Strategies 2

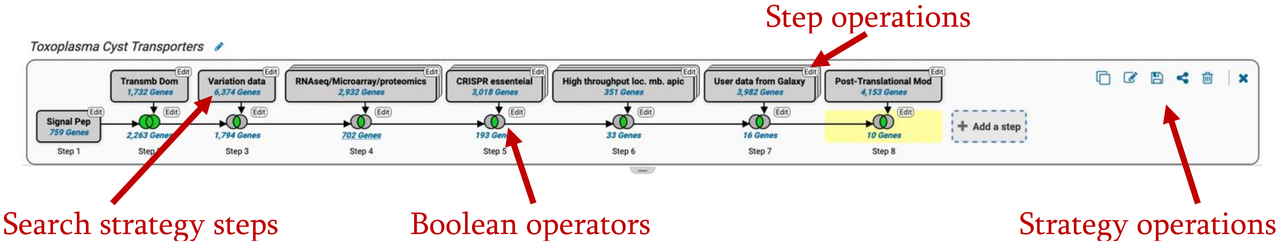
Data Integration Through Search Strategies

This exercise illustrates how to combine search results from different data types and how to effectively explore the results. **Specific objectives include**:

1. Understanding search strategy functions including adding/revising/deleting steps, copying search strategies, and saving and sharing strategies.
2. Interacting with gene results and adding columns
3. Navigating transcriptomic searches
4. Exploring proteomics data
5. Exploring subcellular localization data
6. Exploring genome wide CRISPR data
7. Exploring variation data
8. Leveraging orthology searches
9. Running enrichment analyses

Search strategies

Search strategies in VEuPathDB resources allow you to combine results from different datatype searches using Boolean operators (e.g. Intersect, union, minus). Search strategies enable you to develop *in silico* experiments based on data from the species of interest of from other species (or strains) by leveraging orthology.



Getting started with your first search strategy

There are a few things to consider before developing a search strategy:

1. What is your question? Or what are you trying to find out? (overall strategy)
2. Can you break down your question into smaller components? (strategy steps)

3. What data or analyses can be used to answer the various components of your main question?
4. How will you combine the different components of your question? Ie. Which Boolean operators.

Example question

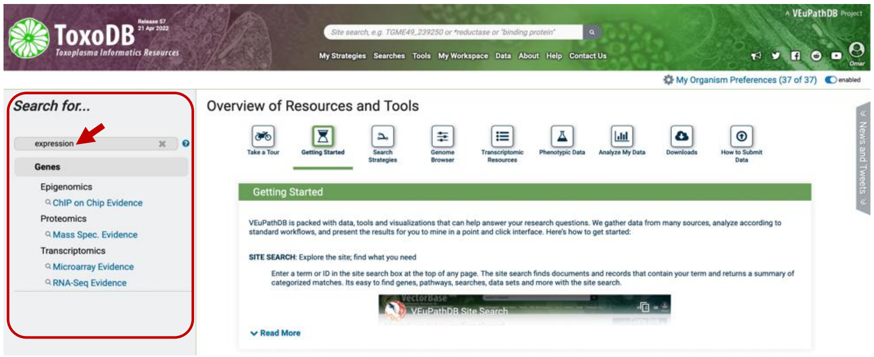
Big question: I would like to identify bradyzoite/tissue cyst specific therapeutic targets.

Let's break it down:

1. How do I identify genes whose expression is upregulated in bradyzoites?
   a. Does upregulated mean a gene is not expressed in other stages?
   b. How do you remove genes that are expressed in other stages?
2. Should I exclude expression from other stages? How can I do this?
3. How do I identify genes that have a specific type of variation?
4. How do I leverage orthology to define phyletic pattern?
5. What about essentiality? How do I find the genes that are important for parasite fitness?

Running your first search

1. Explore the data available in ToxoDB. What data can tell you about expression timing of genes? Expand the menu on the left-hand side of the home page and look for datatypes that would tell you about expression. Hint: try filtering the searches with a key work like "expression".

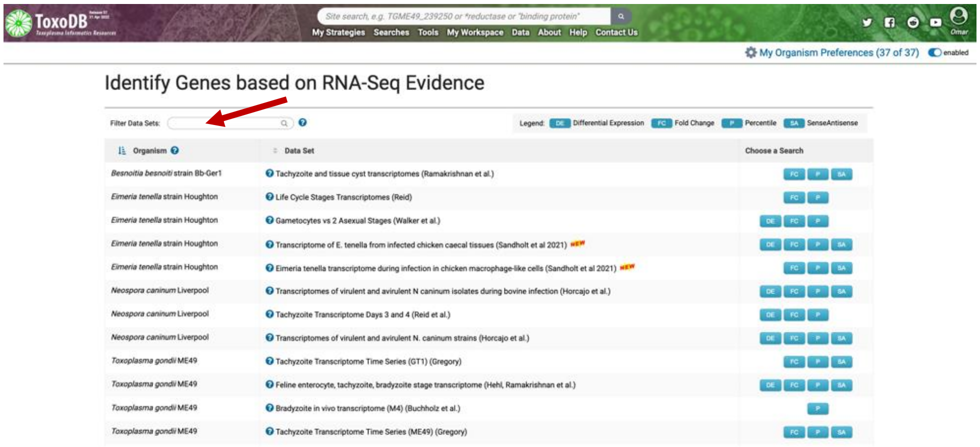


VEuPathDB is packed with data, tools and visualizations that can help answer your research questions. We gather data from many sources, analyze according to standard workflows, and present the results for you to mine in a point and click interface. Here's how to get started:

SITE SEARCH: Explore the site; find what you need

Enter a term or ID in the site search box at the top of any page. The site search finds documents and records that contain your term and returns a summary of categorized matches. Its easy to find genes, pathways, searches, data sets and more with the site search.

2. Explore the RNA-Seq evidence data. Are there any experiments that tell you about bradyzoite expression? Try filtering the datasets using a keywords like "bradyzoite" or "differentiation".


