Hammock: A Hidden Markov model-based peptide clustering to identify protein-interaction consensus motifs in large datasets

Supplementary Information

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S.1 KullbackLeibler divergence calculation

The KullbackLeibler divergence (KLD) is calculated in the way described in Andreatta *et al.* (2012), i.e. using Log-odds (LO) matrices adjusted for small samples.

LO matrix calculation

For each alignment column j, the value of LO matrix for amino acid A is calculated as

$$LO_{A,j} = \frac{n}{n+\sigma} \log \frac{p'_{A,j}}{q_A}$$

where $p'_{A,j}$ is the pseudo-count corrected frequency of amino acid A at position j, q_A is the background frequency of amino acid A, n is the number of sequences in the alignment and σ is a weight factor adjusting LO matrix values for very small clusters. As in Andreatta *et al.* (2012), σ is set to 10.

The background frequencies q_A are defined as follows (values stated are frequencies times 10^3):

A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
74	52	45	54	25	34	54	74	26	68	99	58	25	47	39	57	51	13	32	73

The pseudocount correction is performed as described in Nielsen *et al.* (2004) and Altschul (1997). No sequence weighting is performed. The effective amino-acid frequency $p'_{A,j}$ is calculated as

$$p'_{A,j} = \frac{n \cdot p_{A,j} + \beta \cdot g_{A,j}}{n+\beta}$$

where n is the number of sequences in the alignment, $p_{A,j}$ is the observed frequency of amino acid A in column j, β is the weight on pseudocounts (set to 200, according to Andreatta *et al.* (2012) and $g_{A,j}$ is the pseudocount frequency of amino acid A in column j calculated as

$$g_{A,j} = \sum_m p_{m,j} \cdot q_{A,m}$$

where $p_{m,j}$ is the observed frequency of each amino acid m and $q_{A,m}$ is the target frequency implicit in the (row-normalized) substitution matrix, in this case, Blosum 62 (Henikoff and Henikoff, 1992).

Scoring

The score $S(x, LO)$ of a peptide x aligned to a LO matrix is calculated as the sum of LO values for amino acids from x at match state positions:

$$S(x, LO) = \sum_j LO_{X,j}$$

where j is the index over match state positions and X, j is the amino acid from x at position j.

To evaluate the fitness of a peptide x in a cluster C (where x is part of the cluster's multiple alignment), S_x score is calculated, but peptide x is excluded from the LO matrix used. The fitness of a cluster C is then the average fitness of all its peptides:

$$KLD_C = \frac{\sum_l S(c_l, LO'_l)}{n}$$

where l is the index over peptides in the cluster, c_l is l-th peptide from cluster C, LO'_l is a LO matrix of the alignment without peptide c_l and n is the number of sequences in the cluster.

Finally, the KLD of a whole system of clusters K is calculated as the average fitness of all the peptides in the system.

$$KLD_K = \frac{\sum_k \sum_l S(c_{k,l}, LO'_{k,l})}{\sum_k n_k}$$

where k is the index over clusters in the system.

Scale factor for matrix used is $\frac{2}{\log 2}$, to be consistent with Andreatta *et al.* (2012).

S.2 The antibodies phage display experiment

Antibody preparation

We used mouse monoclonal antibody DO-1 recognizing human p53 protein (uniprot P04637), EEV1-2.1 recognizing human Hsp90 (uniprot P07900 and P08238) and CHIP3.1 recognizing human protein CHIP (uniprot Q9UNE7). The antibodies were dissolved in Phosphate Buffered Saline (PBS) at concentration 1 mg/ml before binding to solid matrices. 20 µl per sample of suspended Protein G Sepharose 4 FF (GE Healthcare) was used to immobilize the antibodies in the first round selection, 1.5 mg of Dynabeads protein A (Life technologies) was used to immobilize the antibodies in the second round and 1.5 mg of Dynabeads protein A (Life technologies) was used to immobilize the antibodies in third round of selection. The bead sediments were resuspended in 100 µl of antibody solution (1 mg/ml) and incubated for 30 min on a rotary shaker at room temperature. The excess unbound antibody was washed by 3 times wash in 1 ml of panning buffer containing bovine serum albumin 10 mg/ml, 50 mM TRIS pH 7.5 and 0.5 % Tween20. The beads were finaly resuspended in 100 µl of panning buffer.

