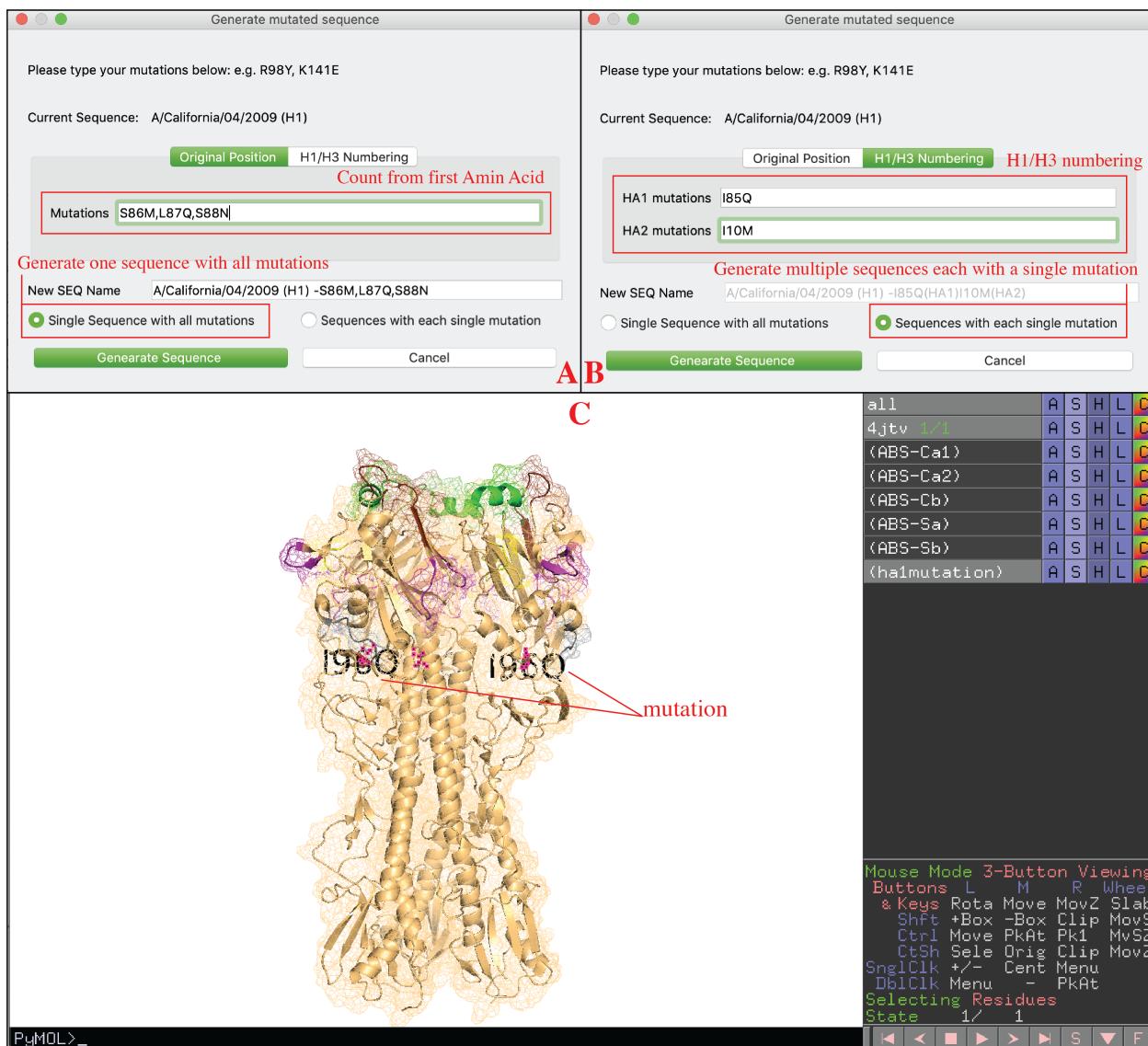
Users can generated new sequences with mutations based on any existing sequence.

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 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).



