bNAber Use Cases

1. Task 1: Compare summary (per clade) of neutralizing activity of selected bNAb in available (different) studies. **HOW TO:** On the front page select Ab you need, e.g., PG16 and click "Submit". On the next, "Search Result" page click on Ab name "PG16". Next page ("Details") has a section with data of "Percent viruses neutralized with an IC50 < 50 µg/ml" and "Median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml" collected from several available studies (see below).

2. Task 2: Compare summary (per clade) of neutralizing activity of several bNAbs in different studies. **HOW TO:** Open several browser windows. In every browser window select Ab of interest and repeat steps described in Task 1. As a result you will have several browser windows with "Percent viruses neutralized with an IC50 < 50 µg/ml" and "Median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml" collected from several available studies. See examples below for two Abs, VRC01 and PG16 (asterisk shows values computed from raw IC50 data).

Screenshot 1 — www.bnaber.org/?q=node/18&detail_mab=VRC01

Percent viruses neutralized with an IC50 < 50 µg/ml:

Study	A	B	C	D	F	G	AE	AG	All clades
Walker2011	93*	100*	89*	84*	100*	93*	100*	90*	93*
Chuang2013	100*	92*	88*	89*	NA	NA	94*	89*	90*
Huang2012	100*	92*	85*	89*	NA	NA	94*	81*	88*
Scheid2011	100*	100*	88*	100*	NA	86*	100*	75*	92*
Liao2013	100*	90*	85*	NA	NA	NA	NA	NA	91*
Georgiev2013	100*	94*	82*	50*	NA	NA	100*	100*	90*
Wu2010	100*	95*	87*	88*	NA	90*	93*	86*	91*

Median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml:

Study	A	B	C	D	F	G	AE	AG	All clades
Walker2011	0.25*	0.24*	0.49*	0.44*	0.39*	0.1*	0.57*	0.12*	0.31*
Chuang2013	0.12*	0.32*	0.24*	0.47*	NA	NA	0.3*	0.2*	0.23*
Huang2012	0.09*	0.36*	0.26*	0.5*	NA	NA	0.33*	0.16*	0.24*
Scheid2011	0.09*	0.22*	0.05*	0.08*	NA	0.17*	0.27*	0.56*	0.09*
Liao2013	0.09*	0.37*	0.26*	NA	NA	NA	NA	NA	0.23*
Georgiev2013	0.12*	0.24*	0.27*	0.11*	NA	NA	0.33*	0.07*	0.2*
Wu2010	0.11*	0.35*	0.34*	0.57*	NA	0.21*	0.38*	0.14*	0.33*

Screenshot 2 — www.bnaber.org/?q=node/18&detail_mab=PG16

Percent viruses neutralized with an IC50 < 50 µg/ml:

Study	A	B	C	D	F	G	AE	AG	All clades
Chuang2013	93*	55*	83*	22*	NA	NA	81*	100*	76*
Huang2012	90*	56*	76*	22*	NA	NA	81*	94*	73*
Walker2009	85*	74*	78*	60*	67*	73*	100*	60*	74*
Georgiev2013	100*	61*	86*	50*	NA	NA	67*	100*	79*

Median IC50 (µg/ml) against viruses neutralized with an IC50 < 50 µg/ml:

Study	A	B	C	D	F	G	AE	AG	All clades
Chuang2013	0.01*	0.14*	0*	2.44*	NA	NA	0.02*	0.1*	0.02*
Huang2012	0.01*	0.1*	0.02*	2.44*	NA	NA	0.02*	0.1*	0.02*
Walker2009	0.11*	0.7*	0.25*	0.02*	0.05*	1.21*	0.04*	0.03*	0.14*
Georgiev2013	0.01*	0.14*	0.01*	0.16*	NA	NA	0.02*	0.05*	0.02*

3. Task 3: Analyze raw data of neutralizing activity in selected study. **HOW TO:** Open browser window with bNAber front page. Go to "Neutralization Heatmaps and Tables" tab and select study of interest. Proceed with selection of neutralization presentation tool (select "Tables"). On the next page (Select Neutralization Table Parameters) select "--any --" and click submit. As a result you will have colored IC50 neutralization table (see example below). You can copy and paste this table into excel spreadsheet (select and paste ad a test) and continue sorting and analyzing the data further.

