Expression Searches: Transcriptomics and Proteomics

Learning Objectives

- Review the types of expression searches in VEuPathDB.
- Use the RNA-Seq differential expression, fold change and percentile searches to explore gene expression in midgut and proventriculus expression of *Trypanosoma brucei* infections.
- Use the Proteomics peptides and quantitative searches to explore expression at the protein level.

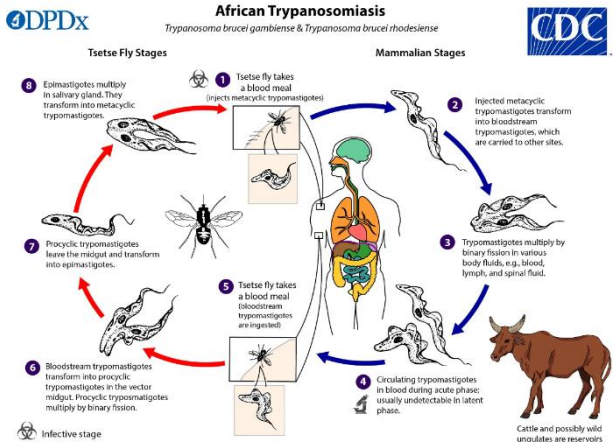
Transcript expression or the abundance of an mRNA, can be determined in the laboratory with several different techniques including RNA-sequence, microarray, and RT-PCR. VEuPathDB supports these data types with several searches (see table below). For RNA sequence data, expression values are graphed on gene pages and mapped reads can be visualized in the genome browser. Proteomics experiments can determine the peptide sequence by mass spectrometry or the abundance value via e.g. isobaric tagging for a peptide in a sample. Each data type is available on gene record pages but only the mass spec peptides have data that can be viewed in JBrowse. Using the search strategy system, it's easy to delve deep into a specific data set and to take advantage of several types of data when combining search results in the strategy system.

Search	Description	RNA-seq	Micro-array	Proteomics
Differential Expression	Statistical analysis of studies whose experimental design includes biological replicates. A differential expression search finds genes based on fold change difference between two samples with a user defined p-value cutoff. Only pairwise comparisons can be made with this search.	✔		
Fold Change	Expression differences between samples are calculated but statistical analyses are not performed. A fold change search finds genes whose expression value differs between samples without considering statistical parameters. This search offers a form of differential expression analysis when the experimental design did not include replicates and allows for comparing groups of samples, e.g. find genes whose expression is up-regulated in the liver time course (2, 24, 36, and 54 hours) vs the control (0 hours).	✔	✔	
Percentile	For each sample in an experiment, each genes' expression value is sorted from lowest to highest and a percentile rank is determined. For example, a percentile search can find genes whose expression is in the highest 10% of expression values within a sample.	✔	✔	
Sense/Antisense	For strand-specific RNA sequence, expression values are determined in the sense and antisense direction. This search finds genes that exhibit simultaneous changes in sense and antisense transcripts. For example, you can look for genes with increasing antisense transcripts and decreasing sense transcripts, as might occur when antisense transcription suppresses sense transcription.	✔		
Splice-site Location	This trypanosome-specific search takes advantage of the 'splice-leader' RNA-seq data which determines transcript abundance within the polycistronic mRNA using splice-leader specific primers. This search identified genes whose 5' splice site location varies between samples.	✔		

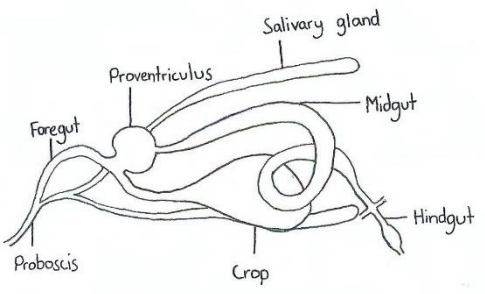
Metacycle	The MetaCycle package detects rhythmic signals from large scale time-series data, such as circadian rhythms within expression time courses, using either ARSER or JTK-Cycle. This search returns genes whose rhythmic signals match the conditions (period and amplitude range) you specify. The search will return the corresponding period, amplitude and p-value of genes that meet your search criteria.	✔	✔	
Similarity	The similarity search returns genes whose expression profile within the experiment follow a similar pattern as the gene you specify.	✔	✔	
Direct Comparison	Microarray data for two samples is often collected on the same glass slide. For these experiments, the direct comparison search returns genes whose expression varies between samples in pairwise comparisons.		✔	
Co-expression	Meta-analysis across multiple microarray experiments defined a co-expression network. This search returns genes within the co-expression network of your gene(s) of interest.		✔	
Mass spec. evidence	Peptides from proteomics experiments are mapped to a reference genome enabling searches for genes based on the mapping			✔
Quantitative mass spec. evidence	Quantitative proteomic experiments produce abundance values for proteins identified in the sample analyzed. (excel spreadsheets of gene/protein IDs and expression intensity values)			✔
Post-translational modification (PTM)	PTM data from proteomics experiments, excel spreadsheets of gene/protein IDs, location and type of modification, are associated with genes in VEuPathDB, enabling searches for genes based on the type and number of the PTM.			✔

1. **Find genes that are up-regulated in the midgut compared to the Proventriculus stages of *Trypanosoma* infection.** TriTrypDB.org

   The life cycle of *Trypanosoma brucei* is split between the tsetse fly and the human host. The fly stage includes a migration from the midgut to the proventriculus and eventually to the salivary gland where it can infect a hose during a blood meal. The cycle in the fly takes approximately 3 weeks. The erythrocytic stages are well studied compared to the liver stages.

   TriTrypDB contains RNA seq data from samples taken during the establishment of infection and progression of *Trypanosoma brucei brucei* through its insect host. (Naguleswaran et al. 2021) This data set includes a time course of midgut samples from 3 to 28 days, as well as proventriculus and salivary gland samples.

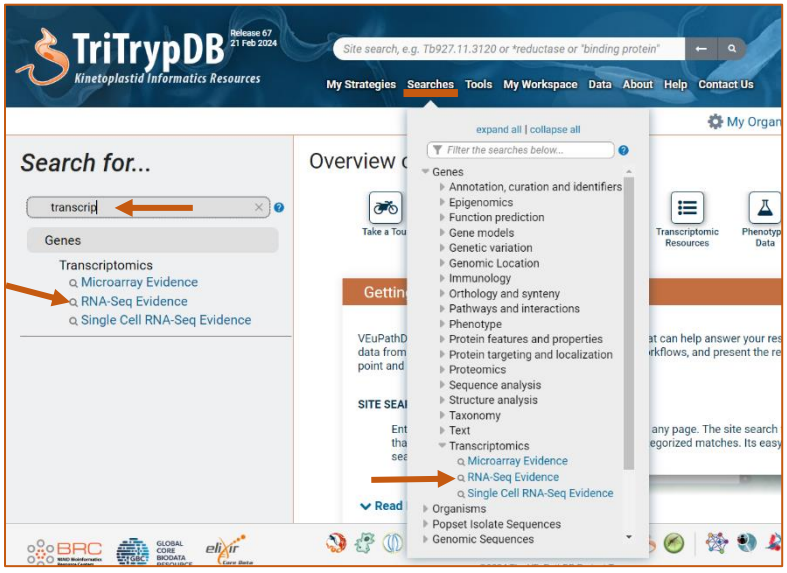
   

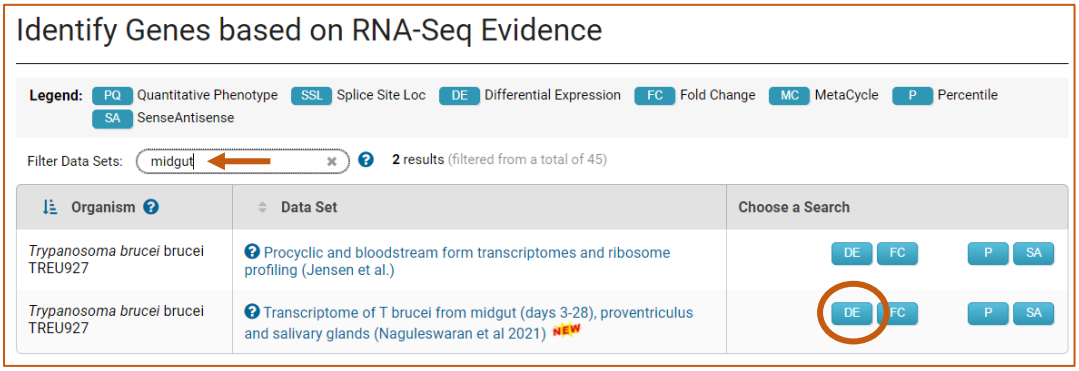
   - Midgut Day 3
   - Midgut Day 7

- Midgut Day 11
- Midgut Day 15
- Midgut Day 28
- Proventriculus Day 28
- Salivary Gland Day 28
- This gene is not statistical https://tritrypdb.org/tritrypdb/app/record/gene/Tb927.8.8280