ToxoDB
Toxoplasma Informatics Resources
Site search, e.g. TGME49_239250 or *reductase or "binding protein"
My Strategies
Searches
Tools
My Workspace
Data
About
Help
Contact Us
Omar
My Organism Preferences (37 of 37)
enabled

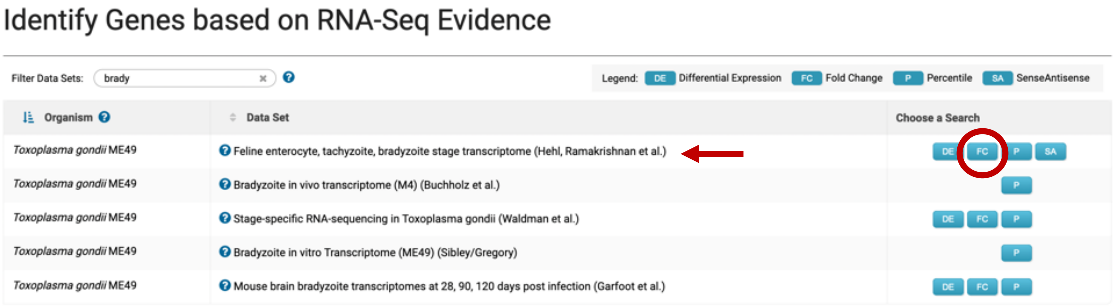
Identify Genes based on RNA-Seq Evidence

Filter Data Sets:

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

Organism	Data Set	Choose a Search
Besnoitia besnoiti strain Bb-Ger1	Tachyzoite and tissue cyst transcriptomes (Ramakrishnan et al.)	FC P SA
Eimeria tenella strain Houghton	Life Cycle Stages Transcriptomes (Reid)	FC P
Eimeria tenella strain Houghton	Gametocytes vs 2 Asexual Stages (Walker et al.)	DE FC P
Eimeria tenella strain Houghton	Transcriptome of E. tenella from infected chicken caecal tissues (Sandholt et al 2021) NEW	DE FC P SA
Eimeria tenella strain Houghton	Eimeria tenella transcriptome during infection in chicken macrophage-like cells (Sandholt et al 2021) NEW	FC P SA
Neospora caninum Liverpool	Transcriptomes of virulent and avirulent N caninum isolates during bovine infection (Horcajo et al.)	DE FC P SA
Neospora caninum Liverpool	Tachyzoite Transcriptome Days 3 and 4 (Reid et al.)	DE FC P
Neospora caninum Liverpool	Transcriptomes of virulent and avirulent N. caninum strains (Horcajo et al.)	DE FC P SA
Toxoplasma gondii ME49	Tachyzoite Transcriptome Time Series (GT1) (Gregory)	FC P SA
Toxoplasma gondii ME49	Feline enterocyte, tachyzoite, bradyzoite stage transcriptome (Hehl, Ramakrishnan et al.)	DE FC P SA
Toxoplasma gondii ME49	Bradyzoite in vivo transcriptome (M4) (Buchholz et al.)	P
Toxoplasma gondii ME49	Tachyzoite Transcriptome Time Series (ME49) (Gregory)	FC P SA

3. Select the fold change search for the "Feline enterocyte, tachyzoite, bradyzoite stage transcriptome (Hehl, Ramakrishnan et al.)".



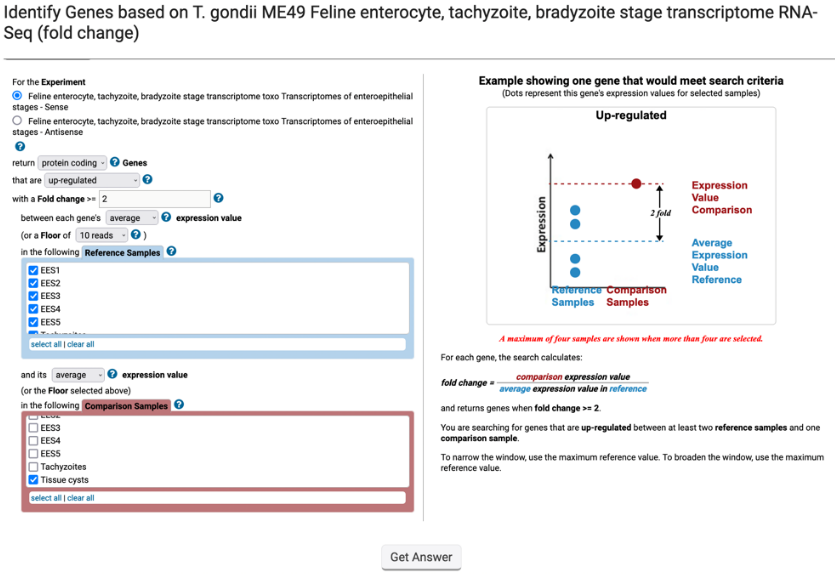
Identify Genes based on RNA-Seq Evidence

Filter Data Sets: brady

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

Organism	Data Set	Choose a Search
Toxoplasma gondii ME49	Feline enterocyte, tachyzoite, bradyzoite stage transcriptome (Hehl, Ramakrishnan et al.)	DE FC P SA
Toxoplasma gondii ME49	Bradyzoite in vivo transcriptome (M4) (Buchholz et al.)	P
Toxoplasma gondii ME49	Stage-specific RNA-sequencing in Toxoplasma gondii (Waldman et al.)	DE FC P
Toxoplasma gondii ME49	Bradyzoite in vitro Transcriptome (ME49) (Sibley/Gregory)	P
Toxoplasma gondii ME49	Mouse brain bradyzoite transcriptomes at 28, 90, 120 days post infection (Garfoot et al.)	DE FC P

4. Configure this search to identify genes that are upregulated by 2-fold in tissue cysts compared to all other stages (use average expression values).



Identify Genes based on T. gondii ME49 Feline enterocyte, tachyzoite, bradyzoite stage transcriptome RNA-Seq (fold change)

For the **Experiment**

● Feline enterocyte, tachyzoite, bradyzoite stage transcriptome toxo Transcriptomes of enteroepithelial stages - Sense

○ Feline enterocyte, tachyzoite, bradyzoite stage transcriptome toxo Transcriptomes of enteroepithelial stages - Antisense

return protein coding **Genes**

that are up-regulated

with a **Fold change** >= 2

between each gene's average **expression value**

(or a **Floor** of 10 reads)

in the following **Reference Samples**

☑ EES1
☑ EES2
☑ EES3
☑ EES4
☑ EES5

select all | clear all

and its average **expression value**

(or the **Floor** selected above)

in the following **Comparison Samples**

☐ EES3
☐ EES4
☐ EES5
☐ Tachyzoites
☑ Tissue cysts

select all | clear all

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

A maximum of four samples are shown when more than four are selected.

For each gene, the search calculates:

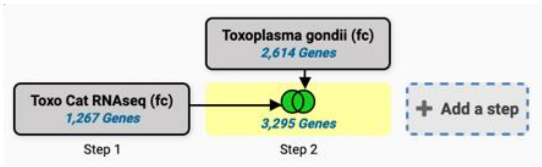
$$\textit{fold change} = \frac{\textit{comparison expression value}}{\textit{average expression value in reference}}$$

and returns genes when **fold change >= 2**.

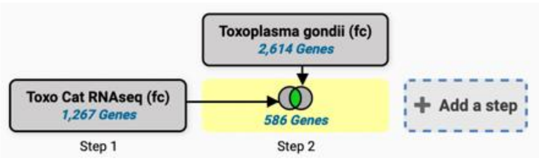
You are searching for genes that are **up-regulated** between at least two **reference samples** and one **comparison sample**.

To narrow the window, use the maximum reference value. To broaden the window, use the maximum reference value.

