Determining optimal cut-off value for TAP-MS datasets

There is a tradeoff between the false negative rate and the false positive rate when determining the optimal cut-off value for the TAP-MS datasets. Because false positives are hard to determine due to the lack of larger reference sets, we have calculated a Receiver-Operator curve based on the agreement of two complex definitions, GO and MIPS. We have defined as positive interacting protein pairs two proteins in the same complex according to both MIPS and GO. Negative interacting protein pairs are defined as two proteins of which each is in another complex according to both MIPS and GO. As our benchmark dataset we have used the intersection dataset. We have also plotted Y2H datasets by Uetz et al. and Ito et al. for comparison to the Intersection dataset based on Krogan et al. and Gavin et al. From this curve we find that a cutoff value of 0 is optimal for our question.

Table: Effect of cut-off on FN and FP

| Cutoff | TP  | FP  | FN  | TN  |
|--------|-----|-----|-----|-----|
| 0      | 7130| 33960| 2481| 185684|
| 1      | 6542| 14431| 3069| 205213|
| 2      | 5223| 3252 | 4388| 216392|
| 3      | 3510| 696  | 6101| 218948|
| 4      | 2834| 199  | 6777| 219445|
| 5      | 2397| 97   | 7214| 219547|
| 6      | 1933| 66   | 7678| 219578|
| 7      | 1614| 48   | 7997| 219596|
| 8      | 1301| 33   | 8310| 219611|
| 9      | 989 | 23   | 8622| 219621|
| 10     | 734 | 15   | 8877| 219629|
| 11     | 514 | 11   | 9097| 219633|
| 12     | 310 | 4    | 9301| 219640|
| 13     | 193 | 2    | 9418| 219642|
| 14     | 111 | 0    | 9500| 219644|
| 15     | 53  | 0    | 9558| 219644|

Ewing et al. HTP IP-HTMS dataset used to calculate conservation between human and yeast interactome

We have chosen Reactome as our reference set in human for calculating the conservation of co-complex membership because it is manually curated and based on expert opinion and therefore is likely to contain fewer errors. A new CoIP dataset for human by Ewing et al. has become available and we show here the same calculations when Reactome is substituted by this dataset below.

The authors state that interactions with a confidence score higher or equal to 0.3 should be regarded as high confidence. When using a higher cut-off value we see a steady rise in conservation (87% for $>= 0.5$ against the intersection dataset) but also see the total
number of conserved protein pairs plummet towards small numbers. The number of conserved protein pairs in Ewing when no cut-off value was used is significantly less than for Reactome and the conservation calculated is therefore less representative.

Ewing shows a much lower preservation of orthologs of protein pairs than Reactome (11% and 32% resp.). It is reported by Ewing et al. explicitly that they have based their bait selection on human disease association. Ewing therefore does not represent the basal conserved eukaryotic machinery as well as Reactome, which would account for the low conservation of protein pairs.

| Cut-off: none | Total interactions: | 5761 | Total conserved: | 650 |
|---------------|---------------------|------|-----------------|-----|
| Dataset       | PPI                 | NO-PPI| Conservation(%) | Covera(%) |
| Uetz          | 6                   | 40    | 13.04           | 0.92 |
| Ito           | 10                  | 256   | 3.76            | 1.54 |
| Uetz Int      | 6                   | 3     | 66.67           | 0.92 |
| Gavin         | 75                  | 292   | 20.44           | 11.54|
| Krogan        | 154                 | 450   | 25.50           | 23.69|
| Intersection  | 117                 | 245   | 32.32           | 18.00|
| Inclusive     | 171                 | 433   | 28.31           | 26.31|

| Cut-off: 0.3 | Total interactions: | 2039 | Total conserved: | 219 |
|---------------|---------------------|------|-----------------|-----|
| Dataset       | PPI                 | NO-PPI| Conservation(%) | Covera(%) |
| Uetz          | 5                   | 10    | 33.33           | 2.28 |
| Ito           | 9                   | 63    | 12.50           | 4.11 |
| Uetz Int      | 5                   | 0     | 100.00          | 2.28 |
| Gavin         | 58                  | 81    | 41.73           | 26.48|
| Krogan        | 99                  | 108   | 47.83           | 45.21|
| Intersection  | 78                  | 59    | 56.93           | 35.62|
| Inclusive     | 105                 | 102   | 50.72           | 47.95|