### 11) Compare sequences and generate screening mutations

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

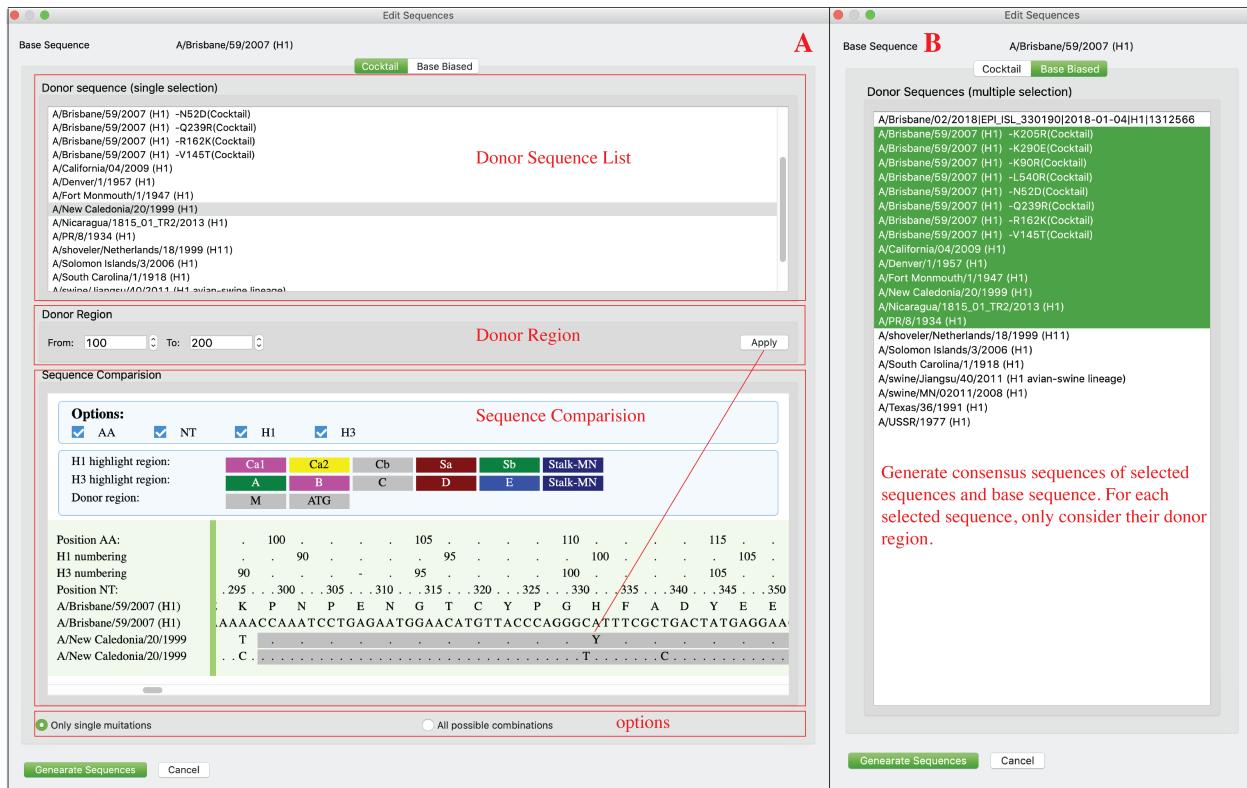
Click *Edit Sequence-> Editing* in menu or just click in *Tool bar*.

#### *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).



## 12) 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).

**Design**

**Select donor sequence:**

A/Brisbane/02/2018/EPI\_ISL\_330190/20  
A/Brisbane/59/2007 (H1)  
A/Corraine/04/2009 (H1)  
A/Corraine/04/2009 (H1)-10M(HA2)  
A/Corraine/04/2009 (H1)-18S(Q/HAI)  
A/Corraine/04/2009 (H1)-S75M(HAI)  
A/Denver/1957 (H1)  
A/Fort Monmouth/1947 (H1)  
A/Nicaragua/1815\_01\_1R2/2013 (H1)  
A/PR/8/1934 (H1)  
A/Solomon Islands/3/2006 (H1)  
A/South Carolina/1918 (H1)  
A/swine/Jiangsu/40/2011 (H1 avian-swine)  
A/swine/MN/02/2012/2008 (H1)  
A/Texas/36/1991 (H1)  
A/US/RR/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....20....25....30....35....40....45...
H1-Numbering: -----1.....5....10....15....20....25....30....35....40....45....
Sequence: MKTIIIALSYIFCLALQGDLPLGNDNSTATLCGLHHAVPNGLTVKLTDDQIEVNATRLVQ
```

