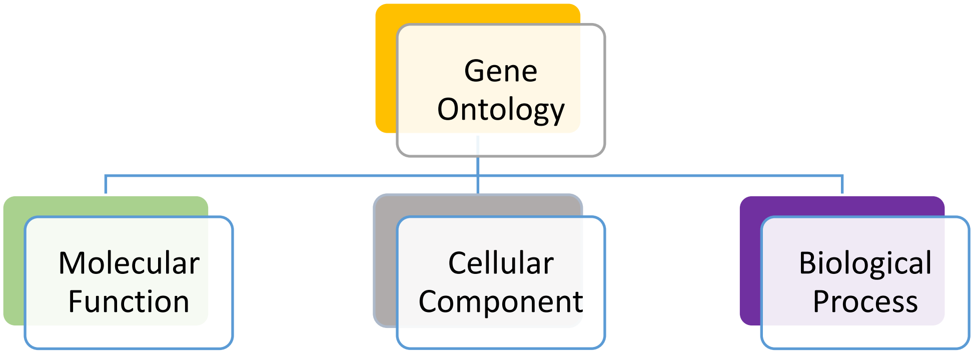
Gene Ontology (GO) Enrichment

Learning objectives:

- Run a GO enrichment analysis
- Explore GO enrichment results

Background:

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

To learn more about Gene Ontology, please visit:
http://geneontology.org/docs/ontology-documentation/

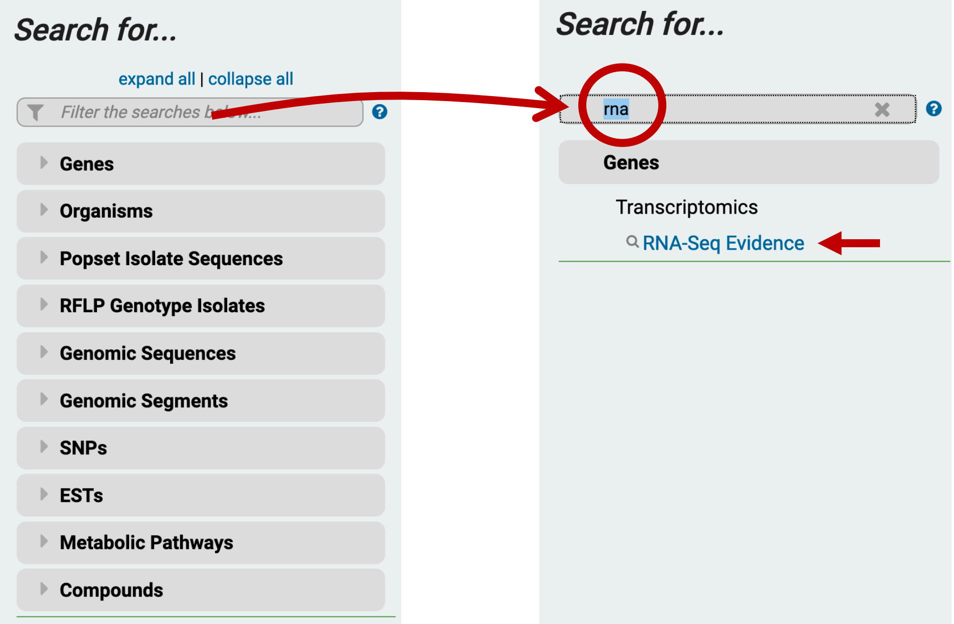
A gene can be assigned a GO term either manually (by an annotator evaluating experimental evidence) or computationally (based on the GO terms of genes that share sequence or functional domains). These GO terms can be used to test whether your set of genes are enriched for a molecular function, cellular component, or biological process.

For example: Does my list of genes have an over-representation of specific GO terms compared to the rest of the genome?

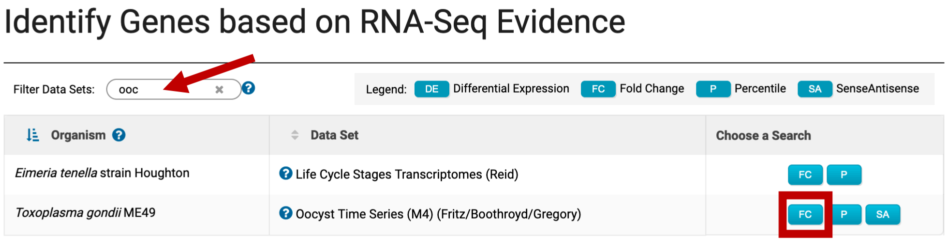
A standard enrichment method employs Fisher's exact test, a statistical test that evaluates a 2x2 contingency table (in this case, number of genes in my set *versus* number of genes from genome not in my set, and number of genes with GO term Z *versus* number of genes without term Z). This test produces 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 that you can upload using 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".



