Case Studies – 1 – ZNF598 interactions

The dataset was taken from "A Novel 4EHP-GIGYF2 Translational Repressor Complex Is Essential for Mammalian Development", Masahiro Morita, et al., Mol Cell Biol. 2012 September; 32(17): 3585–3593.

The study aimed to uncover the interaction between m4EHP complex and GIGYF2. Here, we use one of the bait proteins analyzed as a part of this study, ZNF598. The bait gene was amplified from clone MGC:54362 (BC050477) and sub cloned in the EcoRI and NotI sites of pcDNA3. The FLAG-tagged construct was stably expressed in HEK293 cells (as a pool), and expressed proteins were purified on anti-FLAG M2-agarose beads. Mass spectrometric analysis was conducted in a data-dependent mode (over a 2-h acetonitrile 2 to 40% gradient) on a Thermo LTQ mass spectrometer equipped with a Proxeon Nanosource and an Agilent capillary pump.

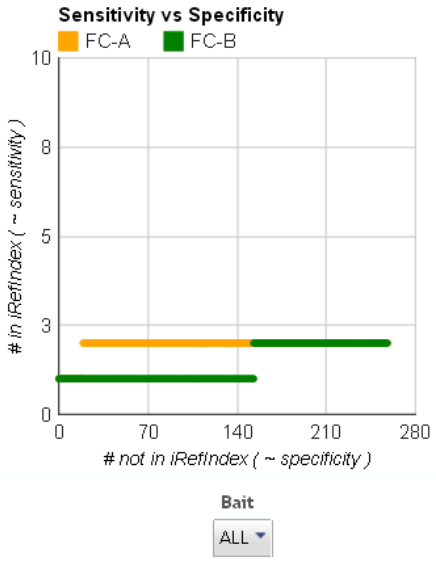
The subset of the dataset used as a case study here consisted of two replicates of ZNF598 protein purifications and two negative controls. The analysis of this dataset is challenging for the following reasons: 1) there were only two negative control runs generated, and they did not capture all contaminant proteins; 2) Bait protein AP-Ms runs contained myosin contamination; 3) Existing protein databases such as iRefIndex were incomplete with respect to protein interactions for this bait and other members of the analyzed protein complex.

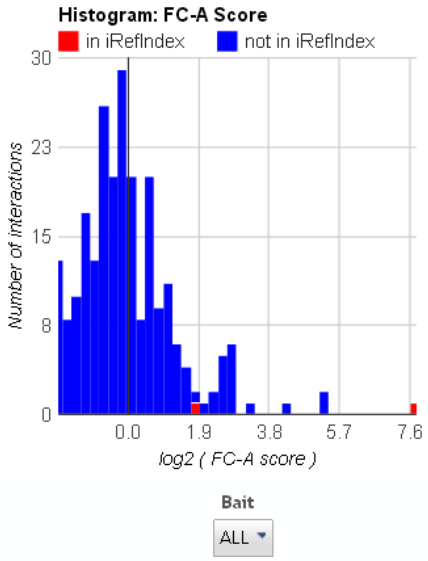
The data was processed using the standard workflow as described in the main text of the CRAPome manuscript (Mellacheruvu et al., 2013). Briefly, RAW files were converted to mzXML using ProteoWizard. The spectra were searched using X!Tandem , and the results were processed using the TPP. The resulting pep.xml and prot.xml files were processed using ABACUS. The resulting spectral count matrix, generated by ABACUS, was uploaded to the CRAPome.

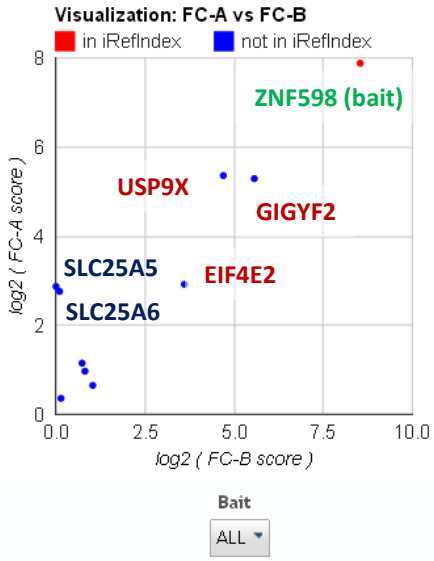
Matching controls were selected using the CRAPome filters which include the cell line, epitope tag, affinity approach. All experiments from protocol 26 were selected because they closely matched the experimental conditions. The analysis was performed using both FC scores and SAINT; SAINT performed sub optimally due to small dataset size and inconsistent spectral counts between the replicates. This was evident from a rapid comparison of the FC and SAINT data, provided on the interface. As such, only the FC score data (standard versus conservative scoring) are displayed here.

The results of the empirical scoring analysis are shown in the Figure below. As mentioned above, due to lack of annotation in iRefWeb and also due to small size of the dataset, the ROC-like curve (Sensitivity vs. Specificity figure) in this dataset was not informative. The histogram of FC-A scores (computed using user controls only) showed a typical background distribution curve, with a number of high scoring interactions in the region of FC-A scores above 4 (2 on the log scale as show in in the Figure). However, the majority of these high scoring interactions scored low using the conservative FC-B score, which was estimated using both the user controls and the selected CRAPome controls. The FC-A vs. FC-B plot nominate the following three proteins as likely interactors of ZNF598: USP9X, GIGYF2, and EIF4E2 (also shown on the figure is the bait itself). Indeed, these were the proteins reported in the original publications as the interaction partners of the bait after an elaborate filtering procedure (interactions between ZNF598, GIGYF2 and EIF4E2 were validated in the original manuscript by orthogonal approaches). The remainder of the prey proteins having high FC-A score are likely to be non-specific

contaminants that appear infrequently across the negative controls, but with high counts when they do (including myosin and myosin- associated proteins). These proteins have low FC-B scores (e.g. SLC25A and SLC25B).







Baits in this data: B1 = ZNF598

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**

Show 10 entries

Search:

ID	PROTID	GENENAMES	B1_FC_A	B1_FC_B	B1_IREF	B1_R1	B1_R2	UC1	UC2	CC10	CC11	CC14	CC15
155	NP_835461	ZNF598	235.37	371.94	1	277	368	0	0	0	0	0	0
288	NP_001034679	USP9X	40.97	25.91	0	5	75	0	0	0	0	0	0
203	NP_001096616	GIGYF2	39.11	47.56	0	41	61	0	0	0	0	0	1
11	NP_004915	ACTN4	20.2	0	0	130	0	0	0	5	0	94	40
62	NP_001093	ACTN1	9.89	0	0	62	0	0	0	1	0	19	7
99	NP_004837	EIF4E2	7.59	12.23	0	9	10	0	0	0	0	0	0
92	NP_001143	SLC25A5	7.3	1.01	0	14	8	0	0	8	4	7	31
30	NP_004990	MYO6	6.86	0	0	42	0	0	0	0	0	0	1
292	NP_001627	SLC25A6	6.78	1.08	0	14	7	0	0	7	1	7	29
73	NP_001106962	AMOT	6.71	0	0	41	0	0	0	0	0	2	0

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