```
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....4....5....10....15....20....25....30....35....40....45....50....
Sequence: SSSSTGTCNNPFLRLGIDCTLILALGPGFLPCDVFQNFTWLVRSKAFNSCPYVDPD
```

```
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....165...
Sequence: YASLRSLVIASGTLFITEETWVITQNGGSNACKRGPGFSRLLNLTKSGSTYPV
```

```
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: 110....115....120....125....130....135....140....145....150....155....160....165...
Sequence: NVTMPMNNDFKLQYIWWVHFPTNQEQTSLYVQASGRVTVSTERRSQQTTIFNIGSRWMV
```

```
Position: 241.....246....251....256....261....266....271....276....281....286....291....296...
H3-Numbering: 220.....230....235....240....245....250....255....260....265....270....275....280....
H1-Numbering: 170....175....180....185....190....195....200....205....210....215....220....225...
Sequence: GLSSRSLVIASGTLFITEETWVITQNGGSNACKRGPGFSRLLNLTKSGSTYPV
```

```
Position: 301....306....311....316....321....326....331....336....341....346....351....356...
H3-Numbering: 280....285....290....295....300....305....310....315....320....325....330....335...
H1-Numbering: 230....235....240....245....250....255....260....265....270....275....280....285...
Sequence: GLLSRSLVIASGTLFITEETWVITQNGGSNACKRGPGFSRLLNLTKSGSTYPV
```

**Current Base sequence:** A/Hong Kong/1/1968 (H3)

**Base sequence name:**

**Replaced region on base sequence:** [60]  [70] **Donor region on donor sequence:** [60]  [70]

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

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

**Legend:**

- H1 numbering
- H3 numbering

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

**Position AA:**

H1 numbering  
H3 numbering  
Original Seq:

```
MKTIIIALSYIFCLALQGDLPLGNDNSTATLCGLHHAVPNGLTVKLTDDQIEVNATRLVQ
```

**Double click to delete**

**Insertion information**

**Confirm** **Cancel**

**Design**

**Select donor sequence:**

A/Brisbane/02/2018/EPI\_ISL\_330190/2011  
A/Brisbane/59/2007 (H1)  
A/Corraine/04/2009 (H1)  
A/Denver/1957 (H1)  
A/Fort Monmouth/1947 (H1)  
A/New Caledonia/201999 (H1)  
A/Nicaragua/1815\_01\_1R2/2013 (H1)  
A/PR/8/1934 (H1)  
A/Switzerland/18/1999 (H1)  
A/Solomon Islands/3/2006 (H1)  
A/South Carolina/1918 (H1)  
A/swine/Jiangsu/40/2011 (H1 avian-swine)  
A/swine/MN/02/2012/2008 (H1)  
A/Texas/36/1991 (H1)  
A/US/RR/1977 (H1)

**Active sequence list**

**Base Sequence: A/Wisconsin/67/2005 (H3)**

**Base sequence**

```
Position: 1.....6....11....16....21....26....31....36....41....46....51....56...
H3-Numbering: -----1.....5....10....15....20....25....30....35....40....45....
H1-Numbering: -----1.....5....10....15....20....25....30....35....40....45....
Sequence: MKTIIIALSYIFCLALQGDLPLGNDNSTATLCGLHHAVPNGLTVKLTDDQIEVNATRLVQ
```

```
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....4....5....10....15....20....25....30....35....40....45....50....
Sequence: SSSSTGICDSFLQHLDGNCNTLIALGFLGCGQFNKWALDFVERSKAYSNCYYVPD
```