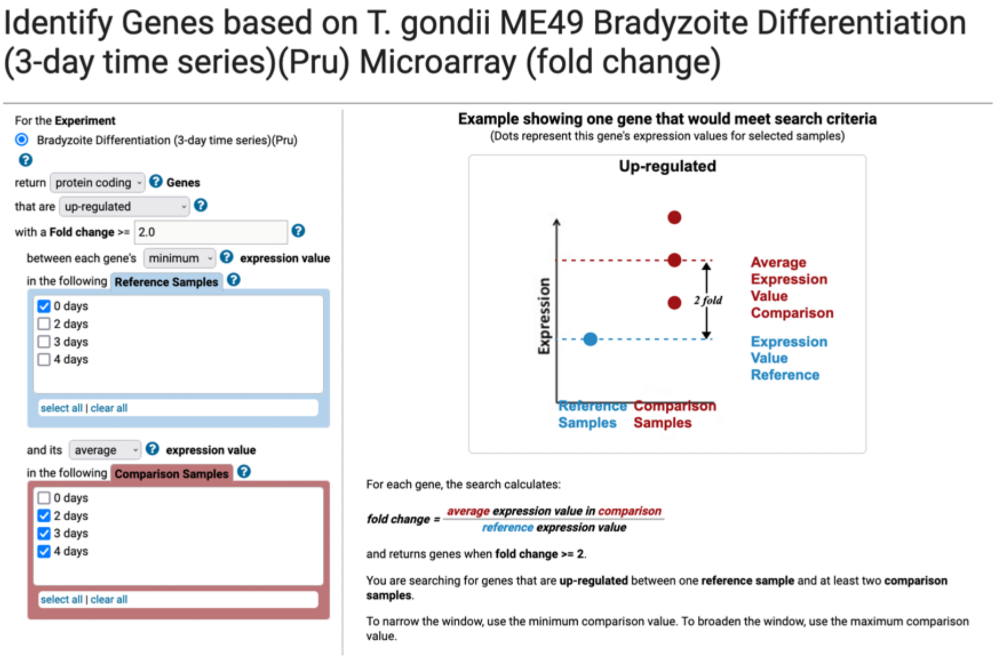
5. Add a step and combine these results with results from another experiments containing bradyzoite samples. For example, try selecting the "Stage-specific RNA-sequencing in Toxoplasma gondii (Waldman et al.)". Configure this search to identify genes that are upregulated by 2-fold when comparing 48hr bradyzoites to 24hr tachyzoites.
   - Did you use an intersect or a union operator? How would your results change if you us one or the other? Is one better than the other in this case?



Vs.



6. How about combining this with data from a microarray experiment? Add a step, go to the microarray data section and select, for example, the "Bradyzoite Differentiation (3-day time series)(Pru) (Buchholz, Fritz and Boothroyd et al.)" experiment. Configure the search to identify genes that are upregulated by 2 fold between time 0 and the other time points.



Identify Genes based on T. gondii ME49 Bradyzoite Differentiation (3-day time series)(Pru) Microarray (fold change)

For the **Experiment**

● Bradyzoite Differentiation (3-day time series)(Pru)

return protein coding **Genes**

that are up-regulated

with a **Fold change** >= 2.0

between each gene's minimum **expression value**

in the following **Reference Samples**

☑ 0 days
☐ 2 days
☐ 3 days
☐ 4 days

select all | clear all

and its average **expression value**

in the following **Comparison Samples**

☐ 0 days
☑ 2 days
☑ 3 days
☑ 4 days

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:

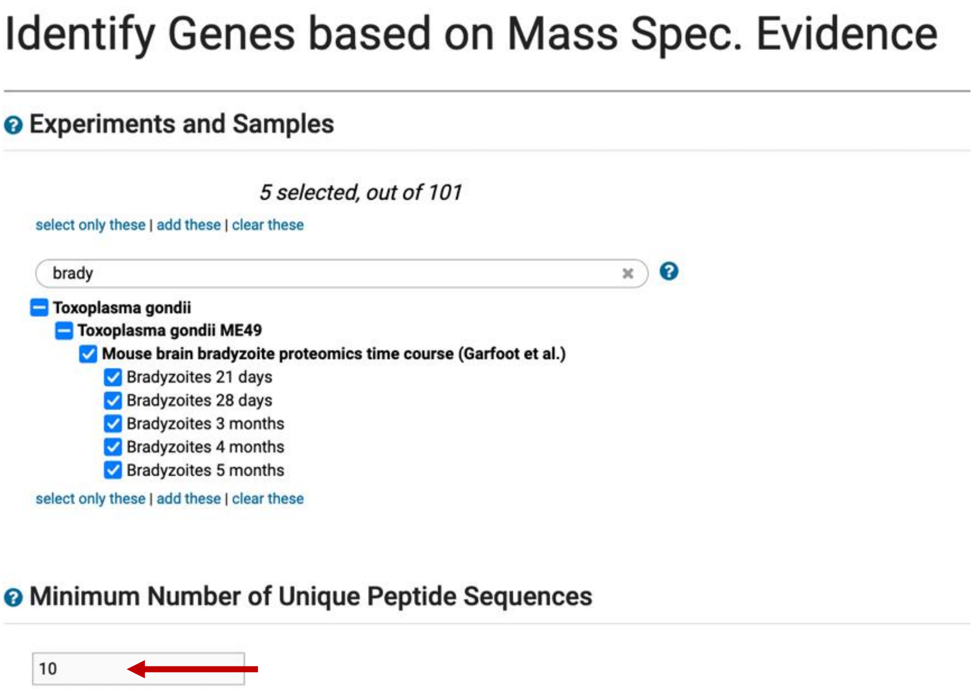
fold change = *average expression value in comparison* / *reference expression value*

and returns genes when **fold change >= 2**.

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

To narrow the window, use the minimum comparison value. To broaden the window, use the maximum comparison value.

7. Add a step and combine any genes that have mass spec evidence from the "Mouse brain bradyzoite proteomics time course (Garfoot et al.)" experiment.