Phage display

The Ph.D.™-12 Phage Display Peptide Library (New Englang Biolabs) was used in all presented experiments. We used 10 µl of original library containing 10^{11} pfu to select the interacting peptides in the first round. The library was panned in a buffer containing bovine serum albumin 10 mg/ml,

50 mM TRIS pH 7.5 and 0.5 % Tween20. The phage library was incubated for 1 hour with antibody-coated beads on a rotary shaker at 4 °C. Unbound phages were removed by 3 washes in the panning buffer and 1 last wash in PBS. Captured phages were eluted by suspending the bead pellets in 100 µl of an elution buffer containing 0.1 M Glycine pH 3.0. The supernatant containing eluted phages was separated into new tubes and was neutralized by the addition of 10 µl of 1 M Tris pH 8.5. This solution of pahges was subsequently used for tittering, amplification, and sequencing. The amplification and tittering of the library was performed according to the original protocol for Ph.D.™-12 Phage Display Peptide Library.

Amplification and sequencing of phage DNA

The sequencing library was prepared by the amplification of a 124 bp region of the phage DNA containing the variable 36 bp sequence. Herculase II fusion polymerase (Agilent) was used to amplify the sequence with forward primer `GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNcgcaattcctttagtggtacc` and reverse primer `ACACGACGCTCTTCCGATCTctgtatgggattttgctaac`, where small letters indicate phage-specific sequence and underlined part stays for the internal index. The PCR reaction of 50 µl contained 10 µl of crude eluted phage or 100 ng of DNA isolated from amplified phage, 300 nM primers, 500 mM Betain, 1x reaction buffer and 1 U of Herculase II enzyme. The PCR reaction was run under these conditions: 95 °C - 60 s; (95 °C - 15 s; 55 °C - 30 s)×30. The purified PCR amplicons were sequenced on the Illumina HiSeq platform at Otogenetics Corporation (Norcross, Georgia, US).

The resulting FASTQ files were filtered on quality and a custom Java application was used to extract the 36 bp regions of interest along with appropriate internal index sequences. The extracted regions were further filtered to satisfy the codon constraints specific for the Ph.D.™-12 Phage Display Peptide Library, translated and formatted into the FASTA format.

S.3 How to reproduce the results, a quick guide

Prerequisities

Java, version at least 1.7.0 is required.
Suppose Hammock was downloaded and extracted to `~/Hammock/`
On Linux-based 64 bit systems, compilation of external tools should not be necessary. On other systems, please download and compile appropriate versions of all tools (Clustal Omega v. 1.2.0, HH-suite v. 2.0.16, Hmmer v. 3.1b1) and move binaries to appropriate locations in Hammock folder (for Clustal Omega: file `Hammock/clustal-omega-1.2.0/clustal0-64bit`, for HH-suite, complete structure of tools' folder in `Hammock/hhsuite-2.0.16`, for Hmmer, files `Hammock/hmmer-3.1b1/src/hmmbuild` and `Hammock/hmmer-3.1b1/src/hmmsearch`). Alternatively, paths to files or commands for installed tools can be changed in `Hammock/settings/settings.prop` file.

To process the SH3 (MUSI) dataset

```
export HHLIB=~/Hammock/hhsuite-2.0.16/lib/hh/
java -jar ~/Hammock/dist/Hammock.jar full -i ~/Hammock/examples/musi.fa
```

This will create a folder `Hammock/dist/Hammock_result_1` containing the results. Output folder can be changed using parameter -o. By default, 4 threads are used. This number can be changed using parameter -t.