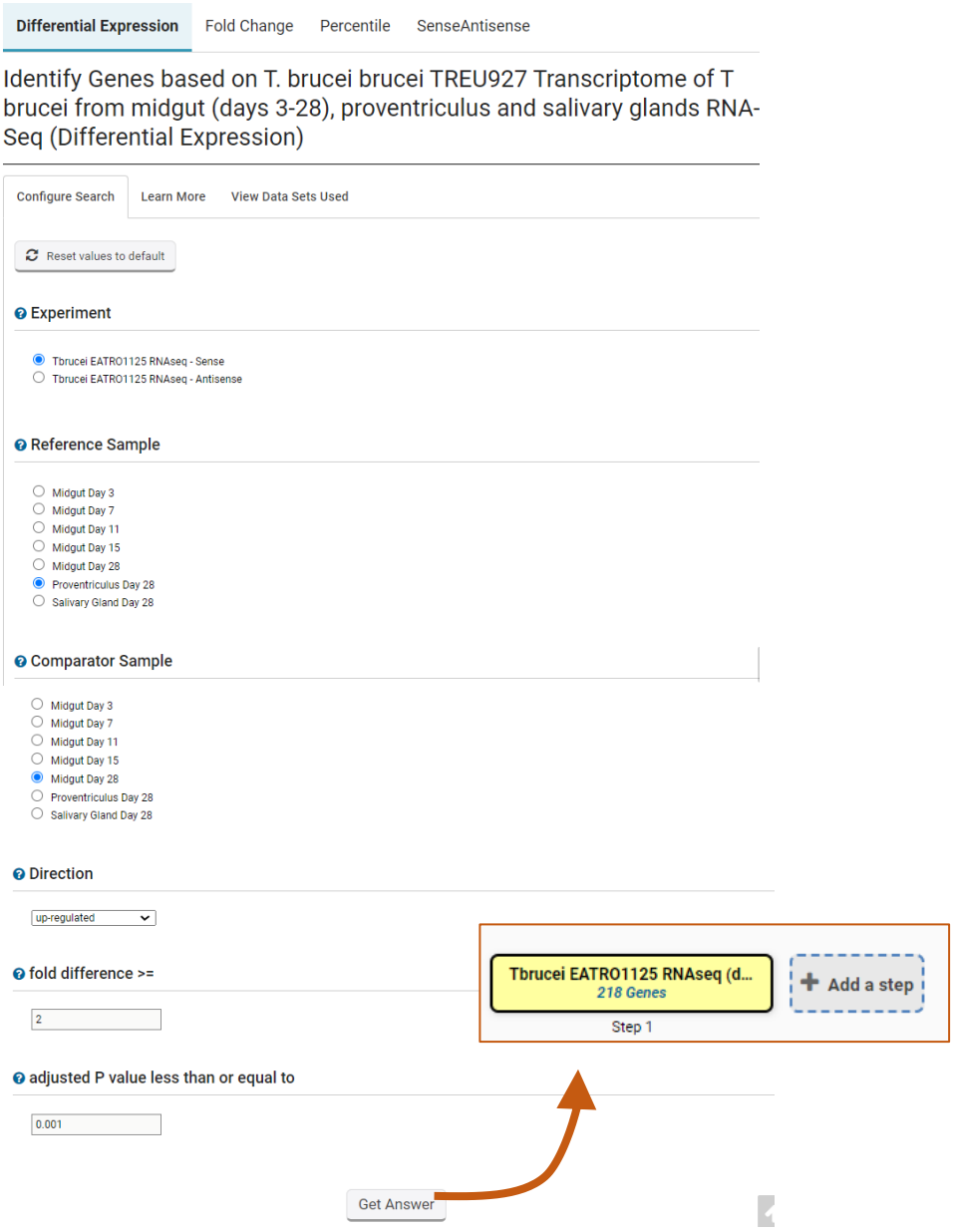
Use this data set to determine what genes are upregulated at least 2 fold (p-value <= 0.001) at Midgut Day 28 vs the proventriculus Day 28.

a. Navigate to the RNA seq search page and find the data set called **Transcriptome of *T brucei* from midgut (days 3-28), proventriculus and salivary glands (Naguleswaran et al 2021).** Searches are available from the Search For… menu on the left side of the home page, as well as the Searches drop down menu in the header.

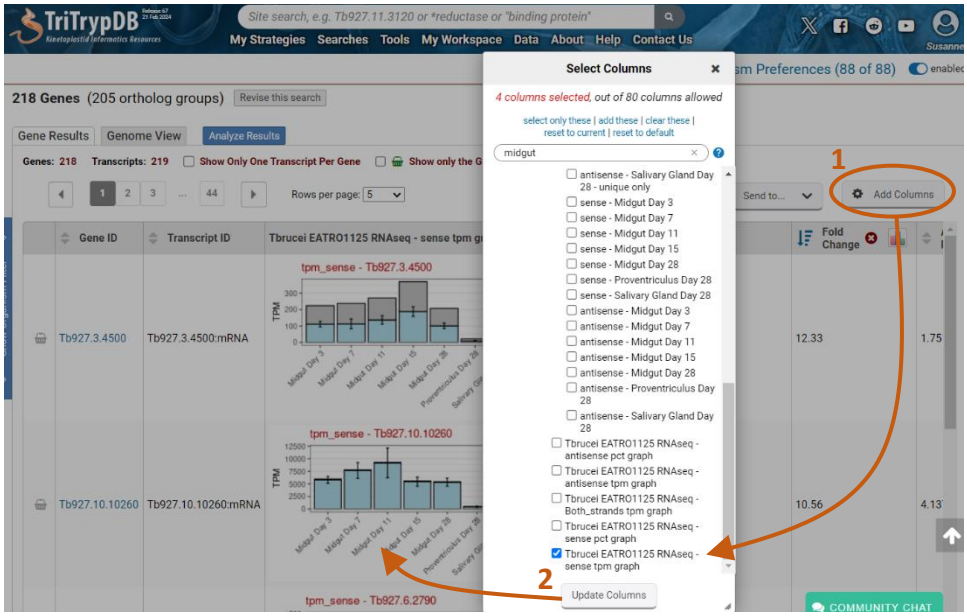




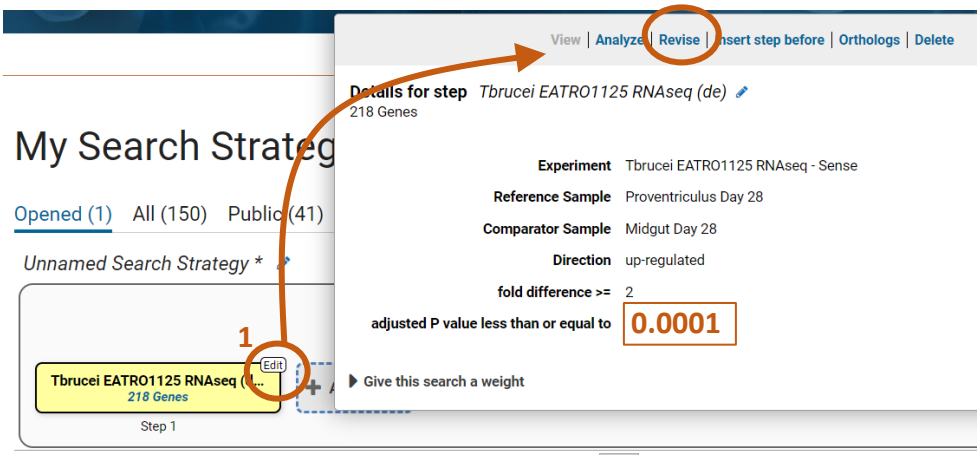
b. Arrange the differential expression search to return genes that are at **least 2-fold up-regulated** in the Midgut Day 28 sample compared to the Proventriculus Day 28 with a p-value of p<0.001.

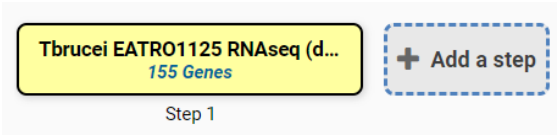


c. How many genes were returned by the search? Do you believe these results? To convince yourself, you could turn on a column with the TPM graph and check if Midgut day 28 is at least 2-fold higher than proventriculus. (The column with the graph might already be showing in the result table.)