Identify Genes based on Mass Spec. Evidence

Experiments and Samples

5 selected, out of 101

select only these | add these | clear these

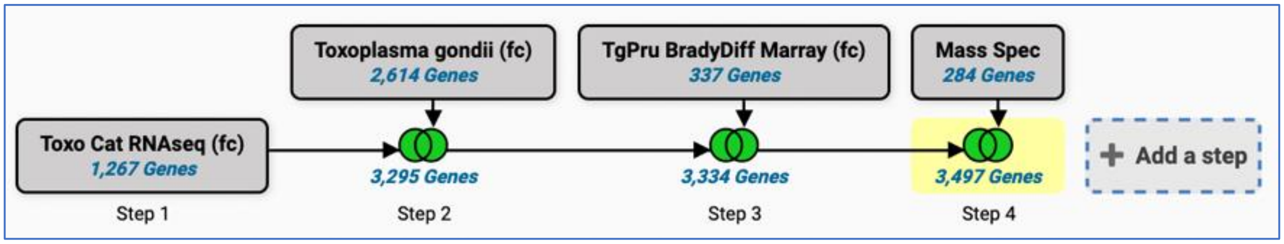
brady

- Toxoplasma gondii
  - Toxoplasma gondii ME49
    - ☑ Mouse brain bradyzoite proteomics time course (Garfoot et al.)
      - ☑ Bradyzoites 21 days
      - ☑ Bradyzoites 28 days
      - ☑ Bradyzoites 3 months
      - ☑ Bradyzoites 4 months
      - ☑ Bradyzoites 5 months

select only these | add these | clear these

Minimum Number of Unique Peptide Sequences

10



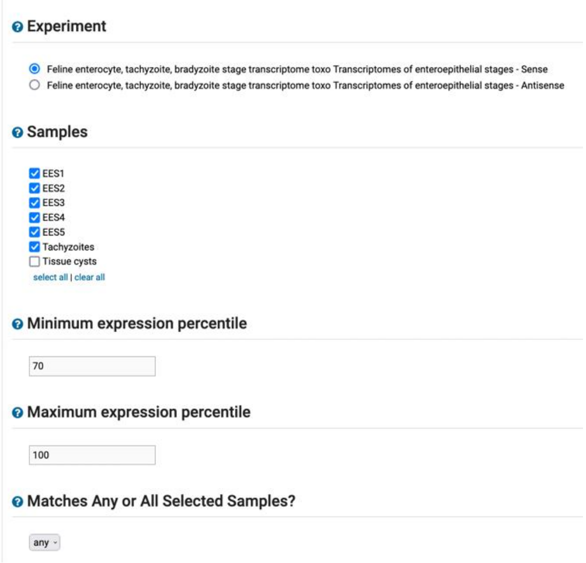
Step 1: Toxo Cat RNAseq (fc) — 1,267 Genes

Step 2: Toxoplasma gondii (fc) — 2,614 Genes; 3,295 Genes

Step 3: TgPru BradyDiff Marray (fc) — 337 Genes; 3,334 Genes

Step 4: Mass Spec — 284 Genes; 3,497 Genes

8. Now let's exclude any gene that is highly expressed in tachyzoite and sexual stages. To do this, add a step and select the **percentile** search for the "Feline enterocyte, tachyzoite, bradyzoite stage transcriptome (Hehl, Ramakrishnan et al.)" dataset. Configure the search to exclude any gene that is expressed in all stages except tissue cyst at 70 or higher percentile.
   - What does the "Matches Any or All Selected Samples?" parameter do? Which option is more stringent, any or all?
   - Which Boolean operator did you use?



Experiment

● Feline enterocyte, tachyzoite, bradyzoite stage transcriptome toxo Transcriptomes of enteroepithelial stages - Sense
○ Feline enterocyte, tachyzoite, bradyzoite stage transcriptome toxo Transcriptomes of enteroepithelial stages - Antisense

Samples

☑ EES1
☑ EES2
☑ EES3
☑ EES4
☑ EES5
☑ Tachyzoites
☐ Tissue cysts

select all | clear all

Minimum expression percentile

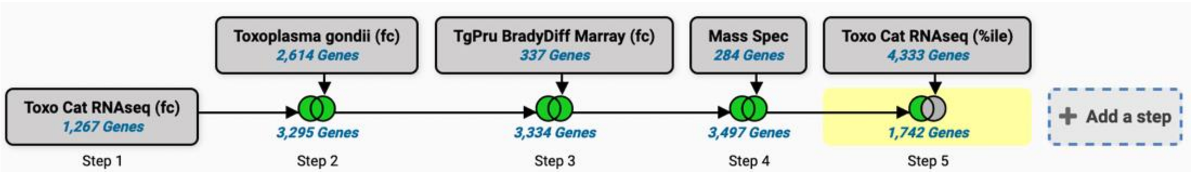
70

Maximum expression percentile

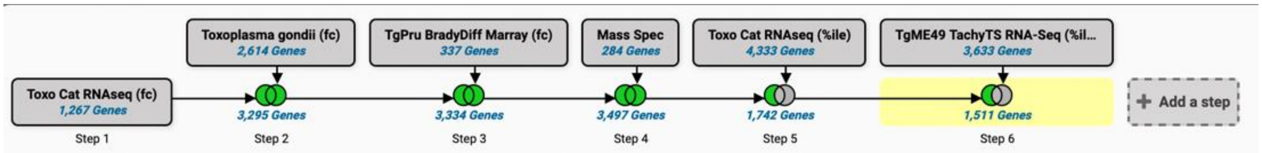
100

Matches Any or All Selected Samples?

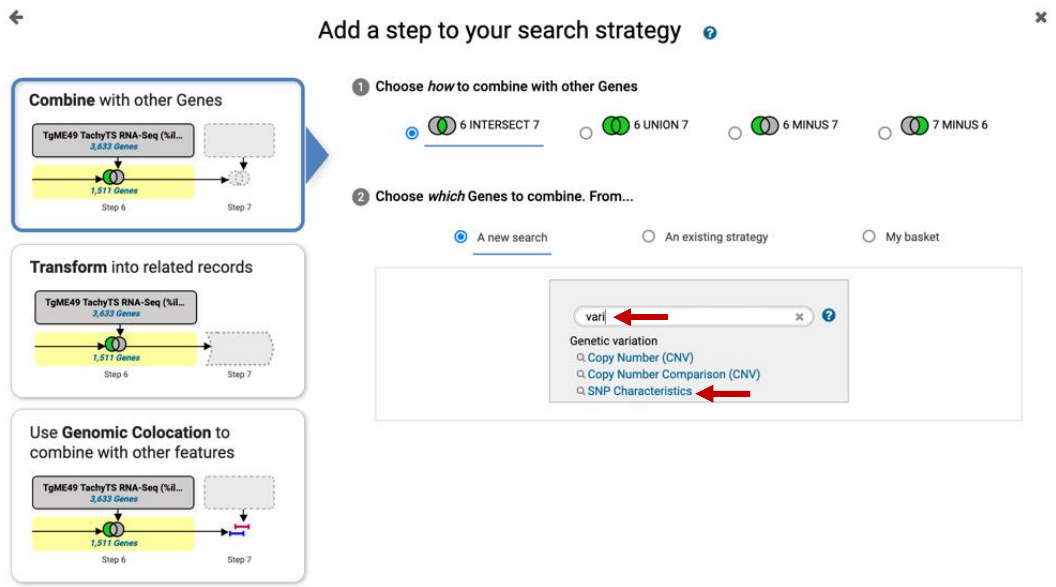
any



9. You can exclude additional genes that show expression in stages you are not interested in from other experiments using the above method. Try this with another experiment – for example the "Tachyzoite Transcriptome Time Series (ME49) (Gregory)".