To process the antibodies dataset

```
export HHLIB=~/Hammock/hhsuite-2.0.16/lib/hh/
java -jar ~/Hammock/dist/Hammock.jar full -i ~/Hammock/examples/antibodies.fa
```

This will create a folder `Hammock/dist/Hammock_result_1` (or `Hammock_result_2`, if `Hammock_result_1` already exists) containing the results. Output folder can be changed using parameter -o. By default, 4 threads are used. This number can be changed using parameter -t. By default, label columns in resulting tables are sorted according to sum of sequences with each label. Default ordering of labels can be manually changed using parameter -l, for example:

```
export HHLIB=~/Hammock/hhsuite-2.0.16/lib/hh/
java -jar ~/Hammock/dist/Hammock.jar full -i ~/Hammock/examples/antibodies.fa -l
chip3.1_S1,chip3.1_A1,chip3.1_S2,chip3.1_A2,chip3.1_S3,hsp90_eev1_S1,hsp90_eev1_A1
,hsp90_eev1_S2,hsp90_eev1_A2,hsp90_eev1_S3,DO-1_S1,DO-1_A1,DO-1_S2,DO-1_A2,DO-1_S3
```

For more details, see the full manual at `http://www.recamo.cz/userfiles/file/Software/Hammock/Hammock-manual.pdf`