Neutralization Table By Study

Virus Strain	Clade	12A12	3BNC117	3BNC55	8ANC195	NIH45-46	VRC01
X2088_c9	G	>50	>50	>50	>50	>50	>50
Du422_1	C	>50	>50	>50	>50	>50	>50
CAP210_2_00_E8	C	>50	17.22	>50	>50	>50	>50
T250_4	AG	>30	>15	0.24	>50	>30	>50
T278_50	AG	>30	>15	>30	>50	>30	>50
620345_c01	CRF01_AE	>30	>15	>30	>50	>30	>50
ZM233M_PB6	C	39.19	0.13	0.64	7.39	1.86	2
3817_v2_c59	CD	23.74	0.15	>50	1	>50	>50
6535_3	B	21.97	0.55	2.6	0.2	0.14	0.54
211_9	CRF02_AG	7.41	0.35	>50	1.34	>50	14.3
6952_v1_c20	CD	4.82	0.15	0.13	1.38	0.02	0.04
THRO4156_18	B	3.05	1.76	>50	>50	1.59	2.3
T251_18	AG	2.62	0.26	>30	0.28	1.45	2.5
1054_07_TC4_1499	B (T/F)	2.55	0.07	4.05	1.02	0.07	0.71
231965_c01	D	2.22	0.05	0.51	0.51	0.02	0.34
SC05_8C11_2344	B (T/F)	2.03	0.15	1.93	0.47	0.14	0.64
HIV_16845_2_22	C	1.93	27.46	>50	3.3	2.05	2.8
QH0692_42	B	1.92	0.13	2.45	2.71	0.55	1.5
703357_c02	CRF01_AE	1.9	33.93	>50	6.55	13.91	4
CAP45_2_00_G3	C	1.83	3.88	>50	28.3	>50	2.3

4. Task 4: Compare raw data of neutralizing activity in two selected studies. **HOW TO:** Open two browser windows. In every browser window go to "Neutralization Heatmaps and Tables" tab perform Task #3. Compare side by side two tables of neutralizing activity in two selected studies (see figure below).

Neutralization Table By Study

Virus Strain	Clade	NIH45-46	NIH45-46 G54W	45-46m2	45-46m7	45-46m25	45-46m28
T278_50	AG	>50	>50	>50	>50	>50	>50
89_F1_2_25	CD	>50	>50	>50	>50	>50	>50
6540_v4_c1	AC	>50	>50	>50	>50	>50	47.3
Ce1172_H1	C (T/F)	>50	>50	>50	>50	>50	>50
620345_c01	CRF01_AE	>50	>50	>50	>50	>50	>50
X2088_c9	G	>50	>50	14.345	>50	>50	23.7
Du422_1	C	>50	>50	5.473	1.647	1.572	0.603
3817_v2_c59	CD	>50	>50	1.138	>50	>50	>50
CAP210_2_00_E8	C	>50	>50	1.945	14.4	24	11.2
CAP45_2_00_G3	C	>50	33.25	0.02	0.44	1.456	0.12
6545_v4_c1	AC	>50	18.92	15.21	0.441	1.636	0.092
211_9	CRF02_AG	>50	16.41	0.253	27.6	26.2	8.414
Du172_17	C	>50	3.65	0.015	0.979	1.548	0.546
3016_v5_c45	D	>50	1.47	0.006	0.023	0.017	0.009
T250_4	AG	>50	1.33	1.554	15.2	8.488	17.1
246F C1G		>50	0.315	0.036	0.067	0.054	0.033
CNE20	BC	7.83	0.04	0.001	0.007	0.01	0.002
CNE21	BC	0.01	0.03	0.001	0.013	0.016	0.009
HIV_16845_2_22	C		0.45	0.38	0.181	0.305	0.213
C2101_c01	CRF01_AE	4.24	0.04	0.044	0.038	0.051	0.041

