Exercises and Solutions

1. Name three limitations of the yeast two-hybrid system that are circumvented by using complementary approaches to identify protein-protein interactions such as AP-MS.

   Answers:

   - To be detected with the yeast two-hybrid system, interactions have to occur within the nucleus of the yeast cell. For most interactions this is not the natural environment while in AP-MS studies the interaction occurs under physiological conditions.

   - Some protein-protein interactions rely on post-translational modifications of either of the interacting proteins. This might require modifying proteins, e.g. protein kinases, not present in the yeast cell. As during AP-MS proteins are expressed in their natural environment they should also be properly modified.

   - Since in the yeast two-hybrid system the read out of interaction relies on transcriptional activation of a reporter gene it is generally difficult to assay proteins with transcriptional activity (activators or repressors), a problem not occurring with AP-MS

2. In this exercise you will be comparing protein interaction data for a small family of proteins called glutaredoxins.

   a) Go to the *Saccharomyces* genome database (SGD) at www.yeastgenome.org and identify the five glutaredoxin genes found in this organism. List the gene names, all aliases, the systematic names, and a brief description for each gene.

Gene name	Alias	Systematic name	Description
GRX1	none	YCL035C	Hydroperoxide and superoxide-radical responsive heat-stable glutathione-dependent disulfide oxidoreductase with active site cysteine pair; protects cells from oxidative damage
GRX2	*TTR1*	YDR513W	Cytoplasmic glutaredoxin, thioltransferase, glutathione-dependent disulfide oxidoreductase involved in maintaining

			redox state of target proteins, also exhibits glutathione peroxidase activity, expression induced in response to stress
GRX3	none	YDR098C	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; monothiol glutaredoxin subfamily member along with Grx4p and Grx5p; protects cells from oxidative damage
GRX4		YER174C	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; monothiol glutaredoxin subfamily member along with Grx3p and Grx5p; protects cells from oxidative damage
GRX5		YPL059W	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; mitochondrial matrix protein involved in the synthesis/assembly of iron-sulfur centers; monothiol glutaredoxin subfamily member along with Grx3p and Grx4p

b) Identify the interactions for each glutaredoxin identified by Ito and coworkers. Go to their web site at http://itolab.cb.k.u-tokyo.ac.jp/Y2H/ and download the full data file. From this file, draw an interaction map that illustrates all interactions identified for each glutaredoxin in this experiment. Identify name or function of each gene product.

See Excel sheet

c) Identify the interactions for each glutaredoxin identified by Uetz and coworkers. Go to their web site at http://depts.washington.edu/sfields/yp_interactions/twohybrid_interaction_data .xls. Try to identify the interactions and draw an interaction map for each glutaredoxin in this experiment. Identify name or function for each gene product.

See Excel sheet

d) Compare the interactions that you identified from the original data sets. Where are overlaps or differences? Discuss the reason for possible discrepancies.

The Ito data set lists 8 interactions for four different glutaredoxins while the Uetz set lists only three interactions for two different glutaredoxins. The only overlap between the two sets is the interaction of GRX5 with YIL105C. There are several fundamental differences between the two data sets concerning the experimental approach to generate them. Both screens were not saturated, meaning that not all bait/prey combinations have been tested. Ito and co-workers made library screens while Uetz et al. tested bait/prey combinations one by one.

e) For this exercise you will take preliminary look at the Cytoscape network visualization tool. To download Cytoscape on your computer go to www.cytoscape.org and follow the instructions for installation. To download the Cytoscape-formatted interaction data for Grx2, go to SGD and klick the "Batch download" link on the left side of the page. Enter Grx2 under "Option 1", select "physical interactions", and then submit. Follow the instructions for downloading the file. Start Cytoscape , in the "File-load-Network" menu, enter the file name of the interaction file that you downloaded from SGD. Under the "Layout" Menu, select "Apply Spring Embedded Layout – all nodes". Find Grx2 in this network. To which nodes is it connected? What is this protein and what is its function. Compare what you see in the Cytoscape network to what you have identified from the Ito and Uetz data, respectively. What is the most highly connected node in the Cytoscape network? Using the functional description for the protein from SGD, provide a biological explanation for this highly connected node.

Grx2 is connected with Pho80, the most highly connected node within the network. Pho80 is a cyclin involved in the adaptation to phosphate stress, Grx2 is also involved in stress defense. Both proteins together could shield the cell from oxidative stress during phosphate deficiency. However, this would need to be experimentally verified.