cluster_id	main_sequence	sum	chip3.1_S1	chip3.1_A1	chip3.1_S2	chip3.1_A2	chip3.1_S3	hsp90_eev1_S1	hsp90_eev1_A1	hsp90_eev1_S2	hsp90_eev1_A2	hsp90_eev1_S3	DO-1_S1	DO-1_A1	DO-1_S2	DO-1_A2	DO-1_S3
1	ALWPPNLHAWVP	61700	665	12707	1286	8775	957	1369	6205	1476	1551	790	1286	13733	3359	6495	1046
21	AHSANNFDVKGI	33640	368	1080	545	697	459	743	2340	6290	4802	11002	608	2258	804	1290	354
2	EAYSDAWKLPNT	33114	412	692	450	560	375	363	362	373	323	464	679	527	3625	10310	13599
4	NLDPITRQYTTP	30944	321	584	3613	7213	15299	326	490	352	315	358	370	434	525	456	288
10	GSFPSDLNRLMR	28015	236	507	456	557	574	280	369	269	271	268	488	462	11769	5977	5532
3	ESVQLPKWMEYE	12796	146	299	259	202	150	252	1341	4048	4669	229	255	199	316	253	178
6	HSHNHPISRGAY	11558	98	1114	802	2050	5690	168	212	120	104	142	204	323	228	192	111
5	HVAPSWWTARLF	10444	194	171	2025	2774	3745	127	135	117	99	124	320	172	204	142	95
9	MPLMSEPALEML	8243	111	1825	185	593	125	314	1564	269	209	158	228	1643	361	565	93
12	YSAHNYIGDSGP	7579	90	2141	184	810	120	184	1446	197	192	114	179	1009	295	521	97
13	TPMVERNYNAAD	6915	72	1718	160	634	106	158	1325	218	191	106	144	1154	305	546	78
17	LRASEWEWKTQR	5074	79	105	143	89	80	153	358	947	708	1414	145	298	254	203	98
40	SVLREYELHTLH	4511	56	64	71	57	56	96	135	1116	726	1787	86	73	78	65	45
14	NELPYRLWHWPR	4383	247	338	308	332	268	293	269	271	251	289	303	297	413	303	201
16	SSYNFGDEFHMK	3544	39	53	55	54	56	67	60	530	321	2053	43	49	67	52	45
19	TLSDLAQHMMSS	2940	26	121	50	84	46	64	155	72	67	325	73	53	1163	370	271
18	HWPGWLERGSPA	2802	128	187	187	194	196	128	327	137	133	152	185	264	248	227	109
22	RLDASSMISGRV	2604	162	408	96	126	37	120	394	67	67	29	108	564	148	250	28
122	IPIANPEFEMLV	2492	32	41	47	33	31	66	116	718	453	744	49	47	44	44	27
37	GSASPWAHEFET	2378	33	78	265	431	48	63	80	463	366	146	67	78	135	72	53
25	NEYHYHRSQLQV	2077	24	40	51	19	28	54	68	714	614	172	73	42	88	49	41
42	TGAPPRLDARPA	2061	434	34	171	188	183	136	35	26	22	21	196	25	309	186	95
53	APPLKEPTSSSI	2038	21	165	60	65	18	93	424	77	72	27	95	266	386	188	81
39	TDHNHNLREFEF	1942	127	37	44	34	21	44	46	242	155	859	181	42	53	33	24
29	YDHSATHLVSLP	1763	46	663	79	163	30	68	124	61	44	33	168	136	70	61	17
50	VDVDETNNQSYP	1501	18	404	55	124	18	66	278	63	22	19	55	212	76	71	20
34	ESHPTLLHWTQA	1470	29	357	116	181	94	55	110	41	29	36	73	164	87	75	23
57	TGTSWESGKVLK	1394	24	35	39	22	24	48	44	322	178	311	48	153	66	60	20
35	GQTVMMRTFGER	1387	11	307	38	154	19	45	468	71	60	28	43	43	43	41	16
33	DDHTFTDAPKLL	1350	15	26	26	18	16	14	12	15	13	14	40	17	529	285	310
38	SGVYKVAYDWQH	1281	190	28	123	30	26	95	22	31	22	45	500	50	68	29	22
98	NHYLSYPSNPGI	1199	28	60	64	32	19	72	207	124	90	148	68	120	84	70	13