10. Now that we have a list of genes that are upregulated in bradyzoites and are likely not highly expressed in other stages, let's find out which of these have more that 10 non-synonymous SNPs. To do this, add a step and find the search for genes by SNP characteristics.


Add a step to your search strategy

Combine with other Genes

Transform into related records

Use Genomic Colocation to combine with other features

1 Choose *how* to combine with other Genes

● 6 INTERSECT 7 ○ 6 UNION 7 ○ 6 MINUS 7 ○ 7 MINUS 6

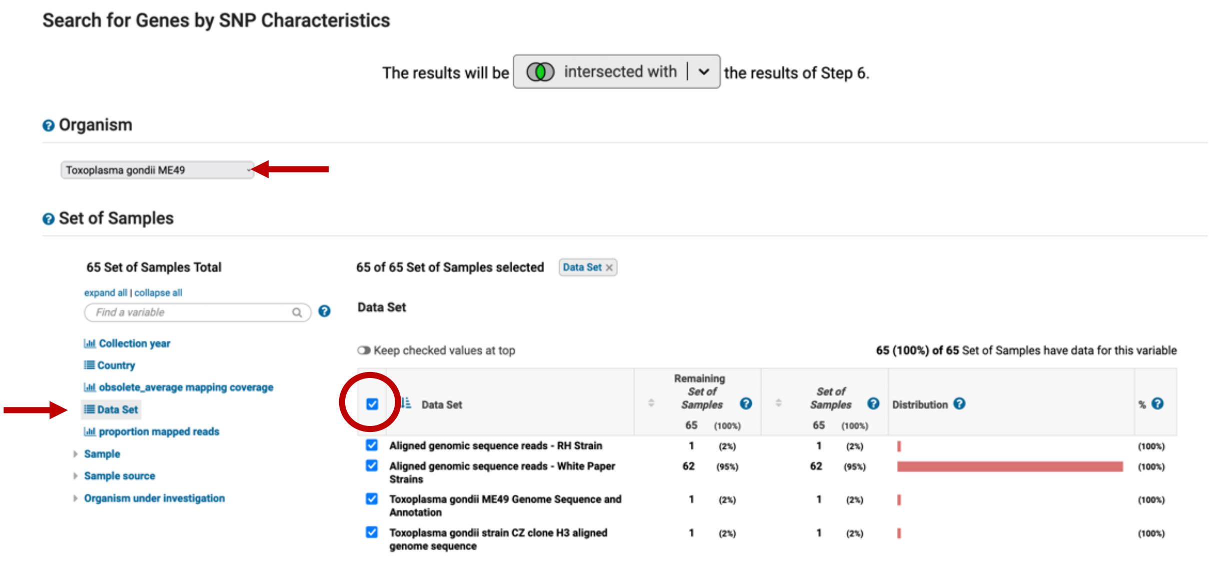
2 Choose *which* Genes to combine. From...

● A new search ○ An existing strategy ○ My basket

vari

Genetic variation
- Copy Number (CNV)
- Copy Number Comparison (CNV)
- SNP Characteristics

- Configure the SNP search to find genes to select all samples aligned to *T. gondii* ME49.



Search for Genes by SNP Characteristics

The results will be intersected with the results of Step 6.

Organism

Toxoplasma gondii ME49

Set of Samples

65 Set of Samples Total

65 of 65 Set of Samples selected Data Set ×

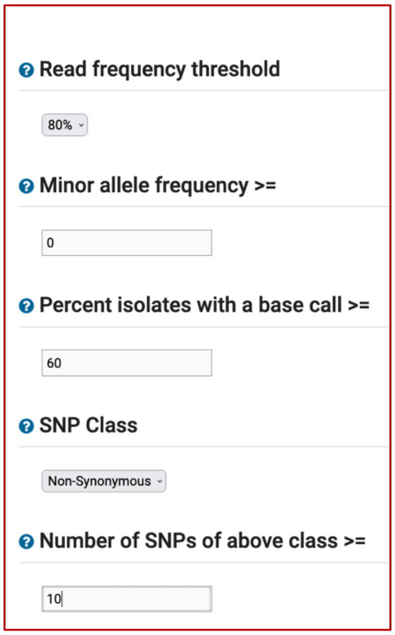
Data Set

Keep checked values at top

65 (100%) of 65 Set of Samples have data for this variable

☑	Data Set	Remaining Set of Samples 65 (100%)	Set of Samples 65 (100%)	Distribution	%
☑	Aligned genomic sequence reads - RH Strain	1 (2%)	1 (2%)		(100%)
☑	Aligned genomic sequence reads - White Paper Strains	62 (95%)	62 (95%)		(100%)
☑	Toxoplasma gondii ME49 Genome Sequence and Annotation	1 (2%)	1 (2%)		(100%)
☑	Toxoplasma gondii strain CZ clone H3 aligned genome sequence	1 (2%)	1 (2%)		(100%)

- Next set the percent isolates with a SNP call to 60, the SNP type to non-synonymous and the number of SNPs of this type to >/= 10.



Read frequency threshold

80%

Minor allele frequency >=

0

Percent isolates with a base call >=

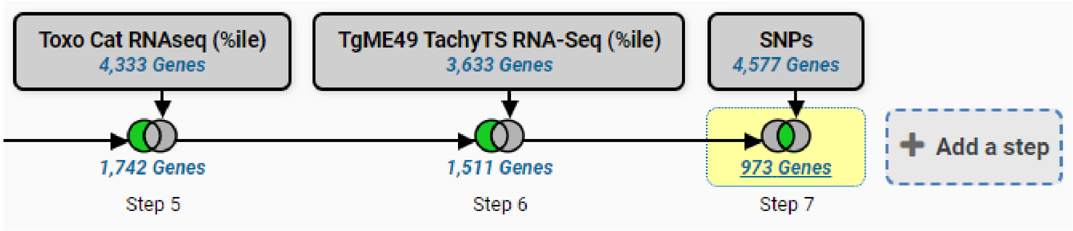
60

SNP Class

Non-Synonymous

Number of SNPs of above class >=

10



Toxo Cat RNAseq (%ile)
4,333 Genes

TgME49 TachyTS RNA-Seq (%ile)
3,633 Genes

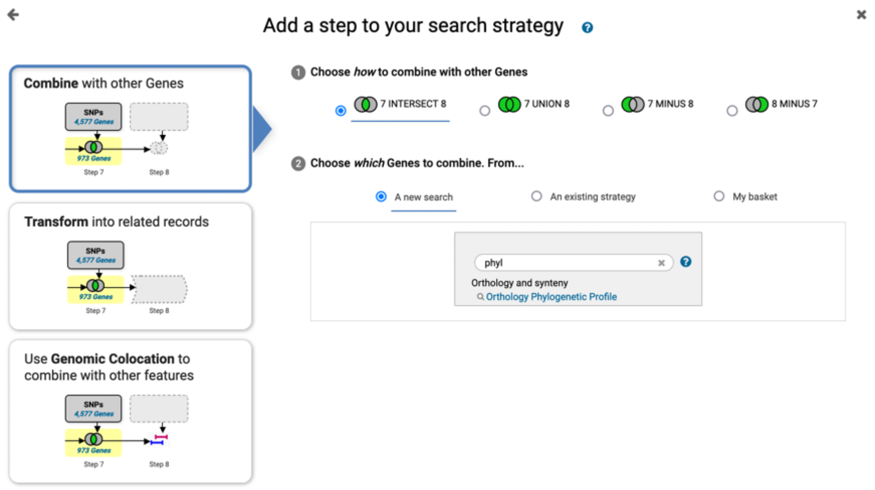
SNPs
4,577 Genes

1,742 Genes
Step 5

1,511 Genes
Step 6

973 Genes
Step 7

11. Now let’s determine how many of these genes do not have orthologs in mammals. Add a step and find the search called “Orthology Phylogenetic Profile”.



