Supplementary information

The CRAPome: a Contaminant Repository for Affinity Purification Mass Spectrometry Data

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The number of interactions in iRefIndex is shown in relation to the different sets of SAINT options employed for the analysis of the four bait benchmark test data. The options tested here were minFold (on/off) and norm (on/off). As described in Choi et al., *Current Protocols in Bioinformatics*, 2012, as well as in the Supplementary Note, minFold "on" forces a separation between the true and false distributions, and norm "on" normalizes the data in relation to the total number of identified spectra in the sample. Both of these options allow for conservative scoring, but they may induce a loss in sensitivity. Systematically testing different parameters allows for a more enlightened selection of the optimal parameters for SAINT filtering in a particular dataset. Here, we found that turning minFold "off" while keeping norm "on" performed slightly better; this is due to the fact that some true interaction partners for MEPCE and EIF4A2 are found at reduced levels in the control runs.

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Controlled vocabularies and values in the CRAPome

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List of the most frequently detected proteins across the entire dataset (*H. sapiens*); reduced list.

Only the top entries are shown; see "Supplementary data" section on the www.crapome.org for full list.

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List of the most frequently detected proteins across the entire dataset (*H. sapiens*); redundant list.

Only the top entries are shown; see "Supplementary data" section on the www.crapome.org for full list

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List of the most enriched GO biological process (level 3) categories (*H. sapiens*)

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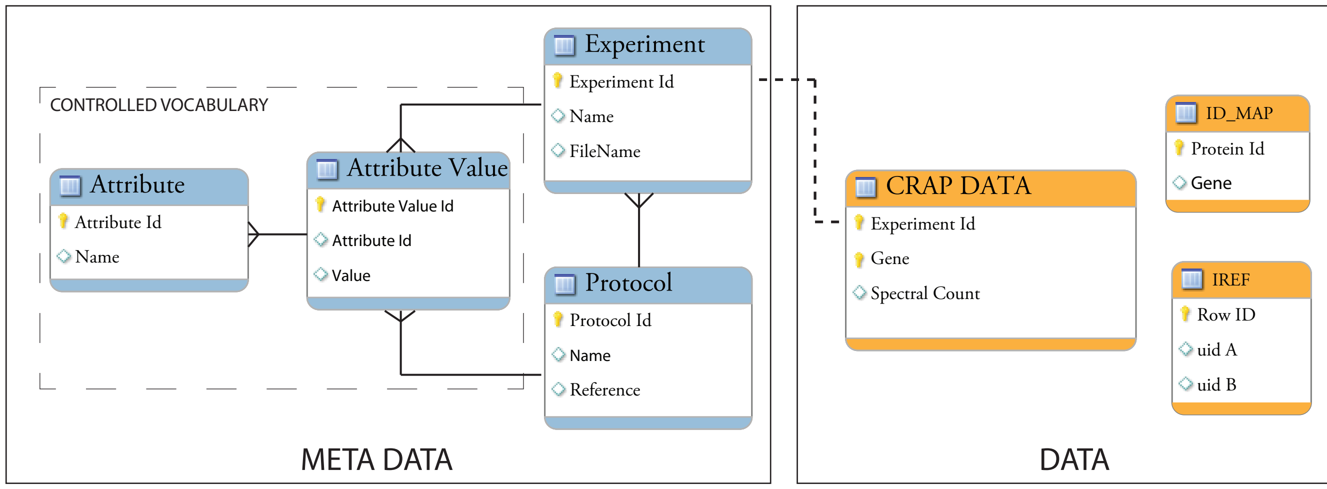
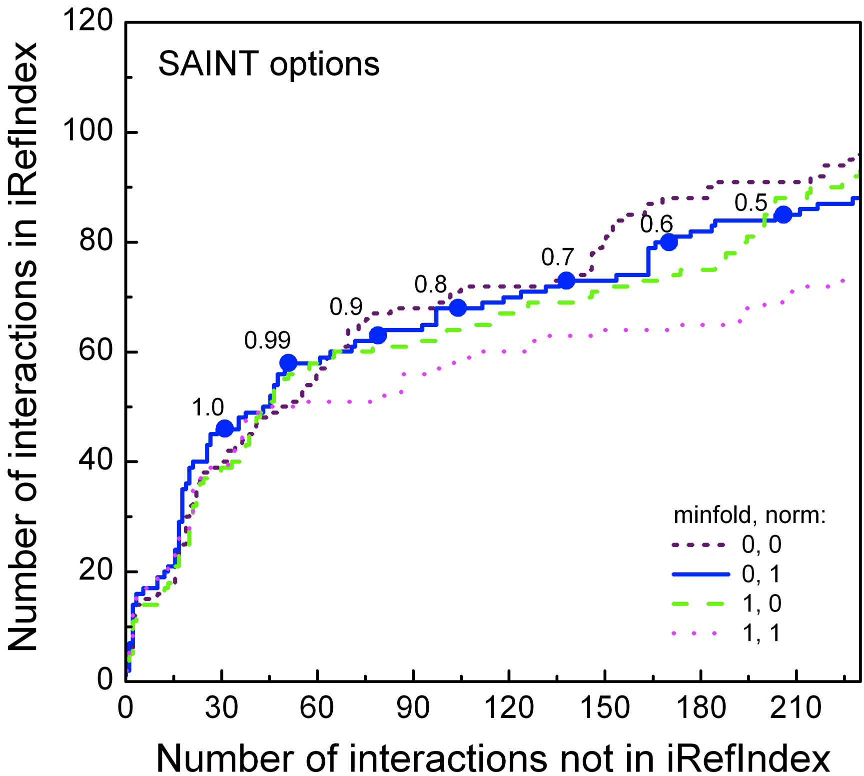


Supplementary Figure 1: Database schema



Supplementary Figure 2: Effect of SAINT options on the results.
The number of interactions in iRefIndex is shown in relation to the different sets of SAINT options employed for the analysis of the four bait benchmark test data. The options tested here were minFold (on/off) and norm (on/off). As described in Choi et al., Current Protocols in Bioinformatics, 2012, as well as on the tutorial, minFold "on" forces a separation between the true and false distributions, and norm "on" normalizes the data in relation to the total number of identified spectra in the sample. Both of these options allow for conservative scoring, but they may induce a loss in sensitivity. Systematically testing different parameters allows for a more enlightened selection of the optimal parameters for SAINT filtering in a particular dataset. Here, we found that turning minFold "off" while keeping norm "on" performed slightly better; this is due to the fact that some true interaction partners for MEPCE and EIF4A2 are found at reduced levels in the control runs.

Supplementary Table 1: Controlled vocabularies and values in the CRAPome

Attribute Name	Attribute Values
Organism	H. sapiens, S. cerevisiae
Cell/tissue type	HEK293, HeLa, U2OS, PBMC, Jurkat, CEM-T, MRC-5, LS174, S288C
Cell/tissue subtype	HEK293T, HEK293 Flp-In T-REx, Jurkat-Flp-In
Drug treatment	aphidicolin, rapamycin, nocodazole, MG132, none, IFN-beta, DMSO, okadaic acid, doxycycline+thymidine, tetracycline+thymidine, thymidine+nocodazole
Subcellular fractionation	total cell lysate, total lysate+chromatin, nuclear fraction, cytosolic fraction
Epitope tag	FLAG, HA, GFP, TAP, HaloTag, Strep-HA
Control protein	RFP, GFP, FLAG, mCherry, tag alone, untransfected, uninduced, NLS-RFP
AP steps	single, tandem
Affinity approach 1	M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, HA-7 anti-HA, HaloLink, IgG, Streptactin, 2xFLAG, SBP, anti-GFP mouse, HA.11 anti-HA
Affinity support 1	agarose, magnetic (dynabead), magnetic (agarose coated), nano-magnetic, microMACS
Affinity approach 2	M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, calmodulin, HA, 2xHA, HA-7 anti-HA, anti-GFP mouse
Affinity support 2	agarose, magnetic bead (dynabead), magnetic beads, agarose coated, nano-magnetic beads, microMACS
Fractionation	SDS-PAGE, 1D LC-MS, MudPIT, RP-RP, GeLC
Instrument type	Velos-Orbitrap, LTQ-Orbitrap, LTQ, LCQ, LTQ-FT, 5600 TripleTOF

Supplementary Table 2: Most frequently detected genes in the CRAPome (reduced list - see Methods). Full Table online at www.crapome.org. This list was processed using ABACUS

Columns are as follows: PROTID, RefSeq protein accession used for mapping in ABACUS; GENEID, Universal Gene Symbol; Num Expt Total, number of experiments in which the gene product was identified; Frequency, the percentage of the experiments in the CRAPome in which the gene product was identified; Max SC, the maximum number of spectral counts with which one gene product was identified across all CRAPome experiments; Ave SC, the average number of spectral counts for the gene product across all experiments; Sum SC, the total spectral counts across the entire CRAPome; Sum SC (unique), the total spectral counts unambiguously assigned to the protein.

PROTID	GENEID	Num Expt Total	Frequency	Max SC	Ave SC	Sum SC	Sum SC (unique)
NP_005336.3	HSPA1A	334	0.97	385	39.89	13641	5355
NP_005518.3	HSPA1L	330	0.96	215	20.67	7065	4
NP_006588.1	HSPA8	330	0.96	395	35.92	12287	9630
NP_006112.3	KRT1	325	0.95	1424	87.44	29294	28989
NP_068814.2	HSPA2	322	0.94	185	14.66	5001	2399
NP_006073.2	TUBA1B	321	0.94	366	33.99	11642	11642
NP_116093.1	TUBA1C	321	0.94	333	32.13	11001	11001
NP_002146.2	HSPA6	318	0.93	173	12.33	4220	312
NP_005992.1	TUBA3C	318	0.93	287	24.88	8519	8519
NP_821133.1	TUBB	317	0.92	355	33.15	11358	11358
NP_005991.1	TUBA4A	313	0.91	284	23.69	8109	8109
NP_006079.1	TUBB4B	313	0.91	330	26.84	9308	9308
NP_001060.1	TUBB2A	309	0.90	319	23.83	8160	8160
NP_821080.1	TUBB2B	309	0.90	321	24.04	8231	8231
NP_005338.1	HSPA5	306	0.89	120	12.99	4422	3237
NP_000414.2	KRT2	303	0.88	723	44.41	15183	13080
NP_001092.1	ACTB	302	0.88	831	35.38	12120	12120
NP_006077.2	TUBB3	302	0.88	175	17.36	5945	5945
NP_006078.2	TUBB4A	302	0.88	264	19.51	6798	6798
NP_000412.3	KRT10	301	0.88	960	68.89	23584	22390
NP_001091.1	ACTA1	295	0.86	426	19.07	6532	6532
NP_001135417.1	ACTA2	293	0.85	344	17.27	5912	5912
NP_001393.1	EEF1A1	290	0.85	168	14.92	5109	1980
NP_001077007.1	POTEE	288	0.84	302	12.10	4148	4148
NP_000217.2	KRT9	287	0.84	834	49.04	16809	16552
NP_001949.1	EEF1A2	283	0.83	113	9.17	3142	13
NP_005546.2	KRT6B	278	0.81	383	20.57	7015	5053
NP_778253.2	KRT77	274	0.80	182	6.93	2353	1952
NP_001017992.1	ACTBL2	266	0.78	171	8.27	2829	2829
NP_115914.1	TUBB6	258	0.75	103	8.71	2985	2985
NP_000517.2	KRT14	255	0.74	356	20.46	7000	5806
NP_005545.1	KRT6A	255	0.74	259	17.38	5908	3946
NP_005548.2	KRT16	251	0.73	310	17.96	6152	4958
NP_004125.3	HSPA9	249	0.73	185	10.43	3545	3538
NP_000415.2	KRT5	244	0.71	279	19.22	6547	4585
NP_005310.1	HIST1H1C	240	0.70	93	6.63	2251	87
NP_005312.1	HIST1H1E	239	0.70	93	6.60	2249	84
NP_005311.1	HIST1H1D	239	0.70	91	6.31	2141	1
NP_002267.2	KRT19	233	0.68	181	8.77	3002	1666
NP_853515.2	KRT27	231	0.67	199	6.54	2235	2235
NP_004684.2	KRT75	230	0.67	104	7.85	2658	997
NP_000413.1	KRT17	228	0.66	159	11.35	3880	2643
NP_001035807.1	HIST2H2AA4	223	0.65	234	7.34	2507	2507
NP_002131.2	HNRNPK	223	0.65	161	8.79	3012	3012
NP_002266.2	KRT15	223	0.65	130	7.98	2723	1529
NP_003504.2	HIST1H2AB	223	0.65	234	7.09	2423	2423
NP_001129064.1	RPS27A	222	0.65	108	4.13	1413	1413
NP_787028.1	KRT79	221	0.64	112	6.78	2297	11

NP_004492.2	HNRNPU	220	0.64	286	10.27	3498	8
NP_114032.2	HNRNPU	220	0.64	286	10.41	3543	53
NP_002264.1	KRT8	218	0.64	592	11.59	3921	3062
NP_705694.2	KRT13	216	0.63	147	8.37	2858	1921
NP_031381.2	HSP90AB1	215	0.63	328	16.69	5306	2620
NP_000215.1	KRT18	213	0.62	365	8.37	2850	1732
NP_001029102.1	UBA52	211	0.62	108	4.00	1371	1371
NP_056932.2	KRT76	211	0.62	97	6.99	2351	1038
NP_001017963.2	HSP90AA1	210	0.61	256	15.62	5028	2561
NP_003371.2	VIM	209	0.61	861	24.16	8260	8075
NP_002127.1	HNRNPA1	207	0.60	54	4.76	1622	1571
NP_000996.2	RPS3	206	0.60	65	7.02	2397	2397
NP_112533.1	HNRNPA2B1	205	0.60	69	6.72	2267	2216
NP_005372.2	NCL	202	0.59	126	8.02	2741	2741
NP_001020241.1	RPS14	201	0.59	41	2.57	880	880
NP_005511.1	HNRNPH1	200	0.58	223	9.18	3142	1041
NP_072045.1	RPS18	199	0.58	45	3.49	1182	1182
NP_001189360.1	PRDX1	198	0.58	53	3.79	1279	803
NP_002037.2	GAPDH	198	0.58	113	5.95	2034	1985
NP_000969.1	RPL23	196	0.57	52	2.97	1014	1014
NP_002263.3	KRT4	193	0.56	61	3.14	1046	729
NP_002645.3	PKM	191	0.56	194	8.96	3064	3064
NP_001165335.1	TUBAL3	189	0.55	25	2.10	716	716
NP_002511.1	NPM1	189	0.55	122	4.25	1442	1442
NP_004550.2	YBX1	189	0.55	112	4.00	1365	1365
NP_001027565.1	HNRNPH2	188	0.55	91	4.52	1539	170
NP_004387.1	DDX5	188	0.55	97	4.89	1662	896
NP_002096.1	H2AFX	186	0.54	157	4.43	1513	1513
NP_000998.1	RPS4X	185	0.54	68	5.51	1874	1874
NP_001091974.1	DDX17	183	0.53	93	4.87	1654	888
NP_000975.2	RPL23A	182	0.53	39	2.95	1006	1006
NP_066402.2	HIST1H2BJ	180	0.52	184	9.03	3088	3088
NP_778235.1	HIST2H2AB	180	0.52	57	2.66	909	909
NP_000968.2	RPL13	179	0.52	60	3.06	1044	1044
NP_005316.1	HIST1H1A	179	0.52	44	2.52	851	30
NP_005314.2	HIST1H1T	178	0.52	38	2.24	770	1
NP_001091674.1	HNRNPF	177	0.52	62	4.29	1464	416
NP_001143.2	SLC25A5	176	0.51	73	3.78	1290	1290
NP_002147.2	HSPD1	174	0.51	177	6.57	2236	2236
NP_005959.2	HNRNPM	174	0.51	179	8.88	3018	3018
NP_001029249.1	HIST2H4B	172	0.50	332	8.52	2923	2923
NP_001203.1	C1QBP	171	0.50	425	5.15	1760	1760
NP_002268.2	KRT31	171	0.50	106	3.58	1222	285
NP_000995.1	RPLP2	170	0.50	134	3.46	1189	1189
NP_001003.1	RPS8	169	0.49	57	3.62	1237	1237
NP_001019.1	RPS25	168	0.49	19	2.14	721	721
NP_476429.2	KRT3	167	0.49	82	5.15	1726	533
NP_001104026.1	FLNA	166	0.48	332	13.65	4651	4651
NP_444513.1	DCD	164	0.48	50	2.28	780	780
NP_001138880.1	NONO	162	0.47	438	9.29	3146	2952
NP_001419.1	ENO1	162	0.47	177	8.21	2707	457
NP_000972.1	RPL19	160	0.47	71	1.97	668	668
NP_002129.2	HNRNPD	160	0.47	33	1.74	645	33
NP_001003810.1	HNRNPD	158	0.46	32	1.66	615	3
NP_000963.1	RPL7A	157	0.46	55	3.29	1121	1121
NP_001011.1	RPS16	157	0.46	56	3.06	1045	1045
NP_000959.2	RPL4	156	0.45	98	4.75	1613	1613