45	FPWKSNWVEFEM	1063	28	15	39	18	13	25	35	193	70	475	83	17	24	17	11
48	HLTHSPIPVRAM	1016	26	244	41	88	13	19	81	27	15	16	62	190	87	79	28
62	SAINTATALHIR	996	13	417	47	133	17	30	41	110	59	28	41	16	16	15	13
93	VIGTPDSSTHGL	891	19	167	59	57	16	81	96	28	28	21	73	118	64	47	17
74	HVVKQAMSNNMM	868	13	88	46	46	12	19	215	26	30	8	31	77	121	88	48
58	YFDWGLSNANDT	845	13	241	35	103	13	29	136	25	23	10	38	84	43	38	14
92	SYSALDHPPHPT	826	21	28	44	13	22	38	185	29	17	11	69	51	171	114	13
85	GLDLNMSTWMLN	801	22	263	43	35	10	63	57	17	12	18	45	57	86	53	20
54	DVFRAATIRTAS	724	224	10	43	18	13	28	13	13	14	9	267	22	27	10	13
109	THLMTEIRWPWY	703	26	19	42	3	9	22	35	129	55	8	38	21	205	65	26
116	SQDIRTWNGTRS	700	50	57	73	25	12	63	45	29	17	31	154	57	53	25	9
123	ADQTHTRNLLRQ	698	122	38	23	20	9	75	78	83	50	32	81	36	27	18	6
60	TNVDQHHTVDWL	680	14	392	37	92	13	23	14	9	4	9	27	15	16	12	3
104	TYTTKPWEEFEV	657	12	11	32	5	12	23	22	155	80	225	31	14	11	14	10
143	YLVPRTPAQHPG	643	21	9	34	20	21	37	15	13	10	11	50	15	217	90	80
84	GATANVYTTQWG	630	10	166	31	62	17	26	70	21	6	5	34	100	44	31	7
94	SWSYDPRQIMMT	595	19	19	127	165	45	19	70	20	11	9	25	7	38	17	4
68	IECCRLVTNTTP	587	10	45	13	21	8	27	115	32	13	5	17	157	47	73	4
71	NPVSMEYGGKQS	547	6	7	6	9	9	15	19	222	194	18	14	6	11	2	9
111	DESPYYVPRTPA	521	7	9	22	7	7	16	16	17	17	39	24	20	149	82	89
106	LDRQLEWMYLSR	515	35	31	41	36	32	32	25	62	38	21	27	33	46	35	21
81	IGSPHQTAPLLT	512	11	44	19	12	6	35	85	16	21	11	32	87	39	86	8
110	EREYDMQKLISY	506	59	12	14	12	9	14	11	49	41	160	89	11	12	9	4
86	SLPILFMPQHYS	480	6	212	20	78	7	16	21	6	7	10	27	24	28	14	4
119	ANYHPWSLMSDK	366	15	113	33	55	13	15	10	5	2	8	37	25	17	13	5
161	NPMLLSMGQKPA	356	13	12	20	11	9	21	65	17	14	4	31	70	38	29	2
180	TLDMHGSSPRQL	334	9	11	24	6	3	14	150	25	18	6	32	7	19	5	5
105	VHLQAGELMNPK	333	5	78	12	33	7	13	45	3	9	0	10	59	26	24	9
158	YTCGAVSTAQPC	332	81	13	22	8	5	34	19	4	3	5	46	52	19	18	3
138	FPIDWYVSTRAT	273	9	3	16	3	4	13	18	4	5	3	15	4	123	46	7
140	DLFGSRHNPGNI	264	93	4	30	4	5	47	8	5	0	7	44	4	7	3	3
232	MPITTYWLSQYR	261	16	37	38	13	4	21	20	6	7	7	31	26	22	6	7
149	STLSPTDYMRHT	260	9	56	23	18	3	10	4	8	7	9	22	41	16	30	4
200	LPSPAGPHDSFE	195	10	3	17	6	4	7	7	49	34	28	12	4	8	3	3
214	AWNNTTPHFESK	186	6	2	12	8	6	15	6	64	23	12	10	7	7	6	2
194	EKQQFDTLKQNP	130	1	2	6	3	3	5	3	42	17	39	3	0	0	2	4
253	GLTMVQGLKEFE	126	1	0	4	2	5	1	5	43	12	39	5	3	2	2	2
250	SALKGLFPADHH	125	13	27	15	6	2	3	4	2	2	4	26	4	11	4	2
216	TQGMCARSPNCT	122	1	5	17	3	0	11	4	5	0	0	15	27	19	14	1
376	QHYWNEYEIPFQ	112	6	3	8	0	2	10	3	23	13	19	9	1	11	1	3
293	WPGLSRAHHGTV	105	21	6	6	1	3	8	32	2	2	1	18	0	3	1	1
279	DRWVARDPASIF	96	15	3	10	1	3	7	1	1	2	2	36	0	8	6	1

