

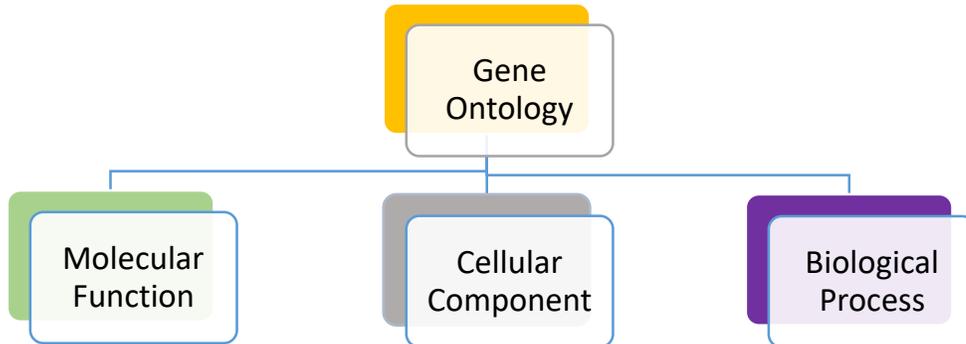
Gene Ontology (GO) Enrichment

Learning objectives:

- Run a GO enrichment analysis
- Explore GO enrichment results
- Port GO enrichment results to Revigo

Background:

Ontologies are a controlled vocabulary of terms and concepts with relationships between them. The Gene Ontology describes the knowledge of biological sciences and divides this knowledge into three broad categories: Molecular function, cellular component and biological process.



Activities at the molecular level performed by gene products, e.g. Toxin activity, catalytic activity of transporter activity

Where a gene product performs its function, e.g. Cilium, Mitochondrion, plastid, Golgi etc...

Processes accomplished by multiple activities, e.g. pyrimidine biosynthesis, gluconeogenesis

To learn more about Gene Ontology, please visit:

<http://geneontology.org/docs/ontology-documentation/>

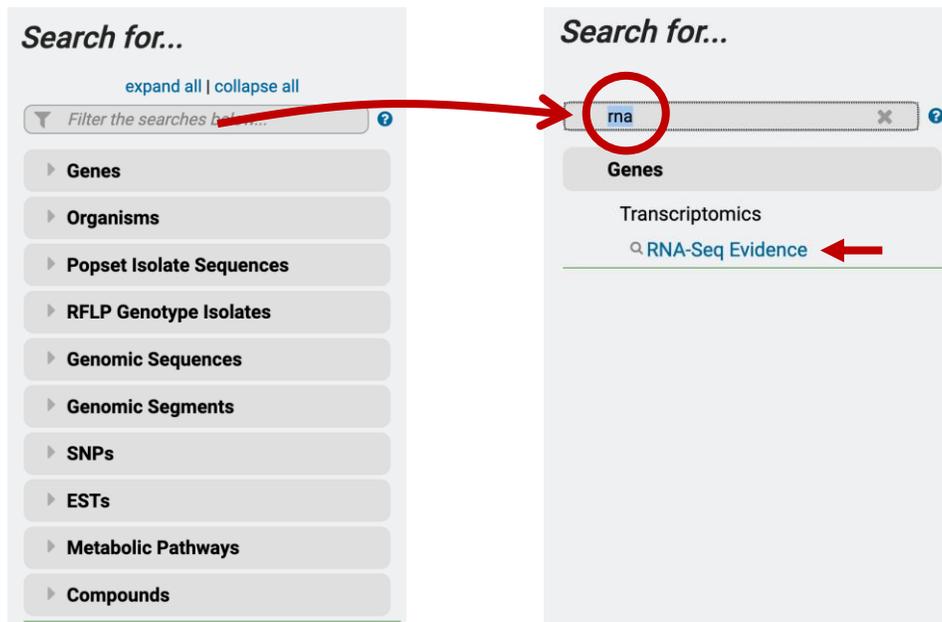
A gene can be assigned a GO term either manually (by an annotator or curator when they evaluate experimental evidence from a publication) or computationally (based on the GO terms of genes that share sequence or functional domains). The origin of the assignment is documented; some researchers believe that manually assigned functional annotations are more accurate than those that are electronically transferred since a researcher has reviewed the manually annotated assignments. GO terms can be used to test whether your set of genes are enriched for a molecular function, cellular component, or biological process.