Add a step to your search strategy

Combine with other Genes

Transform into related records

Use **Genomic Colocation** to combine with other features

1 Choose *how* to combine with other Genes

● 7 INTERSECT 8 ○ 7 UNION 8 ○ 7 MINUS 8 ○ 8 MINUS 7

2 Choose *which* Genes to combine. From...

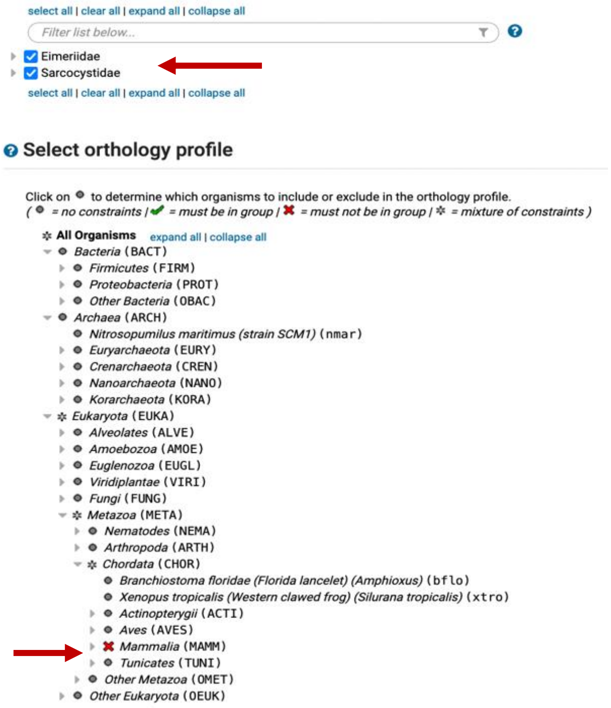
● A new search ○ An existing strategy ○ My basket

phyl

Orthology and synteny

Orthology Phylogenetic Profile

- There are different ways to configure this search depending on which Boolean operator you use. If you use the intersect operator, then configure the search to return all genes in ToxoDB that do not have orthologs in mammals.



select all | clear all | expand all | collapse all

Filter list below...

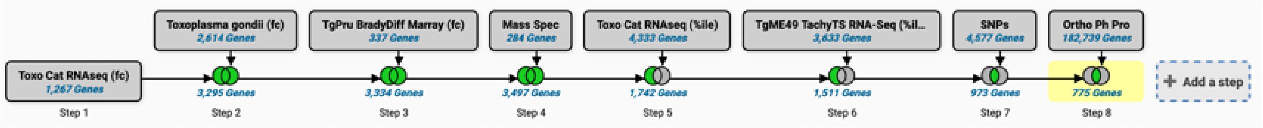
☑ Eimeriidae
☑ Sarcocystidae

select all | clear all | expand all | collapse all

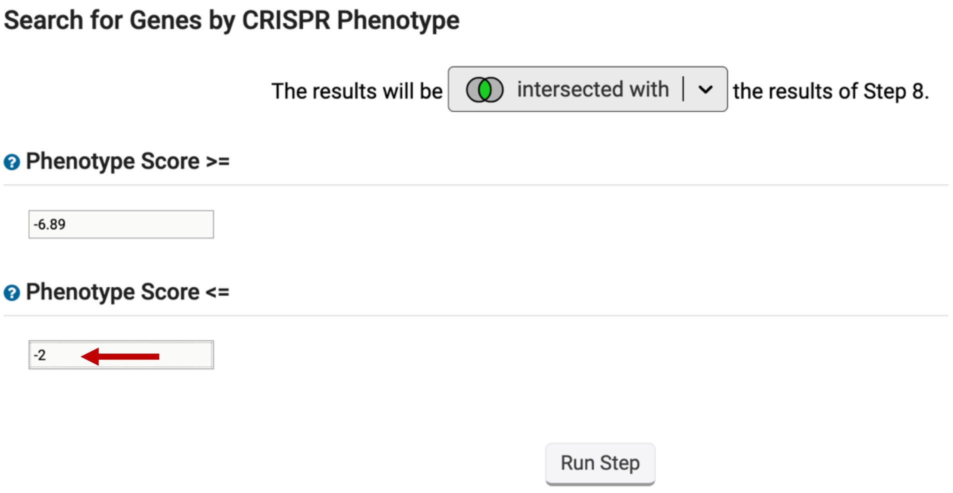
Select orthology profile

Click on ● to determine which organisms to include or exclude in the orthology profile.
(● = no constraints | ✓ = must be in group | ✖ = must not be in group | ✲ = mixture of constraints)

```
✲ All Organisms   expand all | collapse all
  ● Bacteria (BACT)
    ● Firmicutes (FIRM)
    ● Proteobacteria (PROT)
    ● Other Bacteria (OBAC)
  ● Archaea (ARCH)
    ● Nitrosopumilus maritimus (strain SCM1) (nmar)
    ● Euryarchaeota (EURY)
    ● Crenarchaeota (CREN)
    ● Nanoarchaeota (NANO)
    ● Korarchaeota (KORA)
  ✲ Eukaryota (EUKA)
    ● Alveolates (ALVE)
    ● Amoebozoa (AMOE)
    ● Euglenozoa (EUGL)
    ● Viridiplantae (VIRI)
    ● Fungi (FUNG)
    ✲ Metazoa (META)
      ● Nematodes (NEMA)
      ● Arthropoda (ARTH)
      ✲ Chordata (CHOR)
        ● Branchiostoma floridae (Florida lancelet) (Amphioxus) (bflo)
        ● Xenopus tropicalis (Western clawed frog) (Silurana tropicalis) (xtro)
        ● Actinopterygii (ACTI)
        ● Aves (AVES)
        ✖ Mammalia (MAMM)
        ● Tunicates (TUNI)
      ● Other Metazoa (OMET)
    ● Other Eukaryota (OEUK)
```



12. As a final step let's determine which of these genes are essential based on the genome wide CRISPR screen from the Lourido lab. Add a step and find the CRISPR phenotype search. Set the phenotype score < = to -2.



