Enrichment analysis with gProfiler

1. Filter your DESeq2 differential analysis results for absolute value of Log2FC (e.g., 0.58) and FDR (e.g., <0.05).
2. Open gProfiler online platform https://biit.cs.ut.ee/gprofiler/gost and select **g:GOSt Functional profiling** from top banner.
3. Copy the list of identifiers of the resulting list from step 1 and paste in the **Query** box.
4. In the right-side panel, under **Options**, select the **Organism**.
5. Expand the **Advance options** menu.
6. Under Statistical domain scope, select **Only annotated genes**.
7. Under Significance threshold, select **g:SCS threshold or Benjamini-Hochberg FDR.**
8. **User threshold** 0.05
9. **Expand the Data sources** menu and uncheck TRANSFAC, miRTarBase, and CORUM options.
10. Scroll up back to the list input box and click on the **Run query** button.

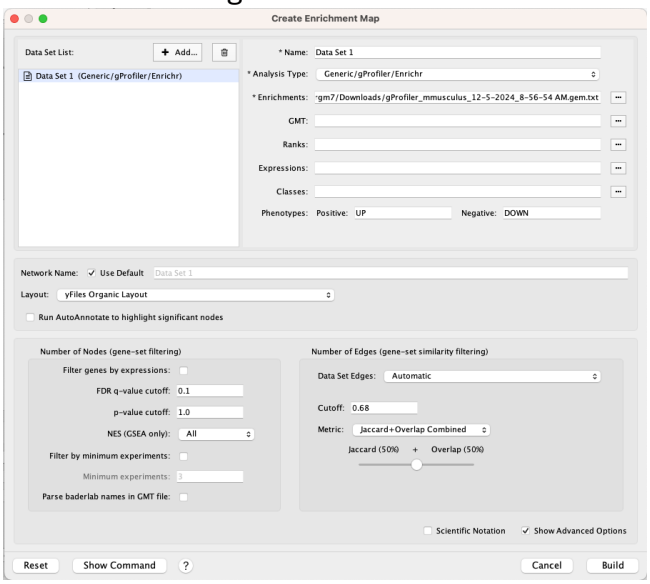
Exporting the results

11. On top of the enrichment graphic, click on the **Detail Results** option
12. Download the enrichment results table by clicking on **csv** button
13. Download the **GEM** file for gene set clustering.

Clustering the enrichment analysis with Enrichment map and labeling clusters with Autoannnotate apps on Cytoscape

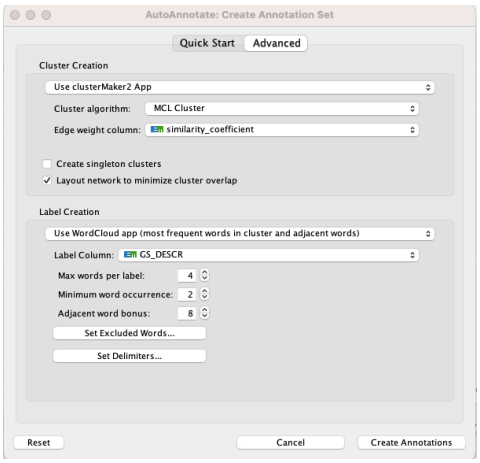
14. Download Cytoscape from https://cytoscape.org/download.html
15. Open Cytoscape
16. From the top menu go to **Apps** -> **App store** -> **Show app store**. A left panel will appear
17. Search the Cytoscape app store by writing Enrichment map on the box of left panel and click enter. A web browser will open with EnrichmentMap download page. Click on the **Download** button to the right.
18. Search the Cytoscape app store for Autoannotate, click on search or enter. A web browser will open with Autoannotate download page. Click on the **Download** button to the right.
19. From Cytoscape top menu click on **Apps** -> **Enrichment map**. The app will open. Click on **+ Add… button** on top -> **+ Add dataset manually**.
20. From ***Analysis Type:** -> **Generic/gProfiler/Enrichr**.
21. From ***Enrichments Pos:** click on the **…** box to the right and upload the downloaded GEM file (*e.g., "gProfiler_mmusculus_12-5-2024_8-56-54 AM.gem.txt from step 13*)

22. Click on Show **Advance Options**. On the right side of the panel set **Cutoff** to the value that better cluster the data (e.g., a value from 0.3 - 0.8) you many need to try different values until you find the optimal.
23. Click on the **Build** button to the right bottom of window.

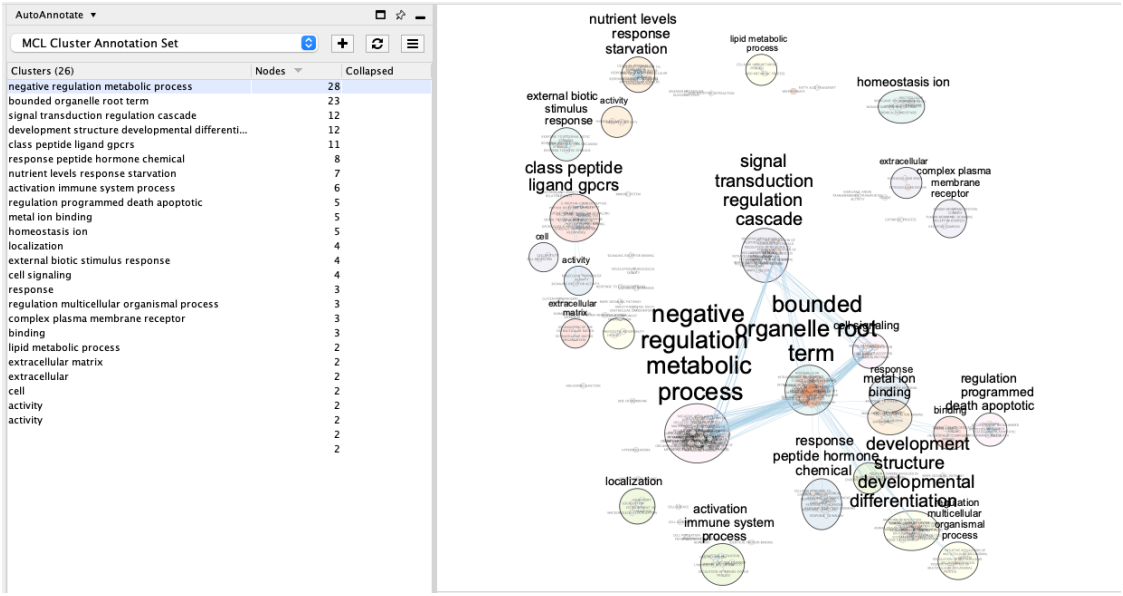


<u>A network of clusters with interconnected nodes (pathways) will appear.</u>

24. From Cytoscape top menu, click on **Apps** -> **Autoannotate** -> **New annotation set.** A new window will open
25. Parameters (modify only these)
    Cluster Creation: select **Use clusterMaker2 App**
    Cluster algorithm: **MCL Cluster**
    Edge weight column: **similarity coefficient**
    Check **Layout network to minimize cluster ovelap**
    Label Column: **GS_DESCR**
    Max words per label: **4**
    Minimum word occurrence: **2**
26. Click on **Create Annotations** button to the right bottom of window.



Nodes (pathways) will be clustered and labeled. A list of those clusters with respective label will show in the left panel. See figure below



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