| Cut-off: 0.4 | Total interactions: | 695  | Total conserved: | 91  |
|---------------|---------------------|------|-----------------|-----|
| Dataset       | PPI                 | NO-PPI| Conservation(%) | Covera(%) |
| Uetz          | 2                   | 3     | 40.00           | 2.20 |
| Ito           | 5                   | 22    | 18.52           | 5.49 |
| Uetz Int      | 2                   | 0     | 100.00          | 2.20 |
| Gavin         | 33                  | 18    | 64.71           | 36.26|
| Krogan        | 52                  | 31    | 62.65           | 57.14|
| Intersection  | 37                  | 14    | 72.55           | 40.66|
| Inclusive     | 53                  | 30    | 63.86           | 58.24|

| Cut-off: 0.5 | Total interactions: | 245  | Total conserved: | 34  |
|---------------|---------------------|------|-----------------|-----|
| Dataset       | PPI                 | NO-PPI| Conservation(%) | Covera(%) |
| Uetz          | 0                   | 1     | 0.00            | 0.00 |
| Ito           | 2                   | 5     | 28.57           | 5.88 |
| Uetz Int      | 0                   | 0     | NA              | 0.00 |
| Gavin         | 18                  | 5     | 78.26           | 52.94|
| Krogan        | 26                  | 5     | 83.87           | 76.47|
| Intersection  | 20                  | 3     | 86.96           | 58.82|
| Inclusive     | 26                  | 5     | 83.87           | 76.47|
Orthology: results are not sensitive to orthology definition.

We also performed our analysis with another orthology definition. We have used inparanoid[1] to calculate orthology between human sequences from the UniProt database and yeast sequences from SGD. Inparanoid is a script which uses BLAST to obtain homology and calculated orthologs taking into account the existence of paralogs and in-paralogs. We have used the standard settings for inparanoid. Below is a table, like table 2 in the publication but based on the inparanoid orthology. We see that the orthology based on inparanoid results in slightly higher conservation and more conserved protein pairs. We feel that the orthology based on Ensembl is more advanced as it is based on reciprocal match, phylogenetic tree construction and tree reconciliation. We therefore used the Ensembl definition in our main analysis as opposed to InParanoid.

### Conservation based on inparanoid (2596 conserved protein pairs)

| Dataset  | PPI   | Non-PPI | Conservation | Coverage |
|----------|-------|---------|--------------|----------|
| Gavin    | 1646  | 274     | 85.73%       | 63.41%   |
| Krogan   | 2084  | 462     | 81.85%       | 80.28%   |
| Inclusive| 2317  | 239     | 90.65%       | 89.25%   |
| Intersection| 1761 | 84      | 95.45%       | 67.84%   |
| Uetz     | 23    | 83      | 21.70%       | 0.89%    |
| Uetz Strict| 23   | 4       | 85.19%       | 0.89%    |
| Ito      | 37    | 634     | 5.51%        | 1.43%    |

### Human Dataset

| Dataset  | PPI   | Non-PPI | Overlap | Coverage |
|----------|-------|---------|---------|----------|
| Rual     | 2     | 9       | 18.18%  | 0.08%    |
| Stelzl   | 5     | 111     | 4.31%   | 0.19%    |
| Ewing    | 46    | 546     | 7.77%   | 1.77%    |

### Conservation based on Ensembl (1916 conserved protein pairs)

**Identical to table 2 in the main text**

| Dataset  | PPI   | Non-PPI | Conservation | Coverage |
|----------|-------|---------|--------------|----------|
| Gavin    | 1305  | 226     | 85.24%       | 68.11%   |
| Krogan   | 1547  | 328     | 82.51%       | 80.74%   |
| Inclusive| 1717  | 167     | 91.14%       | 89.61%   |
| Intersection| 1392 | 75      | 94.89%       | 72.65%   |
| Uetz     | 21    | 63      | 25.00%       | 1.10%    |
| Uetz Strict| 21  | 4       | 84.00%       | 1.10%    |
| Ito      | 36    | 381     | 8.63%        | 1.88%    |

### Human Dataset

| Dataset  | PPI   | Non-PPI | Overlap | Coverage |
|----------|-------|---------|---------|----------|
| Rual     | 3     | 5       | 37.50%  | 0.16%    |
| Stelzl   | 4     | 79      | 4.82%   | 0.21%    |
| Corrected Ewing| 30 | 420     | 6.67%   | 1.57%    |
Errors in orthology, complex definition and neo-functionalisation

Of the 167 non-interactions as found using Reactome and the Inclusive dataset, 139 appear to be potential false negatives. The remaining 28 non-conserved interactions consist of errors in orthology of one gene (5 interactions), incorrect assignment of two proteins to a complex in Reactome (10 interactions) and possible neo-functionalisation after duplication in human (3 proteins, 13 interactions).