Neutralization Table By Study

Virus Strain	Clade	12A12	3BNC117	3BNC55	8ANC195	NIH45-46	VRC01
X2088_c9	G	>50	>50	>50	>50	>50	>50
Du422_1	C	>50	>50	>50	>50	>50	>50
CAP210_2_00_E8	C	>50	17.22	>50	>50	>50	>50
T250_4	AG	>30	>15	0.24	>50	>30	>50
T278_50	AG	>30	>15	>30	>50	>30	>50
620345_c01	CRF01_AE	>30	>15	>30	>50	>30	>50
ZM233M_PB6	C	39.19	0.13	0.64	7.39	1.86	2
3817_v2_c59	CD	23.74	0.15	>50	1	>50	>50
6535_3	B	21.97	0.55	2.6	0.2	0.14	0.54
211_9	CRF02_AG	7.41	0.35	>50	1.34	>50	14.3
6952_v1_c20	CD	4.82	0.15	0.13	1.38	0.02	0.04
THRO4156_18	B	3.05	1.76	>50	>50	1.59	2.3
T251_18	AG	2.62	0.26	>30	0.28	1.45	2.5
1054_07_TC4_1499	B (T/F)	2.55	0.07	4.05	1.02	0.07	0.71
231965_c01	D	2.22	0.05	0.51	0.51	0.02	0.34
SC05_8C11_2344	B (T/F)	2.03	0.15	1.93	0.47	0.14	0.64
HIV_16845_2_22	C	1.93	27.46	>50	3.3	2.05	2.8
QH0692_42	B	1.92	0.13	2.45	2.71	0.55	1.5
703357_c02	CRF01_AE	1.9	33.93	>50	6.55	13.91	4
CAP45_2_00_G3	C	1.83	3.88	>50	28.3	>50	2.3

5. Task 5: bNAbs sequence alignment and phylogenetic tree building. **HOW TO:** These two functions are supported by Jalview (desktop application that uses web services for sequence alignment see http://www.jalview.org/). First, you select all antibodies - go to the bottom of the front page and select "All" next to gp160 picture. On the result page you refine your set for the antibodies you want to use in sequence alignment. Select action "Show sequences and download fasta file) and click Submit. On the next page click on "Download fasta light chain AA" and save result into a file. Start program jalview (either from your desktop or using java web start). In Jalview open saved file with sequences and make alignment using the following commands: Web Service -> Alignment -> T-coffee (other algorithms provided as well). Phylogenetic tree is created next with the commands: Calculate -> Calculate tree -> Neighbor joining Using % Identity (other algorithms provided as well). Figure below shows an average distance

