The search question

Leishmania infantum is the causative agent of infantile visceral leishmaniasis, the most severe form of leishmaniasis, in the Mediterranean region and in Latin America. *Leishmania infantum* has a complex life cycle through sandflies (the vector) and human hosts.

Of the many stages, metacyclic stage parasites are the virulent and disease-inducing form of Leishmania.

To further characterize this infectious parasitic stage, we may ask the question: **What genes show protein expression in the virulent metacyclic stages (A, see figure below) but not in promastigote (B) or amastigote stages (C)?**

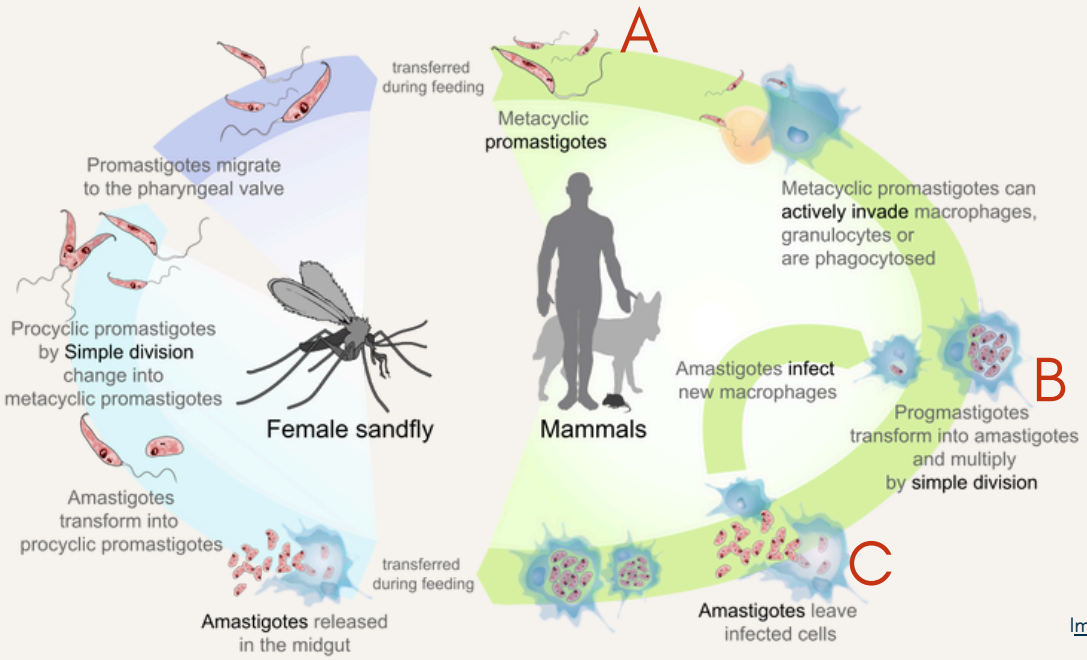


Image credit

What platform is best suited for this question?

TriTrypDB
Kinetoplastid Informatics Resources

This knowledgebase has data on kinetoplastids including Leishmania spp.

What search is best suited for this question?

The **Mass Spec. Evidence** search returns genes whose protein products mapped to peptides found in proteomics experiments.

What is a possible search strategy that will answer this question?

Use **Mass Spec. Evidence** search in two steps- (1) identify genes with protein expression in metacyclic stages, (2) subtract genes with protein expression in promastigote and amastigote stages.