For example: A researcher performs a proteomics experiment on a protein fraction collected during an antimalarial treatment and identifies 100 proteins in total. When

they examine the GO terms assigned to the gene set corresponding to the proteome, they see that 25 genes are assigned GO:0016301, kinase activity. Out of 5000 genes in the genome, only 100 are assigned GO:0016301. There is an overrepresentation of GO:0016301 in the researcher's proteome which is 'enriched' for kinase activity.

A standard enrichment determination method employs Fisher's exact test, a statistical test that evaluates a 2x2 contingency table (in this case, number of genes in my set *versus* number of genes from genome not in my set, and number of genes with GO term Z *versus* number of genes without term Z). This test produces a p-value between 0 and 1, where $p \leq 0.05$ is considered significant (that is, less than 5% probability that the enrichment is due to chance). However, the test is performed for each of the 100s of GO terms, increasing the chances that a GO term will be incorrectly considered enriched (a false positive, or type I, error). Thus, the original p-value must be adjusted for so-called multiple hypothesis testing, resulting in an adjusted p-value such as the Benjamini-Hochberg false discovery rate (FDR) or Bonferroni adjusted p-value.

1. In order to run a GO enrichment analysis, you need a list of genes to test. This can be a list of gene IDs from your experimental results (upload them with the ID search) or a gene list resulting from a search you conducted on a VEuPathDB website. For this example, in [ToxoDB](#), we will identify genes that are differentially regulated over time.
 - a. Navigate to the RNA-Seq searches and find the data set called "**Oocyst Time Series (M4)**" from Fritz *et al*. A quick way of getting to the RNA-Seq searches is to type 'rna' in the filter box on the left of the home page and click on the RNA-Seq Evidence link. See image below.



- b. The RNA-Seq evidence page includes a list of all data sets that are loaded in the website. To quickly find a dataset, you can start typing key words in the “Filter Data Sets” box. For example, start typing the word “oocyst”.

Identify Genes based on RNA-Seq Evidence

Filter Data Sets: ✕ ? Legend: DE Differential Expression FC Fold Change P Percentile SA SenseAntisense

Organism	Data Set	Choose a Search
<i>Eimeria tenella</i> strain Houghton	Life Cycle Stages Transcriptomes (Reid)	FC P
<i>Toxoplasma gondii</i> ME49	Oocyst Time Series (M4) (Fritz/Boothroyd/Gregory)	FC P SA

- c. Once you find the data set of interest, choose the fold-change (FC) search. For this exercise, identify genes that are upregulated by 20-fold in days 4 and 10 compared to the day 0 time point. Parameters to set:
1. Up-regulated
 2. 20-fold
 3. Maximum
 4. Day 0
 5. Minimum
 6. Day 4 and 10

Identify Genes based on T. gondii ME49 Oocyst Time Series (M4) RNA-Seq (fold change)

For the Experiment: Oocyst Time Series (M4) - Sense

return protein coding genes

that are up-regulated down-regulated

with a Fold change \geq 20

between each gene's maximum expression value (or a Floor of 10 reads) in the following Reference Samples:

day 0 day 4 day 10

and its minimum expression value (or the Floor selected above) in the following Comparison Samples:

day 0 day 4 day 10

Example showing one gene that would meet search criteria
(Dots represent this gene's expression values for selected samples)

Up-regulated

Expression

Minimum Expression Value Comparison

Expression Value Reference

20 fold

Reference Samples Comparison Samples

For each gene, the search calculates:

$$\text{fold change} = \frac{\text{minimum expression value in comparison}}{\text{reference expression value}}$$

and returns genes when fold change \geq 20.

You are searching for genes that are up-regulated between one reference sample and at least two comparison samples.

This calculation creates the narrowest window of expression values in which to look for genes that meet your fold change cutoff. To broaden the window, use the average or maximum comparison value.