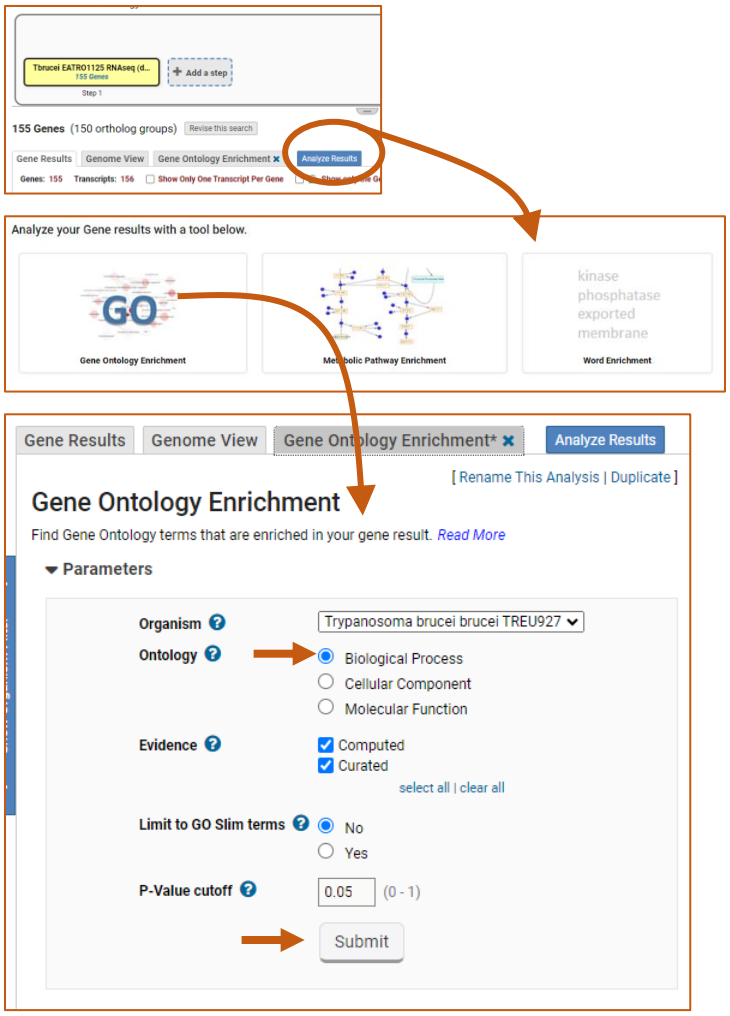


d. Increase the statistical stringency of the search from p <= 0.001 to p<0.0001. How many genes are returned by the search now? Hint: revise the search and change the p-value. Hover over the yellow search box until the Edit icon appears. Click the Edit icon and choose revise from the options panel.





e. Analyze this gene result for enriched GO terms in the Biological Process ontology. The Analyze Results tab offers tools for performing a GO, metabolic pathway and word enrichments. GO terms are a form of annotation that provide information about a gene's biological function, molecular function and cellular location. A GO Enrichment is a statistical analysis that determines the probability that the 155 genes in the result share a term compared to the genome. What Biological processes are shared by the gene set?


Tbrucei EATRO1125 RNAseq (d...
155 Genes
Add a step
Step 1
155 Genes (150 ortholog groups)
Revise this search
Gene Results
Genome View
Gene Ontology Enrichment
Analyze Results
Genes: 155
Transcripts: 156
Show Only One Transcript Per Gene
Analyze your Gene results with a tool below.
GO
Gene Ontology Enrichment
Metabolic Pathway Enrichment
kinase
phosphatase
exported
membrane
Word Enrichment
Gene Results
Genome View
Gene Ontology Enrichment*
Analyze Results
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Gene Ontology Enrichment
Find Gene Ontology terms that are enriched in your gene result. Read More
Parameters
Organism
Trypanosoma brucei brucei TREU927
Ontology
Biological Process
Cellular Component
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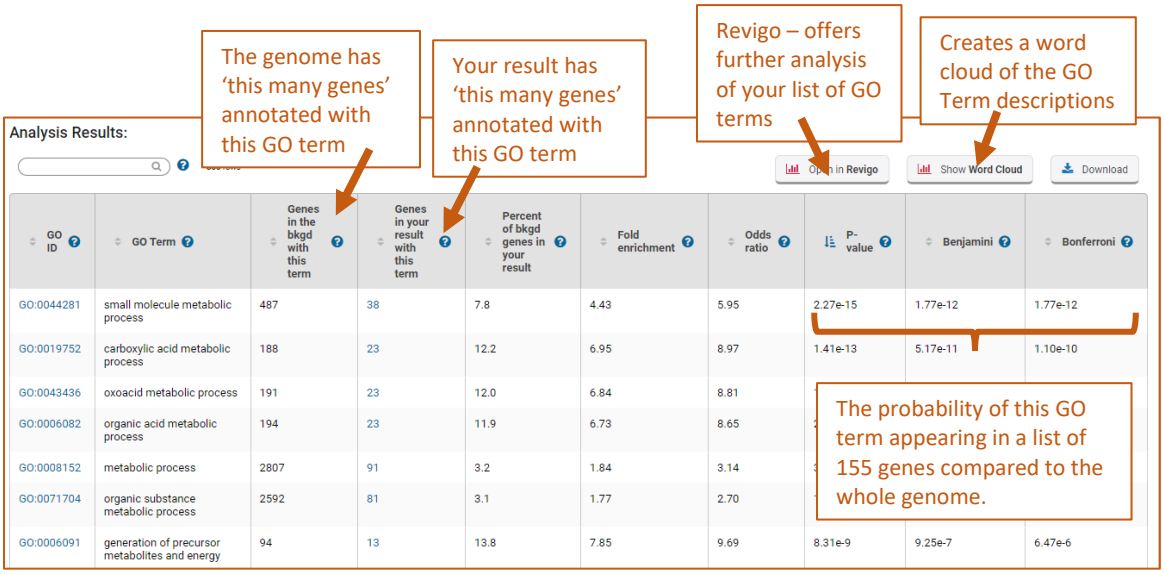
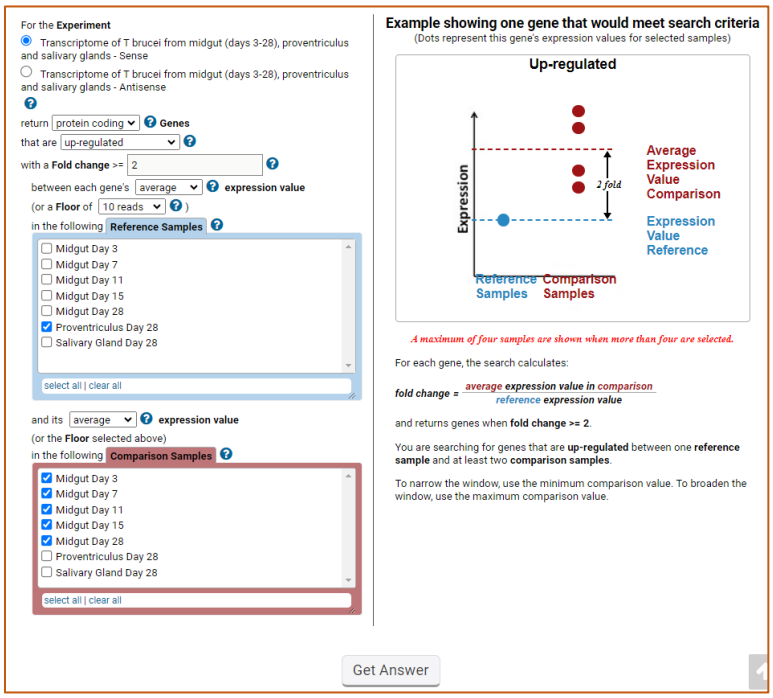
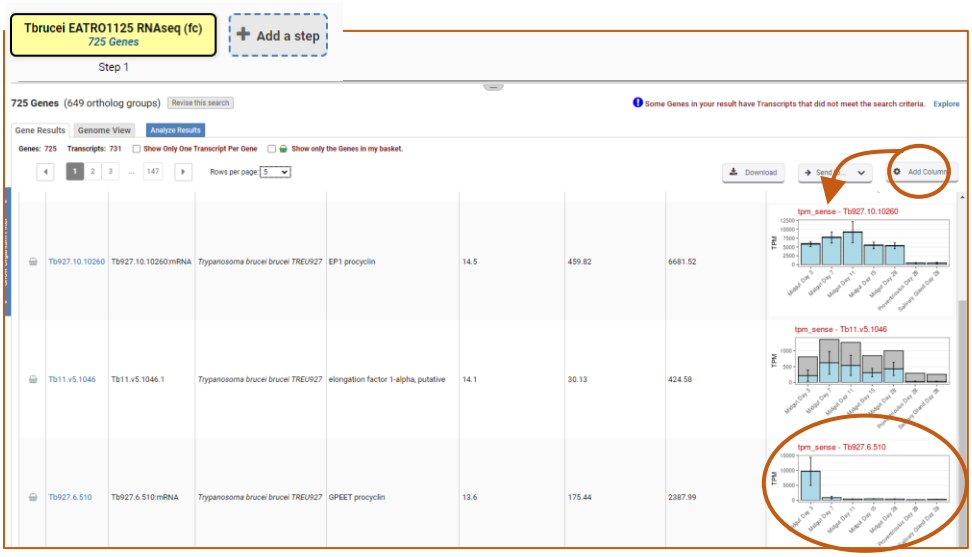

The genome has 'this many genes' annotated with this GO term
Your result has 'this many genes' annotated with this GO term
Revigo – offers further analysis of your list of GO terms
Creates a word cloud of the GO Term descriptions
Analysis Results:
Open in Revigo
Show Word Cloud
Download
GO ID
GO Term
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:0044281 small molecule metabolic process 487 38 7.8 4.43 5.95 2.27e-15 1.77e-12 1.77e-12
GO:0019752 carboxylic acid metabolic process 188 23 12.2 6.95 8.97 1.41e-13 5.17e-11 1.10e-10
GO:0043436 oxoacid metabolic process 191 23 12.0 6.84 8.81
GO:0006082 organic acid metabolic process 194 23 11.9 6.73 8.65
GO:0008152 metabolic process 2807 91 3.2 1.84 3.14
GO:0071704 organic substance metabolic process 2592 81 3.1 1.77 2.70
GO:0006091 generation of precursor metabolites and energy 94 13 13.8 7.85 9.69 8.31e-9 9.25e-7 6.47e-6
The probability of this GO term appearing in a list of 155 genes compared to the whole genome.

