# Enrichment analysis

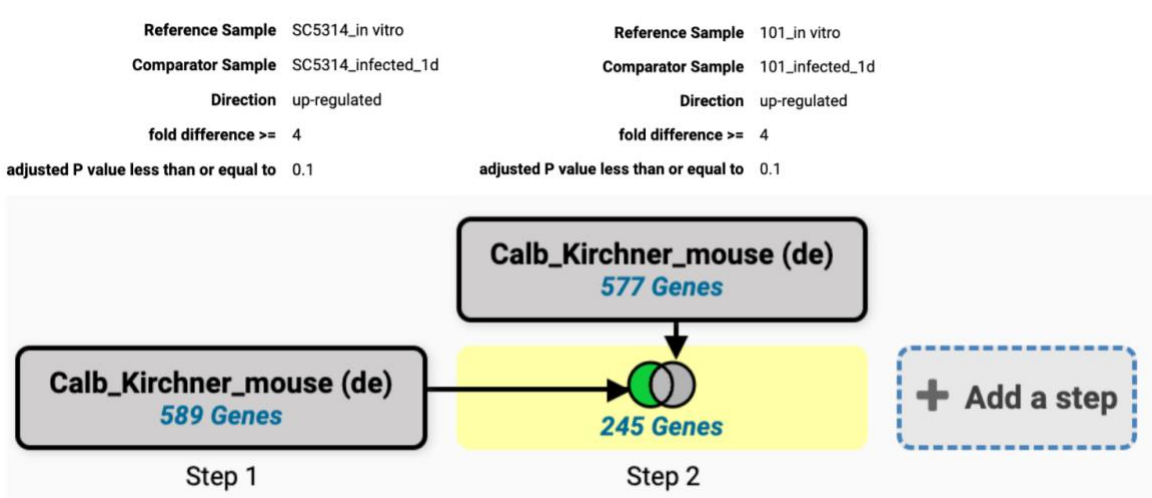
Learning objectives

- Run enrichment analysis on RNA-Seq analysis resuts.

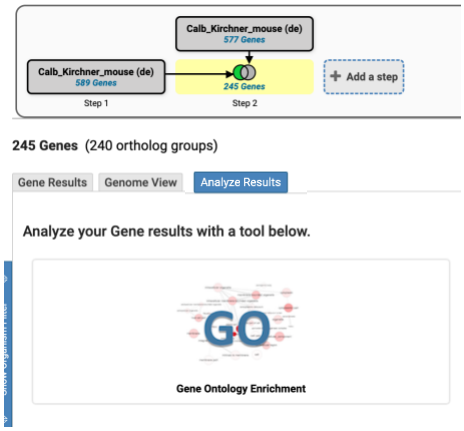
The enrichment analysis tools can be accessed under the blue Analyze Results tab and it includes Gene Ontology, Metabolic Pathway, and Word Enrichment tools. The three types of analysis apply Fisher's Exact test to evaluate ontology terms, over-represented pathways, and product description terms. Enrichemt is carried out with fisher's exact test to assess the proportion of GO terms in one set of genes versus all the genes in the query organism. The test produces P-values which are then corrected for multiple testing with the Benjamini-Hochberg false discovery rate or the Bonferroni test.

In the previous exercise, you examined host response to infection with Candida strains. In this section, we will learn how to perform enrichment analysis on the fungal component of the Kirchner et al dataset. SC5314 induces rapid transcriptional response in the host, while 101 has slower kinetic. The commensal 101 strain has also reduced filamentation when compared to SC5314.

To begin, click on the search strategy link below:
https://fungidb.org/fungidb/app/workspace/strategies/import/34f998f05745cbc3
This strategy identifies genes up-regulated in SC5314 at 1d post infection (Step 1) and subtracts up-regulated genes in common with the persistent 101 strain (Step2).