NP_001001.2	RPS6	156	0.45	74	2.74	931	931
NP_066953.1	PPIA	156	0.45	53	2.40	819	819
NP_001001937.1	ATP5A1	155	0.45	91	4.03	1370	1370
NP_001627.2	SLC25A6	155	0.45	58	3.10	1067	1067
NP_002943.2	RPS2	155	0.45	63	3.50	1194	1194
NP_000981.1	RPL27A	154	0.45	21	1.59	538	538
NP_000997.1	RPS3A	154	0.45	58	4.08	1393	1393
NP_001002.1	RPS7	154	0.45	29	2.30	790	790
NP_001677.2	ATP5B	154	0.45	112	4.01	1373	1373
NP_005800.3	PRDX2	154	0.45	60	2.04	698	426
NP_000993.1	RPLP0	153	0.45	65	3.72	1272	1272
NP_001138898.1	CSDA	153	0.45	53	2.35	804	804
NP_001348.2	DHX9	153	0.45	186	6.37	2173	2173
NP_000966.2	RPL11	152	0.44	25	1.66	559	559
NP_001180345.1	DDX3X	152	0.44	91	4.23	1442	1253
NP_003642.3	CSDA	152	0.44	70	2.56	876	876
NP_733759.1	HIST1H2BA	152	0.44	72	3.23	1106	1106
NP_001188412.1	ENO1	152	0.44	140	6.00	1951	8
NP_000967.1	RPL12	150	0.44	39	2.36	803	803
NP_001021.1	RPS27	150	0.44	35	1.41	482	158
NP_001142.2	SLC25A4	149	0.43	45	2.43	826	826
NP_000970.1	RPL18	148	0.43	82	2.58	884	884
NP_000977.1	RPL24	148	0.43	23	1.74	584	584
NP_001013.1	RPS19	148	0.43	63	2.98	1019	1019
NP_001952.1	EEF2	148	0.43	240	5.72	1939	1885
NP_002464.1	MYH9	148	0.43	754	13.07	4440	4429
NP_006752.1	YWHAE	148	0.43	42	2.95	986	819
NP_000976.1	RPL17	147	0.43	35	2.16	737	737
NP_004490.2	HNRNPAB	147	0.43	25	1.58	538	465
NP_003008.1	SRSF3	146	0.43	14	1.69	575	368
NP_005498.1	CFL1	146	0.43	34	1.50	507	275
NP_008869.1	SNRPD1	145	0.42	82	3.80	1304	1304
NP_001070910.1	HNRNPC	144	0.42	57	3.13	1070	1070
NP_001070911.1	HNRNPC	144	0.42	65	3.29	1125	1125
NP_001116137.1	DDX3Y	143	0.42	70	2.90	984	795
NP_006363.4	SYNCRIP	143	0.42	104	2.83	964	964
NP_005057.1	SFPQ	142	0.41	368	7.76	2645	2460
NP_001004.2	RPS9	139	0.41	55	2.84	961	961
NP_001008.1	RPS13	139	0.41	22	1.86	636	636
NP_001026854.1	SRSF7	139	0.41	36	1.64	557	350
NP_001524.2	HNRNPL	139	0.41	266	4.24	1446	1419
NP_004166.1	SNRPD3	139	0.41	195	4.10	1403	1403
NP_005547.3	KRT7	139	0.41	197	3.48	1164	454
NP_006100.2	PRMT5	139	0.41	3569	94.88	32512	32512
NP_000962.2	RPL7	138	0.40	78	3.42	1169	1169
NP_000974.1	RPL22	138	0.40	26	1.30	442	442
NP_057004.1	RPS27L	137	0.40	21	1.04	358	34
NP_001005.1	RPS10	136	0.40	34	2.22	757	756
NP_001164105.1	FUS	136	0.40	155	6.58	2238	1835
NP_005817.1	HNRNPR	136	0.40	185	3.14	1076	1076
NP_110379.2	TCP1	136	0.40	116	4.36	1492	1492
NP_000961.2	RPL6	135	0.39	296	5.08	1738	1738
NP_000984.1	RPL31	135	0.39	28	1.41	481	481
NP_002130.2	RBMX	135	0.39	79	3.56	1201	1201
NP_005773.3	ALYREF	135	0.39	41	2.42	828	828
NP_000964.1	RPL8	134	0.39	50	2.46	837	837
NP_000979.1	RPL27	134	0.39	62	1.55	530	530

NP_001129171.1	YWHAZ	134	0.39	35	1.82	607	406
NP_003320.2	TXN	134	0.39	15	1.03	350	350
NP_066964.1	XRCC5	134	0.39	78	2.82	956	956
NP_001191397.1	RBM10	133	0.39	277	9.72	3289	3255
NP_001988.1	FAU	133	0.39	14	0.94	322	322
NP_006187.2	PCBP1	133	0.39	120	3.49	1182	1182
NP_524147.2	MYL6	133	0.39	55	1.74	584	584
NP_000994.1	RPLP1	132	0.38	35	1.61	551	551
NP_001395.1	EEF1G	132	0.38	48	2.29	783	783
NP_003082.1	SNRPB	132	0.38	240	5.86	2004	2004
NP_919223.1	HNRNPA3	132	0.38	48	1.90	645	645
NP_001407.1	EIF4A1	131	0.38	92	3.38	1145	1145
NP_003132.2	TRIM21	131	0.38	591	15.90	5435	5435
NP_004719.2	DDX21	131	0.38	93	4.29	1450	1276
NP_005110.2	THRAP3	131	0.38	191	7.13	2415	2320
NP_006422.1	CCT2	131	0.38	98	4.84	1645	1645
NP_114366.1	PCBP2	131	0.38	58	2.28	774	774
NP_001017.1	RPS24	130	0.38	38	1.72	581	581
NP_003118.2	SPTAN1	130	0.38	272	10.46	3526	3526
NP_004588.1	SNRPD2	130	0.38	46	2.31	790	790
NP_004850.1	CLTC	130	0.38	251	5.16	1748	1748
NP_008835.5	PRKDC	130	0.38	206	6.48	2183	2183
NP_009140.1	RPL35	130	0.38	68	1.52	520	520
NP_001182461.1	SPTAN1	129	0.38	274	10.39	3505	3505
NP_003119.2	SPTBN1	129	0.38	269	10.00	3377	3377
NP_009202.1	STK38	129	0.38	515	23.80	8126	7191
NP_036205.1	CCT5	129	0.38	66	4.36	1473	1473
NP_114368.1	PTBP1	129	0.38	114	3.09	1055	1055
NP_001014.1	RPS20	128	0.37	18	1.34	458	458
NP_006421.2	CCT4	128	0.37	74	3.41	1146	1146
NP_000958.1	RPL3	127	0.37	171	4.21	1427	1427
NP_001181883.1	MATR3	127	0.37	109	4.21	1422	1422
NP_006576.2	CCT8	127	0.37	90	4.60	1572	1572
NP_077007.1	WDR77	127	0.37	1271	31.97	10958	10958
NP_000978.1	RPL26	126	0.37	26	1.98	674	674
NP_001000.2	RPS5	126	0.37	21	1.48	500	500
NP_002559.2	PABPC1	126	0.37	123	3.55	1201	1201
NP_000025.1	ALDOA	125	0.36	86	2.55	873	873
NP_001010.2	RPS15A	125	0.36	46	2.44	826	826
NP_001012.1	RPS17	125	0.36	45	2.09	716	716
NP_001022.1	RPS28	125	0.36	15	0.80	272	272
NP_001753.1	CCT6A	125	0.36	70	3.35	1144	1144
NP_005955.1	null	125	0.36	242	6.00	2019	2008
NP_001609.2	PARP1	124	0.36	167	5.10	1729	1729
NP_003290.1	HSP90B1	124	0.36	101	4.51	1524	1305
NP_005989.3	CCT3	124	0.36	71	3.20	1080	1080
NP_009057.1	VCP	124	0.36	121	6.10	2076	2076
NP_842565.2	SPTBN1	124	0.36	243	9.12	3079	3079
NP_000652.2	RPL9	123	0.36	69	2.19	751	751
NP_001156008.1	RBMXL1	123	0.36	65	2.36	804	804
NP_001460.1	XRCC6	123	0.36	87	3.11	1060	1060
NP_000990.1	RPL38	122	0.36	70	2.66	913	913
NP_057177.1	RPL26L1	122	0.36	21	1.63	558	558
NP_001016.1	RPS23	121	0.35	29	1.54	528	528
NP_001030168.1	RPL14	121	0.35	37	1.78	607	607
NP_001408.2	EIF4B	121	0.35	209	7.69	2606	2606
NP_003595.1	IRS4	121	0.35	129	3.36	1139	1139

NP_006420.1	CCT7	121	0.35	83	3.40	1149	1149
NP_036558.3	SF3B3	121	0.35	255	5.34	1822	1822
NP_000983.1	RPL29	120	0.35	71	1.08	371	371
NP_002939.2	RPL15	120	0.35	34	2.34	796	796
NP_006397.1	PRDX4	120	0.35	34	0.97	327	123
NP_001958.2	EIF4A2	119	0.35	42	1.98	669	669
NP_000960.2	RPL5	118	0.34	70	2.32	785	785
NP_001006.1	RPS11	118	0.34	30	1.81	617	617
NP_001018077.1	SERBP1	118	0.34	31	2.14	711	711
NP_001157790.1	FLNB	118	0.34	135	2.78	931	931
NP_004506.2	ILF2	118	0.34	57	2.05	702	702
NP_006704.3	SUB1	118	0.34	27	2.04	693	693
NP_001284.1	CLNS1A	117	0.34	353	9.85	3378	3378
NP_006004.2	RPL10	117	0.34	40	2.02	687	687
NP_001020.2	RPS26	116	0.34	25	0.96	322	322
NP_001167568.1	LDHB	116	0.34	105	2.92	992	992
NP_001814.2	CKB	116	0.34	116	3.13	1072	1053
NP_005753.1	TRIM28	116	0.34	56	2.47	836	836
NP_001180432.1	ENO3	115	0.34	30	1.36	463	9
NP_036565.2	SF3B1	115	0.34	177	5.42	1840	1840
NP_055554.1	BCLAF1	115	0.34	119	5.65	1915	1820
NP_056444.3	PRPF31	115	0.34	142	3.44	1170	1170
NP_057103.2	LUC7L2	115	0.34	110	4.88	1668	1668
NP_001349.2	DHX15	114	0.33	201	4.70	1597	1540
NP_001427.2	FBL	114	0.33	29	1.38	468	468
NP_003478.1	TAF15	114	0.33	38	1.85	609	206
NP_004095.4	FASN	114	0.33	404	8.06	2736	2736
NP_001032752.1	EEF1B2	113	0.33	22	1.09	366	360
NP_004514.2	KIF11	113	0.33	777	20.10	6853	6853
NP_036555.1	RPL13A	112	0.33	87	1.91	642	642
NP_001966.1	ENO2	111	0.32	30	1.35	461	12
NP_060090.2	ILF3	111	0.32	123	3.67	1231	1231
NP_068733.1	CFL2	111	0.32	17	0.83	279	47
NP_001012321.1	RPSA	110	0.32	59	1.71	584	584
NP_004332.2	CAD	110	0.32	116	2.65	894	889
NP_004893.1	RBM39	110	0.32	68	3.10	1054	1054
NP_112740.1	HNRPDL	110	0.32	20	1.12	380	212
NP_003555.1	TAGLN2	109	0.32	15	1.13	384	384
NP_006817.1	YWHAQ	109	0.32	95	1.42	468	260
NP_001136077.1	EFTUD2	108	0.31	90	3.77	1275	1221
NP_001138416.1	MYL12B	108	0.31	85	1.32	453	453
NP_001734.1	CALM2	108	0.31	233	2.17	739	739
NP_002256.2	KPNB1	108	0.31	69	2.36	798	798
NP_002257.1	KPNA2	108	0.31	36	1.56	523	523
NP_003395.1	YWHAB	108	0.31	27	1.49	493	256
NP_006462.1	MYL12A	108	0.31	85	1.30	446	446
NP_008855.1	SRSF1	108	0.31	69	2.53	863	836
NP_001005464.1	HIST2H3A	107	0.31	56	2.07	685	685
NP_003520.1	HIST1H3A	107	0.31	44	2.08	688	688
NP_006316.1	RAN	107	0.31	39	1.12	383	383
NP_006436.3	PRPF8	107	0.31	136	5.70	1917	1917
NP_006796.1	HNRNPA0	107	0.31	31	1.07	365	365
NP_055815.1	STK38L	106	0.31	375	10.31	3658	2723
NP_000973.2	RPL21	105	0.31	38	1.64	557	557
NP_001007.2	RPS12	105	0.31	19	1.22	417	417
NP_001182356.1	SRSF2	105	0.31	10	0.73	251	251
NP_002893.1	RCN2	105	0.31	27	1.11	378	378

Supplementary Table 3: Most frequently detected genes in the CRAPome (redundant list - see Methods). Full Table online at www.crapome.org. This list was processed using a program developed in house.
Columns are as follows: GENEID, Universal Gene Symbol; Num Expt Total, number of experiments in which the gene product was identified; Frequency, the percentage of the experiments in the CRAPome in which the gene product was identified; Max SC, the maximum number of spectral counts with which one gene product was identified across all CRAPome experiments; Ave SC, the average number of spectral counts for the gene product across all experiments; Sum SC, the total spectral counts across the entire CRAPome; IsMapped, whether the protein accession number was successfully mapped to a Gene. Note that only mapped entries were used for the calculations Table 1.

GENEID	Num Expt Total	Frequency	Max SC	Ave SC	Sum SC	IsMapped
HSPA8	328	0.96	332	30.27	10381	Yes
HSPA1B	328	0.96	331	33.44	11471	Yes
HSPA1A	328	0.96	331	33.44	11471	Yes
HSPA1L	322	0.94	189	17.28	5926	Yes
TUBA1C	321	0.94	281	27.99	9600	Yes
TUBA1B	321	0.94	314	29.78	10216	Yes
TUBA1A	321	0.94	299	27.22	9337	Yes
KRT1	320	0.93	1178	75.65	25949	Yes
TUBA3D	318	0.93	242	21.60	7410	Yes
TUBA3C	318	0.93	242	21.60	7410	Yes
HSPA2	318	0.93	167	11.94	4096	Yes
TUBA3E	316	0.92	227	18.79	6446	Yes
TUBB	314	0.92	338	28.72	9850	Yes
HSPA6	313	0.91	159	10.67	3659	Yes
TUBA4A	309	0.90	242	20.47	7020	Yes
TUBB2C	308	0.90	310	22.97	7879	Yes
TUBA8	308	0.90	175	15.21	5218	Yes
TUBB2B	304	0.89	301	20.23	6938	Yes
TUBB2A	304	0.89	299	20.04	6873	Yes
TUBB4	298	0.87	248	16.73	5739	Yes
KRT2	298	0.87	629	38.62	13245	Yes
KRT10	297	0.87	824	58.80	20169	Yes
TUBB3	296	0.86	172	15.18	5206	Yes
ACTG1	295	0.86	716	30.12	10332	Yes
ACTB	295	0.86	716	30.12	10332	Yes
HSPA5	292	0.85	108	10.86	3726	Yes
ACTC1	286	0.83	357	15.91	5457	Yes
ACTA1	286	0.83	357	15.87	5445	Yes
gi|134133226|ref|NP_001077007.1|	284	0.83	252	10.53	3612	No
POTEF	283	0.83	193	8.84	3033	Yes
ACTG2	283	0.83	293	14.37	4930	Yes
ACTA2	283	0.83	294	14.42	4946	Yes
EEF1A1	282	0.82	160	13.06	4481	Yes
KRT9	281	0.82	699	40.96	14050	Yes
EEF1A2	275	0.80	109	8.09	2775	Yes
KRT77	268	0.78	163	5.75	1972	Yes
KRT6B	266	0.78	320	16.77	5753	Yes
ACTBL2	261	0.76	129	6.67	2289	Yes
gi|42558279|ref|NP_817124.1|	254	0.74	155	8.08	2772	No
POTEI	252	0.73	158	5.69	1952	Yes
TUBB6	249	0.73	94	7.48	2565	Yes
POTEJ	240	0.70	134	5.05	1733	Yes
HSPA9	239	0.70	159	9.15	3138	Yes
KRT28	235	0.69	227	7.75	2657	Yes
KRT6C	234	0.68	212	13.76	4720	Yes
KRT6A	234	0.68	215	14.05	4820	Yes
HIST1H1E	234	0.68	76	5.78	1983	Yes
HIST1H1C	234	0.68	76	5.79	1986	Yes
HIST1H1D	233	0.68	75	5.52	1893	Yes

KRT5	232	0.68	223	15.93	5463	Yes
KRT14	230	0.67	282	16.45	5644	Yes
TUBB1	224	0.65	62	4.08	1399	Yes
KRT75	221	0.64	80	6.39	2192	Yes
KRT16	220	0.64	243	14.30	4904	Yes
HNRNPK	220	0.64	143	7.62	2615	Yes
KRT27	218	0.64	156	5.56	1906	Yes
KRT25	218	0.64	156	5.51	1891	Yes
RPS27A	213	0.62	90	3.38	1160	Yes
HNRNPU	212	0.62	256	8.71	2989	Yes
HSP90AB1	211	0.62	278	14.03	4813	Yes
HSP90AA1	206	0.60	219	12.90	4423	Yes
HIST3H2A	206	0.60	217	6.08	2086	Yes
HIST2H2AC	206	0.60	218	6.28	2154	Yes
HIST2H2AA4	206	0.60	218	6.28	2154	Yes
HIST2H2AA3	206	0.60	218	6.28	2154	Yes
HIST1H2AM	206	0.60	217	6.22	2134	Yes
HIST1H2AL	206	0.60	217	6.22	2134	Yes
HIST1H2AK	206	0.60	217	6.22	2134	Yes
HIST1H2AJ	206	0.60	217	6.22	2134	Yes
HIST1H2AI	206	0.60	217	6.22	2134	Yes
HIST1H2AH	206	0.60	217	6.22	2134	Yes
HIST1H2AG	206	0.60	217	6.22	2134	Yes
HIST1H2AE	206	0.60	217	6.08	2086	Yes
HIST1H2AD	206	0.60	217	6.15	2110	Yes
HIST1H2AC	206	0.60	217	6.15	2110	Yes
HIST1H2AB	206	0.60	217	6.08	2086	Yes
H2AFJ	206	0.60	217	6.22	2134	Yes
KRT79	205	0.60	83	5.30	1818	Yes
UBA52	202	0.59	90	3.26	1119	Yes
VIM	201	0.59	754	20.30	6962	Yes
HNRNPA1	201	0.59	48	3.99	1368	Yes
gi|310114721|ref|XP_003119987.1|	201	0.59	50	3.16	1084	No
UBC	200	0.58	90	3.22	1106	Yes
UBB	200	0.58	90	3.22	1106	Yes
RPS3	200	0.58	56	5.96	2044	Yes
RPS14	198	0.58	33	2.38	815	Yes
KRT17	198	0.58	121	8.45	2898	Yes
HNRNPA2B1	197	0.57	56	5.57	1912	Yes
NCL	195	0.57	99	6.64	2276	Yes
HNRNPH1	195	0.57	201	8.22	2821	Yes
KRT19	190	0.55	151	6.21	2130	Yes
GAPDH	190	0.55	97	5.18	1777	Yes
HNRNPH2	189	0.55	83	4.09	1404	Yes
KRT15	188	0.55	102	5.69	1951	Yes
KRT13	188	0.55	126	6.28	2154	Yes
RPS18	187	0.55	40	2.91	997	Yes
PRDX1	186	0.54	44	3.09	1060	Yes
HNRNPA1L2	186	0.54	29	2.78	953	Yes
gi|55770868|ref|NP_064424.3|	186	0.54	47	2.49	855	No
YBX1	184	0.54	101	3.58	1229	Yes
NPM1	184	0.54	100	3.56	1220	Yes
KRT18	182	0.53	311	6.66	2283	Yes
H2AFZ	182	0.53	156	3.61	1237	Yes
H2AFV	182	0.53	156	3.61	1237	Yes
DDX5	182	0.53	86	4.14	1420	Yes
PKM2	181	0.53	181	7.86	2696	Yes
KRT76	181	0.53	87	5.28	1811	Yes