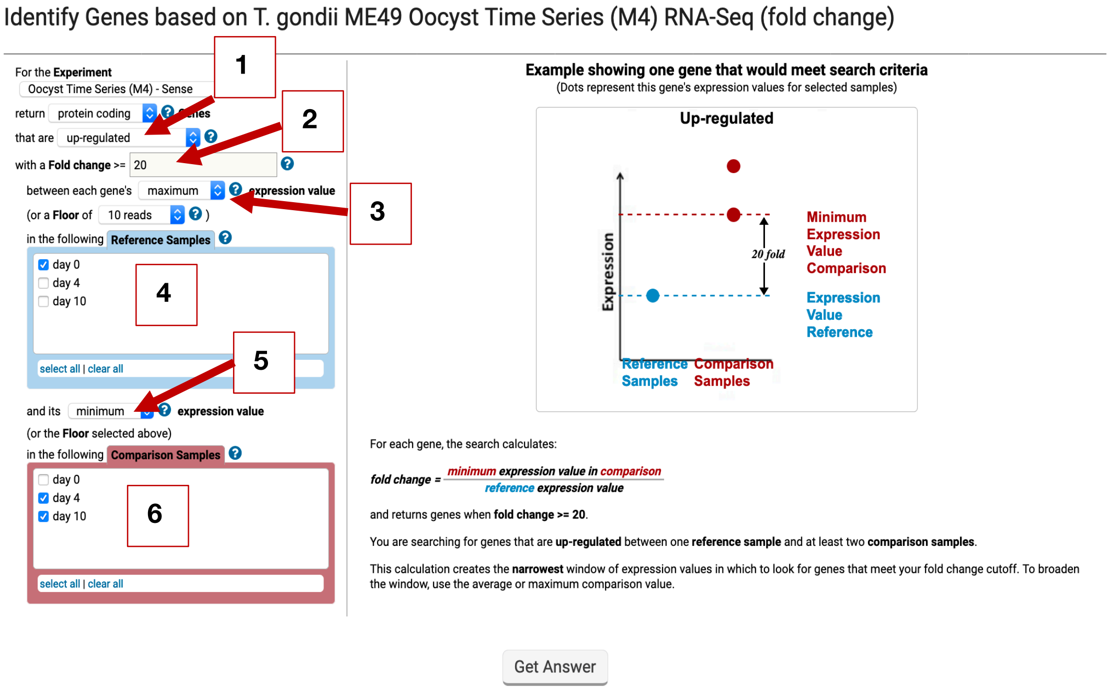
Identify Genes based on RNA-Seq Evidence

Filter Data Sets: ooc

Legend: DE Differential Expression FC Fold Change P Percentile SA SenseAntisense

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

c. Once you find the data set of interest, click on the fold-change (FC) option. This will open a search page that contains the parameters that you can manipulate to search this data set. For this exercise, identify genes that are upregulated by 20-fold in the day 4 and day 10 time points 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 ?

with a **Fold change** >= 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

select all | clear all

and its minimum ? **expression value**

(or the **Floor** selected above)

in the following **Comparison Samples** ?

☐ day 0
☑ day 4
☑ day 10

select all | clear all

Example showing one gene that would meet search criteria

(Dots represent this gene's expression values for selected samples)

For each gene, the search calculates:

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

and returns genes when **fold change >= 20**.

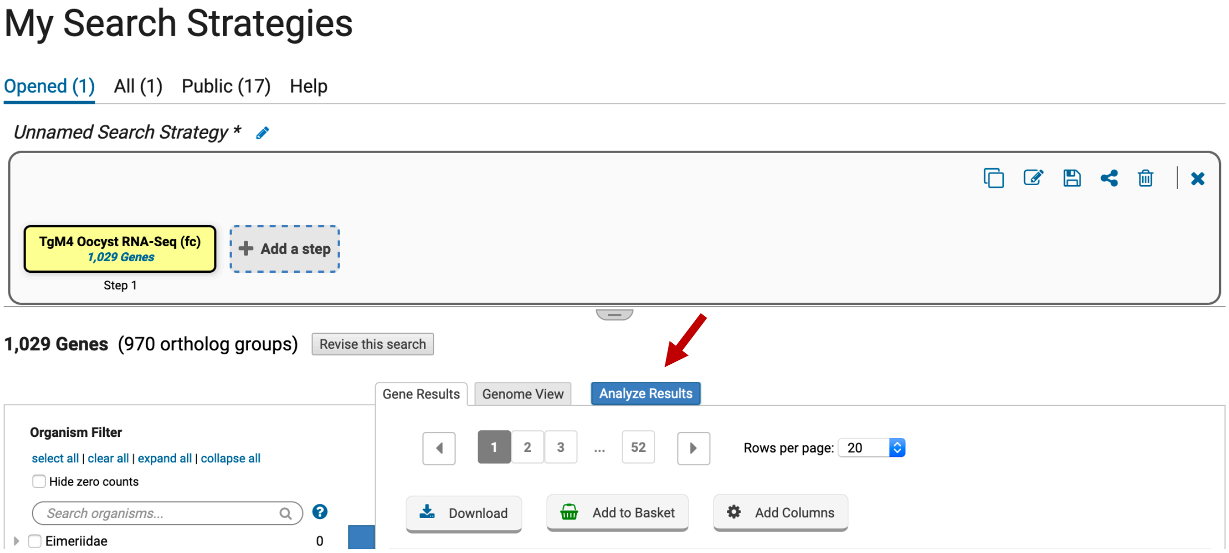
You are searching for genes that are **up-regulated** between one **reference sample** and at least two **comparison samples**.