- **Perform GO Enrichment analysis (Molecular function)**
  1. Select Step 2 results (they will become highlighted in yellow).
  2. Click on the Analyze Results button located about the gene results table.

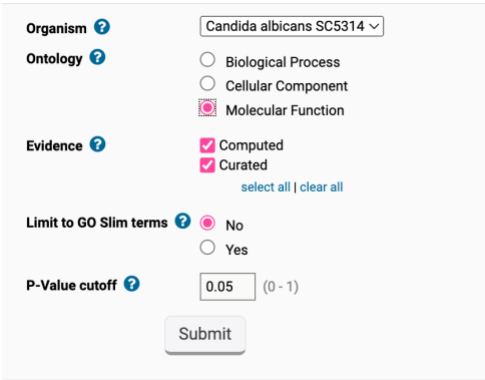



GO enrichment analysis can be performed on the following ontology groups: molecular function, cellular component, and biological processes. Also, other parameters allow users to limit their analysis on either "Curated" or "Computed" annotations, or both. Those with a GO evidence code inferred from electronic annotation (IEA) are denoted "Computed", while all others have some degree of curation. The default P-value is set to 0.05 but can be adjusted manually.

3. Select the "Molecular function" option.

4. Run the enrichment analysis on both computed and curated evidence (GO terms) and leave other parameters at default.

   Note: When the GO Slim option is chosen, both the genes of interest and the background are limited to GO terms that are part of the generic GO Slim subset




5. Click on the "Submit" button.

- Examine your results. Looking at the enriched terms, do they make sense in terms of what you know about the Kirchner et al. 2019 dataset?