RPL23	178	0.52	48	2.48	851	Yes
KRT8	178	0.52	528	8.76	3004	Yes
RPL23A	176	0.51	30	2.40	824	Yes
HNRNPF	176	0.51	58	3.85	1321	Yes
gi|310128732|ref|XP_003120650.1|	176	0.51	116	5.13	1758	No
gi|310128730|ref|XP_003120648.1|	176	0.51	116	5.13	1758	No
DDX17	174	0.51	80	4.17	1431	Yes
HIST1H1A	173	0.50	32	2.18	748	Yes
HIST1H1T	172	0.50	32	2.03	696	Yes
TUBAL3	171	0.50	22	1.63	559	Yes
H2AFX	171	0.50	142	3.65	1251	Yes
HIST1H2AA	170	0.50	139	3.49	1197	Yes
gi|310128736|ref|XP_001719942.2|	169	0.49	110	4.60	1578	No
gi|310128734|ref|XP_003120649.1|	169	0.49	110	4.60	1578	No
KRT24	167	0.49	97	3.45	1185	Yes
RPS4X	166	0.48	58	4.44	1523	Yes
RPLP2	166	0.48	117	3.13	1073	Yes
RPL13	166	0.48	49	2.55	873	Yes
C1QBP	165	0.48	381	4.63	1588	Yes
HIST4H4	164	0.48	316	7.28	2496	Yes
HIST2H4B	164	0.48	316	7.28	2496	Yes
HIST2H4A	164	0.48	316	7.28	2496	Yes
HIST2H2AB	164	0.48	50	2.24	769	Yes
HIST1H4L	164	0.48	316	7.28	2496	Yes
HIST1H4K	164	0.48	316	7.28	2496	Yes
HIST1H4J	164	0.48	316	7.28	2496	Yes
HIST1H4I	164	0.48	316	7.28	2496	Yes
HIST1H4H	164	0.48	316	7.28	2496	Yes
HIST1H4F	164	0.48	316	7.28	2496	Yes
HIST1H4E	164	0.48	316	7.28	2496	Yes
HIST1H4D	164	0.48	316	7.28	2496	Yes
HIST1H4C	164	0.48	316	7.28	2496	Yes
HIST1H4B	164	0.48	316	7.28	2496	Yes
HIST1H4A	164	0.48	316	7.28	2496	Yes
RPS8	163	0.48	51	3.19	1094	Yes
HSPD1	163	0.48	165	5.67	1946	Yes
SLC25A5	162	0.47	58	2.95	1012	Yes
HNRNPM	161	0.47	149	7.33	2514	Yes
HIST3H2BB	160	0.47	145	7.01	2406	Yes
HIST2H2BF	160	0.47	154	7.53	2583	Yes
HIST2H2BE	160	0.47	150	7.20	2468	Yes
HIST1H2BO	160	0.47	150	7.20	2468	Yes
HIST1H2BN	160	0.47	154	7.53	2583	Yes
HIST1H2BM	160	0.47	154	7.53	2583	Yes
HIST1H2BL	160	0.47	153	7.49	2569	Yes
HIST1H2BK	160	0.47	154	7.52	2579	Yes
HIST1H2BJ	160	0.47	150	7.18	2464	Yes
HIST1H2BI	160	0.47	154	7.53	2583	Yes
HIST1H2BH	160	0.47	154	7.53	2583	Yes
HIST1H2BG	160	0.47	154	7.53	2583	Yes
HIST1H2BF	160	0.47	154	7.53	2583	Yes
HIST1H2BE	160	0.47	154	7.53	2583	Yes
HIST1H2BD	160	0.47	154	7.53	2583	Yes
HIST1H2BC	160	0.47	154	7.53	2583	Yes
HIST1H2BB	160	0.47	150	7.20	2468	Yes
FLNA	158	0.46	305	11.62	3986	Yes
KRT4	156	0.45	43	2.23	764	Yes
ENO1	155	0.45	163	7.04	2414	Yes

DCD	155	0.45	45	2.00	687	Yes
RPS25	154	0.45	18	1.69	579	Yes
HNRNPD	153	0.45	28	1.50	513	Yes
RPL19	152	0.44	67	1.75	599	Yes
NONO	152	0.44	386	7.87	2701	Yes
KRT26	150	0.44	92	3.19	1095	Yes
RPLP0	149	0.43	47	3.27	1122	Yes
RPL4	148	0.43	81	3.97	1361	Yes
RPL11	148	0.43	20	1.36	465	Yes
RPS16	147	0.43	42	2.47	848	Yes
RPL7A	147	0.43	48	2.78	953	Yes
ATP5A1	147	0.43	83	3.43	1175	Yes
ATP5B	145	0.42	106	3.56	1220	Yes
SNRPD1	144	0.42	63	3.26	1117	Yes
KRT35	144	0.42	62	2.24	770	Yes
KRT3	144	0.42	62	3.90	1337	Yes
HNRNPAB	144	0.42	20	1.32	454	Yes
DDX3X	144	0.42	87	3.63	1246	Yes
RPS6	143	0.42	63	2.35	807	Yes
RPS2	143	0.42	52	2.80	961	Yes
KRT31	143	0.42	83	2.55	876	Yes
DHX9	143	0.42	169	5.45	1870	Yes
CSDA	143	0.42	64	2.22	760	Yes
SLC25A6	142	0.41	47	2.43	835	Yes
RPS19	142	0.41	58	2.56	879	Yes
RPL27A	142	0.41	19	1.37	471	Yes
KRT33B	142	0.41	63	2.36	810	Yes
ALB	142	0.41	93	4.50	1545	Yes
RPS3A	141	0.41	47	3.35	1149	Yes
KRT32	141	0.41	62	2.08	714	Yes
RPL12	140	0.41	30	2.10	720	Yes
PRDX2	140	0.41	54	1.70	584	Yes
YBX2	139	0.41	29	1.50	513	Yes
RPL18	139	0.41	64	2.26	776	Yes
MYH9	139	0.41	670	11.09	3804	Yes
gi|310128738|ref|XP_003120651.1|	139	0.41	94	3.56	1221	No
EEF2	139	0.41	219	4.78	1638	Yes
RPS7	138	0.40	27	1.90	653	Yes
KRT38	138	0.40	62	1.98	679	Yes
KRT37	138	0.40	62	1.98	678	Yes
KRT36	138	0.40	62	2.03	697	Yes
SLC25A4	136	0.40	40	1.89	648	Yes
PRMT5	136	0.40	2950	78.36	26876	Yes
DDX3Y	136	0.40	66	2.45	841	Yes
SRSF3	135	0.39	14	1.38	475	Yes
RPL24	135	0.39	22	1.45	497	Yes
SYNCRIP	133	0.39	97	2.45	842	Yes
SFPQ	133	0.39	336	6.68	2291	Yes
RPL7	133	0.39	66	2.87	985	Yes
RPL22	133	0.39	26	1.20	413	Yes
RPL17	133	0.39	29	1.79	615	Yes
HNRNPL	133	0.39	236	3.69	1267	Yes
gi|228008398|ref|NP_001153146.1|	133	0.39	95	2.42	830	No
CFL1	133	0.39	30	1.32	452	Yes
RPS10	132	0.38	25	1.80	616	Yes
gi|228008295|ref|NP_001153147.1|	132	0.38	97	2.41	826	No
RPS27	131	0.38	28	1.10	379	Yes
PPIA	131	0.38	44	1.92	658	Yes

FUS	131	0.38	129	5.59	1916	Yes
SRSF7	130	0.38	34	1.40	479	Yes
RPLP1	130	0.38	35	1.55	530	Yes
RBM10	130	0.38	240	8.21	2817	Yes
YWHAE	129	0.38	37	2.33	798	Yes
HNRNPR	129	0.38	173	2.78	952	Yes
STK38	128	0.37	381	18.09	6204	Yes
PCBP1	128	0.37	108	3.04	1043	Yes
HNRNPC	127	0.37	57	2.82	967	Yes
EEF1G	127	0.37	41	1.85	634	Yes
THOC4	126	0.37	38	2.09	717	Yes
TCP1	126	0.37	106	3.64	1247	Yes
SNRPD3	126	0.37	152	3.08	1055	Yes
PCBP2	126	0.37	53	1.96	673	Yes
MYL6	126	0.37	42	1.43	489	Yes
WDR77	125	0.36	1143	27.62	9472	Yes
SNRPN	125	0.36	196	4.80	1646	Yes
SNRPB	125	0.36	196	4.80	1647	Yes
RPL31	125	0.36	21	1.08	369	Yes
XRCC5	124	0.36	71	2.28	782	Yes
THRAP3	124	0.36	176	6.05	2074	Yes
RPL6	124	0.36	249	4.15	1423	Yes
RBMX	124	0.36	68	3.05	1045	Yes
HNRNPA3	124	0.36	43	1.61	551	Yes
EIF4A1	124	0.36	87	2.82	966	Yes
CLTC	124	0.36	234	4.36	1497	Yes
CCT4	124	0.36	62	2.84	975	Yes
SPTAN1	123	0.36	239	8.89	3048	Yes
HIST1H2BA	123	0.36	45	2.13	732	Yes
DDX21	123	0.36	75	3.51	1205	Yes
TXN	122	0.36	10	0.84	287	Yes
TRIM21	122	0.36	473	12.99	4456	Yes
RPS24	122	0.36	36	1.47	504	Yes
CCT8	122	0.36	82	3.95	1356	Yes
RPL8	121	0.35	42	2.09	716	Yes
RPL27	121	0.35	42	1.24	425	Yes
PRPH	121	0.35	98	1.87	642	Yes
HSP90B1	121	0.35	96	3.84	1318	Yes
CCT5	121	0.35	62	3.76	1289	Yes
RPS13	120	0.35	17	1.50	513	Yes
RPS28	119	0.35	14	0.70	240	Yes
RPS17L	119	0.35	42	1.87	642	Yes
RPS17	119	0.35	42	1.87	642	Yes
RPS15A	119	0.35	36	2.01	689	Yes
PARP1	119	0.35	151	4.31	1480	Yes
CCT2	119	0.35	95	4.19	1436	Yes
SNRPD2	118	0.34	40	1.98	679	Yes
SLC25A31	118	0.34	13	0.88	303	Yes
RPL35	118	0.34	56	1.20	411	Yes
RPL29	117	0.34	65	0.96	328	Yes
PABPC1	117	0.34	104	2.95	1013	Yes
CCT6A	117	0.34	65	2.84	973	Yes
ALDOA	117	0.34	72	2.13	730	Yes
YWHAZ	116	0.34	26	1.44	493	Yes
SPTBN1	116	0.34	232	8.52	2922	Yes
RPS27L	116	0.34	18	0.80	275	Yes
RPS20	116	0.34	15	1.17	400	Yes
MATR3	116	0.34	96	3.59	1230	Yes

Supplementary Table 4: List of the most enriched GO Biological Process level 3 categories from the top most frequently detected proteins in the CRAPome

Term	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0010467~gene expression	446	32.91512915	4.81E-37	1179	2999	13463	1.698190604	3.46E-34	3.46E-34	7.28E-34
GO:0044260~cellular macromolecule metabolic process	651	48.04428044	2.17E-33	1179	5214	13463	1.425732345	1.56E-30	7.81E-31	3.29E-30
GO:0006139~nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	448	33.06273063	3.80E-24	1179	3409	13463	1.500648759	2.73E-21	9.10E-22	5.75E-21
GO:0034641~cellular nitrogen compound metabolic process	467	34.46494465	2.67E-22	1179	3670	13463	1.453044306	1.92E-19	4.80E-20	4.04E-19
GO:0042254~ribosome biogenesis	50	3.6900369	3.32E-21	1179	122	13463	4.679917685	2.39E-18	4.78E-19	5.03E-18
GO:0065003~macromolecular complex assembly	135	9.963099631	3.94E-21	1179	665	13463	2.318142685	2.83E-18	4.72E-19	5.96E-18
GO:0034621~cellular macromolecular complex subunit organization	89	6.568265683	6.46E-20	1179	357	13463	2.84675329	4.65E-17	6.64E-18	9.78E-17
GO:0034622~cellular macromolecular complex assembly	82	6.051660517	2.39E-19	1179	318	13463	2.944521794	1.72E-16	2.15E-17	3.61E-16
GO:0006403~RNA localization	38	2.804428044	6.32E-15	1179	100	13463	4.339219678	4.55E-12	5.06E-13	9.58E-12
GO:0022402~cell cycle process	108	7.970479705	6.62E-15	1179	565	13463	2.182746741	4.79E-12	4.79E-13	1.01E-11
GO:0051276~chromosome organization	96	7.084870849	2.84E-14	1179	485	13463	2.260255502	2.04E-11	1.85E-12	4.28E-11
GO:0050658~RNA transport	36	2.656826568	8.19E-14	1179	97	13463	4.237979067	5.89E-11	4.91E-12	1.24E-10
GO:0051236~establishment of RNA localization	36	2.656826568	8.19E-14	1179	97	13463	4.237979067	5.89E-11	4.91E-12	1.24E-10
GO:0019538~protein metabolic process	349	25.75645756	1.19E-13	1179	2812	13463	1.417222868	8.57E-11	6.59E-12	1.80E-10
GO:0000278~mitotic cell cycle	79	5.830258303	1.21E-13	1179	370	13463	2.43811063	8.71E-11	6.22E-12	1.83E-10
GO:0015931~nucleobase, nucleoside, nucleotide and nucleic acid transport	38	2.804428044	5.14E-13	1179	113	13463	3.840017414	3.69E-10	2.46E-11	7.78E-10
GO:0022618~ribonucleoprotein complex assembly	28	2.066420664	6.21E-12	1179	69	13463	4.633796757	4.46E-09	2.79E-10	9.39E-09
GO:0051439~regulation of ubiquitin-protein ligase activity during mitotic cell cycle	28	2.066420664	1.37E-11	1179	71	13463	4.503267271	9.87E-09	5.81E-10	2.08E-08
GO:0046907~intracellular transport	109	8.044280443	5.93E-11	1179	657	13463	1.894476267	4.26E-08	2.37E-09	8.97E-08
GO:0006461~protein complex assembly	88	6.494464945	4.47E-10	1179	505	13463	1.989845397	3.22E-07	1.69E-08	6.77E-07
GO:0042273~ribosomal large subunit biogenesis	10	0.73800738	2.69E-09	1179	10	13463	11.41899915	1.94E-06	9.69E-08	4.08E-06
GO:0042274~ribosomal small subunit biogenesis	10	0.73800738	1.37E-08	1179	11	13463	10.38090832	9.82E-06	4.68E-07	2.07E-05
GO:0007010~cytoskeleton organization	74	5.461254613	4.00E-08	1179	436	13463	1.938087012	2.88E-05	1.31E-06	6.05E-05
GO:0009059~macromolecule biosynthetic process	320	23.61623616	1.28E-07	1179	2832	13463	1.29028239	9.20E-05	4.00E-06	1.94E-04
GO:0034728~nucleosome organization	26	1.918819188	2.46E-07	1179	93	13463	3.192408365	1.77E-04	7.37E-06	3.73E-04
GO:0051649~establishment of localization in cell	118	8.708487085	4.19E-07	1179	852	13463	1.581504577	3.01E-04	1.20E-05	6.34E-04
GO:0044249~cellular biosynthetic process	373	27.52767528	7.26E-07	1179	3442	13463	1.237445289	5.22E-04	2.01E-05	0.001098184
GO:0010605~negative regulation of macromolecule metabolic process	104	7.675276753	7.67E-07	1179	734	13463	1.617950834	5.51E-04	2.04E-05	0.00116091
GO:0031497~chromatin assembly	24	1.771217712	1.02E-06	1179	87	13463	3.150068732	7.32E-04	2.62E-05	0.001542051
GO:0070727~cellular macromolecule localization	67	4.944649446	1.11E-06	1179	414	13463	1.848002278	8.00E-04	2.76E-05	0.001685409
GO:0022403~cell cycle phase	66	4.870848708	2.30E-06	1179	414	13463	1.820420155	0.001650138	5.50E-05	0.003476287
GO:0009892~negative regulation of metabolic process	107	7.896678967	2.51E-06	1179	780	13463	1.566452448	0.001803654	5.82E-05	0.003799979
GO:0051128~regulation of cellular component organization	71	5.239852399	2.52E-06	1179	458	13463	1.770194192	0.001812485	5.67E-05	0.003818599
GO:0046164~alcohol catabolic process	22	1.623616236	4.18E-06	1179	81	13463	3.10145656	0.003004298	9.12E-05	0.00633325
GO:0006986~response to unfolded protein	20	1.47601476	7.21E-06	1179	71	13463	3.216619479	0.005172002	1.53E-04	0.010914519
GO:0006974~response to DNA damage stimulus	59	4.354243542	1.08E-05	1179	373	13463	1.806222386	0.007737026	2.22E-04	0.016348136
GO:0000387~spliceosomal snRNP biogenesis	12	0.885608856	1.17E-05	1179	28	13463	4.893856779	0.008345291	2.33E-04	0.01763867
GO:0051789~response to protein stimulus	25	1.84501845	1.34E-05	1179	107	13463	2.667990456	0.009589573	2.60E-04	0.020281034
GO:0031324~negative regulation of cellular metabolic process	97	7.158671587	1.65E-05	1179	720	13463	1.538392941	0.011761673	3.11E-04	0.024901517
GO:0016052~carbohydrate catabolic process	25	1.84501845	1.86E-05	1179	109	13463	2.619036503	0.013311429	3.44E-04	0.028204233
GO:0008104~protein localization	113	8.339483395	2.91E-05	1179	882	13463	1.462978349	0.020700953	5.23E-04	0.044022357
GO:0033554~cellular response to stress	78	5.756457565	5.84E-05	1179	566	13463	1.573642993	0.04110355	0.001023188	0.088310821
GO:0033043~regulation of organelle organization	38	2.804428044	6.03E-05	1179	217	13463	1.999640404	0.042427365	0.001031698	0.091216246
GO:0010639~negative regulation of organelle organization	20	1.47601476	6.48E-05	1179	82	13463	2.785121744	0.045551737	0.001083641	0.09808904
GO:0000226~microtubule cytoskeleton organization	29	2.140221402	6.54E-05	1179	147	13463	2.252727724	0.045917146	0.00106772	0.098894281
GO:0010033~response to organic substance	94	6.937269373	7.98E-05	1179	721	13463	1.488746075	0.05577324	0.001274497	0.120728552
GO:0043244~regulation of protein complex disassembly	15	1.10701107	8.41E-05	1179	51	13463	3.358529162	0.05868355	0.00131383	0.127218459
GO:0007018~microtubule-based movement	24	1.771217712	1.01E-04	1179	113	13463	2.425274156	0.070088306	0.001544883	0.152841474