Search for Genes by CRISPR Phenotype

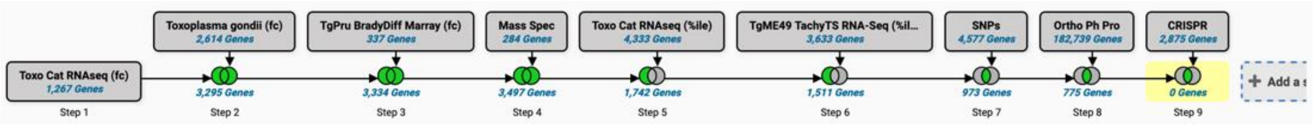
The results will be intersected with the results of Step 8.

Phenotype Score >=

-6.89

Phenotype Score <=

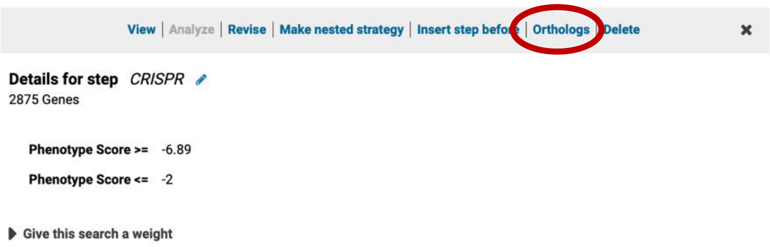
-2



- ○ How many results did you get? Is this surprising? Why do you think you got 0 results?
- ○ How can you get over the problem observed above? Is there a tool that would allow you to convert *T. gondii* GT1 genes to *T. gondii* ME49 genes?

13. Hover over the CRISPR step and click on the edit icon. In the popup click on the "orthologs" option and select ME49 from the list of organisms to transform to.

- ○ Did this improve the results?

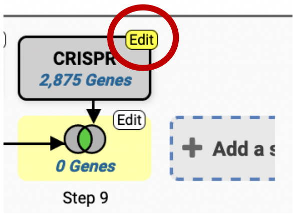

View | Analyze | Revise | Make nested strategy | Insert step before | Orthologs | Delete ×

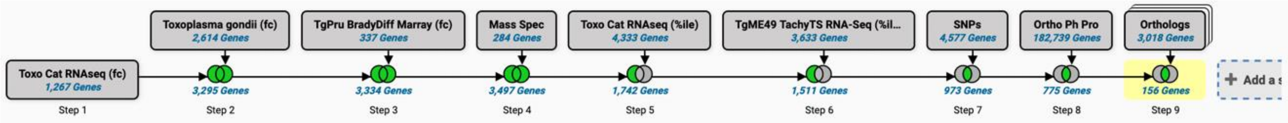
Details for step CRISPR
2875 Genes

Phenotype Score >= -6.89

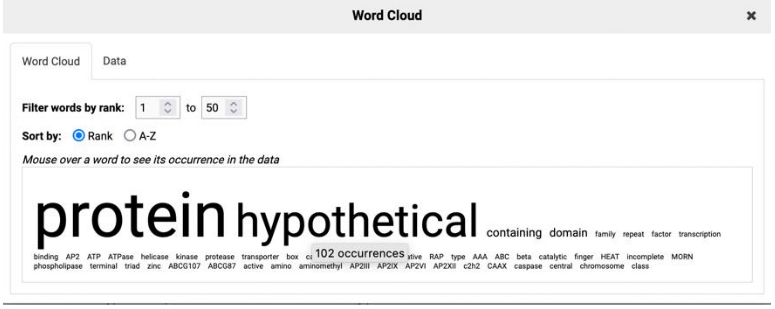
Phenotype Score <= -2

Give this search a weight





14. Explore the genes in your result list. Are there any interesting genes that you might purpsue further in the lab?
15. How many hypothetical genes are in your results? A quick way to find out is to click on the graph icon in the Product Description column heading. This generates and interactive word cloud. Hover over the word hypothetical to see the number.

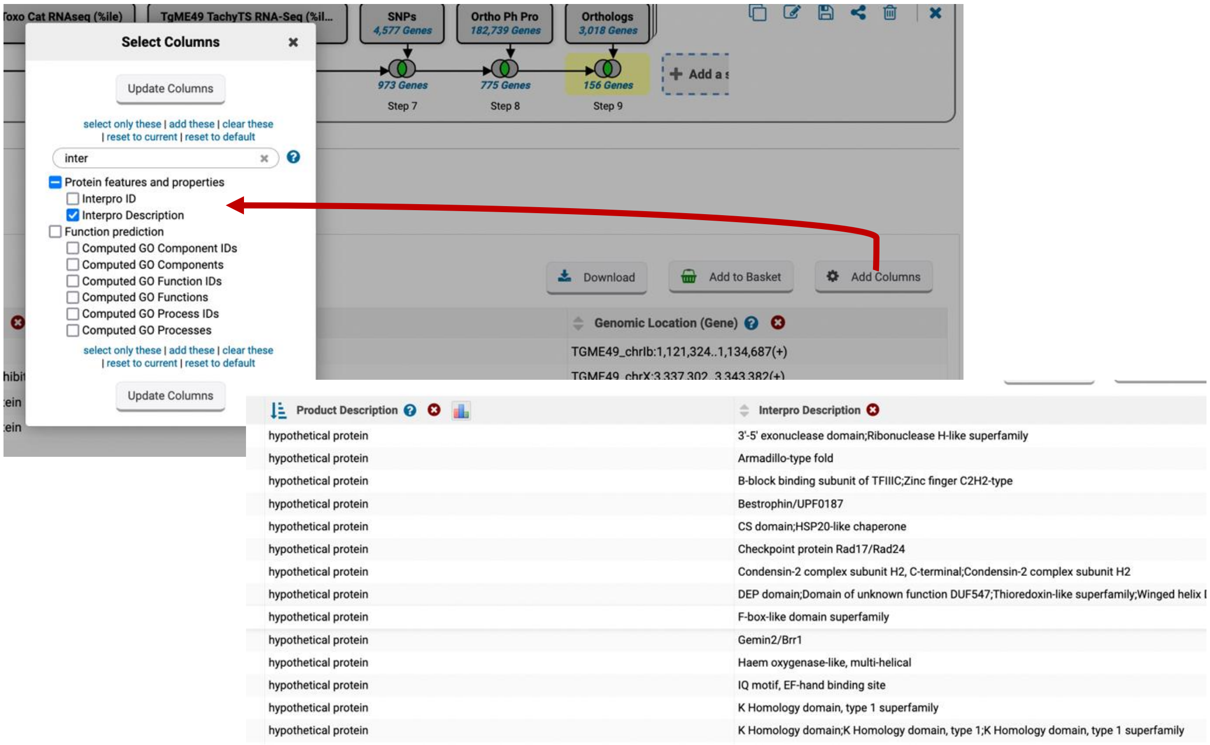


Filter words by rank: 1 to 50

Sort by: ● Rank ○ A-Z

Mouse over a word to see its occurrence in the data

16. What can you do to figure out what some of these hypothetical genes do? Here are a couple of suggestions:
   - Add a column for InterPro domain descriptions. Click on add column, search for InterPro and add the appropriate column. Did this give you an idea for possible functions for some of the hypothetical genes?