Five protein pairs do not show an interaction due to incorrect orthology assignment in Ensembl. The human protein TF2H4 [Swiss-Prot:Q92759] is annotated as orthologous to VAS1 [SGD:YGR094W] and is present in five conserved protein pairs in Reactome. We could not confirm any homology between these proteins (let alone orthology) and it seems unlikely as well from the annotation: TF2H4 is a subunit of Transcription Factor IIH complex whereas VAS1 is a valyl-tRNA synthetase.

Ten protein pairs are probably erroneously assigned to the spliceosome complex in Reactome, based on our re-analysis of the available literature. Of these ten pairs, five protein pairs contain NFX1 [Swiss-Prot:Q12986] and five protein pairs contain SMC1 alpha [Swiss-Prot:Q14683]. Human NFX1 [Swiss-Prot:Q12986] is associated with the spliceosome complex and “Export Receptor bound mature mRNA Complex” according to Reactome (internal id’s 72022, 72074, 72057, 159329, 159259, 113815). NFX1 however is a transcription factor for MHCII genes and is not implicated in pre-mRNA modifications or nuclear export. Confusingly the NXF1 protein (mind the spelling of N XF1) is a known nuclear export factor. NXF1 is not listed as part of the “Export Receptor bound mature mRNA Complex”. A misspelling of NXF1 might have caused a mix-up in Reactome. (for example NXF1 [Swiss-Prot:Q9UBU9] is misspelled in Cohen and Panning[2] as NFX1.)

SMC1 alpha (human [Swiss-Prot:Q14683], yeast [SGD:YFL008W]), responsible for another five protein pairs, is part of the cohesin complex but also takes part in the spliceosome formation according to Reactome (internal id’s 72159, 72022, 72074, 77505, 72057). However, we could not find any literature which linked SMC1 alpha directly to the spliceosome. The link between the cohesin complex and the spliceosome is one of its alleged co-complex members CD2B2 [Swiss-Prot:O95400] of which LIN1 [SGD:YHR156C is its ortholog in yeast. LIN1 is implicated to link chromatine modification and the cohesin complex to the spliceosome complex[3]. But the similarity between CD2B2 and LIN1 is weak, and both have very different functions. CD2B2 is involved in immunity and binds to antibodies, whereas LIN1 is a non-essential component of U5 snRNP. CD2B2 and the spliceosome are mentioned together in an article by Monos et al.[4] because an antibody raised against CD2B2 also reacted with the spliceosomal Sm B/B’ proteins. The experimental link between SMC1 alpha and the spliceosome is weak and it can therefore be argued that SMC1 is not part of the spliceosome complex.

We identified 13 protein pairs which could be possible new interactions. Each of these
pairs contain one of three proteins: PCBP1 [Swiss-Prot:Q15365], PABP2 [Swiss-Prot:Q86U42] and XAB2 [Swiss-Prot:Q9HCS7]. The human PCBP1 is involved in regulating the spliceosome[5]. Its yeast ortholog PBP2/HEK1 [SGD:YBR233W] is involved in the regulation of telomere position effect and telomere length[6]. However PCBP1 is not the only ortholog of PBP2. 14 human proteins are orthologs to PBP2. These are active in different processes, some of them still perform the ancestral function[7]. So whereas PBP2 solely has a function in the regulation of telomere position effect and telomere length in yeast, the human PCBP family of inparalogs has gained many other functions and interaction partners after several rounds of duplications in the course of evolution (neofunctionalization of inparalogs).

The human PABP2 is a poly(A)-binding protein and is part of the “3’ end cleaved, ligated exon containing complex” in the nucleus according to Reactome. Its ortholog in yeast, SGN1 [SGD:YIR001C], is a poorly characterized poly(A)-binding protein that localizes to the cytoplasm and not to the nucleus[8]. Hence some degree of functional differentiation took place in either human or yeast.

The human protein XAB2 is involved in transcription coupled-nucleotide excision repair (TC-NER)[9] and also in mRNA splicing (spliceosome)[10] albeit indirectly. The ortholog of XAB2 in yeast, SYF1 [SGD:YDR416W], is a component of the spliceosome[11, 12]. SYF1 however has not been implied with nucleotide excision repair. XAB2 apparently has gained a new function, and new interaction partners, in human TC- NER, but also seemingly retained its ancestral function (or some of it), like its yeast ortholog SYF1, in the spliceosome.

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