



## Step 1

Start Cytoscape and load the network *galFiltered.cys* from the *sampleData* folder in the Cytoscape directory. Select a cluster of genes from the network (indicated in yellow). Select *BiNGO* from the *Plugins* menu.



## Step 2

The *BiNGO Settings* panel pops up. Start by filling in a name for your cluster. This name will be used for creating the output file and the visualization of the results in Cytoscape. Check the box *Get Cluster from Network* (see below for an example with text input). We want to assess *overrepresentation* of GO categories, and we want to *visualize* the results in Cytoscape. The corresponding boxes are checked accordingly by default. Then select a statistical test (the *Hypergeometric Test* is exact and equivalent to an exact Fisher test, the *Binomial Test* is less accurate but quicker) and a multiple testing correction (we recommend *Benjamini & Hochberg's FDR correction*, the *Bonferroni* correction will be too conservative in most cases), and choose a significance level, e.g. 0.05. Since we only want to visualize those GO categories that are overrepresented after multiple testing correction, and their parents in the GO hierarchy, select the corresponding visualization option. We're interested in assessing the overrepresentation of functional categories in our cluster with respect to the whole yeast genome, which is why we choose the *Complete Annotation* as the reference set. Select *GO\_Biological\_Process* from the ontology list, and *Saccharomyces cerevisiae* from the organism list. We want to consider all evidence codes, so don't fill in anything in the evidence code box. Finally, select a directory to save the output file in (the file will be named *test.bgo* if you filled in *test* as a cluster name), and press *Start BiNGO...* 

00	BiNGO Settings	
BiNGO settings		
Save settings as default		Help
Cluster name:		
test		