

Introduction to Pathway Analysis and Visualization – Day 1

Hands-on instructions

The example data represents lists of differentially expressed genes, corresponding to up- and down-regulated genes for ORA analysis (UP: $\log_2FC > 1$ AND $\text{adj.P.Value} < 0.05$, DOWN: $\log_2FC < -1$ AND $\text{adj.P.Value} < 0.05$), and a datafile all genes in the dataset with associated rank, for GSEA.

Functional Enrichment Analysis

EnrichR


Introduction

1. Go to the **EnrichR** website: <https://amp.pharm.mssm.edu/Enrichr/>
2. To explore available libraries, go to the **Libraries** tab to browse.
3. You can also browse libraries by term, for example “cancer”. From the results, you can download individual gene sets in gmt format.

Gene Ontology

1. Open the file with upregulated genes, **lung.expr.up.txt**, and copy the full list of symbols.
2. On the **Analyze** tab, paste the list of gene symbols in the input box.
3. *Note:* There is an option to contribute your gene list, which makes it available to others. If you select this option, you will be prompted to assign a name and provide a brief description of the list.
4. Click **Submit**.
5. When the results load, go to **Ontologies -> GO Biological Process**.
6. By default, the terms are sorted by p-value ranking. Clicking on any of the bars representing terms will re-sort the bars based on either combined score or rank-based ranking.
7. To save the bar chart, click either of the **SVG / PNG / JPG** buttons just above the bar graph.
8. It is also possible to change the color scheme by clicking the **Settings** icon at the top right.
9. Switch to the **Table** view at the top. To export the table, click the **Export entries to table** link at the bottom.
10. Open the downloaded table. Each row has statistics for a go term, and a list of changed genes.

WikiPathways

1. At the top of the page at EnrichR, click **Pathways** to see results for pathway sets.
2. Click the heading for **WikiPathways**.
3. Again, we can export the bar graph and table.
4. To share or archive the results, a permanent link is available by clicking the link icon  at the top of the page.
5. *Optional*: Repeat for the down-regulated list, **DE_down.txt**.

WebGestalt

GSEA Pathways

1. Go to the **WebGestalt** website: <http://www.webgestalt.org/>
2. Select **Homo sapiens** as species, **GSEA** and for Functional Database select **pathway** and then **WikiPathways**.
3. Under Gene List, select **Gene symbol**.
4. Open the **lung.expr.entrez.gsea.txt** file and copy the contents.
5. In the WebGestalt **Gene List** box, paste the GSEA data.
6. Skip the Advanced Parameters for now and click **Submit**. The results may take several minutes to load.
7. The top of the results page includes a job summary and a link to download the results.
8. Results are displayed as a bar chart by default. Right-clicking on the bar chart lets you download in either PNG or SVG format. Clicking on the bars updates the pathway-specific display at the bottom of the page. The **Table** and **Volcano plot** views have similar interactivity.
9. To cluster the gene sets by content, select the **Affinity propagation** option.