Table S1: **Overview of clustering results for the antibodies dataset**. Each line represents one resulting cluster. The column `main_sequence` contains the most abundant sequence in each cluster. The total size of each cluster and amounts of sequences in each category are stated. The table is sorted from the largest clusters to the smallest.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.419	1.284	1.306	1.264	1.272	1.313	1.320	1.325	1.089	1.329	1.288	1.326	1.263
2	0.419	0	1.259	1.278	1.248	1.230	1.296	1.287	1.299	1.083	1.345	1.307	1.311	1.265
3	1.284	1.259	0	0.052	0.111	1.262	1.255	1.296	1.327	1.056	1.350	1.293	1.365	1.262
4	1.306	1.278	0.052	0	0.255	1.153	1.124	1.175	1.203	1.068	1.372	1.322	1.399	1.293
5	1.264	1.248	0.111	0.255	0	1.217	1.216	1.267	1.281	0.842	1.351	1.292	1.322	1.262
6	1.272	1.230	1.262	1.153	1.217	0	0.183	0.147	0.154	0.991	1.330	1.429	1.371	1.255
7	1.313	1.296	1.255	1.124	1.216	0.183	0	0.015	0.072	0.941	1.233	1.331	1.258	1.133
8	1.320	1.287	1.296	1.175	1.267	0.147	0.015	0	0.045	0.994	1.213	1.326	1.244	1.112
9	1.325	1.299	1.327	1.203	1.281	0.154	0.072	0.045	0	0.798	1.179	1.297	1.171	1.119
10	1.089	1.083	1.056	1.068	0.842	0.991	0.941	0.994	0.798	0	1.017	1.000	0.857	1.017
11	1.329	1.345	1.350	1.372	1.351	1.330	1.233	1.213	1.179	1.017	0	0.013	0.020	0.513
12	1.288	1.307	1.293	1.322	1.292	1.429	1.331	1.326	1.297	1.000	0.013	0	0.025	0.506
13	1.326	1.311	1.365	1.399	1.322	1.371	1.258	1.244	1.171	0.857	0.020	0.025	0	0.457
14	1.263	1.265	1.262	1.293	1.262	1.255	1.133	1.112	1.119	1.017	0.513	0.506	0.457	0