inter

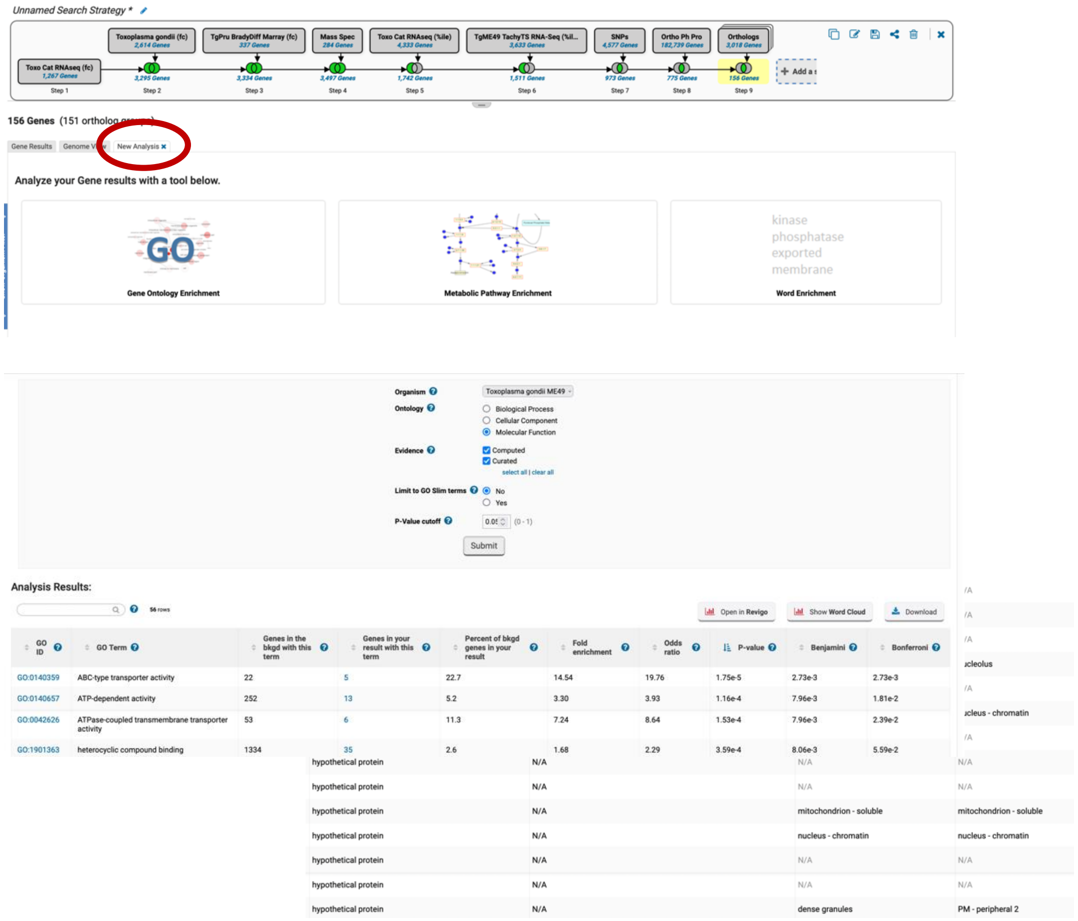
- ▬ Protein features and properties
  - □ Interpro ID
  - ☑ Interpro Description
- □ Function prediction
  - □ Computed GO Component IDs
  - □ Computed GO Components
  - □ Computed GO Function IDs
  - □ Computed GO Functions
  - □ Computed GO Process IDs
  - □ Computed GO Processes

TGME49_chrIb:1,121,324..1,134,687(+)
TGME49_chrX:3,337,302..3,343,382(+)

Product Description	Interpro Description
hypothetical protein	3'-5' exonuclease domain;Ribonuclease H-like superfamily
hypothetical protein	Armadillo-type fold
hypothetical protein	B-block binding subunit of TFIIIC;Zinc finger C2H2-type
hypothetical protein	Bestrophin/UPF0187
hypothetical protein	CS domain;HSP20-like chaperone
hypothetical protein	Checkpoint protein Rad17/Rad24
hypothetical protein	Condensin-2 complex subunit H2, C-terminal;Condensin-2 complex subunit H2
hypothetical protein	DEP domain;Domain of unknown function DUF547;Thioredoxin-like superfamily;Winged helix I
hypothetical protein	F-box-like domain superfamily
hypothetical protein	Gemin2/Brr1
hypothetical protein	Haem oxygenase-like, multi-helical
hypothetical protein	IQ motif, EF-hand binding site
hypothetical protein	K Homology domain, type 1 superfamily
hypothetical protein	K Homology domain;K Homology domain, type 1;K Homology domain, type 1 superfamily

- Add another column for the hyper_LOPIT subcellular localization data. Add the column called “Predicted Location (TAGM-MAP)”. Did this reveal some possible clues about the some of the other hypotheticals?

17. Do your results contain an enrichment of certain functions? Try some of the analysis tools available in the analysis tab.



Analyze your Gene results with a tool below.

Organism: Toxoplasma gondii ME49

Ontology: ○ Biological Process ○ Cellular Component ● Molecular Function

Evidence: ☑ Computed ☑ Curated select all | clear all

Limit to GO Slim terms: ● No ○ Yes

P-Value cutoff: 0.05 (0 - 1)

Analysis Results:

56 rows

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:0140359	ABC-type transporter activity	22	5	22.7	14.54	19.76	1.75e-5	2.73e-3	2.73e-3
GO:0140657	ATP-dependent activity	252	13	5.2	3.30	3.93	1.16e-4	7.96e-3	1.81e-2
GO:0042626	ATPase-coupled transmembrane transporter activity	53	6	11.3	7.24	8.64	1.53e-4	7.96e-3	2.39e-2
GO:1901363	heterocyclic compound binding	1334	35	2.6	1.68	2.29	3.59e-4	8.06e-3	5.59e-2

hypothetical protein	N/A	N/A	N/A
hypothetical protein	N/A	N/A	N/A
hypothetical protein	N/A	mitochondrion - soluble	mitochondrion - soluble
hypothetical protein	N/A	nucleus - chromatin	nucleus - chromatin
hypothetical protein	N/A	N/A	N/A
hypothetical protein	N/A	N/A	N/A
hypothetical protein	N/A	dense granules	PM - peripheral 2

Link to strategy:

https://toxodb.org/toxo/app/workspace/strategies/import/ce05740b75a965d9