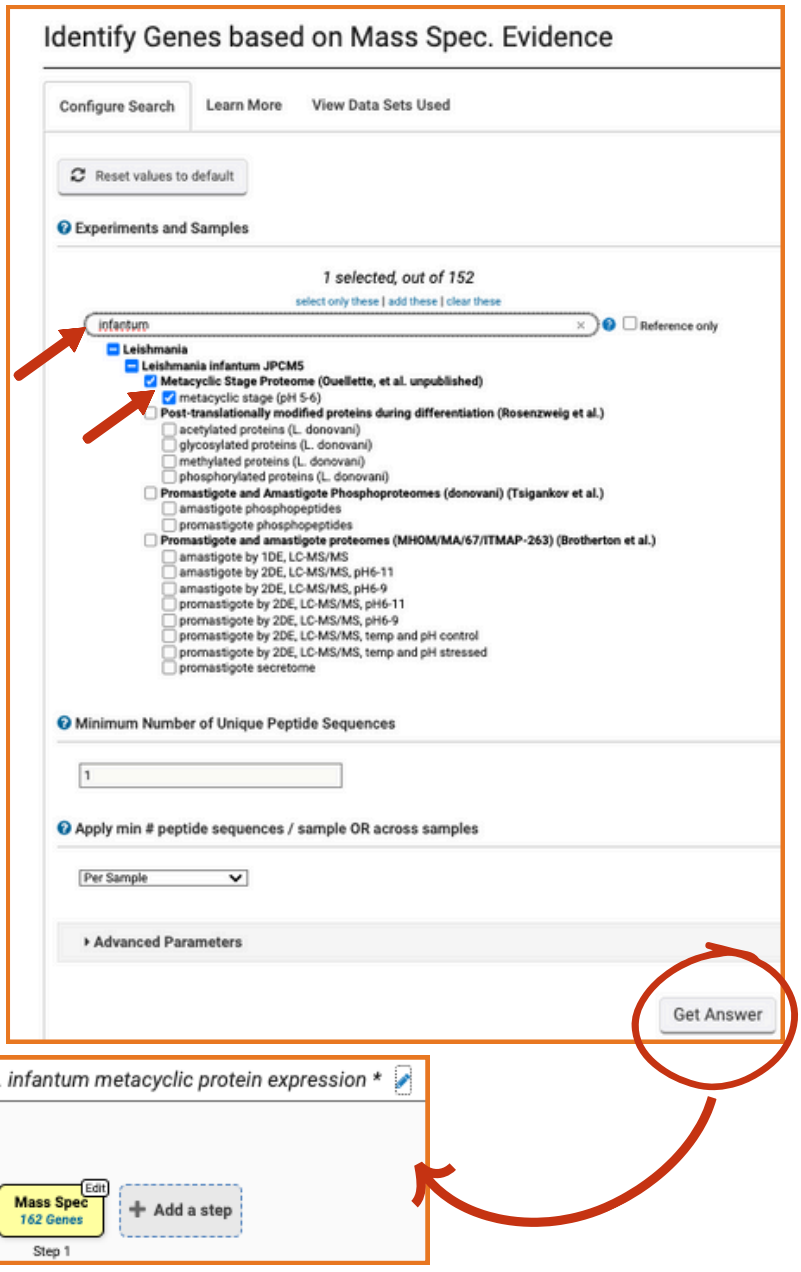
1. Navigate to the appropriate search

- Navigate to TriTrypDB.org
- From the sidebar or header, search or scroll for "proteomics" and click on **Mass Spec. Evidence**

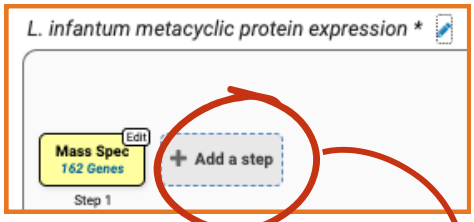
2. Choose appropriate experiments/ samples & parameters within the search

- Filter the experiment and sample tree by typing a word in the filter box: **"infantum"**.
- The first step in our strategy is to identify genes that show protein expression in the metacyclic stages.
- Select all *L. infantum* samples that come from the metacyclic stage proteome.
- Kepe the default search parameters and click on the **Get Answer** button.

How many genes did you get?