- d. Click “Get Answer” to initiate the search. This will return a one-step search strategy. How many genes did you get?



2. To run a GO enrichment analysis on these results, do the following:
 - a. Click on the Analyze Results tab just above the list of genes (arrow in image below) to open the enrichment tools. Besides GO enrichment, what other analyses are available?

TgM4 Oocyst RNA-Seq (fc)
1,073 Genes
Step 1

+ Add a step

1,073 Genes (1,018 ortholog groups) [Revise this search](#)

Gene Results | Genome View | **Analyze Results**

Rows per page: 1000

Gene ID	Transcript ID	Organism	Product Description
TGME49_210682	TGME49_210682-t26_1	<i>Toxoplasma gondii</i> ME49	hypothetical protein

Organism Filter

select all | clear all | expand all | collapse all

Hide zero counts

Search organisms...

Eimeriidae 0

Sarcocystidae 1,073

select all | clear all | expand all | collapse all

Hide zero counts

- b. Click on the GO enrichment option. This will reveal the parameters that you can modify. For the purpose of this exercise, keep all the defaults and click on "Submit".

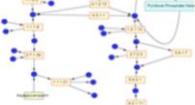
Organism = *T. gondii* ME49
 Ontology = Cellular Component
 Evidence = Computed and Curated
 Limit to GO Slim terms? = NO

Gene Results Genome View New Analysis ✕

Analyze your Gene results with a tool below.



Gene Ontology Enrichment



Metabolic Pathway Enrichment

kinase
phosphatase
exported
membrane

Word Enrichment

Hide Organism Filter

- c. What is the top enriched GO term from this analysis? Does this make sense for an enrichment analysis of the cellular component of your Oocyst expressed genes? Notice that the p-value is a rather low, 10^{-24} .

Gene Ontology Enrichment

Find Gene Ontology terms that are enriched in your gene result. [Read More](#)

▼ Parameters

Organism ?

Ontology ?
 Cellular Component
 Molecular Function
 Biological Process

Evidence ?
 Computed
 Curated
 select all | clear all

Limit to GO Slim terms ?
 No
 Yes

P-Value cutoff ? (0 - 1)

Analysis Results:

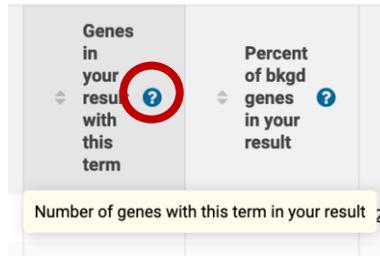
GO ID ?	GO Term ?	Genes in the bkgd with this term ?	Genes in your result with this term ?	Percent of bkgd genes in your result ?	Fold enrichment ?	Odds ratio ?	P-value ?	Benjamini ?
GO:0045177	apical part of cell	90	54	60.0	4.71	11.15	1.27e-26	2.00e-24
GO:0070258	inner membrane pellicle complex	37	19	51.4	4.03	7.42	1.47e-8	7.69e-7
GO:0020039	pellicle	37	19	51.4	4.03	7.42	1.47e-8	7.69e-7

COMMUNITY CHAT

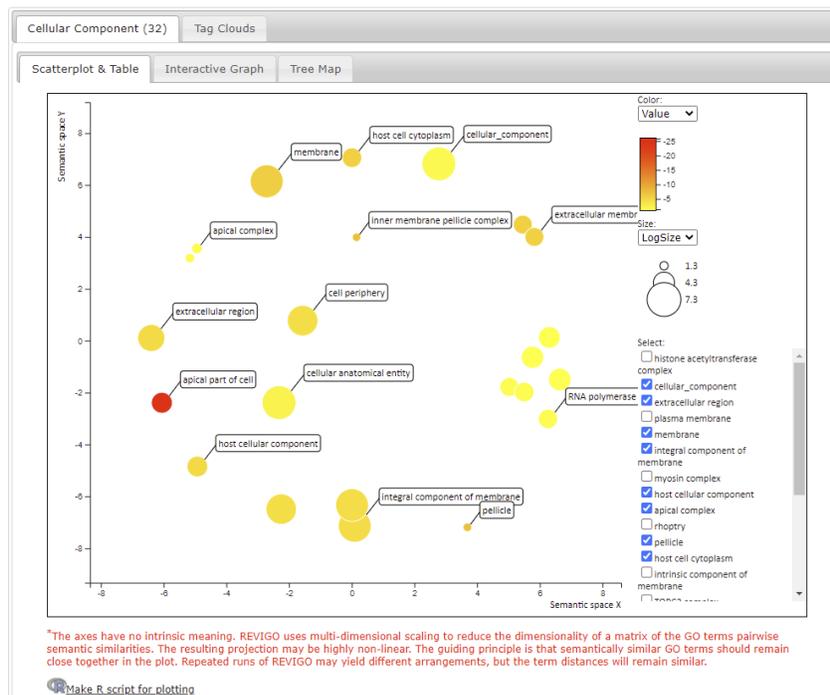
- d. What do each of the columns in the analysis table represent? (Hint: move your mouse over the question mark next to each column header)