Supplementary Table 5: List of the most enriched GO Molecular Function level 3 categories from the top most frequently detected proteins in the CRAPome

Term	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0003723~RNA binding	262	19.33579336	1.53E-106	1090	718	13051	4.369121668	3.09E-104	3.09E-104	1.92E-103
GO:0016817~hydrolase activity, acting on acid anhydrides	146	10.77490775	2.74E-22	1090	764	13051	2.288109419	5.53E-20	2.77E-20	3.44E-19
GO:0032553~ribonucleotide binding	266	19.63099631	1.08E-21	1090	1836	13051	1.734707481	2.18E-19	7.25E-20	1.35E-18
GO:0017076~purine nucleotide binding	274	20.22140221	1.67E-21	1090	1918	13051	1.710484928	3.38E-19	8.45E-20	2.10E-18
GO:0001883~purine nucleoside binding	238	17.56457565	1.09E-20	1090	1601	13051	1.779929975	2.21E-18	4.41E-19	1.37E-17
GO:0008092~cytoskeletal protein binding	101	7.453874539	6.21E-17	1090	504	13051	2.399430246	2.24E-14	3.77E-15	1.44E-13
GO:0008135~translation factor activity, nucleic acid binding	36	2.656826568	2.81E-14	1090	98	13051	4.398389815	5.67E-12	8.11E-13	3.53E-11
GO:0051082~unfolded protein binding	35	2.58302583	2.94E-11	1090	115	13051	3.644076586	5.94E-09	7.42E-10	3.69E-08
GO:0003743~translation initiation factor activity	24	1.771217712	2.13E-10	1090	61	13051	4.710843736	4.30E-08	4.77E-09	2.67E-07
GO:0019899~enzyme binding	84	6.199261993	6.32E-09	1090	523	13051	1.923069097	1.28E-06	1.28E-07	7.93E-06
GO:0016875~ligase activity, forming carbon-oxygen bonds	19	1.402214022	1.48E-08	1090	47	13051	4.840308413	2.99E-06	2.72E-07	1.86E-05
GO:0008022~protein C-terminus binding	34	2.509225092	3.85E-08	1090	141	13051	2.88720151	7.77E-06	6.47E-07	4.83E-05
GO:0016885~ligase activity, forming carbon-carbon bonds	6	0.442804428	7.30E-05	1090	7	13051	10.26290957	0.014634866	0.001133435	0.091586475
GO:0042802~identical protein binding	82	6.051660517	8.92E-05	1090	640	13051	1.53409117	0.017865503	0.001286815	0.111975936
GO:0051920~peroxiredoxin activity	6	0.442804428	1.81E-04	1090	8	13051	8.980045872	0.035943862	0.002437408	0.227247685
GO:0005516~calmodulin binding	26	1.918819188	2.15E-04	1090	140	13051	2.223630406	0.042572615	0.002715394	0.270022665
GO:0008565~protein transporter activity	18	1.328413284	7.44E-04	1090	87	13051	2.477254034	0.139586491	0.008804668	0.930031186
GO:0051287~NAD or NADH binding	12	0.885608856	0.001370911	1090	47	13051	3.057036892	0.242031979	0.015277326	1.707529298
GO:0008134~transcription factor binding	63	4.649446494	0.001856408	1090	513	13051	1.470416868	0.312946347	0.01956104	2.305773093
GO:0002060~purine binding	4	0.295202952	0.002173309	1090	4	13051	11.9733945	0.355632614	0.021734636	2.694459373
GO:0003746~translation elongation factor activity	8	0.590405904	0.002696061	1090	24	13051	3.991131498	0.420355162	0.025634275	3.33251561
GO:0042393~histone binding	11	0.811808118	0.002826456	1090	44	13051	2.993348624	0.435464734	0.025653946	3.491071024
GO:0032403~protein complex binding	29	2.140221402	0.00346242	1090	196	13051	1.771573675	0.503723659	0.030002556	4.2609548
GO:0030674~protein binding, bridging	17	1.254612546	0.004580104	1090	94	13051	2.165401132	0.604382021	0.037900843	5.600317149
GO:0009374~biotin binding	4	0.295202952	0.005095595	1090	5	13051	9.578715596	0.643685561	0.040437346	6.212219441
GO:0033293~monocarboxylic acid binding	11	0.811808118	0.012780778	1090	54	13051	2.439024805	0.925604312	0.095105412	14.91241079
GO:0016835~carbon-oxygen lyase activity	11	0.811808118	0.014487973	1090	55	13051	2.394678899	0.947554332	0.103434883	16.7413405
GO:0016903~oxidoreductase activity, acting on the aldehyde or oxo group of donors	8	0.590405904	0.01460093	1090	32	13051	2.993348624	0.948754712	0.100675941	16.86106648
GO:0050662~coenzyme binding	25	1.84501845	0.015696616	1090	181	13051	1.65378377	0.959068883	0.104347012	18.01421726
GO:0019904~protein domain specific binding	40	2.95202952	0.017638799	1090	331	13051	1.446935891	0.972534806	0.112926874	20.02215711
GO:0047485~protein N-terminus binding	13	0.959409594	0.018843036	1090	74	13051	2.103434168	0.978562451	0.116580585	21.24432971
GO:0043023~ribosomal large subunit binding	3	0.221402214	0.019711058	1090	3	13051	11.9733945	0.982072119	0.11809302	22.11459372
GO:0016614~oxidoreductase activity, acting on CH-OH group of donors	17	1.254612546	0.031290416	1090	116	13051	1.754721607	0.998374053	0.176832767	32.90825408
GO:0051087~chaperone binding	6	0.442804428	0.038048729	1090	23	13051	3.123494216	0.999604706	0.205837395	38.55349411
GO:0016859~cis-trans isomerase activity	8	0.590405904	0.040246695	1090	39	13051	2.456080922	0.999750975	0.211075395	40.29304022
GO:0002039~p53 binding	5	0.36900369	0.047714235	1090	17	13051	3.521586616	0.999948596	0.239917178	45.87020123
GO:0003713~transcription coactivator activity	26	1.918819188	0.053957471	1090	214	13051	1.454711481	0.999986387	0.261270865	50.16055951
GO:0042153~RPTP-like protein binding	3	0.221402214	0.05868428	1090	5	13051	7.184036697	0.999995051	0.274925813	53.19815362
GO:0032182~small conjugating protein binding	6	0.442804428	0.079014189	1090	28	13051	2.565727392	0.99999994	0.347097159	64.41904174
GO:0005048~signal sequence binding	5	0.36900369	0.079768827	1090	20	13051	2.993348624	0.999999949	0.342827243	64.78332924
GO:0003916~DNA topoisomerase activity	3	0.221402214	0.083246103	1090	6	13051	5.986697248	0.999999976	0.34833287	66.41799089
GO:0016860~intramolecular oxidoreductase activity	7	0.516605166	0.092740036	1090	38	13051	2.205625302	0.999999997	0.373804663	70.53217549

Supplementary Table 6: List of the most enriched GO Cellular Component level 3 categories from the top most frequently detected proteins in the CRAPome

Term	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GO:0044424~intracellular part	1226	90.4797048	1.15E-140	1273	10624	15283	1.385423716	3.02E-138	3.02E-138	1.51E-137
GO:0044446~intracellular organelle part	760	56.0885609	7.02E-139	1273	4225	15283	2.159569019	1.84E-136	9.20E-137	9.19E-136
GO:0005622~intracellular	1233	90.99631	1.40E-130	1273	10995	15283	1.34631924	3.68E-128	1.23E-128	1.84E-127
GO:0043232~intracellular non-membrane-bounded organelle	555	40.9594096	1.16E-121	1273	2596	15283	2.566660958	3.04E-119	7.61E-120	1.52E-118
GO:0030529~ribonucleoprotein complex	229	16.900369	1.62E-112	1273	515	15283	5.338367437	4.23E-110	8.47E-111	2.12E-109
GO:0044428~nuclear part	432	31.8819188	3.41E-105	1273	1822	15283	2.84652881	8.93E-103	1.49E-103	4.46E-102
GO:0070013~intracellular organelle lumen	402	29.6678967	1.79E-89	1273	1779	15283	2.712878317	4.69E-87	6.70E-88	2.34E-86
GO:0043233~organelle lumen	406	29.9630996	1.23E-88	1273	1820	15283	2.678149737	3.23E-86	4.04E-87	1.61E-85
GO:0043229~intracellular organelle	1063	78.4501845	3.51E-87	1273	8977	15283	1.421615823	9.19E-85	1.02E-85	4.59E-84
GO:0005737~cytoplasm	878	64.797048	8.72E-57	1273	7319	15283	1.440200569	2.29E-54	2.29E-55	1.14E-53
GO:0005681~spliceosome	78	5.75645756	5.62E-49	1273	132	15283	7.094158395	1.47E-46	1.34E-47	7.35E-46
GO:0033279~ribosomal subunit	70	5.16605166	7.02E-41	1273	128	15283	6.565507168	1.84E-38	1.53E-39	9.18E-38
GO:0005840~ribosome	90	6.64206642	1.85E-40	1273	215	15283	5.025557646	4.83E-38	3.72E-39	2.41E-37
GO:0043231~intracellular membrane-bounded organelle	880	64.9446494	1.10E-37	1273	7982	15283	1.323582932	2.87E-35	2.05E-36	1.43E-34
GO:0044444~cytoplasmic part	604	44.5756458	2.90E-33	1273	4895	15283	1.481373093	7.61E-31	5.07E-32	3.80E-30
GO:0044430~cytoskeletal part	161	11.8819188	6.48E-19	1273	952	15283	2.030341712	1.70E-16	1.06E-17	8.47E-16
GO:0044427~chromosomal part	88	6.49446494	2.27E-18	1273	386	15283	2.737004913	5.95E-16	3.50E-17	2.97E-15
GO:0030530~heterogeneous nuclear ribonucleoprotein complex	17	1.25461255	7.58E-17	1273	17	15283	12.00549882	2.91E-14	1.67E-15	1.44E-13
GO:0046930~pore complex	36	2.65682657	9.14E-15	1273	95	15283	4.549452185	2.39E-12	1.26E-13	1.19E-11
GO:0005643~nuclear pore	32	2.36162362	4.11E-14	1273	79	15283	4.862986864	1.08E-11	5.38E-13	5.38E-11
GO:0005635~nuclear envelope	53	3.91143911	1.61E-13	1273	205	15283	3.103860671	4.23E-11	2.01E-12	2.11E-10
GO:0031967~organelle envelope	106	7.82287823	9.79E-13	1273	620	15283	2.052553024	2.56E-10	1.17E-11	1.28E-09
GO:0000502~proteasome complex	26	1.91881919	3.79E-12	1273	61	15283	5.117097858	9.93E-10	4.32E-11	4.96E-09
GO:0009295~nucleoid	16	1.18081181	4.92E-09	1273	31	15283	6.196386489	1.29E-06	5.37E-08	6.44E-06
GO:0044429~mitochondrial part	87	6.42066421	2.64E-07	1273	595	15283	1.755425878	6.92E-05	2.77E-06	3.46E-04
GO:0005852~eukaryotic translation initiation factor 3 complex	10	0.73800738	5.88E-07	1273	15	15283	8.003665881	1.54E-04	5.93E-06	7.70E-04
GO:0005625~soluble fraction	53	3.91143911	1.11E-06	1273	313	15283	2.032879992	2.90E-04	1.07E-05	0.00144894
GO:0030532~small nuclear ribonucleoprotein complex	12	0.88560886	1.15E-06	1273	24	15283	6.002749411	3.01E-04	1.07E-05	0.00150321
GO:0030684~preribosome	9	0.66420664	1.98E-06	1273	13	15283	8.311499184	5.20E-04	1.79E-05	0.00259582
GO:0005832~chaperonin-containing T-complex	7	0.51660517	2.14E-06	1273	7	15283	12.00549882	5.60E-04	1.87E-05	0.0027983
GO:0031988~membrane-bounded vesicle	79	5.8302583	6.82E-06	1273	568	15283	1.669778885	0.001785608	5.77E-05	0.00892658
GO:0016585~chromatin remodeling complex	19	1.40221402	1.43E-05	1273	71	15283	3.212739121	0.003735985	1.17E-04	0.01869421
GO:0019866~organelle inner membrane	51	3.76383764	2.24E-05	1273	329	15283	1.861034772	0.005846417	1.78E-04	0.0292839
GO:0016591~DNA-directed RNA polymerase II, holoenzyme	20	1.47601476	2.74E-05	1273	81	15283	2.964320697	0.007165559	2.11E-04	0.03591393
GO:0031410~cytoplasmic vesicle	84	6.19926199	3.37E-05	1273	642	15283	1.57081293	0.008796337	2.52E-04	0.04412181
GO:0005844~polysome	9	0.66420664	4.62E-05	1273	18	15283	6.002749411	0.012019572	3.36E-04	0.06038252
GO:0031252~cell leading edge	27	1.99261993	6.30E-05	1273	138	15283	2.348901943	0.016382312	4.46E-04	0.0824723
GO:0005839~proteasome core complex	9	0.66420664	1.14E-04	1273	20	15283	5.40247447	0.02948591	7.87E-04	0.14938362
GO:0000786~nucleosome	16	1.18081181	1.57E-04	1273	63	15283	3.049015574	0.040364036	0.001055885	0.20558621
GO:0042995~cell projection	86	6.34686347	2.10E-04	1273	697	15283	1.481309754	0.053583238	0.001375859	0.27470454
GO:0005853~eukaryotic translation elongation factor 1 complex	5	0.36900369	2.23E-04	1273	5	15283	12.00549882	0.056766215	0.001424378	0.29148413
GO:0016459~myosin complex	16	1.18081181	2.28E-04	1273	65	15283	2.95519971	0.057997568	0.001421546	0.29798985
GO:0012505~endomembrane system	92	6.7896679	6.24E-04	1273	782	15283	1.412411626	0.150841959	0.003795337	0.81339613
GO:0000796~condensin complex	5	0.36900369	6.25E-04	1273	6	15283	10.00458235	0.15104583	0.003714676	0.81458572
GO:0005732~small nucleolar ribonucleoprotein complex	8	0.5904059	8.01E-04	1273	20	15283	4.802199529	0.189284034	0.004652201	1.04265184
GO:0031965~nuclear membrane	16	1.18081181	8.52E-04	1273	73	15283	2.631342207	0.200126286	0.004842615	1.10917966

User manual – CRAPome version 1.1 (March 2013)

Prepared by Datta Mellacheruvu, Anne-Claude Gingras and Alexey Nesvizhskii,

1. Introduction

This tutorial describes the Contaminant Repository for Affinity Purification, its web interface, and related tools, collectively referred to as CRAPome (www.crapome.org). The contaminant repository contains the lists of proteins identified in negative control experiments collected using affinity purification followed by mass spectrometry (AP-MS). Original MS data for each experiment are obtained from the data creator(s), generally as .raw or mzXML/mzML files (mgf files are also accepted if raw/mzXML data cannot be obtained for any reason). MS/MS data are processed by the repository administrator using a uniform data analysis pipeline consisting of an X!Tandem database search against the RefSeq protein sequence database (*H. sapiens* data) or SGD (*S. cerevisiae*), followed by PeptideProphet and ProteinProphet analysis (part of the Trans-Proteomic Pipeline). Each experiment in the CRAPome represents a biological replicate (technical replicates, i.e. repeated LC-MS/MS runs on the same affinity purified sample, or multiple fractions as in the case of 1D SDS-PAGE separation, are combined into a single protein list). Protein identifications are mapped to genes and stored in a database along with their abundance information (spectral counts). CRAPome controls are associated with an experimental description via text-based protocols and controlled vocabularies. Users query the database via a web interface at www.crapome.org, using different user workflows (described below). Some functionality in workflow 2 and workflow 3 require user registration. The database currently contains data from *H. sapiens* and *S. cerevisiae* AP-MS experiments only: as the database expands, additional species will be added. As of March 2013, the database contains ~350 experiments generated using ~75 unique protocols that were deposited by 12 laboratories.

2. CRAPome welcome screen

Step 1: Choose Organism
Choose Organism
H. sapiens
Your currently selected organism is: H. sapiens
Step 2: Choose Workflow
Workflow 1: Query Proteins (against CRAPome)
Start | Query Proteins → View Profile
Workflow 2: Download Background Contaminant Lists
Start | Select Controls → Download Data
Workflow 3: Analyze Your Data
Start | Select Controls → Upload Data → Analyze Online → Visualize/Download Results

Figure 2.1. CRAPome welcome screen. The three current user workflows are displayed; select the organism and then select the desired workflow by pressing "Start".

The users of the repository access information stored in the database by first selecting the organism (currently *H. sapiens* or *S. cerevisiae*) and then one of the three workflows shown in **Fig. 2.1**, and described in detail below. A number of additional options are available from the menu bar; visible options depend on user status

(e.g. end user, data contributor/annotator, admin). Selecting an organism sets the context and filters the data appropriately throughout the application. *H. sapiens* is selected for this tutorial.

3. Workflow 1: Query selected proteins

This workflow allows the user to query for selected protein(s) of interest and view their profiles across different negative control experiments.

Step 1: Paste a list of protein(s) or gene entries in a tab, comma or new-line separated format and click on 'submit' as shown in **Fig. 3.1** (compatible formats are shown in the figure legend).

Search Protein Profiles

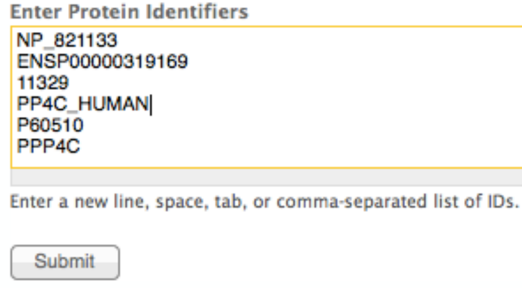


Figure 3.1. Protein/Gene identifiers compatible with the CRAPome. *From top to bottom*: RefSeq protein ID, Ensembl protein ID, NCBI Gene ID, Uniprot entry name, Uniprot entry ID, and gene symbol are supported for *H. sapiens*. In addition to the above identifiers, systematic names as per the Saccharomyces Genome Database (referred to as SGD ID in the CRAPome; e.g. YGR192C) and the standard names as per SGD (e.g. TDH3) are also supported for *S. cerevisiae*.