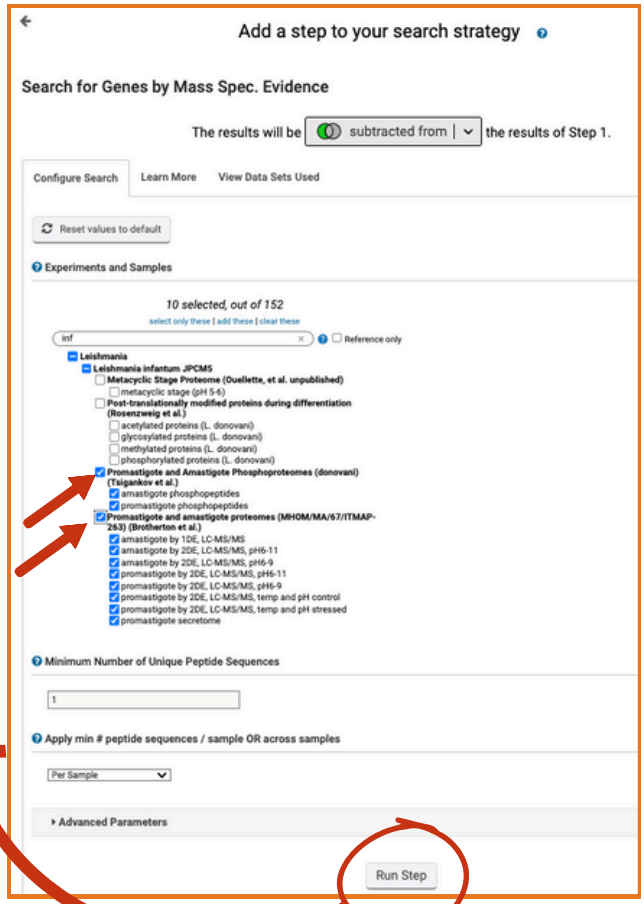


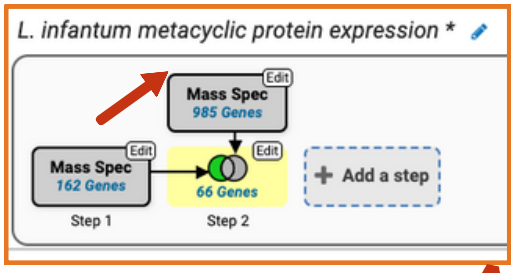
3. Add a step to your search strategy



- Now subtract the genes that have protein expression in the promastigote and amastigote stages.
- To do this, **add a step** to your strategy that combines the first step with other genes using a **1 minus 2 operator**.
- To choose which genes to combine, click on **Mass Spec. Evidence**