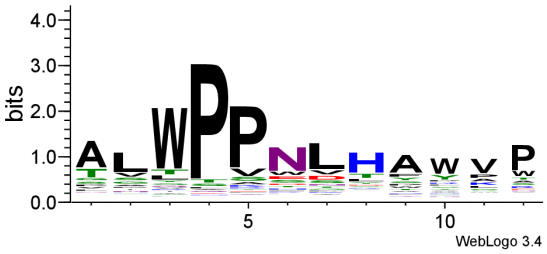
Table S2: **Table of correlation-based distance between 14 largest clusters from the antibodies dataset**. Normalized amounts of sequences within each of 15 categories form a vector of length 15 for each cluster. The distance between vectors v_1 and v_2 is then computed as $1-cor(v_1, v_2))$ where *cor* is the Pearson correlation coefficient.

unique sequences	time pseudorandom (s)	time matochko (s)
1000	10.592	9.35
2000	18.046	17.139
5000	38.666	36.451
10000	62.945	60.648
20000	106.41	100.417
30000	141.639	144.286
40000	180.092	179.805
50000	215.931	222.831
60000	253.4	265.263
70000	283.461	309.756
80000	321.459	349.715
90000	366.695	411.717
100000	399.213	426.257
150000	727.446	NA
200000	834.747	918.438
250000	1243.978	NA
300000	1308.411	1660.36
350000	1845.977	NA
400000	1805.957	2414.153
450000	2419.692	NA
500000	2375.185	3197.307
600000	2892.586	3404.279
700000	3444.12	4293.217
800000	4050.89	4470.755
900000	4719.001	5948.539
1000000	5310.038	6301.207

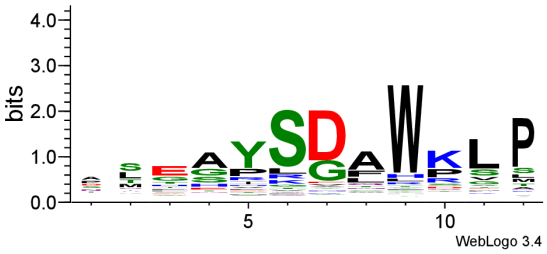
Table S3: **Table of run times displayed in Fig. 5 in the article.** Time values are stated in seconds.

order	# of clusters	# of sequences	# of cl1 sequences	KLD match	KLD all
size	74	14421	753	17.897	17.635
alphabetic	71	12294	661	18.065	18.1
random 42	70	12898	626	18.709	18.656
random 198	63	12742	902	17.86	17.52
random 9827	73	12899	631	18.6	17.96

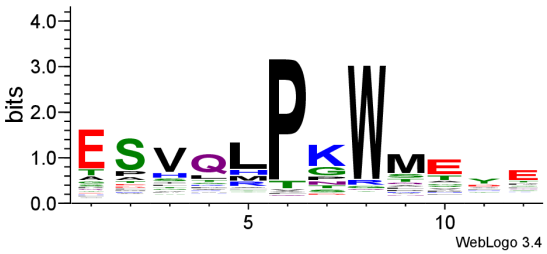
Table S4: **Comparison of sorting strategies for the greedy clustering step**. The antibodies dataset was clustered using Hammock with default parameters and different sorting strategies. For random sorting, 3 different seeds of the pseudorandom number generator were used. Column *# of cl1 sequences* states the number of unique sequences in the largest cluster containing the most frequent sequence ALWPPNLHAWVP.