```
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....165...
Sequence: YASLRSLVIASGTLFITEETWVITQNGGSNACKRGPGFSRLLNLTKSGSTYPV
```

```
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: 110....115....120....125....130....135....140....145....150....155....160....165...
Sequence: NMYWTLEPDGVIIFEANGNLIAPRYAPALSRGFGSGCINSNAMDKDCAKQCQPGAI
```

```
Position: 241.....246....251....256....261....266....271....276....281....286....291....296...
H3-Numbering: 220.....230....235....240....245....250....255....260....265....270....275....280....
H1-Numbering: 170....175....180....185....190....195....200....205....210....215....220....225...
Sequence: INYWTLEPDGVIIFEANGNLIAPRYAPALSRGFGSGCINSNAMDKDCAKQCQPGAI
```

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

**Base sequence name:**

**Replaced region on base sequence:** [0]  [0] **Donor region on donor sequence:** [0]  [0]

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

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

**Legend:**

- H1 numbering
- H3 numbering

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

**Position AA:**

H1 numbering  
H3 numbering  
Original Seq:

```
MKTIIIALSYIFCLALQGDLPLGNDNSTATLCGLHHAVPNGLTVKLTDDQIEVNATRLVQ
```

**Double click to delete**

**Insertion information**

**Confirm** **Cancel**

**Design**

**Select donor sequence:**

A/Brisbane/02/2018/EPI\_ISL\_330190/2011  
A/Brisbane/59/2007 (H1)  
A/Corraine/04/2009 (H1)  
A/Denver/1957 (H1)  
A/Fort Monmouth/1947 (H1)  
A/New Caledonia/201999 (H1)  
A/Nicaragua/1815\_01\_1R2/2013 (H1)  
A/PR/8/1934 (H1)  
A/Switzerland/18/1999 (H1)  
A/Solomon Islands/3/2006 (H1)  
A/South Carolina/1918 (H1)  
A/swine/Jiangsu/40/2011 (H1 avian-swine)  
A/swine/MN/02/2012/2008 (H1)  
A/Texas/36/1991 (H1)  
A/US/RR/1977 (H1)

**Active sequence list**

**Base Sequence: A/Nicaragua/1815\_01\_1R2/2013 (H1)**

**Base sequence**

```
Position: 1.....6....11....16....21....26....31....36....41....46....51....56...
H3-Numbering: -----1.....5....10....15....20....25....30....35....40....45....
H1-Numbering: -----1.....5....10....15....20....25....30....35....40....45....
Sequence: KATKCKALWLVLTYFATANADPFLCIGHAQNTEVTVLKVNTVTHSVNLLDKEDKG
```

```
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....4....5....10....15....20....25....30....35....40....45....50....
Sequence: KATKCKALWLVLTYFATANADPFLCIGHAQNTEVTVLKVNTVTHSVNLLDKEDKG
```

```
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....165...
Sequence: YASLRSLVIASGTLFITEETWVITQNGGSNACKRGPGFSRLLNLTKSGSTYPV
```

```
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: 110....115....120....125....130....135....140....145....150....155....160....165...
Sequence: NMYWTLEPDGVIIFEANGNLIAPRYAPALSRGFGSGCINSNAMDKDCAKQCQPGAI
```

```
Position: 241.....246....251....256....261....266....271....276....281....286....291....296...
H3-Numbering: 220.....230....235....240....245....250....255....260....265....270....275....280....
H1-Numbering: 170....175....180....185....190....195....200....205....210....215....220....225...
Sequence: INYWTLEPDGVIIFEANGNLIAPRYAPALSRGFGSGCINSNAMDKDCAKQCQPGAI
```

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

**Base sequence name:**

**Replaced region on base sequence:** [0]  [0] **Donor region on donor sequence:** [0]  [0]

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

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

**Legend:**

- H1 numbering
- H3 numbering

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

**Position AA:**

H1 numbering  
H3 numbering  
Original Seq:

```
MKTIIIALSYIFCLALQGDLPLGNDNSTATLCGLHHAVPNGLTVKLTDDQIEVNATRLVQ
```

**Double click to delete**

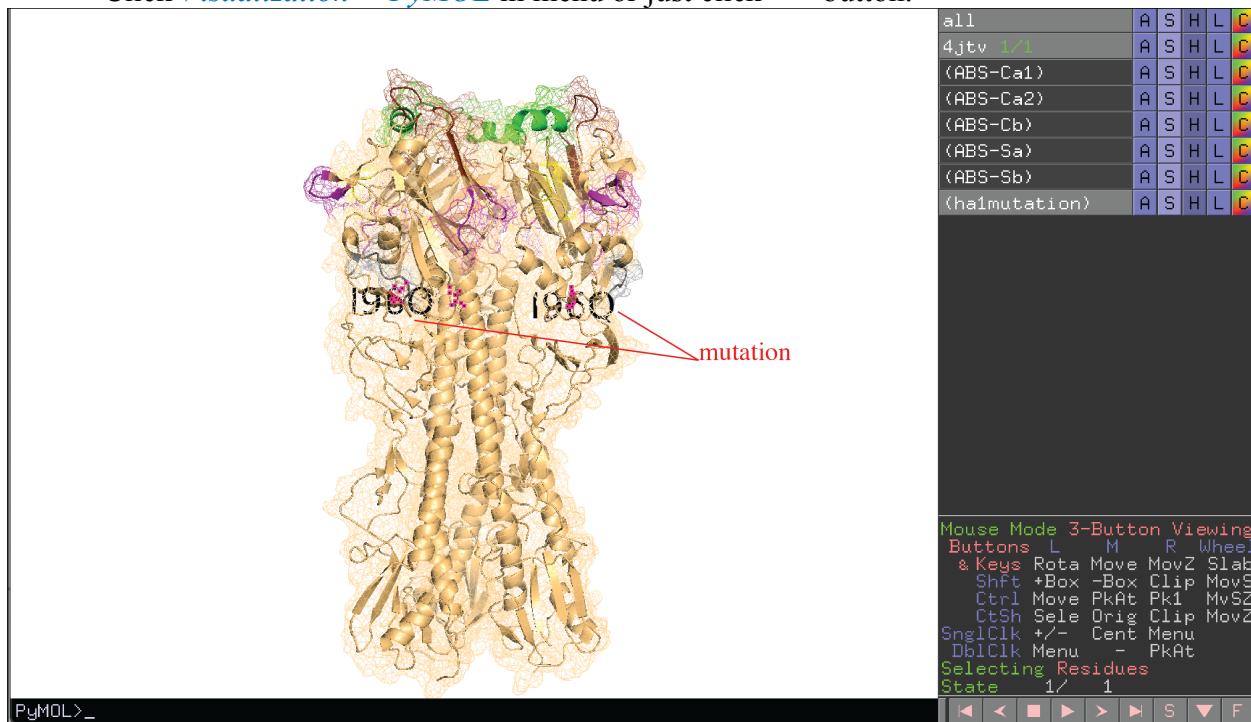
**Insertion information**

**Confirm** **Cancel**

### 13) 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.



#### 14) 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: MKTIIIAFSCILCLIAQAKLPGSDNSMATICLIGHHHAAPNGTLVKITDDQIEVTNATELVOSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFWVERSTAYSNCPYYFPDYA  
 Fragment 2: MKTIIIALSYIPLCALQDLPQNNDNSTATLIGHHHAAPNGTLVKITDDQIEVTNATELVOSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFWVERSKAFNSCNYPYDVPDYA  
 Fragment 3: MKTIIIALSYIPLSCPFCLAPCONSENNNTATLIGHHHAAPNGTLVKITDDQIEVTNATELVOSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFWVERSKAFNSCNYPYDVPDYA  
 Fragment 4: MKTIIIALSYIPLSYMLCVAQNLPGNDNSTATLIGHHHAAPNGTLVKITDDQIEVTNATELVOSSSTGRICNSPHQILDGKNTLIDALLGDPHCDLNQKEDWLFWVERSKAFNSCNYPYDVPDYA  
 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