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



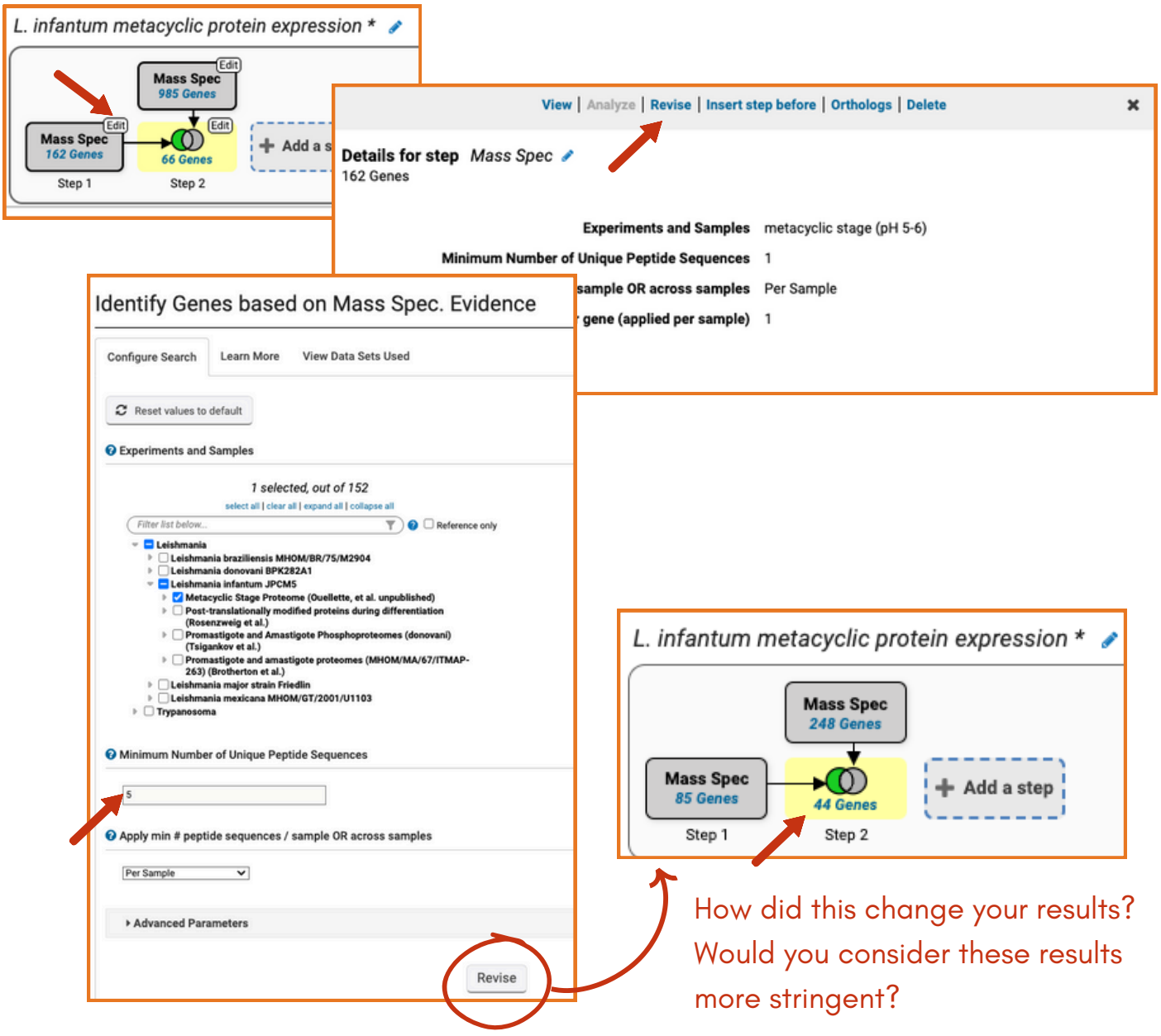


How many genes did you get that have evidence of protein expression in metacyclic stages but not in promastigote and amastigote stages?