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. Once you have set the parameters, click the "Get Answer" button at the bottom of 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).

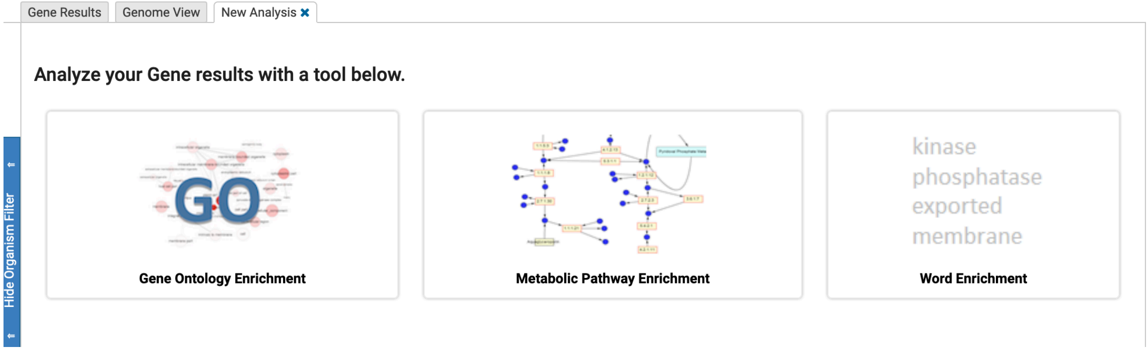


My Search Strategies

Unnamed Search Strategy *

1,029 Genes (970 ortholog groups)

b. Click on the "Analyze Results" tab to reveal the different analyses that you can run on your results. Besides GO enrichment, what other analyses are available?

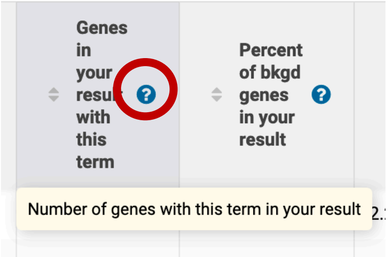


Analyze your Gene results with a tool below.

c. 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".

d. What is the top enriched GO term from this analysis?

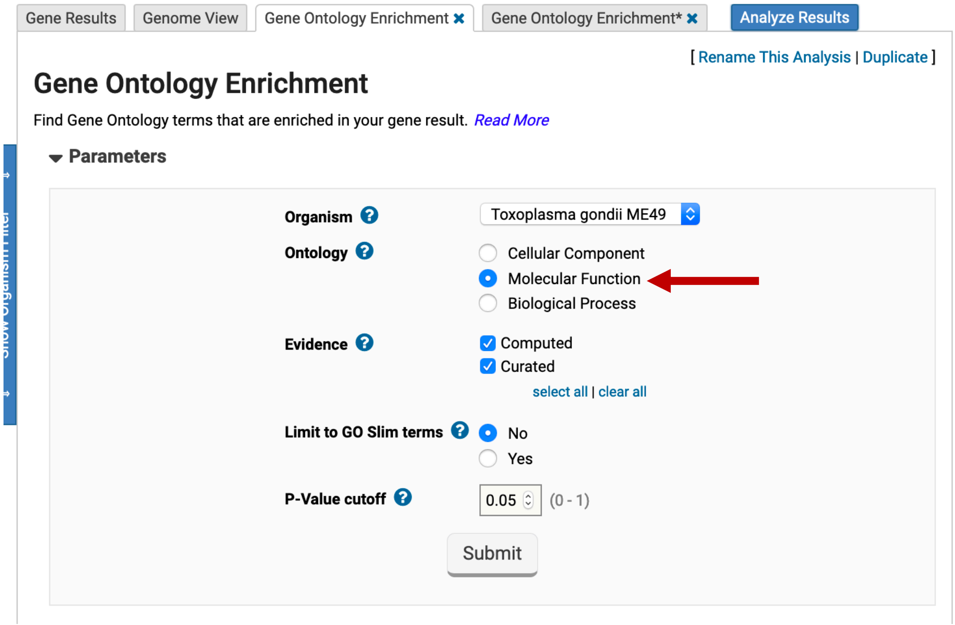
e. What do each of the columns in the analysis table represent? (Hint: move your mouse over the question mark next to each column header to get more information.)