Step 2: The query results are displayed as shown in **Fig. 3.2**.

Query Results

Download as tab-delimited file

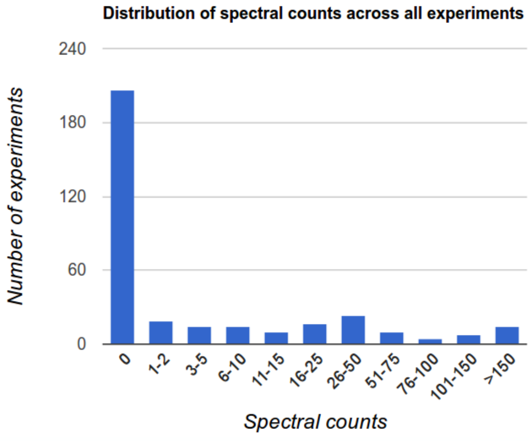
User Input	Mapped Gene Symbol	Num of Expt. (found/total)	Ave SC	Max SC	Detail
NP_821133	TUBB	314 / 343	31.4	338	detail
ENSP00000319169	PRMT5	136 / 343	197.6	2950	detail
11329	STK38	128 / 343	48.5	381	detail
PP4C_HUMAN	PPP4C	3 / 343	1.3	2	detail
P60510	PPP4C	3 / 343	1.3	2	detail
PPP4C	PPP4C	3 / 343	1.3	2	detail

Ave SC: Average Spectral Counts

Figure 3.2. Query results. The first column shows the list of entries submitted by the user, while the second column lists the Gene Symbols mapped to the entries. The third column details the number of experiments in the database in which the selected gene/protein was detected (with at least one peptide having PeptideProphet probability of 0.9 or higher); the total number of experiments in the CRAPome is also listed. The fourth and fifth columns list the averaged spectral counts and maximal spectral counts for the selected gene/protein across the experiments in which it was identified. The last column provides a link to the detailed profile for each of the selected genes/proteins. Note that this summary page can be downloaded as a tab-delimited file. From a quick survey of the results, PPP4C (protein phosphatase 4, catalytic subunit) does not appear in a high percentage of experiments (only 3 out of 343 in total), nor with high spectral counts, across the CRAPome, suggesting that it is unlikely to be a common contaminant in AP-MS studies. By contrast, TUBB (tubulin), PRMT5 (protein arginine methyltransferase 5) and STK38 (serine threonine kinase 38, also known as NDR1) are frequently detected, and often with a high number of spectral counts, indicating that they may be contaminants.

Step 3: When the user clicks on the 'detail' link, a profile of the protein (in the CRAPome repository) is shown, as in **Fig. 3.3** and **3.4**. At the top of the page are graphical summary views of the data. The profile on the left shows the abundance distribution across all experiments (i.e., how many CRAPome controls report this protein in the spectral count ranges 1-2, 3-5, 5-15, and so on; **Fig. 3.3**, left panel). If there are many experiments that report a protein in the higher spectral count ranges, one can suspect that it has a greater propensity to be a contaminant. Also shown (on the right) are the frequency of identification across groups of experiments selected based on the controlled vocabularies used to organize the data. This figure helps to provide an overview of (experimental) conditions in which this protein is likely to be a background contaminant (**Fig. 3.3;** right panel).

Profile Detail for PRMT5



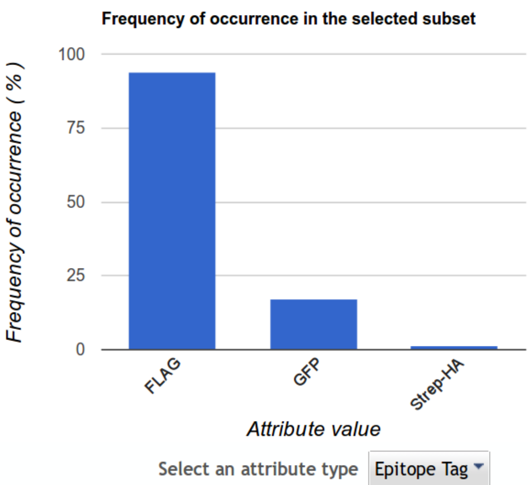
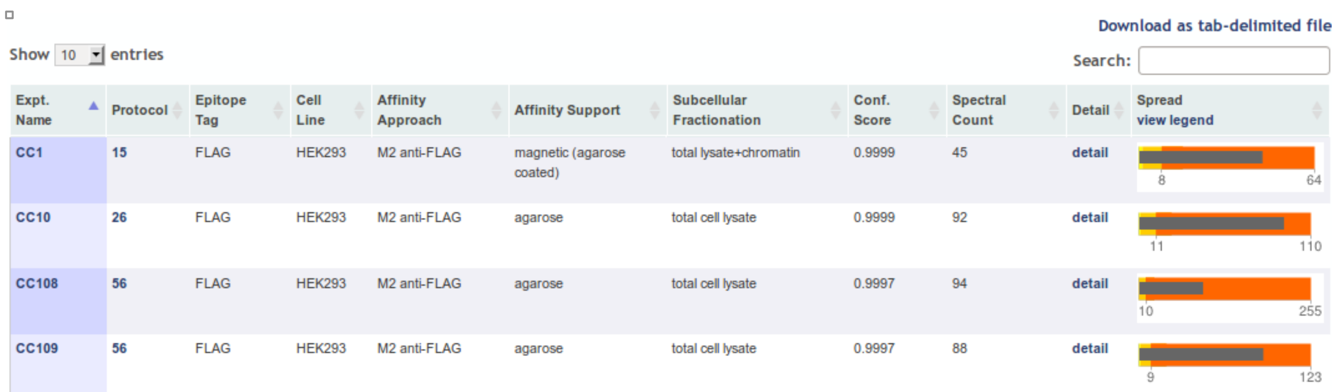


Figure 3.3. Detailed view of PRMT5 as a contaminant across the CRAPome. *Left*: abundance distribution of PRMT5 (spectral counts) across all experiments. While ~200 AP-MS analyses revealed no peptides for PRMT5, this protein was detected with a large spread of spectral counts across the remaining experiments. *Right*: distribution of the identification of PRMT5 across different epitope tag purifications. In this case, PRMT5 is frequently detected across FLAG purifications (94%; mouse over the bar to see the frequency listed as fraction of total, 123/131), but much less so across GFP (11/64) or Strep-HA (2/136) purifications, indicating that its contaminant propensity may be linked specifically to the FLAG epitope or the anti-FLAG antibody. Only those attribute values are shown on the plot that have at least one associated experiment in CRAPome where the queried protein was identified (e.g. PRMT5 was not identified in any TAP experiments, and thus TAP is not shown). Note that this graph can also be redrawn to show the distribution for the type of affinity support, cell line, or subcellular fractionation, to further explore contaminant behavior.

In addition to these summary views, the actual identifications of the protein in each experiment in the CRAPome repository (along with the identification scores and spectral counts) are listed below the figures in a tabular format (**Fig. 3.4**). The protein abundance distribution for each control (with protein abundances measured by their spectral counts) is also shown in a small box plot-like figure. The grey bar represents the spectral counts for the protein of interest. The background bands, from light yellow to dark orange colors, represent the 1st, 2nd, 3rd and 4th quartiles, respectively. When the grey bar representing the protein spectral counts is in the dark orange area, this protein is amongst the most abundant proteins in the corresponding CRAPome control.



Download as tab-delimited file

Show 10 entries

Search:

Expt. Name	Protocol	Epitope Tag	Cell Line	Affinity Approach	Affinity Support	Subcellular Fractionation	Conf. Score	Spectral Count	Detail	Spread view legend
CC1	15	FLAG	HEK293	M2 anti-FLAG	magnetic (agarose coated)	total lysate+chromatin	0.9999	45	detail	8 64
CC10	26	FLAG	HEK293	M2 anti-FLAG	agarose	total cell lysate	0.9999	92	detail	11 110
CC108	56	FLAG	HEK293	M2 anti-FLAG	agarose	total cell lysate	0.9997	94	detail	10 255
CC109	56	FLAG	HEK293	M2 anti-FLAG	agarose	total cell lysate	0.9997	88	detail	9 123

Figure 3.4. Detailed view of the Experiments (column "Expt. Name") in which PRMT5 was detected, alongside its spectral counts, linked protocol and controlled vocabularies. The column "Conf. Score" refers to the identification of PRMT5 in the mass spectrometry experiment (the values for this score is the maximum PeptideProphet probability across all peptides mapping to that protein). Lastly, the column "Spread" shows the protein identified with the maximal spectral counts as a colored bar (separated in quartiles; max counts are shown – click on "view legends" for details) and the spectral counts for PRMT5 are shown as a grey bar. Click on the "View legend" button to see the color mapping (**Fig. 3.5**).

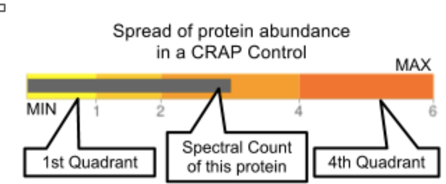
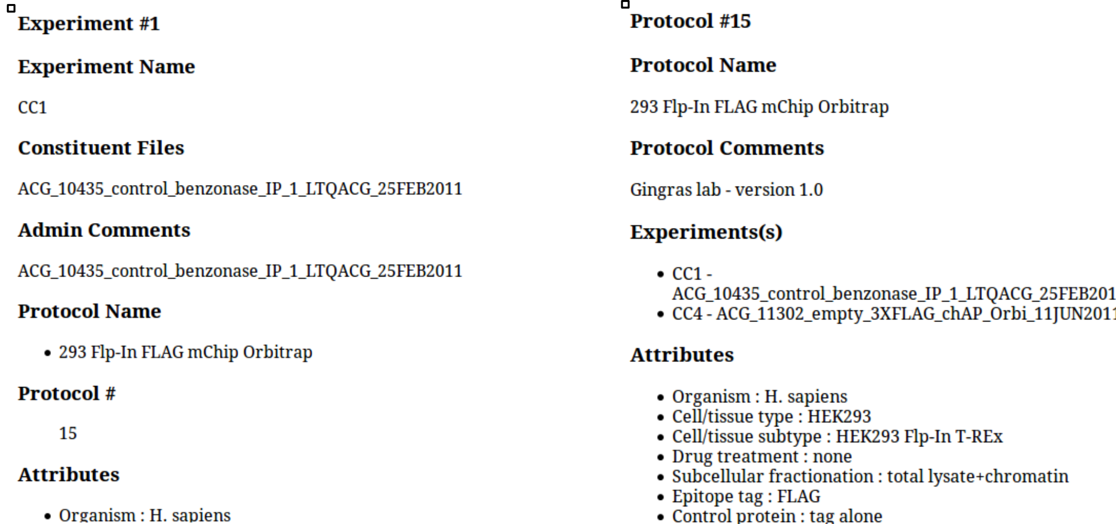


Figure 3.5. Color-coding map of the quartile information shown in the "Spread" column of Fig. 3.4.

The numbers in the Experiment Name and Protocol columns (as shown in **Fig. 3.4**) are hyperlinks. By clicking on these links, information about the experiment and the experimental protocol are shown in new windows as in **Fig. 3.6**.



Experiment #1

Experiment Name

CC1

Constituent Files

ACG_10435_control_benzonase_IP_1_LTQACG_25FEB2011

Admin Comments

ACG_10435_control_benzonase_IP_1_LTQACG_25FEB2011

Protocol Name

- 293 Flp-In FLAG mChip Orbitrap

Protocol #

15

Attributes

- Organism : H. sapiens

Protocol #15

Protocol Name

293 Flp-In FLAG mChip Orbitrap

Protocol Comments

Gingras lab - version 1.0

Experiments(s)

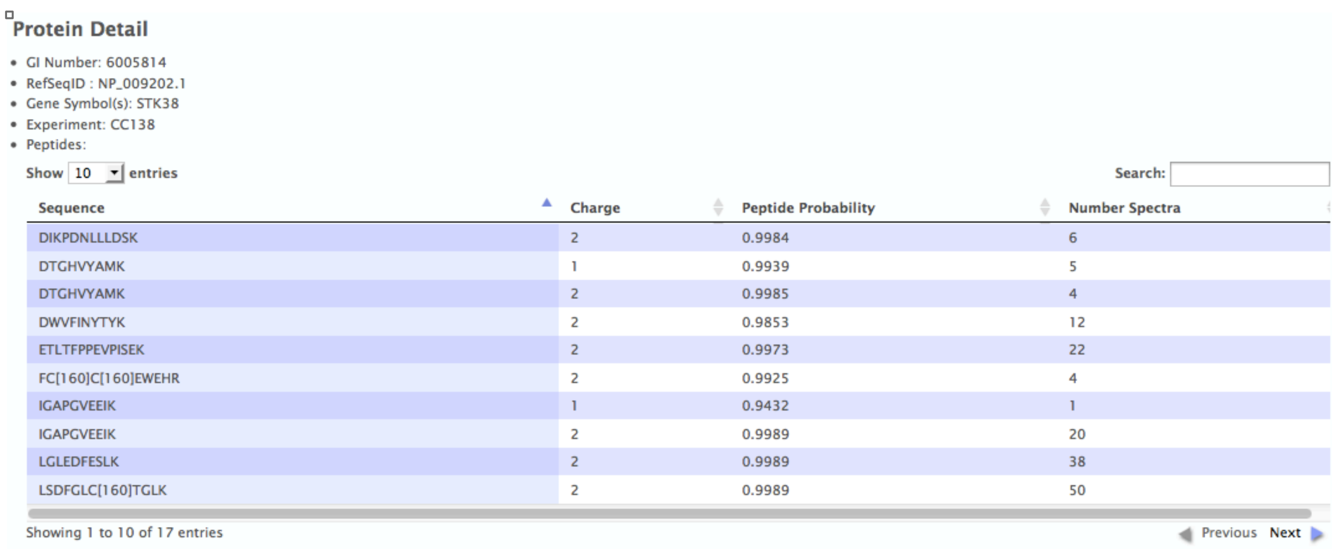
- CC1 - ACG_10435_control_benzonase_IP_1_LTQACG_25FEB2011
- CC4 - ACG_11302_empty_3XFLAG_chAP_Orbi_11JUN2011

Attributes

- Organism : H. sapiens
- Cell/tissue type : HEK293
- Cell/tissue subtype : HEK293 Flp-In T-REx
- Drug treatment : none
- Subcellular fractionation : total lysate+chromatin
- Epitope tag : FLAG
- Control protein : tag alone

Figure 3.6. Experiment (left) and Protocol (right) information. Only the top of the page is show; scroll down for additional information.

By clicking on the 'detail' link in the Spectral Count column, more detailed information about the protein is shown, including the list of identified peptides, along with their probabilities and spectral counts (**Fig. 3.7**).



Protein Detail

- GI Number: 6005814
- RefSeqID : NP_009202.1
- Gene Symbol(s): STK38
- Experiment: CC138
- Peptides:

Show 10 entries Search:

Sequence	Charge	Peptide Probability	Number Spectra
DIKPDNLLLDSK	2	0.9984	6
DTGHVYAMK	1	0.9939	5
DTGHVYAMK	2	0.9985	4
DWVFINYTYK	2	0.9853	12
ETLTFPPEVPISEK	2	0.9973	22
FC[160]C[160]EWEHR	2	0.9925	4
IGAPGVEEIK	1	0.9432	1
IGAPGVEEIK	2	0.9989	20
LGLEDFESLK	2	0.9989	38
LSDFGLC[160]TGLK	2	0.9989	50

Showing 1 to 10 of 17 entries Previous Next

Figure 3.7. Peptide summary view for a selected protein (STK38) in one experiment (CC138)

The summary table can be downloaded as an Excel-compatible table (**Fig. 3.7**).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	id	ipName	protID	numSpec	exptSum	exptFreq	exptMin	expt2ndQ	expt3rdQ	exptMax	protocol	epitopeTag	cellLine	affinitySupport	subcellularFrac	proteinProb	maxUniqPepProb	maxIniProb
2	81608	CC189	PRMT5	2950	10077	137	1	60	24	2950	66	FLAG	HEK293	agarose	total cell lysate	0.9998	0.9998	0.9998
3	83271	CC192	PRMT5	2853	10744	137	0	68	26	2853	66	FLAG	HEK293	agarose	total cell lysate	0.9998	0.9998	0.9998
4	81480	CC190	PRMT5	2830	9861	137	1	70	26	2830	66	FLAG	HEK293	agarose	total cell lysate	0.9998	0.9998	0.9998
5	81060	CC191	PRMT5	2521	7887	137	0	46	15	2521	66	FLAG	HEK293	agarose	total cell lysate	0.9998	0.9998	0.9998

Figure 3.8. Summary table. The columns are as follows: A) ID (a unique identifier for the detection of a given protein across the database; B) ipName, the unique identifier for the experiment in the CRAPome; C) protID, the mapped Official Gene Symbol; D) numSpec, the spectral counts associated with PRMT5 in the experiment; E) exptSum, the sum of all spectra assigned in the experiment; F) exptFreq, how often PRMT5 has been detected across the CRAPome; G-J) quartiles (defined by their spectral count boundaries); K) protocol used for the experiment; L-O) Selected controlled vocabulary values; P-R) protein probability scores.

4. Workflow 2: Create contaminant lists

This workflow allows the user to download subsets of data from the CRAPome repository. Each control experiment (CRAPome Control, or CC) is assigned a unique identifier (CC1-CCx), linked to a protocol, and annotated with standard vocabulary (such as the epitope tag type, cell line, affinity matrix, etc.). These attributes can be used to filter the list of available CRAPome controls. These filters are available on the left as shown in **Fig. 4.1**.

Step 1: Use the filters on the left (**Fig. 4.1**) to narrow down the list of negative controls.
Step 2: "Add" each desired CRAPome control of interest by clicking the button in the table. If desired, select the "Add All" button instead. Added controls will appear in the "Selected controls" box on the right. *There is a limit of 30 controls that can be selected at the same time*. A link at the top of the home page provides an option to download the entire database content as a tab-delimited text.
Step 3 (*optional*): Give this list of selected controls a name and save it for future use (note that this option is restricted to registered users). If you wish to reload a previously saved list, you can do so by clicking the "load" link.