2. **Using the same data set, find genes that are upregulated 2-fold in any midgut stage compared to proventriculus.**

a. Navigate to the RNA Seq search page and choose the **Fold Change** search for the **Transcriptome of *T brucei* from midgut (days 3-28), proventriculus and salivary glands (Naguleswaran et al 2021).** data set as in 1a above.

b. Arrange the fold change search to return **protein coding** genes that are **up-regulated** in the **average** expression across the midgut samples (comparator) compared to the proventriculus sample (reference).

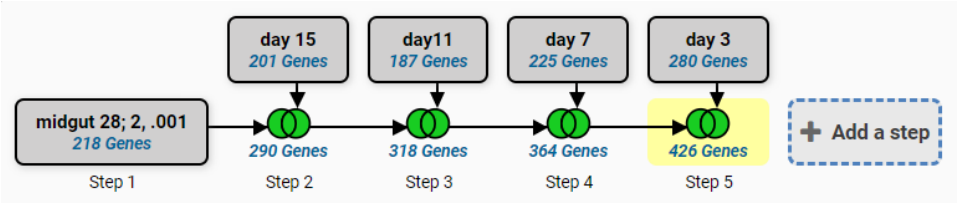




c. Explore your results. Did the search return more genes or fewer genes than the differential expression search? How confident are you that this gene list?

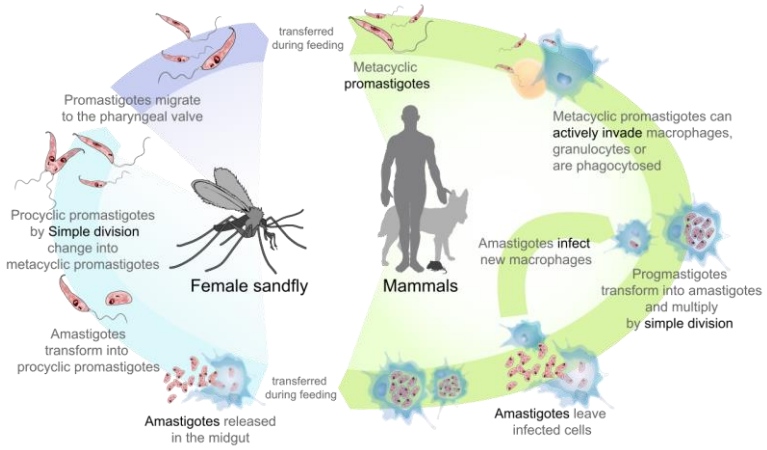
d. Use the Add Columns to turn on the TPM graph for the 'Ex-erythrocytic stages' data set. Notice the error bars and fold change values for the Tb927.6.510 GPEET procyclin gene. Would GPEET procyclin gene be returned by the Differential Expression search for with pairwise comparisons of Midgut Days 7, 11, 15, 28 with proventriculus? (You may need to sort the Fold Change column in the results, to display the gene with the highest FC value at the top. Then GPEET is about the 4th gene down)

e. How would you use the DE search to find genes upregulated in any midgut sample compared to proventriculus? Hint: Use the strategies to sum the results of several pairwise comparisons.



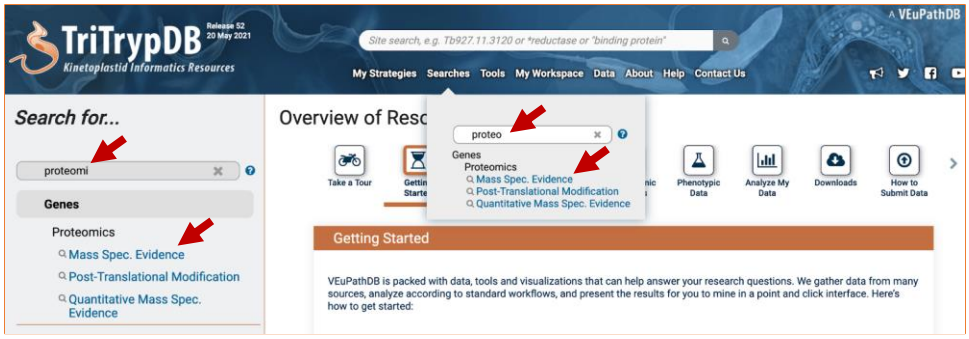
https://tritrypdb.org/tritrypdb/app/workspace/strategies/import/d432e56011aae73e

3. Find *Leishmania infantum* genes that have protein expression evidence from metacyclic stages but not amastigote or promastigote stages. Note: for this exercise use http://tritrypdb.org

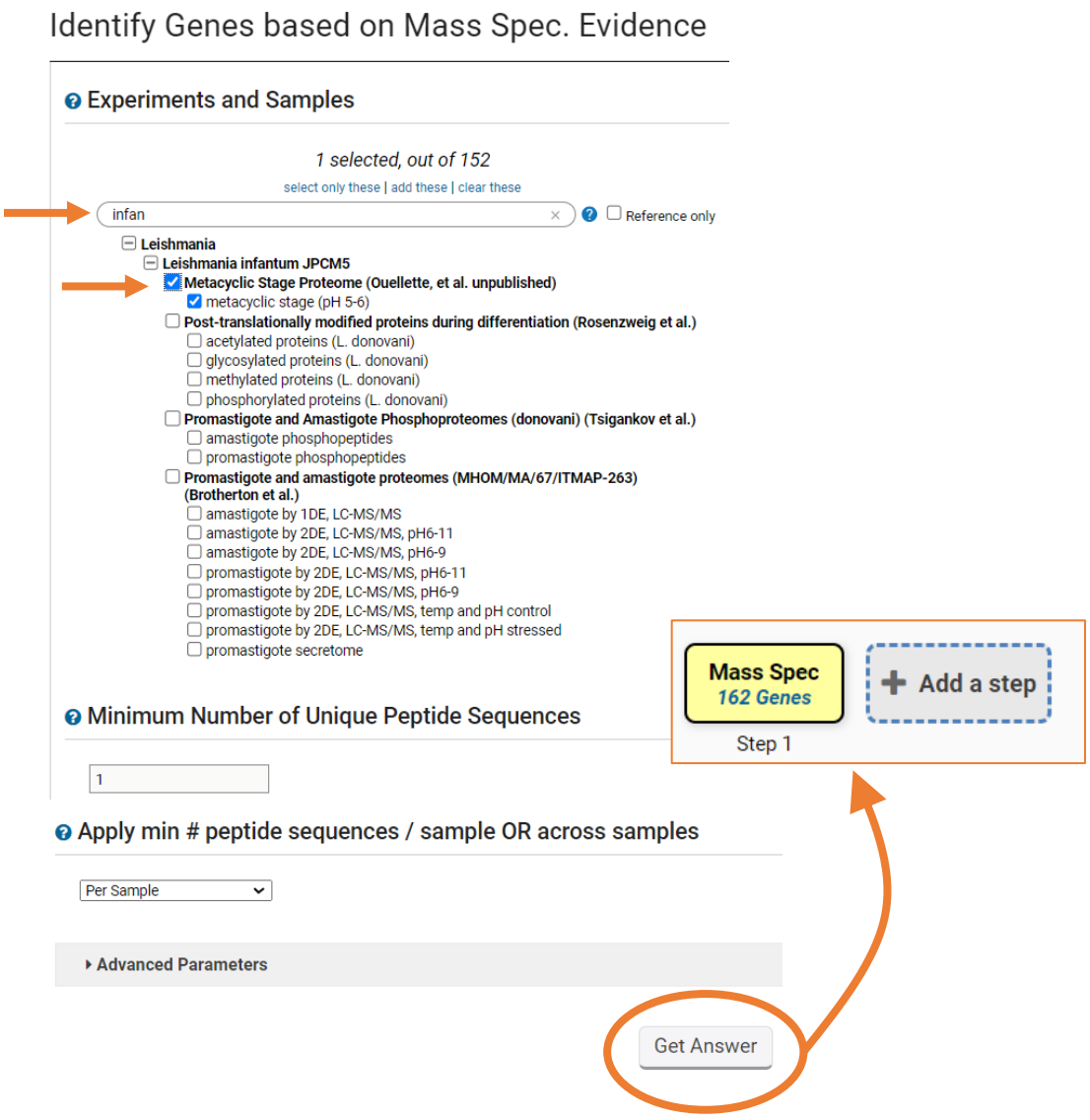


Life cycle of Leishmania. https://commons.wikimedia.org/wiki/File:Leishmaniasis_life_cycle_diagram_en.svg