Number of genes with this term in your result

Genes in your result with this term
Percent of bkgd genes in your result

f. Try rerunning the GO enrichment analysis, but this time select the Molecular Function ontology. What is the top enriched GO term?



[Rename This Analysis | Duplicate]

Gene Ontology Enrichment

Find Gene Ontology terms that are enriched in your gene result. *Read More*

▾ **Parameters**

Organism ? Toxoplasma gondii ME49

Ontology ?
○ Cellular Component
● Molecular Function
○ Biological Process

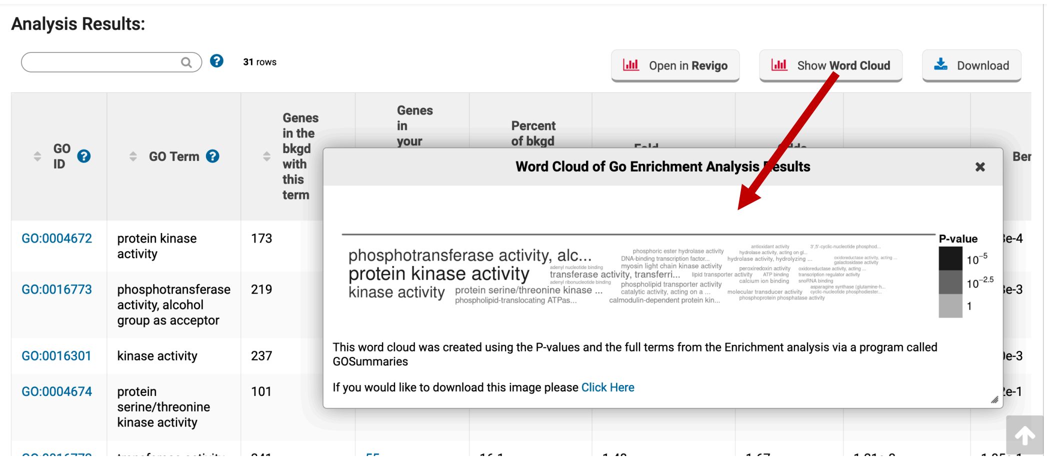
Evidence ?
☑ Computed
☑ Curated
select all | clear all

Limit to GO Slim terms ?
● No
○ Yes

P-Value cutoff ? 0.05 (0 - 1)

Submit

g. Click on the "Word Cloud" button above the analysis results. What does this do? (See image below).



Analysis Results:

? 31 rows

GO ID ?	GO Term ?	Genes in the bkgd with this term	Genes in your	Percent of bkgd	Fold	Odds		Ber
GO:0004672	protein kinase activity	173						e-4
GO:0016773	phosphotransferase activity, alcohol group as acceptor	219						e-3
GO:0016301	kinase activity	237						e-3
GO:0004674	protein serine/threonine kinase activity	101						e-1

Word Cloud of Go Enrichment Analysis Results ✖

phosphotransferase activity, alc...
protein kinase activity
kinase activity
protein serine/threonine kinase ...
phospholipid-translocating ATPas...
transferase activity, transferri...
adenyl nucleotide binding
adenyl ribonucleotide binding
phosphoric ester hydrolase activity
DNA-binding transcription factor...
myosin light chain kinase activity
phospholipid transporter activity
catalytic activity, acting on a ...
calmodulin-dependent protein kin...
lipid transporter activity
antioxidant activity
hydrolase activity, acting on gl...
hydrolase activity, hydrolyzing ...
peroxiredoxin activity
ATP binding
calcium ion binding
molecular transducer activity
phosphoprotein phosphatase activity
3',5'-cyclic-nucleotide phosphod...
oxidoreductase activity, acting ...
galactosidase activity
oxidoreductase activity, acting ...
transcription regulator activity
snoRNA binding
asparagine synthase (glutamine-h...
cyclic-nucleotide phosphodiester...
P-value
10^-5
10^-2.5
1

This word cloud was created using the P-values and the full terms from the Enrichment analysis via a program called GOSummaries

If you would like to download this image please Click Here

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