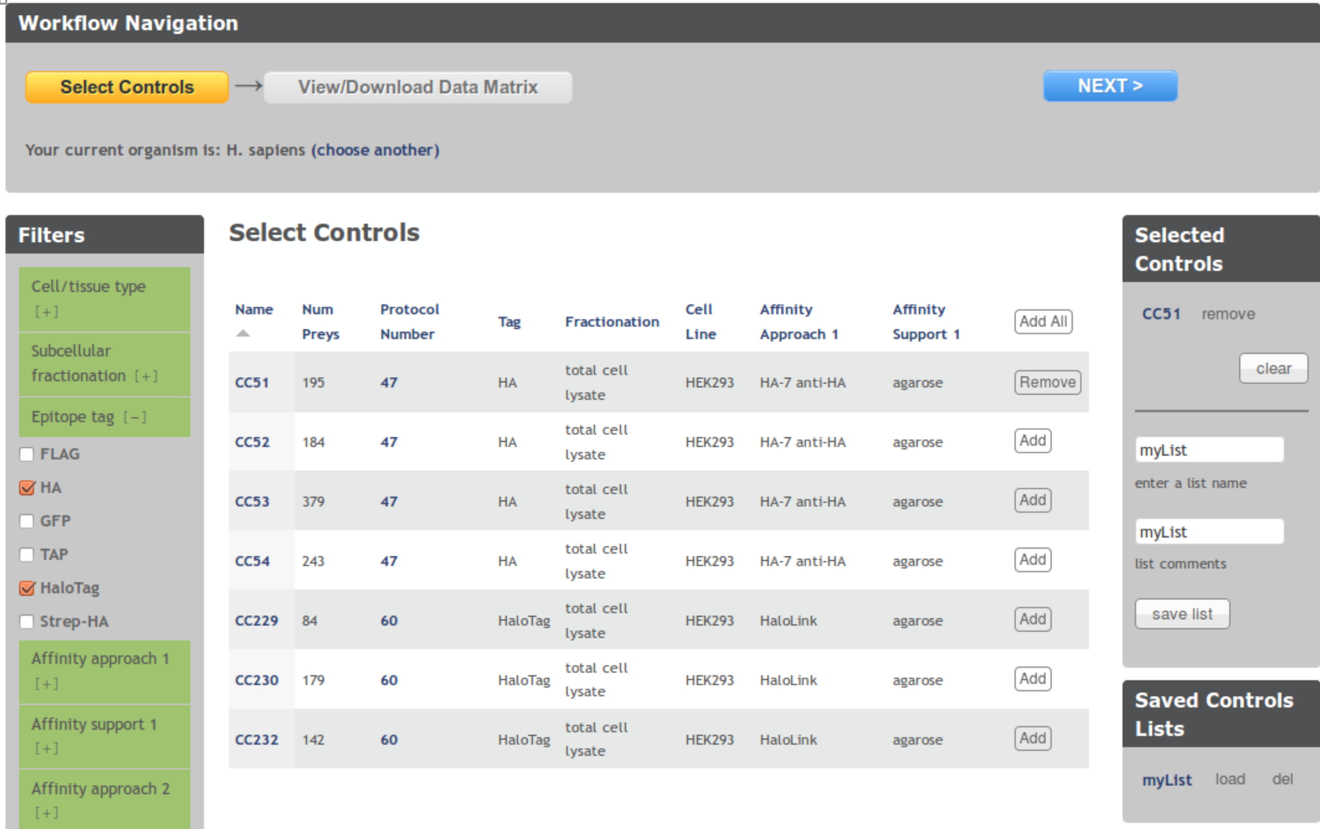
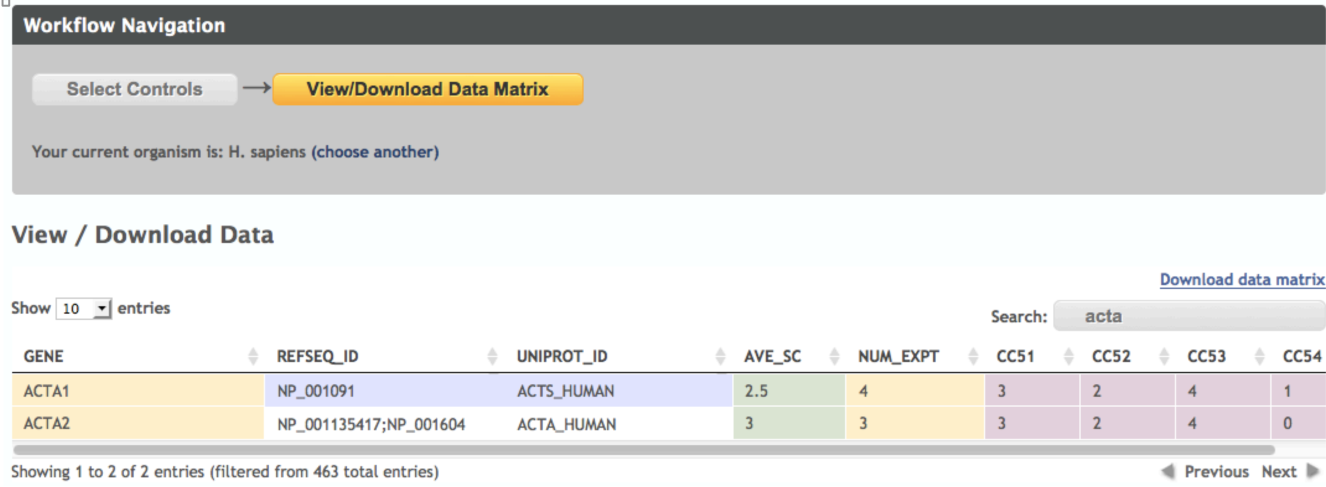


Figure 4.1. Selection of CRAPome controls in User Workflow 2. *Left panel*: available controlled vocabularies to filter the list of controls (selecting different options across categories is equivalent to an "*and*" function; selecting multiple boxes within a category corresponds to an "*or*" function, as shown here with the selection of HA tag and HaloTag experiments from the database. *Middle*: table of the controls that passed the selected filters. Clicking on the control name (first column) or the protocol name (third column) displays extended information. The second column lists the number of proteins identified in each of the controls. A limited view of the controlled vocabularies is shown in columns 4-8. Controls are added to the list by clicking on the "Add" or "Add All" buttons in the last column (note that added controls can also be manually removed). Alternatively, controls can be added by loading a previously saved list (*right, bottom*). Once the desired controls are selected, the data table can be generated by pressing the blue "Next" button at the top right of the page.

Step 4: Click on "Next" button at the top of the page to view and/or download the data matrix (**Fig. 4.2**). The data matrix can be downloaded as an Excel compatible table using the "download data matrix" option.

Step 5 (*optional*): Specific proteins can be queried in the data matrix by typing partial or complete gene name (wild cards are automatically added at the beginning and end).



GENE	REFSEQ_ID	UNIPROT_ID	AVE_SC	NUM_EXPT	CC51	CC52	CC53	CC54
ACTA1	NP_001091	ACTS_HUMAN	2.5	4	3	2	4	1
ACTA2	NP_001135417;NP_001604	ACTA_HUMAN	3	3	3	2	4	0

Figure 4.2. Detailed table output for the User Workflow 2 – limited for search term "ACTA" across all experiments selected based on "Epitope tag = HA" in the previous step. The complete list of the proteins identified across the selected CRAPome controls is shown by default. It can be also restricted to a selected search term (here: "ACTA"). NUM_EXPT is the number of experiments (among the experiments selected in the previous step) in which the protein was identified (4 and 3 experiments for ACTA1 and ACTA2, respectively). Also shown are the averaged spectral counts (AVE_SC) across the experiments in which the protein was identified, and the spectral counts in each of the selected experiments (CC51, CC52, CC53, CC54; highlighted in pink cells). Mapped IDs (RefSeq ID and Uniprot ID) are also provided in the table.

5. Workflow 3: Use the CRAPome to analyze your data.

This workflow allows the user to process his/her data online using the CRAPome controls and the scoring tools implemented within the system. This workflow is only available to registered users. The minimum requirement is for the user to submit information regarding one bait (one sample), though we strongly advocate the use of biological replicates for the bait, and recommend that the user also uploads his/her own negative control runs.

Step 1: Select the CRAPome database controls that are most similar to the user data using controlled vocabularies and detailed protocols as shown for workflow 2 above (see **Fig. 4.1**). Selected controls can be saved as a list and reloaded as needed as in workflow 2. Press the blue "Next" button to navigate to the next page.

Step 2: Upload user data (See **Fig. 5.1**). The data should be formatted as per instructions on the webpage (also see **Fig. 5.2**). Once uploaded, the data appear in the 'user data' section below.

Step 3 (optional): If the user would like to exclude some of his/her data from the analysis, it can be done at this stage by clicking on 'remove' button. Similarly, one can go back and add/remove CRAPome controls. For a quick preview of the data matrix, click on 'Preview Data Matrix'. After the analysis is complete, the data can be deleted by clicking on 'clear uploaded user data' (See **Fig. 5.1**).

Step 4: Proceed to the analysis section by clicking on 'Next'. Here, Fold Change calculations and SAINT probability scoring can be used to generate ranked lists of bait-prey interactions.

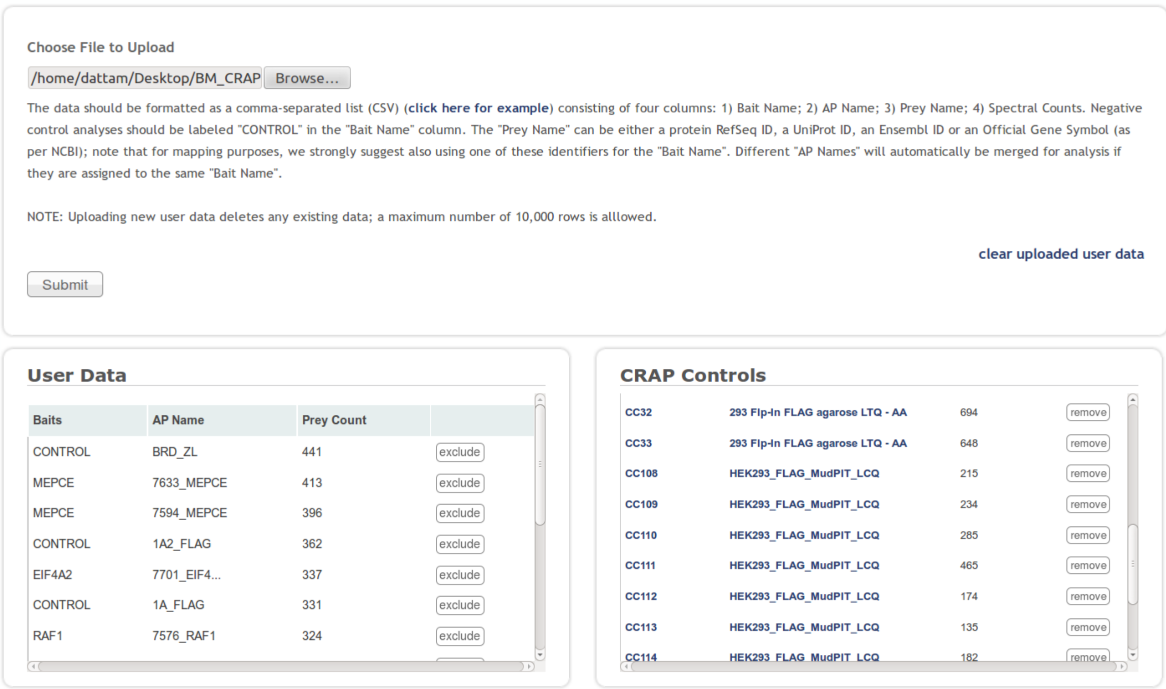


Figure 5.1. Upload user data. The top of the page enables browsing to upload the user data prepared in a comma-separated values (CSV) file (see **Fig. 5.2**). The table should not have headers and should consist of four columns: 1) Bait Name; 2) AP Name (the name you are giving to this particular affinity purification); 3) Prey Name; 4) Spectral Counts. Negative control analyses should be labeled "CONTROL" in the "Bait Name" column. The "Prey Name" can be either a RefSeq protein ID, Ensembl protein ID, Uniprot entry name, Uniprot entry ID or gene symbol (SGD systematic gene name or standard name for *S. cerevisiae*). For mapping purposes, we strongly suggest also using one of these identifiers for the "Bait Name". Different "AP Names" will automatically be merged for analysis if they are assigned to the same "Bait Name". The bottom left of the page lists the User data that was uploaded while the bottom right lists the selected CRAPome controls.

	A	B	C	D
1	RAF1	7626_RAF1	NP_006752	406
2	RAF1	7576_RAF1	NP_006752	398
3	RAF1	7576_RAF1	NP_002871	388

Figure 5.2. Sample column format for upload. Column A is the BAIT name, column B the identifier for the experiment (AP name), column C the PREY identifier (here RefSeq protein ID) and column D is the spectral count for the PREY in the AP.

Step 5: Select desired scoring options for Fold Change calculations (**Fig. 5.3**). Two different Fold Change calculations are generated by default. The first one (FC-A; standard) estimates the background by averaging the spectral counts across the selected controls while the second one (FC-B; stringent) estimates the background by combining the top 3 values for each prey. Combining scores from biological replicates of a bait purification is performed in FC-A by a simple averaging, while FC-B performs a more stringent geometric mean calculation. These parameters are preselected by default, but may be modified by the user as required. The user can also specify what set of controls to use (user controls alone or in combination with selected CRAPome controls). A series of worked examples of the use of the CRAPome for scoring interactions is made available on the CRAPome site, under “Supplementary data”.

Step 6 (optional): The user can specify whether to run SAINT or not, and which SAINT options (‘lowMode’, ‘minFold’, ‘norm’) should be employed. Briefly, “lowMode” is an option useful when looking at interconnected datasets (i.e. datasets in which the baits share interactors). In the “lowMode” default setting (“off”), interactions which are detected with multiple baits are penalized, since SAINT expect them to be frequent fliers. Turning lowMode “on” partially alleviates this penalty, enabling more

sensitive detection. “minFold” defines the quantitative separation (minimum quantitative fold change) between the test samples and the controls before an interaction is deemed significant. In the default “minFold = on” setting, a minimum 10 fold separation rule between false and true interaction distributions is enforced; this increases the stringency of the filtering, especially towards proteins which are frequently detected across the negative control runs used in the modeling. Finally, “norm” is a normalization step, which takes into account the total number of spectra identified in an experiment (typically, negative controls have less such counts than a test experiments, and “norm = on” is therefore conservative in that it relatively boosts the quantitative values for the controls as compared to the test samples). For additional details regarding SAINT and the options, please refer to the Choi et al., Current Protocols in Bioinformatics (PMID 22948730 – a pdf version of the SAINT protocol paper is provided in the “Supplementary data” section of the CRAPome). As with the Fold Change calculations, the user may select which controls to use, and how replicates should be combined. Note that if the number of controls is greater than 10, SAINT generates 10 “virtual controls” by selecting the 10 highest counts for each protein. This accelerates the data processing, but also provides a more conservative analysis of the dataset.

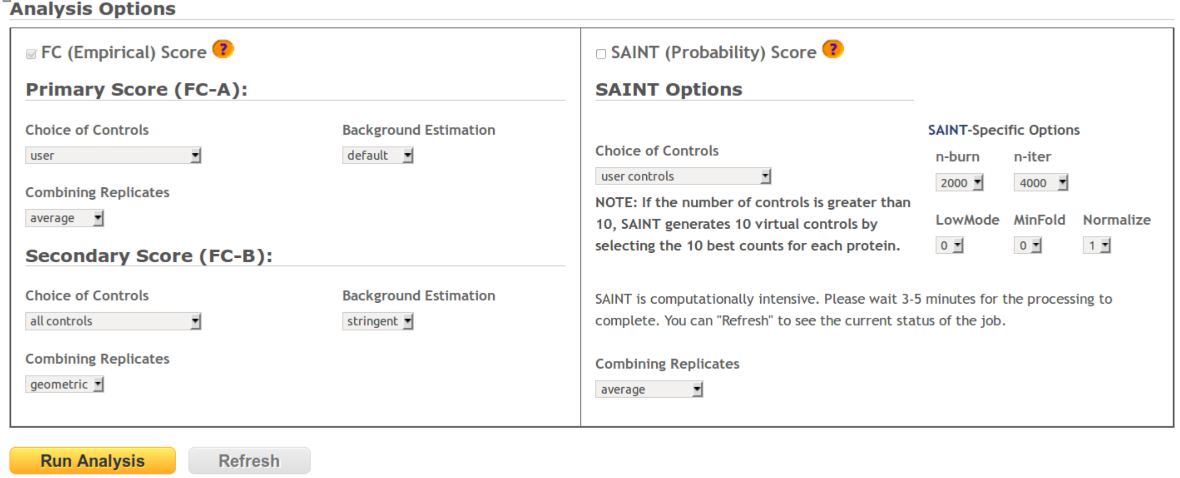


Figure 5.3. Interface for data analysis using CRAPome. The left panel displays the interface for running the Fold Change empirical score; the CRAPome automatically generates two FC-scores as part of the analysis, the second one being designed as a more stringent score by default. Parameters can be modified as described above. The right panel displays the optional SAINT scoring, associated with model options.

Step 6: Once the desired options are selected, press “Run Analysis”. The new entry will appear at the top of the ‘Analysis Results’ list (the list includes all previous jobs run by the user). Initially, the Status (last column) of the new job will be shown as ‘queued’ (followed by ‘running’ and then ‘complete’). The column “Score Options” lists selected options for the Fold Change calculations for both the primary (FC-A; here labeled S1) and the secondary, more stringent (FC-B, here labeled S2) scores. SAINT options (when applicable) are listed in the next column.

Analysis Results

Job ID	Date	Score Options	SAINT Options	Status	Actions	
682	2012-12-07 14:59:56	S1(user,default); S2(all,stringent)	C(User);iter(2000,4000); LM=0,MF=0,Norm=1	complete	view results	delete
681	2012-12-07 14:55:48	S1(user,default); S2(all,stringent)	C(User);iter(2000,4000); LM=0,MF=0,Norm=1	complete	view results	delete
666	2012-12-06 16:05:41	S1(user,default); S2(all,stringent)	-	complete	view results	delete

Figure 5.4. Job status. This table lists all analyses performed by the user, alongside the selected options for the scoring.

Step 7: Refresh the web page periodically by clicking on 'Refresh'. When the job is finished and the results are ready to be viewed, the Status will change to 'complete'. A link called 'view results' will appear. The user will also receive an email notification with a link to the results page.