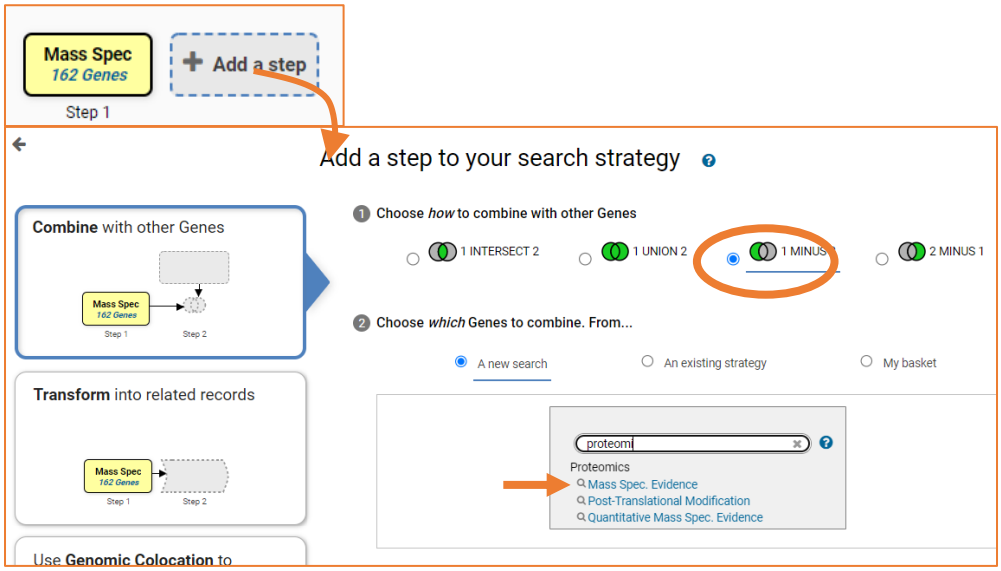
a. Navigate to the mass spec. evidence search. This search returns genes whose protein products mapped to peptides found in proteomics experiments.



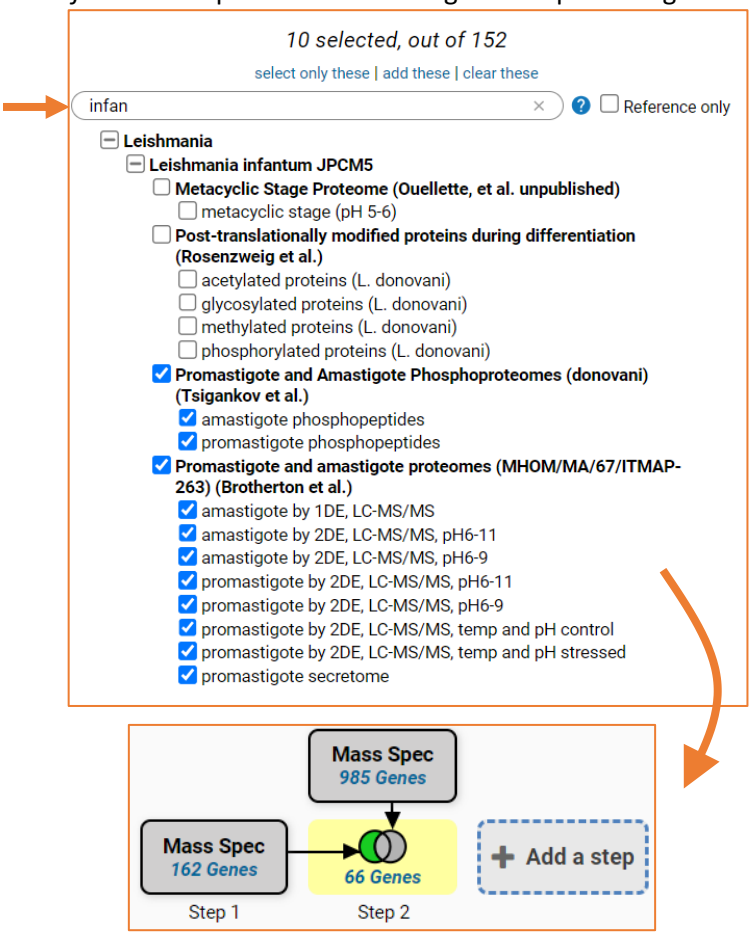
b. Filter the experiment and sample tree by typing a word in the filter box. **Select all *L. infantum* samples that come from the metacyclic stages.** Keep the default search parameters and click on the Get Answer button.



c. Now subtract the genes that have protein expression in the amastigote and promastigote stages. Add a step to your strategy that returns amastigote and promastigote genes and choose the 1 minus 2 operator to combine the searches.

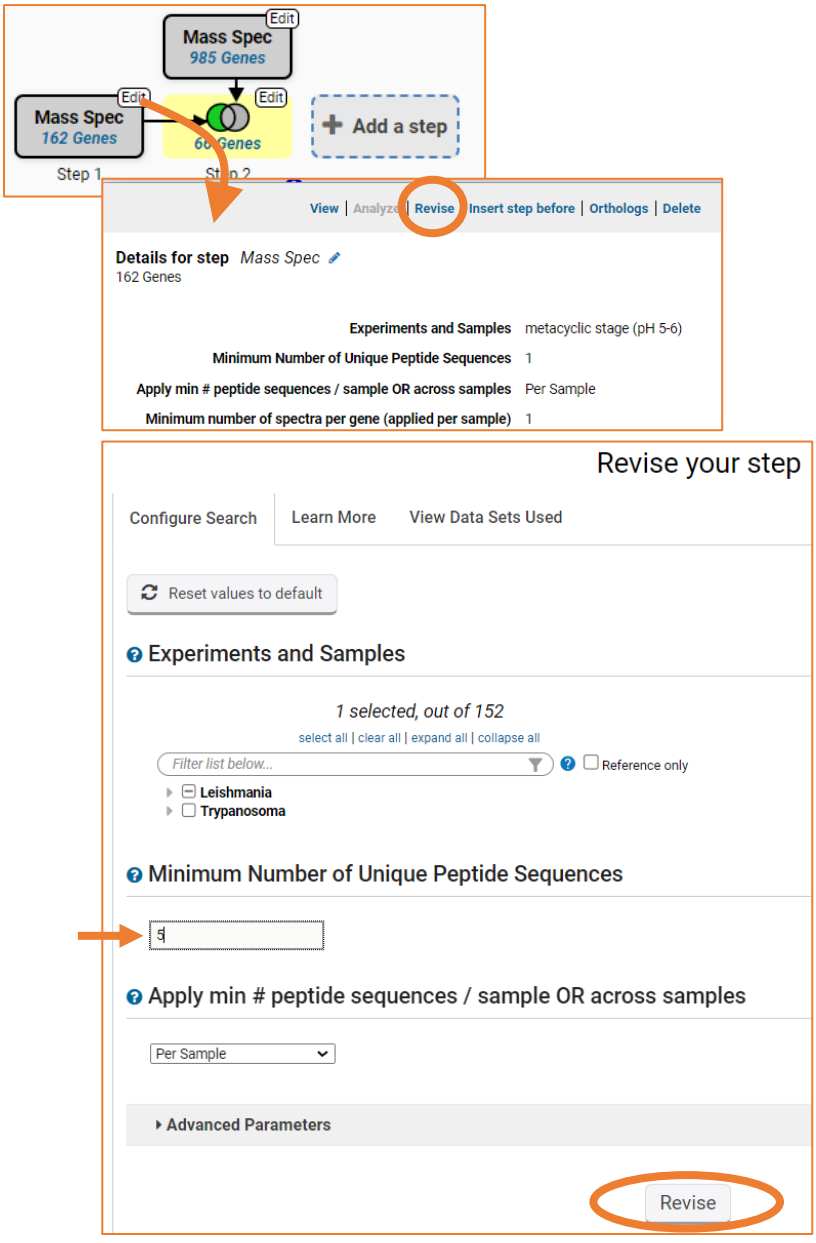


d. Choose all the *L. infantum* samples labeled amastigote and promastigote and run the search

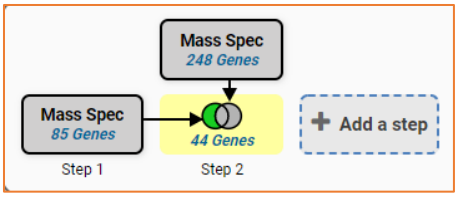


e. Visit the gene pages of some of your results. There you can view mapped peptides and data from other experiments. (https://tritrypdb.org/tritrypdb/app/record/gene/LINF_340044200#ProteinExpressionPBrowse)

f. How can you increase the stringency of your results? One way is to increase the minimum number of unique peptides that are required to map to a gene before it is returned by the search. The default settings that we used above return any gene with a minimum of one peptide. Edit each search to look for genes that are supported by 5 peptide sequences instead of just one.