4. Increase the stringency of your search

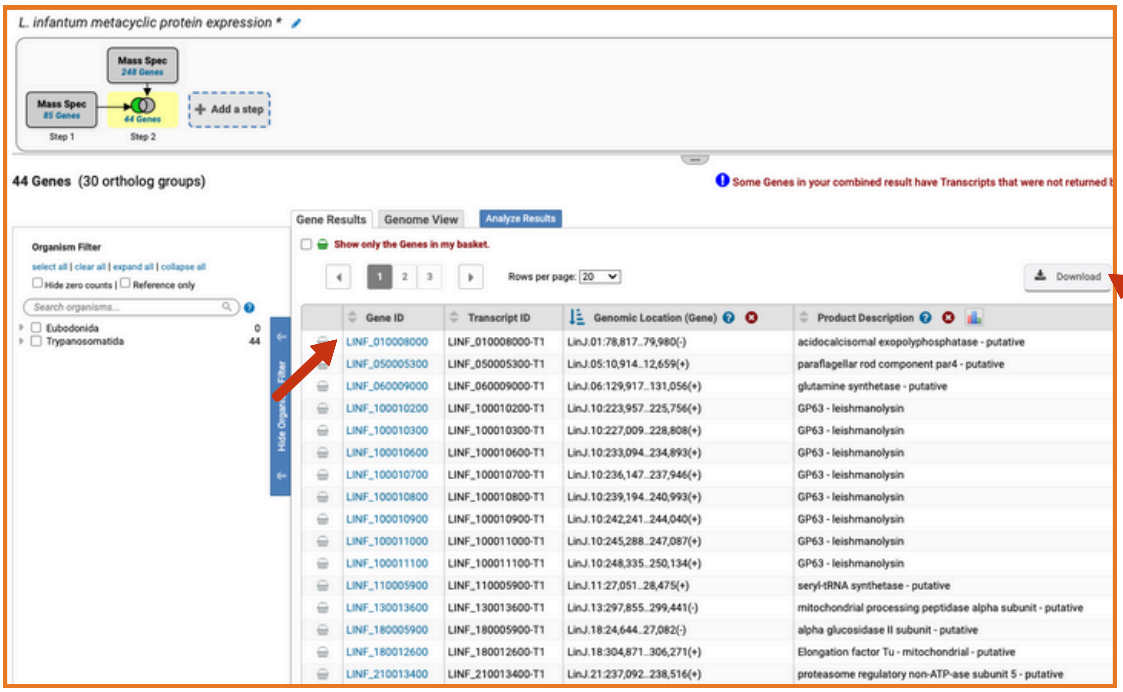
One way to increase stringency is to increase the minimum number of unique peptides that are required to map to a gene before it is returned by the search. The default settings that we used above return any gene with a minimum of 1 peptide.

- Click on the edit button in the first step of the search
- Click on the Revise option in the popup
- Change the value of the "Minimum Number of Unique Peptide Sequences" search parameter from 1 to 5 and click on the revise button
- Remember to do this for each step!

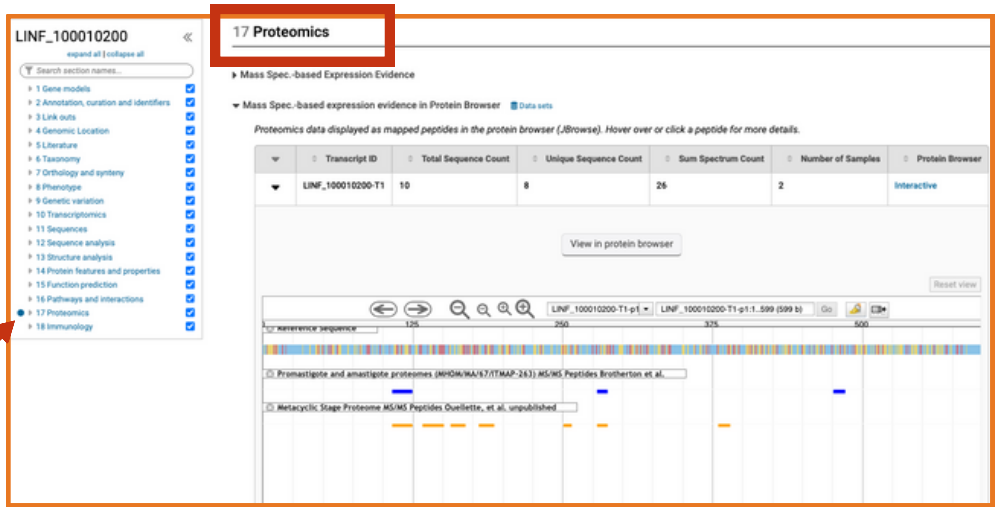


5. Explore search results

Examine the gene results. Do you see any genes that might be associated with parasite virulence? View the gene pages of some of your results by clicking on the **Gene ID**. You can download the gene results by clicking on the button on the right.



Gene pages have a **Proteomics** section where you can view mapped peptides and data from other experiments.



Questions? Comments? Write to help@veupathdb.org