Step 8: Click on 'view results' link to view the results. At the top of the page, you will see graphical views of the data that summarize the results for each of the baits analyzed, or for all baits at once. The left panel compares SAINT (when run) to FC-A; when SAINT is not used, this panel displays a comparison between FC-A and FC-B (see **Fig. 5.5**). In both cases, the left panel describes the Receiver Operating Characteristic (ROC) analysis of the scoring (benchmarked to the interactions reported in iRefIndex). This visualization can assist in deciding which scoring function to use on the data. The middle panel displays a histogram of the interactions reported in iRefIndex versus those not reported, at different bins of SAINT probability or FC-A score when SAINT was not run. Finally, the panel on the right compares two different scores (by default, SAINT and FC-B if SAINT is used; FC-A vs. FC-B otherwise) at the level of individual proteins. Mousing over any of the graphs will display relevant information (e.g. gene names).

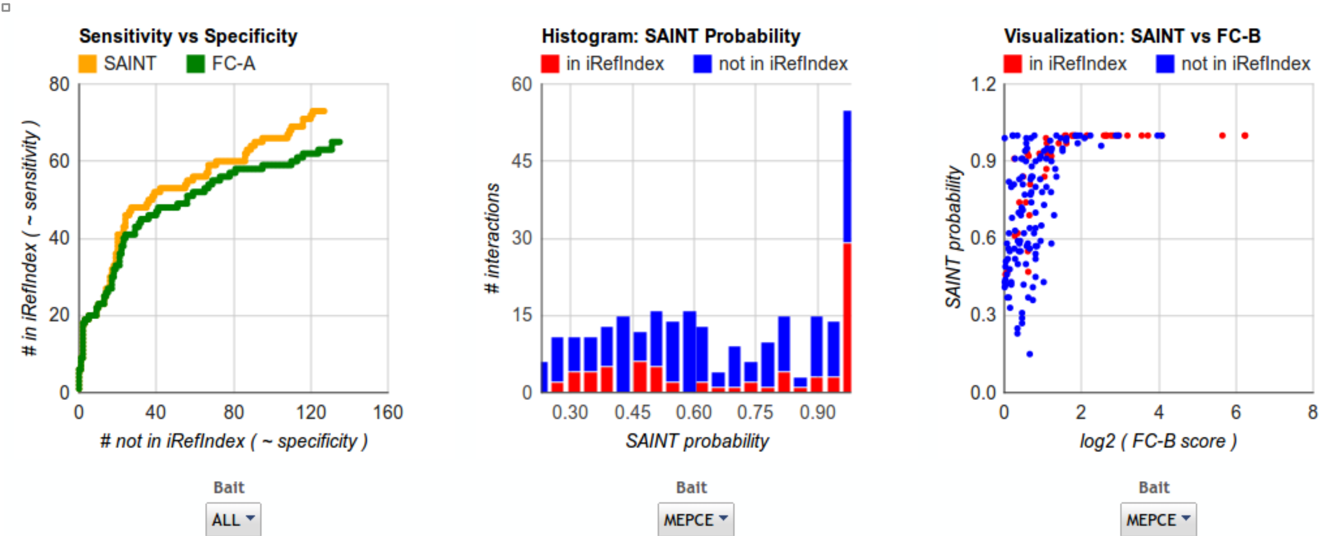
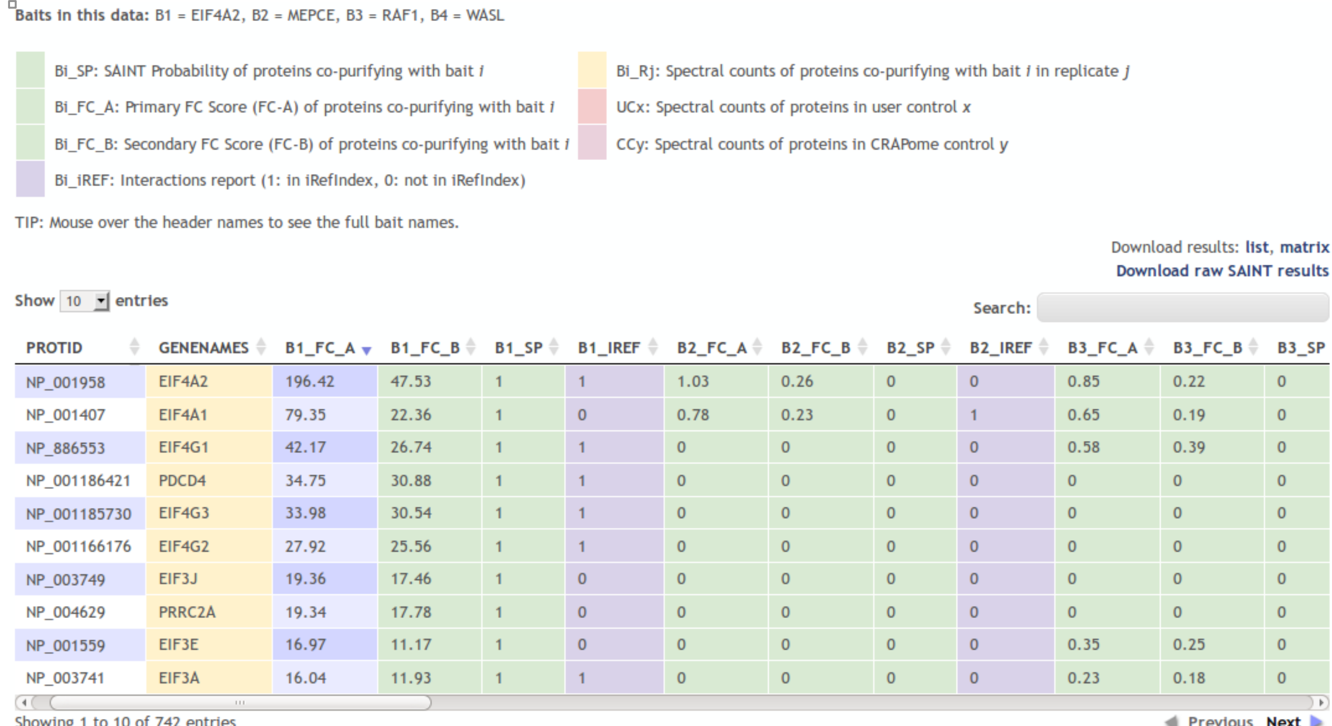


Figure 5.5. Graphical views of the data. Three different graphs are drawn for each analysis. Color coding on the middle and right graphs corresponds to the information present (red) or absent (blue) from the iRefIndex. When more than one bait was employed, use the dropdown menu below each graph to generate figures for the individual baits, or for all baits combined.

Step 9: The results can be viewed online in a matrix form or downloaded in a tabular format (see **Fig. 5.6**)



Baits in this data: B1 = EIF4A2, B2 = MEPCE, B3 = RAF1, B4 = WASL

- Bi_SP: SAINT Probability of proteins co-purifying with bait *i*
- Bi_FC_A: Primary FC Score (FC-A) of proteins co-purifying with bait *i*
- Bi_FC_B: Secondary FC Score (FC-B) of proteins co-purifying with bait *i*
- Bi_IREF: Interactions report (1: in iRefIndex, 0: not in iRefIndex)
- Bi_Rj: Spectral counts of proteins co-purifying with bait *i* in replicate *j*
- UCx: Spectral counts of proteins in user control *x*
- CCy: Spectral counts of proteins in CRAPome control *y*

TIP: Mouse over the header names to see the full bait names.

Download results: **list**, **matrix**
Download raw SAINT results

Show 10 entries

Search:

PROTID	GENENAMES	B1_FC_A	B1_FC_B	B1_SP	B1_IREF	B2_FC_A	B2_FC_B	B2_SP	B2_IREF	B3_FC_A	B3_FC_B	B3_SP
NP_001958	EIF4A2	196.42	47.53	1	1	1.03	0.26	0	0	0.85	0.22	0
NP_001407	EIF4A1	79.35	22.36	1	0	0.78	0.23	0	1	0.65	0.19	0
NP_886553	EIF4G1	42.17	26.74	1	1	0	0	0	0	0.58	0.39	0
NP_001186421	PDCD4	34.75	30.88	1	1	0	0	0	0	0	0	0
NP_001185730	EIF4G3	33.98	30.54	1	1	0	0	0	0	0	0	0
NP_001166176	EIF4G2	27.92	25.56	1	1	0	0	0	0	0	0	0
NP_003749	EIF3J	19.36	17.46	1	0	0	0	0	0	0	0	0
NP_004629	PRRC2A	19.34	17.78	1	0	0	0	0	0	0	0	0
NP_001559	EIF3E	16.97	11.17	1	0	0	0	0	0	0.35	0.25	0
NP_003741	EIF3A	16.04	11.93	1	1	0	0	0	0	0.23	0.18	0

Showing 1 to 10 of 742 entries — Previous **Next**

Figure 5.6. Results table. Preys are listed in rows. The columns are as follows: PROTID, the protein name in the upload file; GENENAMES, the mapped Gene Name. The rest of the columns describe the data: B1 indicates Bait 1 (in this case, EIF4A2). FC_A and FC_B are Fold Changes A and B, respectively; SP is the SAINT probability, and IREF denotes an interaction reported in iRefIndex (1 = reported; 0 = not reported). The table has many more columns than can fit in this window, including spectral counts for every replicate (R1 is replicate 1) of a bait purification, and for every selected control. The table can be downloaded in matrix and list formats (compatible with Cytoscape). SAINT results files can also be downloaded.

Annotator Manual – CRAPome version 1.0

Prepared by Datta Mellacheruvu; Edited by Anne-Claude Gingras, December 2012

1: Overview

The CRAPome is a repository of negative controls performed in affinity purification coupled with mass spectrometry (AP-MS) experiments. Negative controls are collected from various studies (published or unpublished), processed, annotated and made available for download and analysis via an online interface. See User Manual for details.

An Annotator is usually the contributor of mass spectrometry data to the CRAPome. Contributors first submit raw mass spectrometry files to the CRAPome administrator. The administrator processes them to yield protein identifiers and spectral counts, assigns an experiment number to each of the files that passed a quality control step (these experiments are labeled CCx; CRAPome Control x), and releases them for annotation. The Annotator defines protocols to describe the experimental procedures and links the protocols to each experiment. Protocols include controlled vocabularies and free text.

2. Accessing the system as an Annotator and viewing existing experiments and protocols

Annotators are assigned a higher level of privileges than regular registered users. They can create protocols and link protocols to experiments. Annotator-level login access can be requested by emailing the CRAPome administrator. Use the login credentials to enter the CRAPome (**Fig. 2.1**). The Annotator menu bar will look like **Fig. 2.2**.

CRAPome
Contaminant Repository for Affinity Purification
An online database of common contaminants in AP-MS experiments
Home About Tutorial API Login/Register
User account
Create new account | Log in | Request new password
Username *
Enter your CRAPome username.
Password *
Enter the password that accompanies your username.
Log in

Figure 2.1. Welcome screen at the www.CRAPome.org database. Enter username and password as prompted.

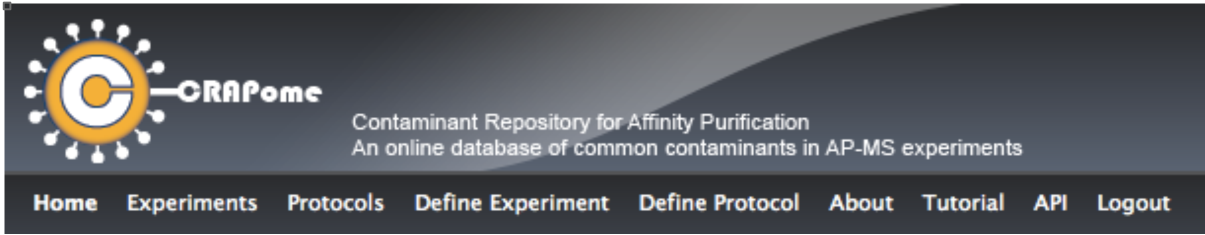


Figure 2.2. Annotator menu bar. "Experiments" lists all the experiments that the annotator has access to (those that have been contributed by their laboratory). "Protocols" lists all the protocols in the CRAPome, but enables editing only those protocols belonging to the Annotator. "Define Experiment" and "Define Protocol" enable the creation of new data.

Select the "Experiments" tab to view the list of all experiments contributed by the Annotator laboratory (**Fig. 2.3**). Click on an Experiment Name (here CC40) to view the associated details (**Fig. 2.4**). Similarly, select the "Protocols" tab to view the list of all protocols available in the CRAPome (only those protocols contributed by the Annotator laboratory can be edited; **Fig. 2.5**). Clicking on the name or protocol number opens a new window with the protocol details (**Fig. 2.6**).

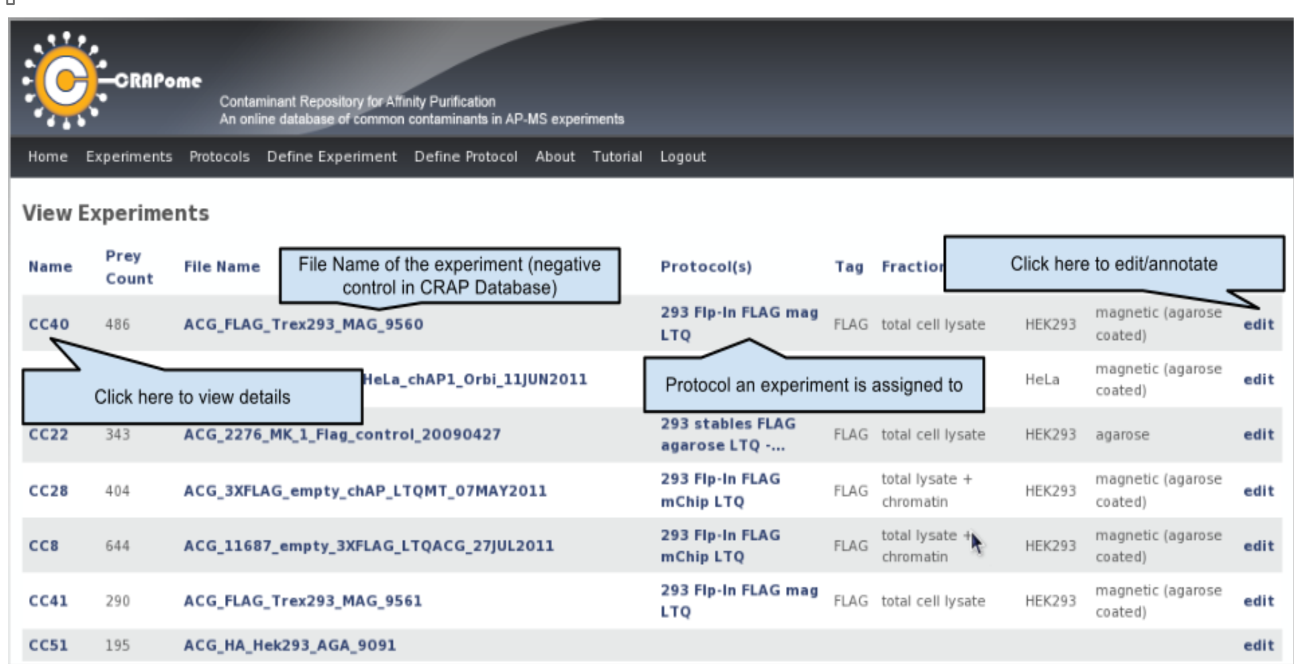


Figure 2.3. Experiment View. The procedure for creating and editing protocols will be described in section 3.

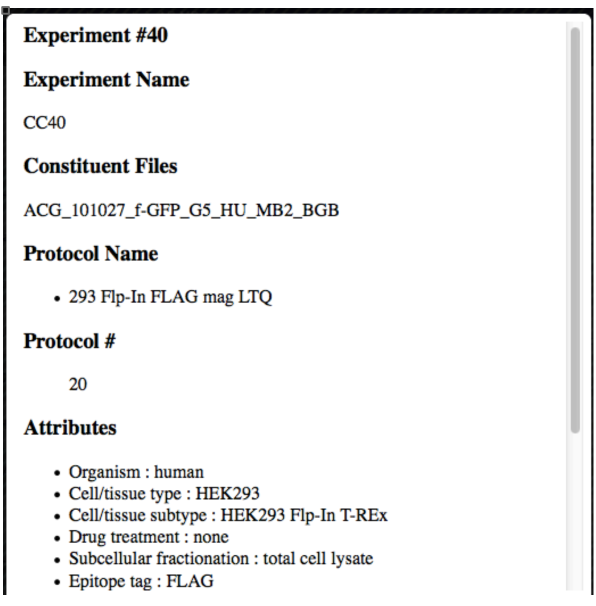


Figure 2.4. Experiment details. Only the top portion of the Experimental details view is shown.

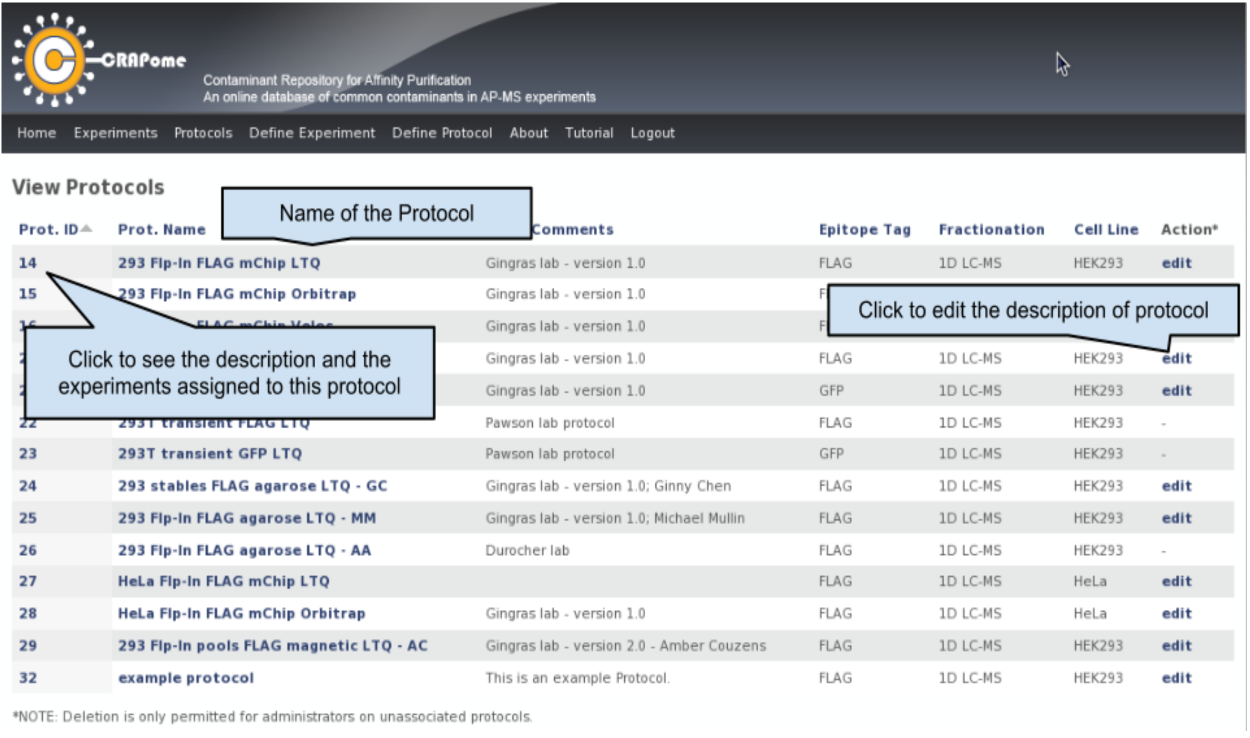


Figure 2.5. Protocol View. The procedure for creating and editing protocols will be described in section 3.