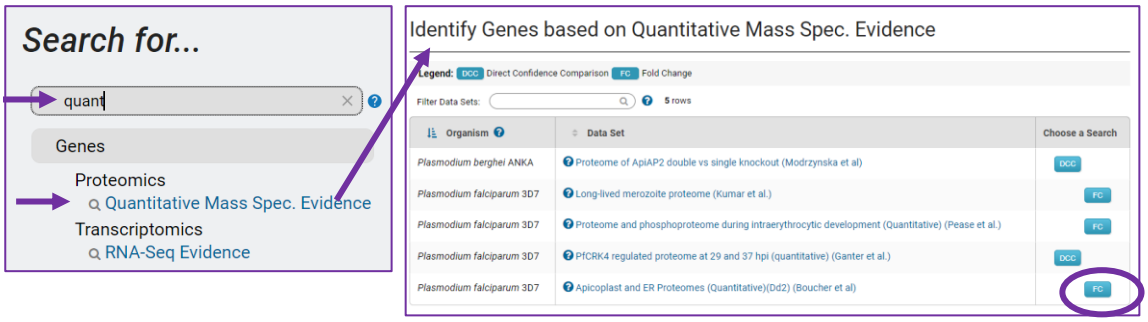
g. How did this change your results? Are you more or less confident in saying that these genes are expressed at the protein level?



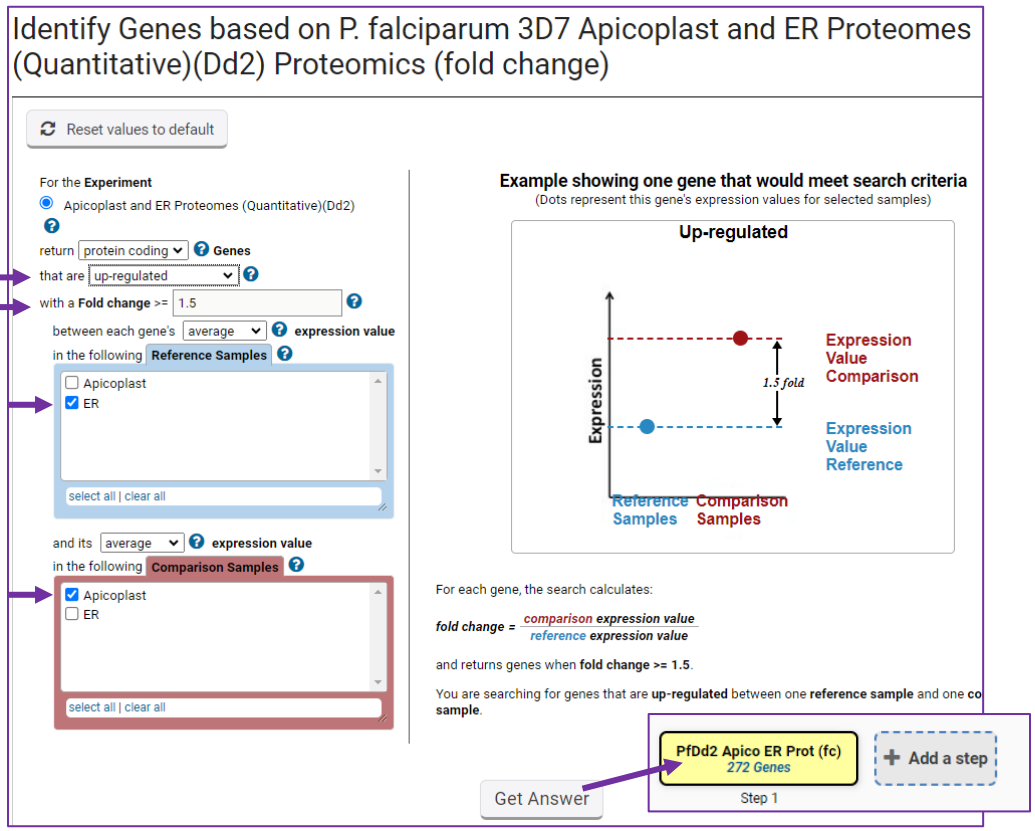
4. **Find genes in *Plasmodium falciparum* that are present at a higher concentration in the apicoplast compared to the endoplasmic reticulum (ER). Note for this exercise use https://plasmodb.org**

a. Go to the **quantitative** mass spec evidence searches

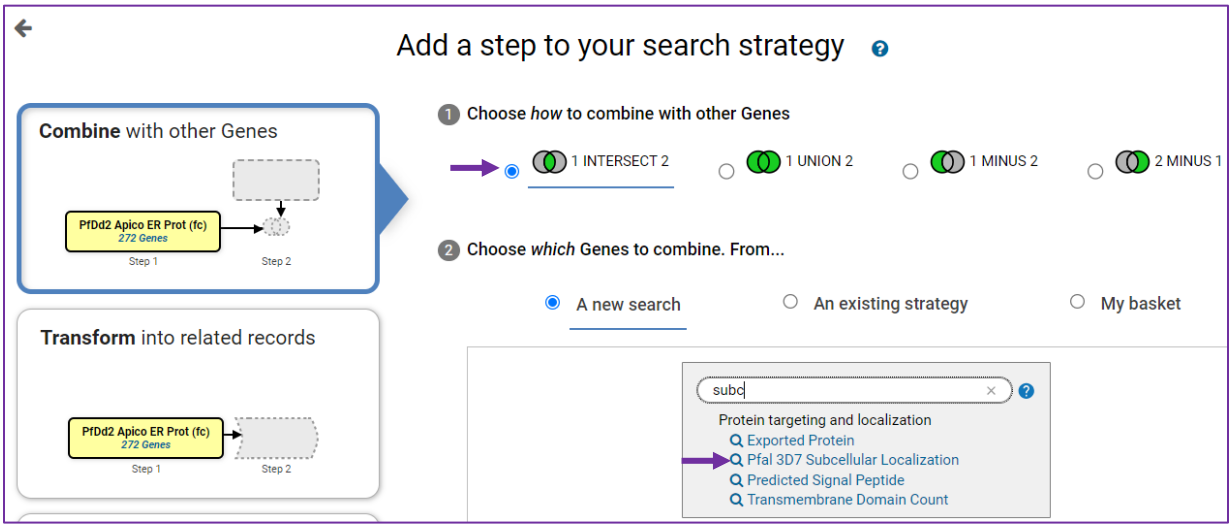
b. Select the experiment called Apicoplast and ER Proteomes (Quantitative)(Dd2) (Boucher et al)



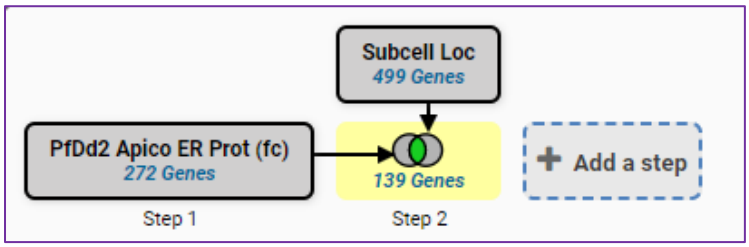
c. Configure this search to return all genes that are upregulated by 1.5 fold in the apicoplast sample compared to the ER sample.



d. Can you leveraging other data about apicoplast biology to validate your results? For example, it is well known that proteins with transit peptides are targeted to the apicoplast. PlasmoDB has a search that returns genes with the transit peptides. Add a step to your strategy that increases the stringency of evidence for these genes being apicoplast genes. The search is called 'Pfal 3D7 Subcellular Localization'