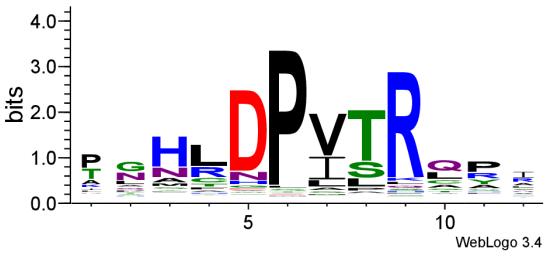
(a) cluster 1



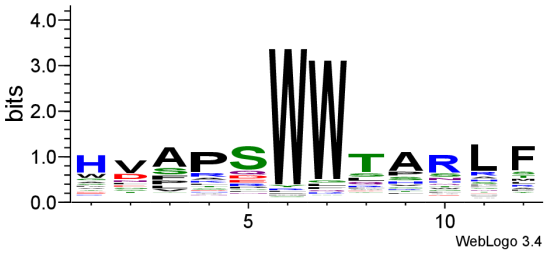
(b) cluster 2



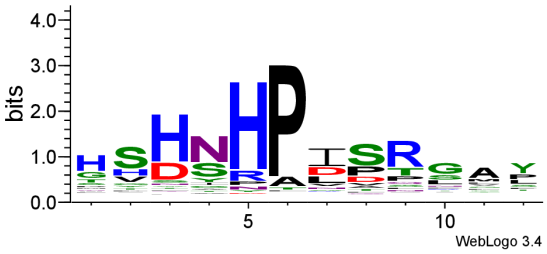
(c) cluster 3



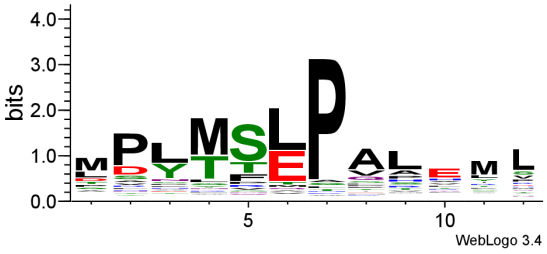
(d) cluster 4



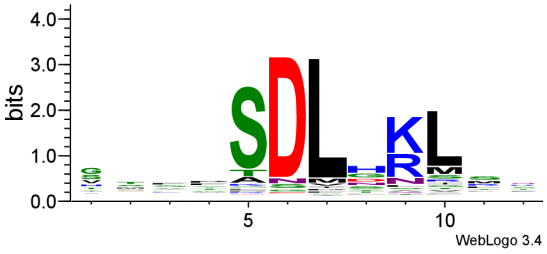
(e) cluster 5



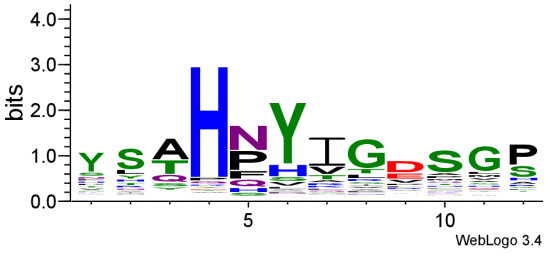
(f) cluster 6



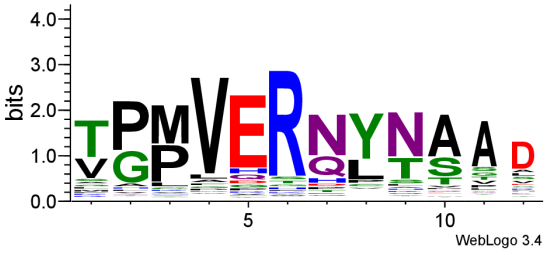
(g) cluster 9



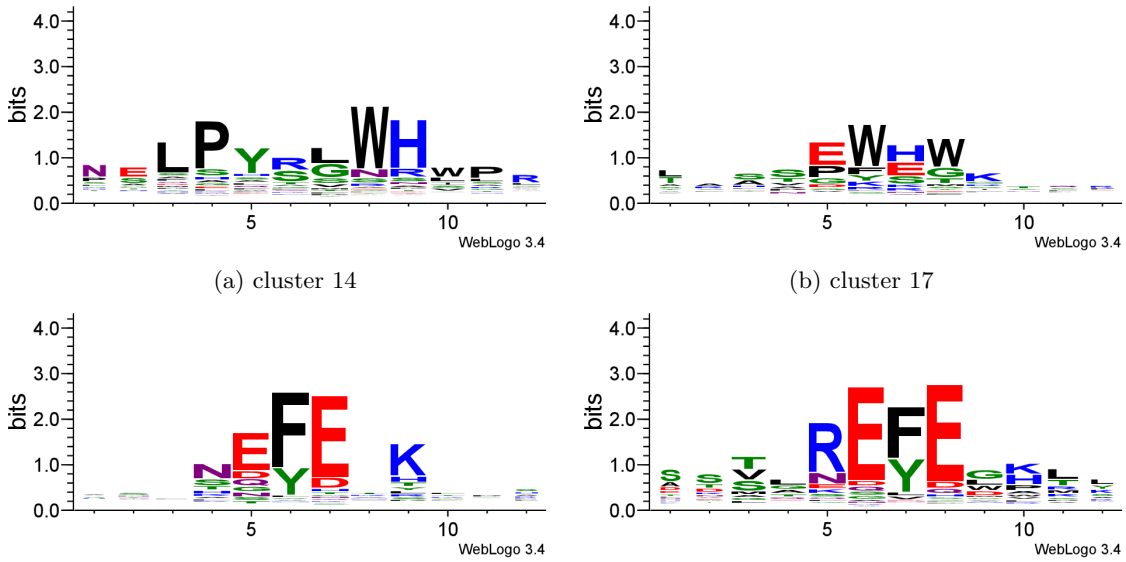
(h) cluster 10



(i) cluster 12



(j) cluster 13



(a) cluster 14

(b) cluster 17

(c) cluster 21

(d) cluster 40

Figure S1: **Sequence logos of all 14 largest clusters from the antibodies dataset.**

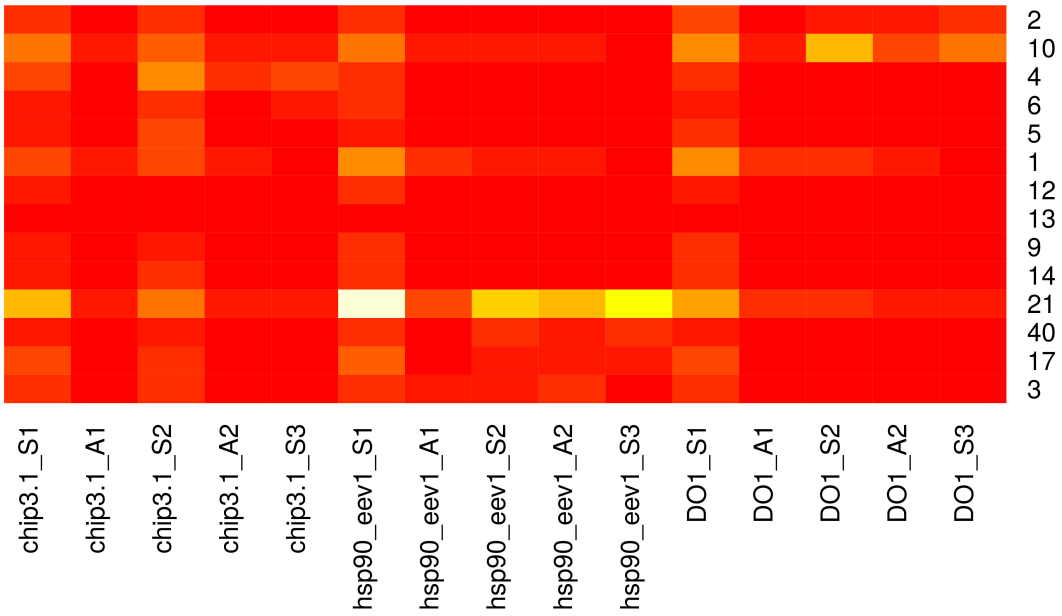


Figure S2: **A heatmap of diversity (=unique sequence counts) in all categories for 14 of the largest clusters.** Each row represents a category profile corresponding to one cluster (cluster ids are listed on the right side) and each column represents one category. There are 15 categories—3 antibodies, for each antibody 3 selection rounds (S1, S2, S3) and 2 amplification rounds (A1, A2). The heatmap is normalized for total read count in each category.

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