Protocol #14

Protocol Name

293 Flp-In FLAG mChip LTQ

Protocol Comments

Gingras lab - version 1.0

Experiments(s)

- CC2 - ACG_10783_empty_3XFLAG_chAP_LTQACG_08APR2011
- CC3 - ACG_10986_empty_3xFLAG_chAP_3_LTQMT_07MAY2011
- CC8 - ACG_11687_empty_3XFLAG_LTQACG_27JUL2011
- CC28 - ACG_3XFLAG_empty_chAP_LTQMT_07MAY2011

Attributes

- Organism : human
- Cell/tissue type : HEK293
- Cell/tissue subtype : HEK293 Flp-In T-REx
- Drug treatment : none

Figure 2.6. Protocol Details. Only the top portion of the Experimental details view is shown.

3. Creating and editing protocols and experiments

The main responsibility of the Annotator is to define Protocols and link Experiments to the protocols. An annotator can also edit protocols and experiments that belong to their research group. **Fig. 3.1** summarizes the different steps of the annotation process.

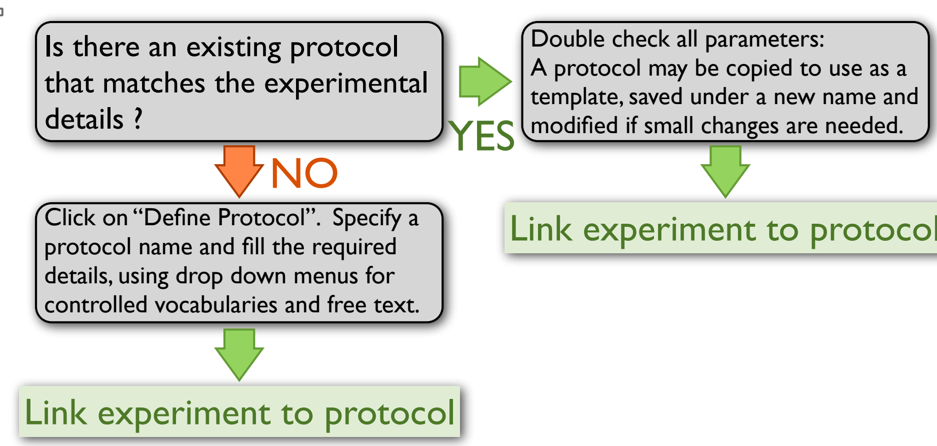


Figure 3.1. Flowchart of the Annotator tasks.

Task 1: Define/Edit Protocol

The first task of the Annotator is to define a protocol that corresponds to the experiments to be annotated. Create a new protocol by clicking the "Define Protocol" tab: Fill in the requested information, including a descriptive name for the protocol associated with optional protocol notes. Select the controlled vocabulary by using the drop down "Attributes" (**Fig. 3.2;** See **Fig. 3.3** for current CV terms; contact the CRAPome administrator if the controlled vocabulary is inadequate), and add text-based experimental details (see **Fig. 3.4** for an example). Before creating a new protocol, review the list of the existing protocols to prevent duplication. Note, however, that since even minor changes in experimental procedures can lead to observable changes in the composition of the background contaminants, new protocols should be created that fully describe protocol details without creating obvious redundancies.

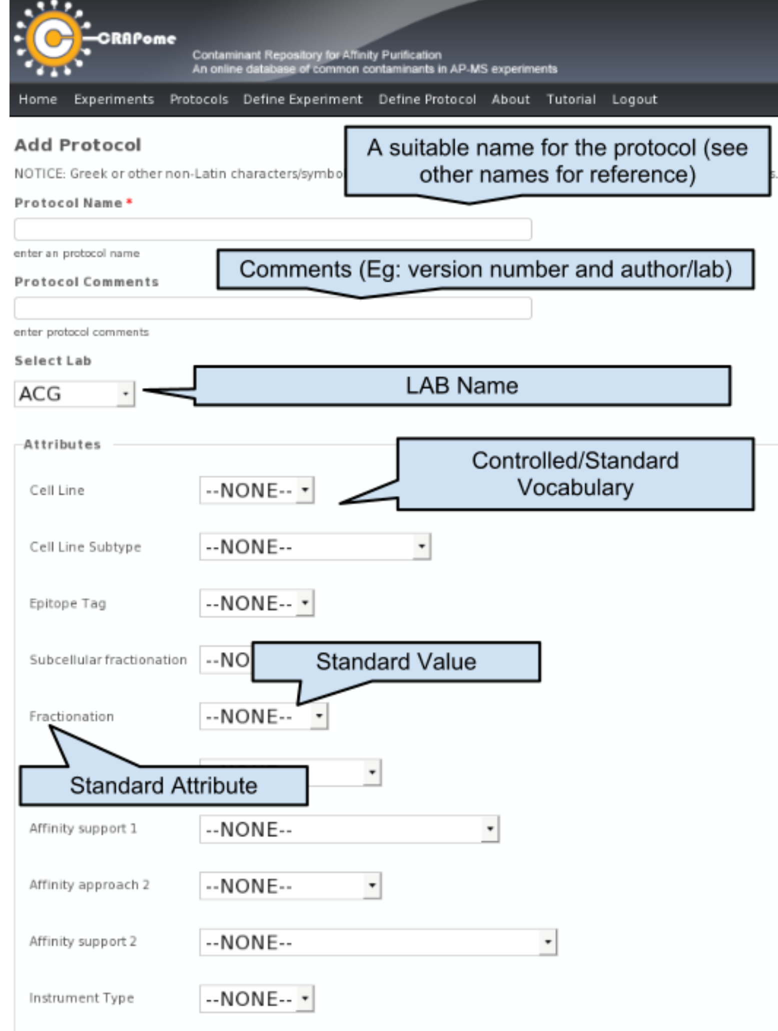


Figure 3.2. Creating a new CRAPome protocol / part A, define controlled vocabulary.

Current Attributes

Attribute Name	Attribute Values
Organism	human
Cell/tissue type	HEK293, HeLa, U2OS, PBMC, Jurkat, CEM-T, MRC-5, LS174
Cell/tissue subtype	--, HEK293T, HEK293 Flp-In T-REx, Jurkat-Flp-In
Drug treatment	aphidicolin, rapamycin, nocodazole, MG132, none, IFN-beta, DMSO, okadaic acid, doxycycline+thymidine, tetracycline+thymidine, thymidine+nocodazole
Subcellular fractionation	total cell lysate, total lysate+chromatin, nuclear fraction, cytosolic fraction
Epitope tag	FLAG, HA, GFP, TAP, HaloTag, Strep-HA
Control protein	RFP, GFP, FLAG, mCherry, tag alone, untransfected, uninduced, RFP
AP steps	single, tandem
Affinity approach 1	M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, HA-7 anti-HA, HaloLink, IgG, Streptactin, 2xFLAG, SBP, anti-GFP mouse
Affinity support 1	agarose, magnetic (dynabead), magnetic (agarose coated), nano-magnetic, microMACS
Affinity approach 2	--, M2 anti-FLAG, anti-GFP camel, anti-GFP rabbit, calmodulin, HA, 2xHA, HA-7 anti-HA, anti-GFP mouse
Affinity support 2	--, agarose, magnetic bead (dynabead), magnetic beads, agarose coated, nano-magnetic beads, microMACS
Fractionation	SDS-PAGE, 1D LC-MS, MudPIT, RP-RP, GeLC
Instrument type	Velos-Orbitrap, LTQ-Orbitrap, LTQ, LCQ, LTQ-FT, 5600 TripleTOF

Figure 3.3. Currently available controlled vocabularies (Attributes)

Biological Material

Stable pools, HEK293. Transfection of low passage HEK293 (CRL1573) with vector using lipofectamine PLUS; selection for ~14 days with 750ug/ml active G418. Amplification off cells in 5-8 x 150mm plates; harvesting at 80-95% confluence. Harvest through scraping, followed by 3 washes with PBS. Cell pellet either frozen on dry ice and stored dry at -80C, or processed immediately.

Affinity Purification

Cells were lysed by [passive lysis assisted by freeze-thaw]. Briefly, to the frozen cell pellet, 1:4 or 1:5 pellet weight:volume ratio of lysis buffer was added. Lysis buffer was 50 mM Hepes-NaOH pH 8.0, [100 mM KCl], 2 mM EDTA, [0.1% NP40], 10% glycerol, 1 mM PMSF, 1 mM DTT and Sigma protease inhibitor cocktail, P8340, 1:500. No phosphatase inhibitors were added. Resuspended pellets were incubated on ice (or on a nutator at 4°C) for 10 min to assist lysis, then pipetted up and down to break up pellet. Tubes were frozen and thawed once (liquid nitrogen or dry ice ~5min, 37°C with agitation, then put on ice, and the lysate transferred to 2 ml Eppendorf tubes. An aliquot (20ul) was taken to monitor solubility (This aliquot was spun down, the supernatant transferred to a fresh tube, and 6 μl 4X Laemmli sample buffer added to the supernatant. The pellet was resuspendended in 26 μl 2X Laemmli sample buffer). The 2 ml tubes were centrifuged at 14000 rpm for 20 min at 4°C, and the supernatant transferred to fresh 15 ml conical tubes. The protein concentration was measured (using BSA as a control). To the rest of the lysate, 25-30 μl packed [FLAG M2 agarose beads] pre-washed 4X in lysis buffer were added, and the mixture incubated 2 hours at

Peptide Preparation

Trypsin (1 μg Sigma Trypsin Singles, T7575) dissolved in 70 μl of 50 mM ammonium bicarbonate pH 8 was added each sample. Tubes were vortexed, briefly centrifuged, and incubated at 37°C overnight. After quickly centrifugating the samples, an additional amount of trypsin (0.25 μg) was added, and the samples incubated for another 3-4 hours. The samples were acidified by adding 2 μl of 50% formic acid, and lyophilized in the speed-vac. The samples were stored at –40°C. When ready for mass spectrometry, 20 μl 5% formic acid was added to the samples and the samples were centrifuged at max speed for 10 min.

LC-MS

The ammonium bicarbonate was evaporated, and the samples were resuspended in HPLC buffer A (2% acetonitrile, 0.1% formic acid), then directly loaded onto capillary columns packed in-house with Magic 5 um, 100A, C18AQ. MS/MS data was acquired in data-dependent mode (over a 65min - 2 hr acetonitrile 2-40% gradient) on a ThermoFinnigan LTQ, equipped with a Proxeon NanoSource and an Agilent 1100 capillary pump.

Publication Reference

Chen et al., J Biol Chem, 2008. PMID:18715871; Chen and Gingras, Methods, 2007. PMID: 17532517; Goudreault et al., Mol Cell Proteomics, 2009. PMID: 18782753; Kean et al., J Biol Chem.,PMID: 21561862.

Figure 3.4. Creating a new CRAPome protocol / part B, adding protocol details. Add information details pertaining to the biological material (How were the cells grown and harvested? How was the recombinant protein expressed? Has a subcellular fractionation been performed?), the affinity purification step, the procedure for preparing the peptides (including fractionation at the protein or peptide level when applicable), and details of the LC-MS/MS analysis. If the Method has been published, add citations in the “Publication reference” box.

Task 2: Link protocols to experiments

The general pipeline for the addition of experiments to the CRAPome database begins by the processing of the raw mass spectrometry files by the CRAPome administrator. The CRAPome administrator then defines the experiments with some basic information (such as the name of the spectrum file and the laboratory that deposited the data) and initiates the processing of data.

The role of the Annotator is to "edit" such experiments by linking protocols to them. To do so, the Annotator access the list of his/her experiments by selecting the "Experiments" tab at the top of the page (as in **Fig. 2.3**). Experiments which are already linked to a protocol (e.g. CC5 in **Fig. 3.4**) already have controlled vocabularies associated with them, in addition to the protocol number and protocol name. Experiments which are not yet associated are missing this information (see CC40 in **Fig. 3.4**).

To associate protocols and controlled vocabularies, select the "edit" link on the right. This will open a new window: information entered by the administrator is displayed, but not editable (please contact the CRAPome administrator to report any errors). The Annotator should change the "Experiment Status" to "Annotated" (from the default "newly added"), and link the experiment to a protocol via the drop-down menu. When the annotator selects a protocol for an experiment (see **Fig. 3.5**), all the attributes of the experiment ("controlled vocabularies") are populated with the attributes of the protocol (see **Fig. 3.6**).

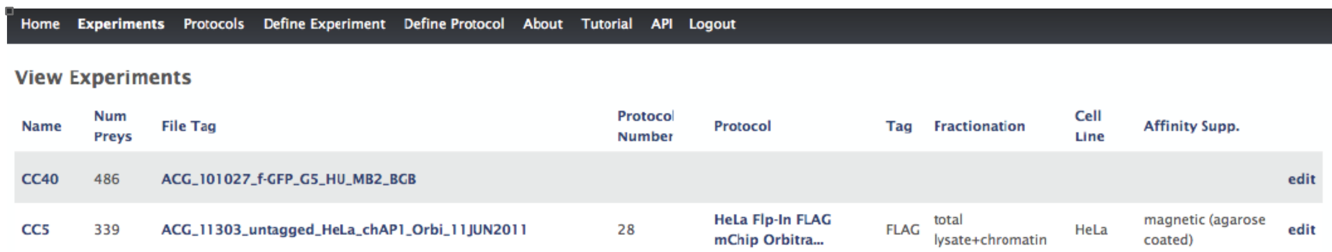


Figure 3.4. Experiments view. Clicking on "edit" on the right enable linking a protocol to the experiment.

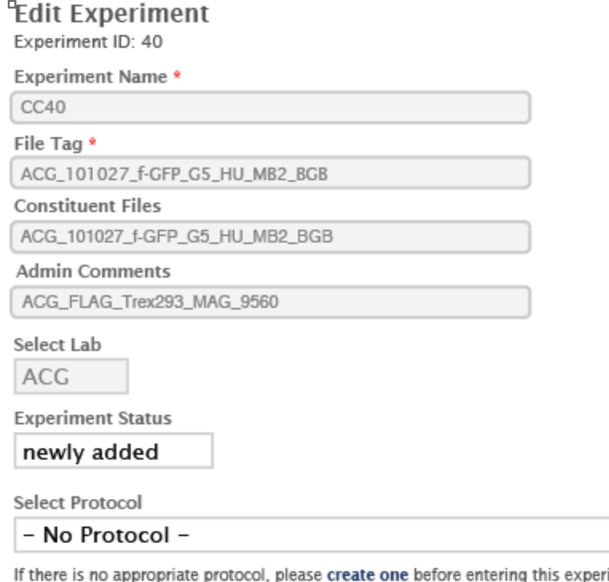


Figure 3.5. Edit experiment view. Data entered by the administrator is greyed out. Select a protocol to link to the experiment. Create new protocols as needed, as described above.

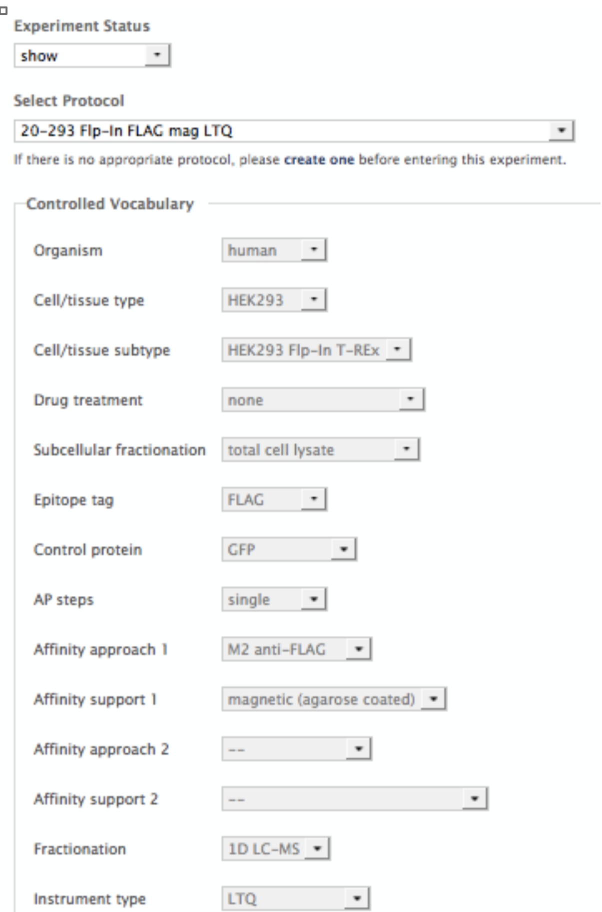


Figure 3.6. Controlled vocabularies are populated from the selected protocols.

Deleting experiments and protocols:

The annotator can only define or edit new experiments (or protocols) but cannot delete them. Each newly defined experiment has an attribute called 'status' (see **Fig. 3.4**) which can be one of a) newly added, b) annotated, c) ready for release, d) show, or e) retire. If the CRAPome administrator adds a new experiment, he/she sets the status to "newly added". The annotator can change the status to "annotated", once the annotation is complete. The status is set to "ready for release" once the spectrum file(s) are processed and the database is updated. Finally, the status is set to "show" when the data is released to the end user. Only those experiments with a status "show" can be viewed by the end user. If the annotator accidently created a wrong entry, he/she can set the status to retire. All retired experiments will be periodically purged from the database by the CRAPome administrator.

Requesting new controlled vocabularies:

The annotator can only use pre-defined controlled vocabularies (attributes): new CVs can be requested to the CRAPome administrator.