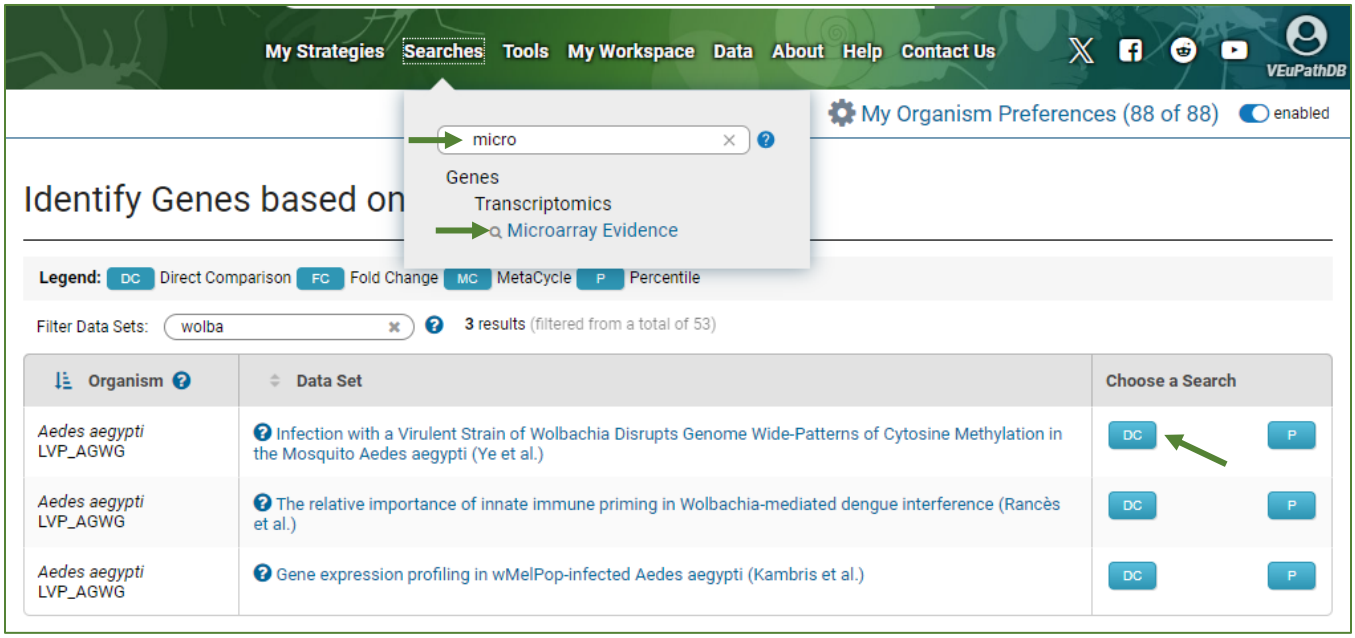


e. Make sure Apicoplast localization is selected and click on the Run Step button. How many genes did you identify? Are you more confident that these genes are apicoplast genes? How would you use the PlasmoDB tools to boost your confidence in these so called apicoplast genes?

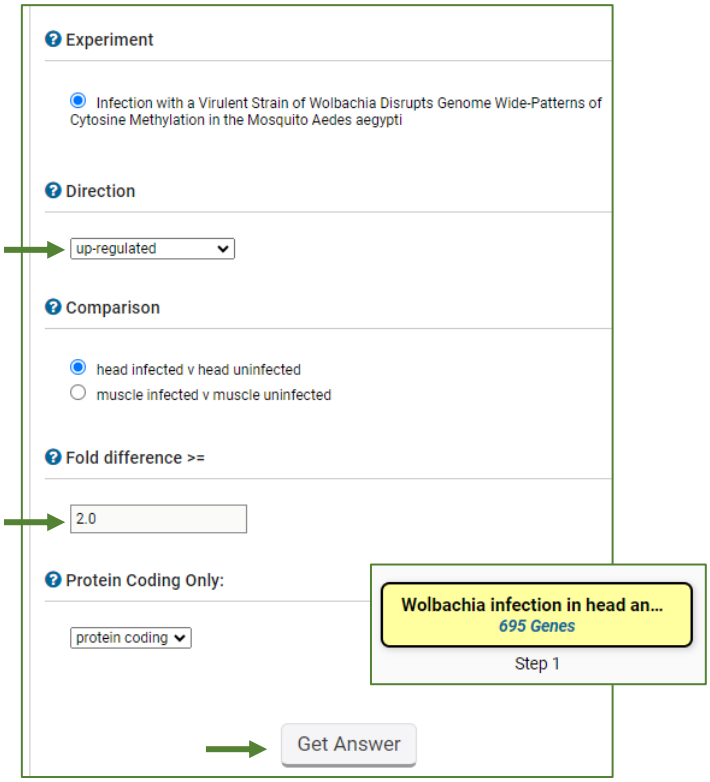


5. **Find *Aedes aegypti* genes that are upregulated in both head and muscle during infection with *Wolbachia***. The *Wolbachia* strain wMelPop, which reduces longevity in *Drosophila melanogaster*, has been introduced into the Dengue virus mosquito vector, *Aedes aegypti* as a strategy to reduce disease transmission. VectorBase has a **microarray** data set that compared *Wolbachia* infected and uninfected mosquito head and muscle. This exercise uses VectorBase.org.

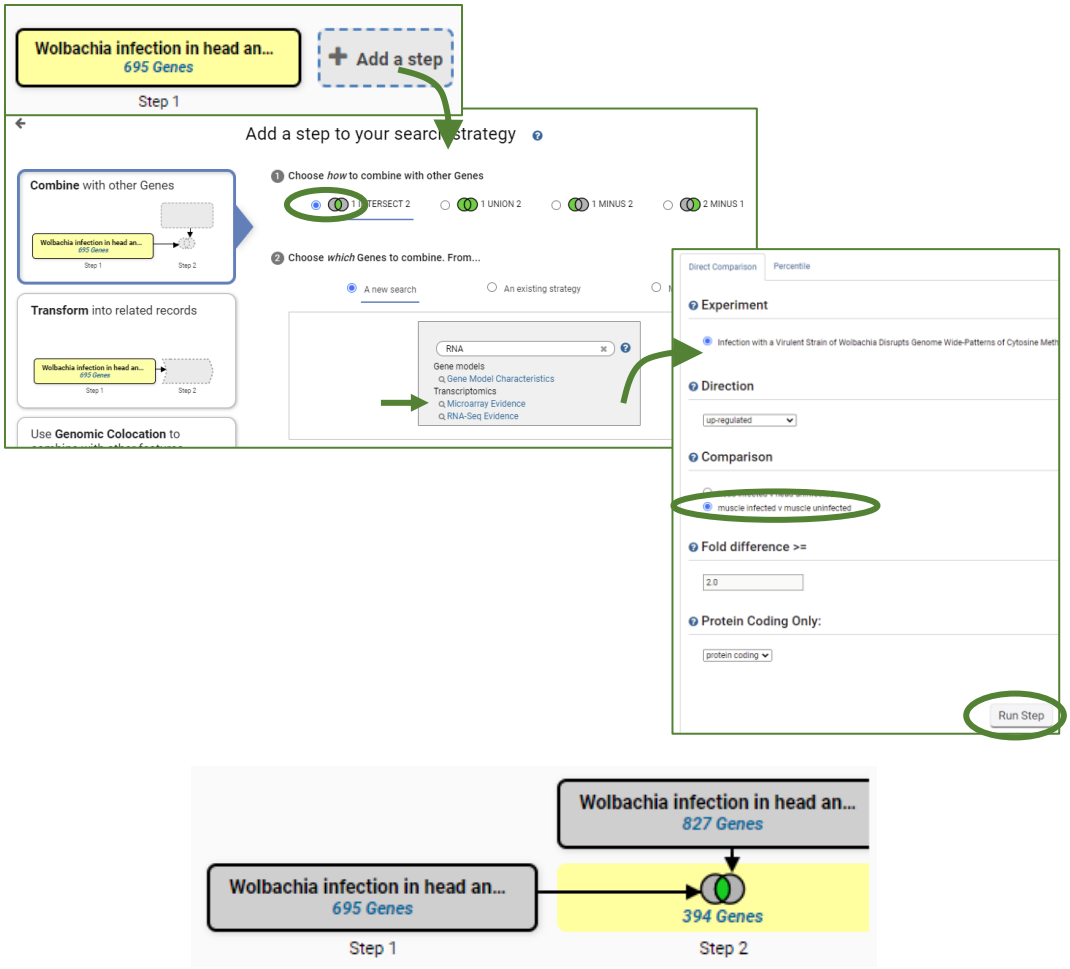
a. Navigate to the microarray search and choose the **Direct Comparison** search for the dataset titled **'Infection with a Virulent Strain of Wolbachia Disrupts Genome Wide-Patterns of Cytosine Methylation in the Mosquito Aedes aegypti (Ye et al.)'**