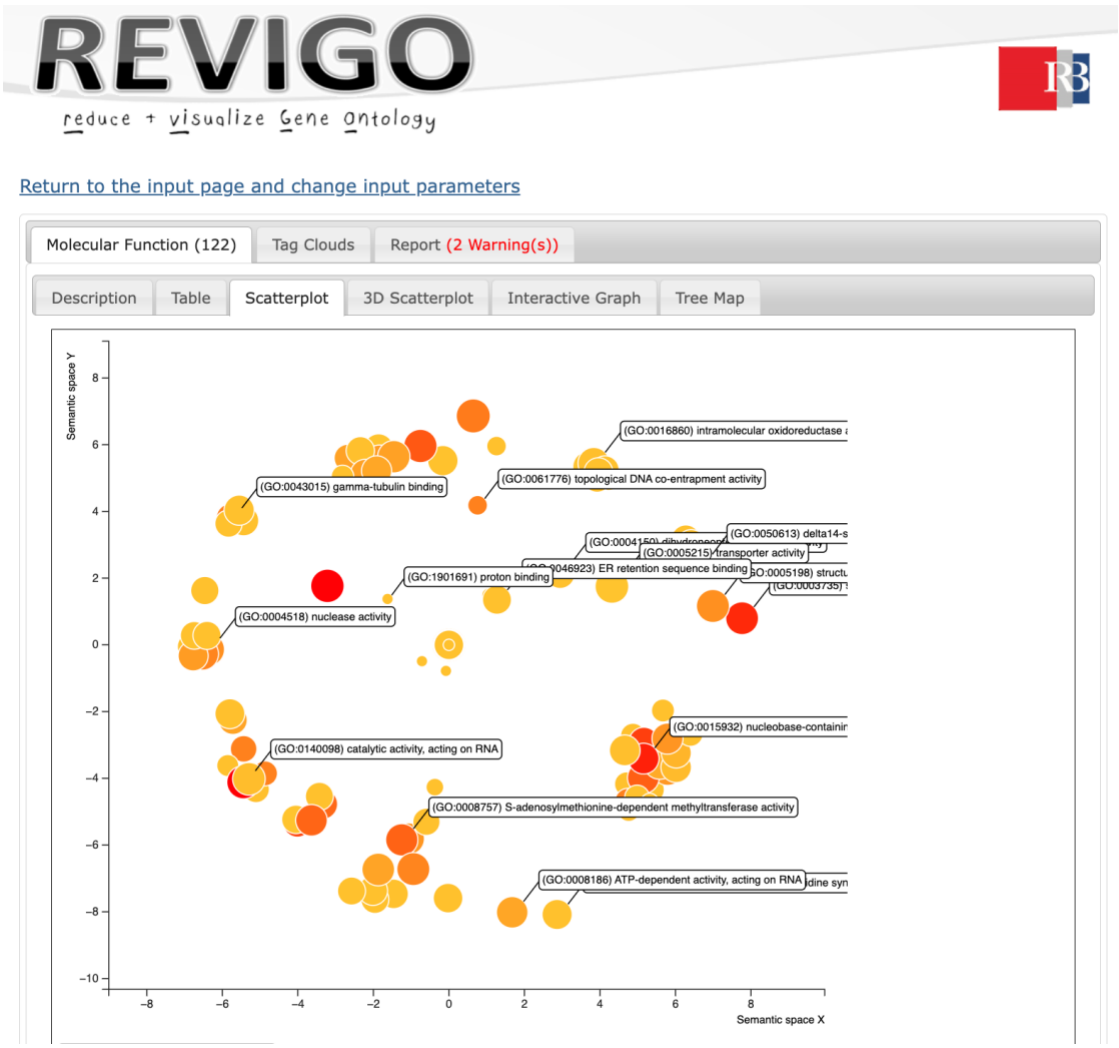

| GO ID | GO Term | Genes in the bkgd with this term | Genes in your result with this term | Percent of bkgd genes in your result | Fold enrichment | Odds ratio | P-value | Benjamini | Bonferroni |
|---|---|---|---|---|---|---|---|---|---|
| GO:0140098 | catalytic activity, acting on RNA | 262 | 28 | 10.7 | 2.70 | 3.15 | 1.19e-6 | 6.18e-4 | 6.18e-4 |
| GO:0140640 | catalytic activity, acting on a nucleic acid | 403 | 36 | 8.9 | 2.26 | 2.62 | 2.56e-6 | 6.68e-4 | 1.34e-3 |
| GO:0015932 | nucleobase-containing compound transmembrane transporter activity | 40 | 9 | 22.5 | 5.68 | 7.28 | 1.92e-5 | 2.26e-3 | 1.00e-2 |
| GO:0005347 | ATP transmembrane transporter activity | 23 | 7 | 30.4 | 7.69 | 10.90 | 1.99e-5 | 2.26e-3 | 1.04e-2 |
| GO:0015215 | nucleotide transmembrane transporter activity | 32 | 8 | 25.0 | 6.31 | 8.33 | 2.48e-5 | 2.26e-3 | 1.29e-2 |
| GO:0003735 | structural constituent of ribosome | 166 | 19 | 11.4 | 2.89 | 3.32 | 2.60e-5 | 2.26e-3 | 1.36e-2 |

The results table includes several additional statistical measurements:

- **Fold enrichment** - The ratio of the proportion of genes in the list of interest with a specific GO term over the proportion of genes in the background with that term.
- **Odds ratio** - Determines if the odds of the GO term appearing in the list of interest are the same as that for the background list.
- **P-value** - The probability of a result occurring by chance under a null hypothesis.
- **Benjamini-Hochburg false discovery rate** - A method for controlling false discovery rates for type 1 errors.
- **Bonferroni adjusted P-values** - A method for correcting significance based on multiple comparisons.

Note: you can sort genes in your results using the sort options within a column.

- **Visualize enrichment in REVIGO.**
  1. Click on the "Open in Revigo" button and follow prompts to complete this step.




The table tab provides a detailed overview of the GO terms, P-values and also parent GO terms used to describe a group of related GO terms (http://geneontology.org/docs/ontology-relations/).
More about REVIGO:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0021800