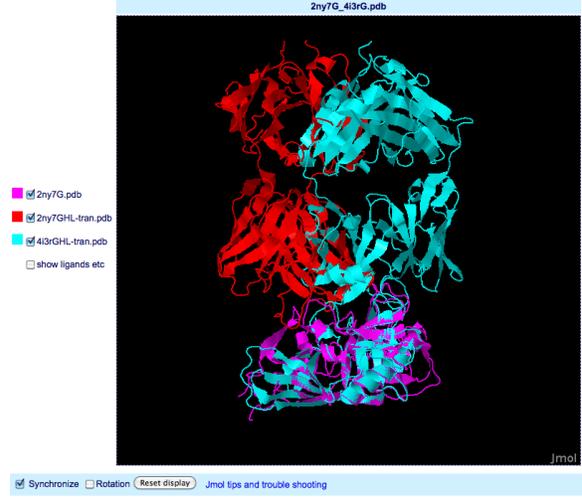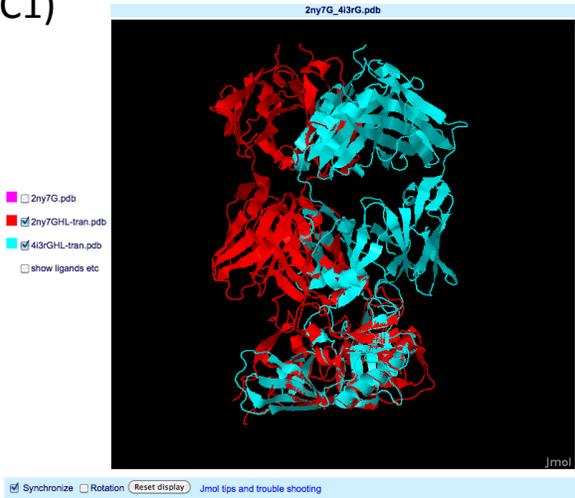tree and multiple sequence alignment of variable regions of heavy and light chains of selected bNAbs.
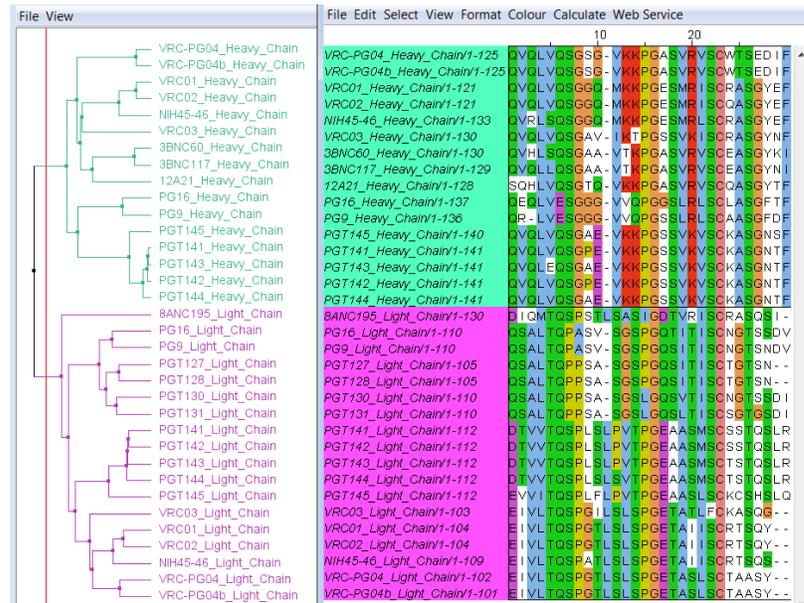
6. Task 6: Cluster Abs based on neutralization properties and compare with clustering by sequence similarity (phylogenetic tree). **HOW TO:** Go to "Neutralization heatmaps and tables" and create clustered neutralization heatmap for particular study. In a separate web browser repeat Task #5 and make phylogenetic tree for the same set of sequences.

7. Task 7: Analyze difference in binding mode of bNAbs that recognize the same epitope on gp160. **HOW TO:** On the front page select antibodies binding to CD4bs (click on the pointer near the picture). On the next page select two antibodies – b12 and VRC-PG04 and select action "Overlay structure (based on Ab or gp120)" - see Figure A below. Result page shows option for structure comparisons of any pair of PDB structures (two available structures of b12 and three available structures for VRC-PG04), Figure B below. For each pair of structures, structure comparisons based on antigen chain ("G" link), heavy chain ("H" link) and light chain ("L" link) are available. We select comparison of 4i3r (VRC-PG04) with 2ny7 (b12) using superposition of gp120 antigens by clicking on "G" link for this pair of structures (Figure B below). The structure comparison 3D viewer page (Figure C) shows that the antigen chain of 2ny7 (ENVELOPE GLYCOPROTEIN GP120) and antigen chain of 4i3r (OUTER DOMAIN OF HIV-1 GP120) aligned well with RMSD of 2.67Å and identity of 51.46% (Figure C1, created by clicking on "Show Alignment" on the top of the screen). The most of antibody-antigen contacts are between the heavy chain and the antigen. However the binding site between the heavy chain and antigen chain are not completely identical in two

complexes. The antigen chain, which structure was used as a base for comparison, is colored in magenta. It can be turned off by un-checking the check mark lebled "2ny7G.pdb" (Figure C2). Ligands and other small molecules can be turned on and off (Figure C3).

(A)

(B)

(C)

(C1)

(C2)

(C3)