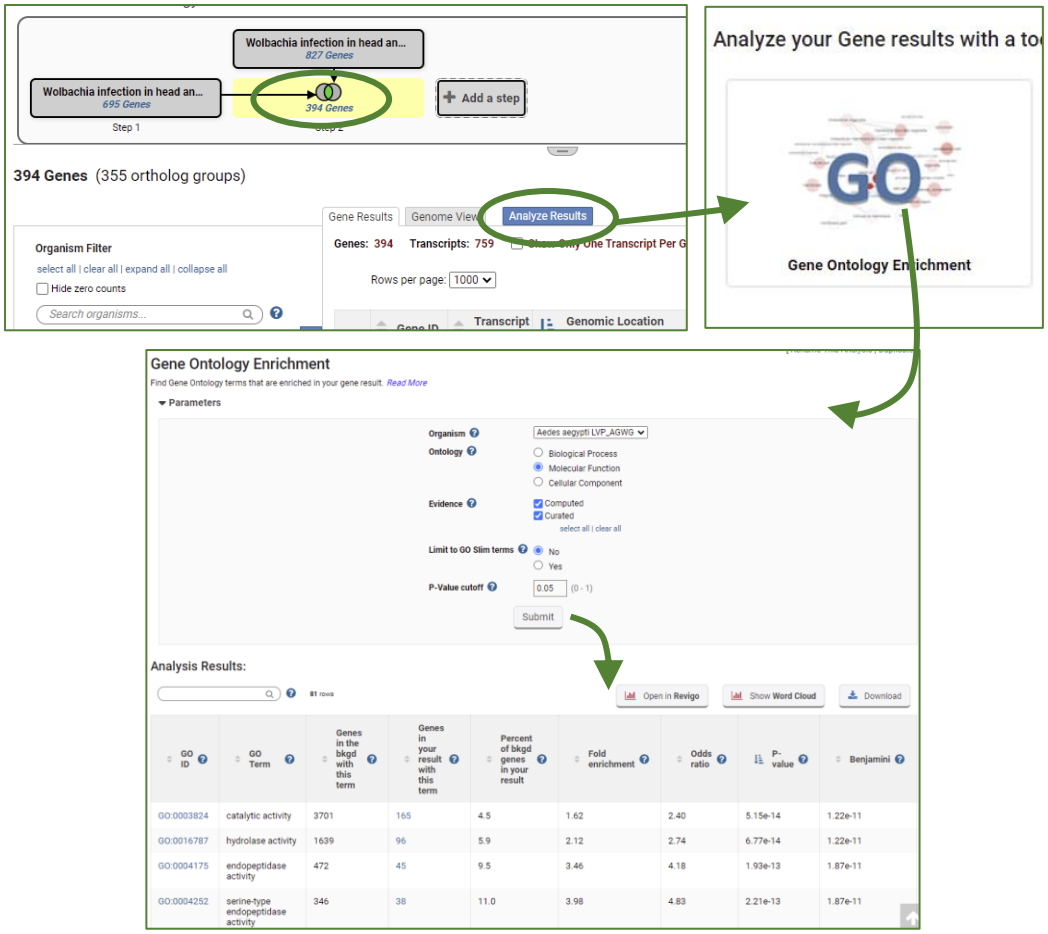
b. Initiate a search that returns genes that **are upregulated 2 fold in infected head vs uninfected**.



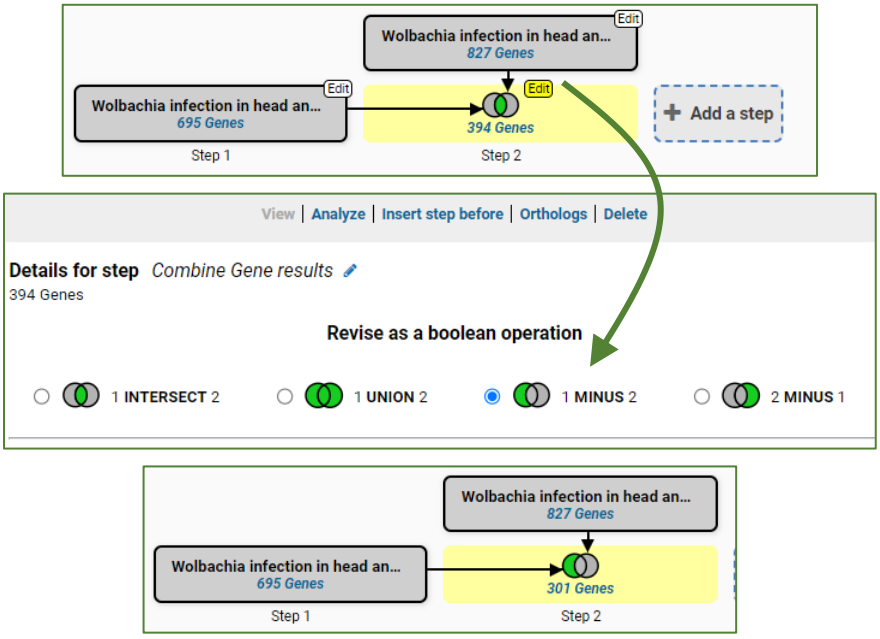
c. Intersect your search result with another search that returns genes upregulated 2-fold in muscle vs uninfected. Your combined result will be genes that are upregulated in head and muscle in response to *Wolbachia* infection.



d. Determine enriched Molecular Function GO terms for the upregulated genes. Make sure you are viewing the combined result (the Step 2 result will be highlighted yellow) and click Analyze Result to open the Enrichment Tool. What gene functions are shared by the combined result? What biological role can you envision for these mosquito genes during the *wolbachia* infection?



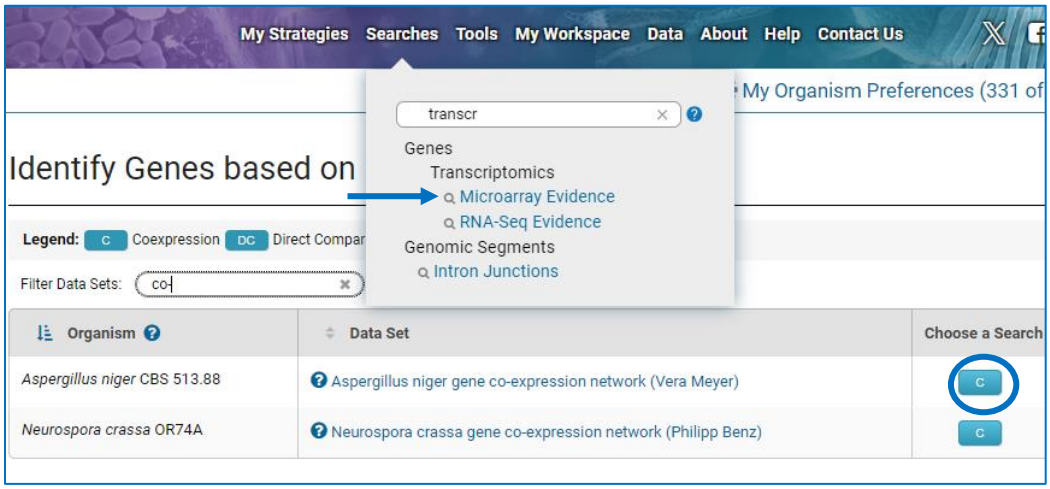
e. Modify the strategy to find genes that are upregulated in head but not in muscle. Do these genes have any enriched Molecular Function GO terms?



6. **Find genes that are likely co-expressed with An04g07430, an *Aspergillus niger* protein coding gene with little functional annotation.** By finding genes that are expressed at the same time as An04g07430, we may find clues about its function and the biological processes that it participates in. This exercise uses FungiDB.

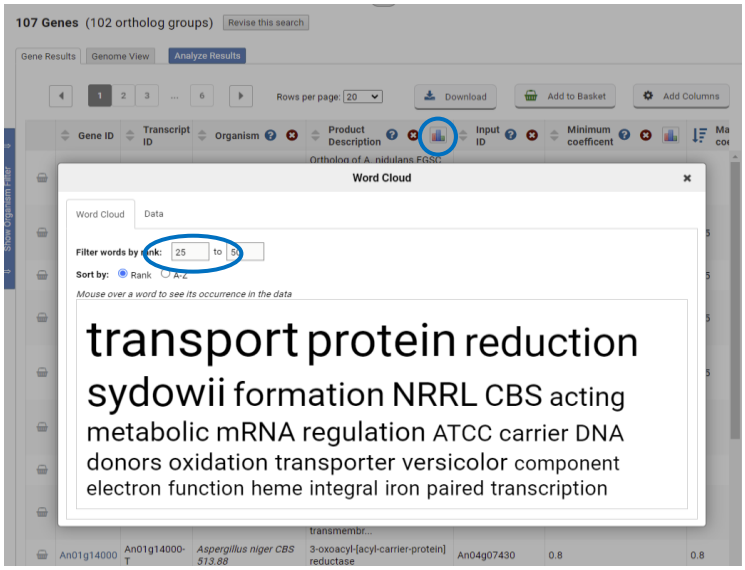
a. Navigate to the microarray searches in FungiDB and choose the Co-expression search for the data set titled ***Aspergillus niger* gene co-expression network (Vera Meyer).** Schape et al Nucleic Acids Research 2019. These data are the results of a meta-analysis of 155 publicly available transcriptomics analyses for *A. niger*, which were used to generate a genome-level co-expression network and sub-networks for >9,500 genes.

b. Run the search to find the co-expression network for An04g07430.



c. What genes share the co-expression profile of An04g07430? Four of these genes have a correlation coefficient of 0.85. What are these genes? Can you tell from the product descriptions in the result table? The Orthologs table or the Attributes and Protein Browser section on their respective gene pages might offer more clues.

d. Scan the product description column for genes with known functions. Use the Column Histogram tool to view a word cloud of the product descriptions in the column. Set the rank range to 25-50. What words occur most often in the product descriptions of An04g07430 co-expressed genes?



e. Run the enrichment analyses for Molecular Function, Cellular Component and Biological Processes. Do these provide information about what this group of co-expressed genes might be doing?