- 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 -The odds of the GO term appearing in the gene list are the same as that for the background list

- P-value –The null hypothesis or the probability of getting a result that is equal or greater than what was observed-
- 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

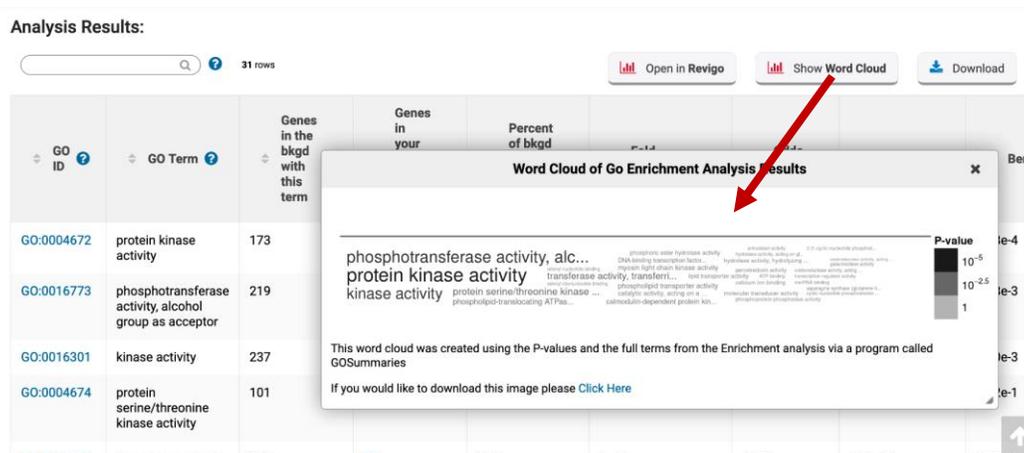


- e. Click the Open in Revigo button to port the results to Revigo, the Reduce and Visualize Ontology tool. Once at Revigo, you may need to scroll down to click Start Revigo to run the analysis with default parameters. Revigo provides a scatterplot and table, an integrative map and a tree map to supplement the table provided in the VEuPathDB site. [Revigo publication](#)



- f. Try rerunning the GO enrichment analysis, but this time select the Molecular Function ontology. What is the top enriched GO term? What is the p-value for the enrichment? Do you have more or less confidence than in 2c that this function is enriched in your gene set?

- g. Click on the “Word Cloud” button above the analysis results. What type of analysis is this? What information can you (See image below).



Additional resources:

Gene Ontology:

<http://geneontology.org/docs/ontology-documentation/>

Enzyme Commission numbers:

<https://www.qmul.ac.uk/sbcs/iubmb/enzyme/>

More info on Fischer’s exact test:

<http://www.biostathandbook.com/fishers.html>

Fisher's Exact Test and the Hypergeometric Distribution (the M&M example):

<https://youtu.be/udyAvvaMjfM>

Some more info about Odds ratios:

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2938757/>

False discovery rates and P value correction:

<http://brainder.org/2011/09/05/fdr-corrected-fdr-adjusted-p-values/>

GO Slim:

<http://www-legacy.geneontology.org/GO.slims.shtml>

REVIGO:

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0021800>