

Introduction to Pathway Analysis and Visualization

Hands-on instructions

We have two files with lists of differentially expressed genes, corresponding to up- and down-regulated genes and also a datafile representing the whole dataset with log fold changes and p values.

Functional Enrichment Analysis

EnrichR

Gene Ontology

1. Open the file with up-regulated genes, and copy the full list of symbols
2. Go to the EnrichR website: <https://amp.pharm.mssm.edu/Enrichr/>
3. Paste the list of gene symbols in the input box
4. Click "Submit"
5. When the results load, go to "Ontologies" -> "GO Biological Process"
6. To save the bar chart, click either of the svg / png / jpg buttons just above the bar graph
7. To export the table, click the "Export entries to table" link at the bottom

WikiPathways

1. At the top of the page, click "Pathways" to see results for pathway sets
2. Click the box for "WikiPathways"
3. Again, we can export the bar graph and table. We will use the gene sets from the table later on.

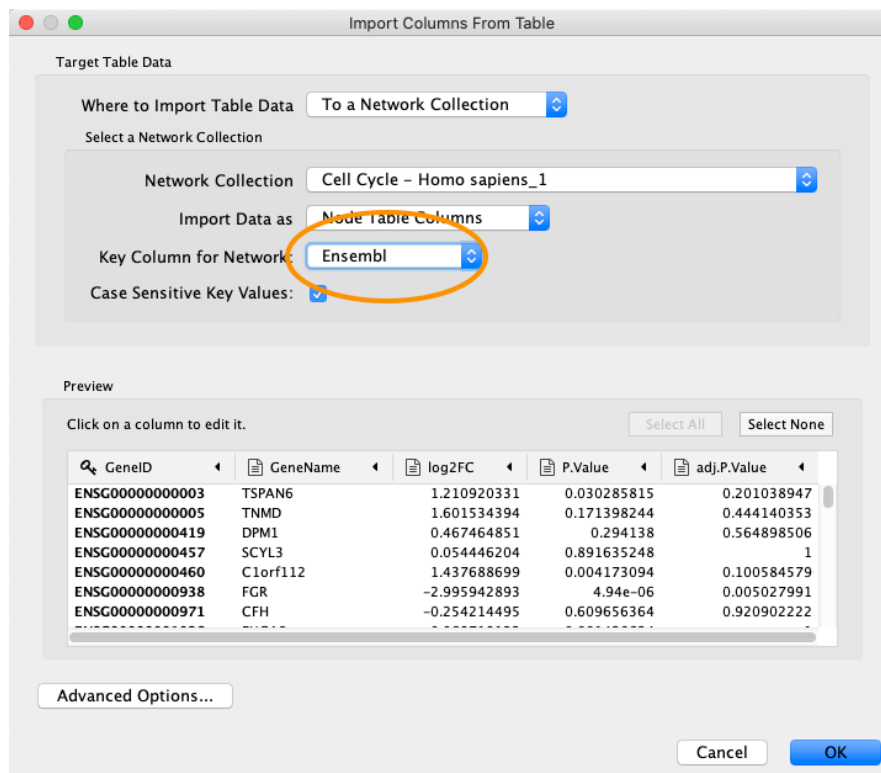
WebGestalt

1. Go to WebGestalt website: <http://www.webgestalt.org/>
2. Select species, GSEA and for Functional Database select pathway and then WikiPathways
3. Under Gene List, select Gene symbol
4. Upload (or copy/paste) the lung.expr.entrez.gsea.csv example file
5. Skip the Advanced Parameters for now and click Submit.
6.

Pathway Visualization

Cytoscape

1. Launch Cytoscape
2. In the network tab, select WikiPathways, and type in the name of one of the top pathways in the EnrichR results, for example cell cycle.
3. WikiPathways will return many pathways as results, find the relevant one by WPID
4. Double-click on the pathway in the list to open, or click "Import as Pathway"
5. Now we can add data. Go to "File -> Import Table from File" and select the example datafile (TBD).
6. Next, an import dialog will open. The data is annotated with Ensembl IDs, which are also present in the pathway, but in order for the data and pathway to map we have to specify which column in the pathway has Ensembl IDs. In the "Key Column for Network" select Ensembl:



Now, let's use the Log fold change to color the gene boxes. In Cytoscape, this is done by creating a mapping between log fold change and fill color in the Style interface.

7. In the Style tab, click the triangle to the right of Fill Color. Before we create a new mapping we need to delete the current mapping. Click the trash can symbol.
8. In the Column drop-down, select "log2FC".
9. In the Mapping Type, select "Continuous"

10. This will create a default color gradient from blue to red.
11. Open pathway as network.....
12. Export.....

GO Term Visualization

Cytoscape

1. STRING Protein query for gene names for specific GO term.....
2. Import data....
3. Styles.....
- 4.