

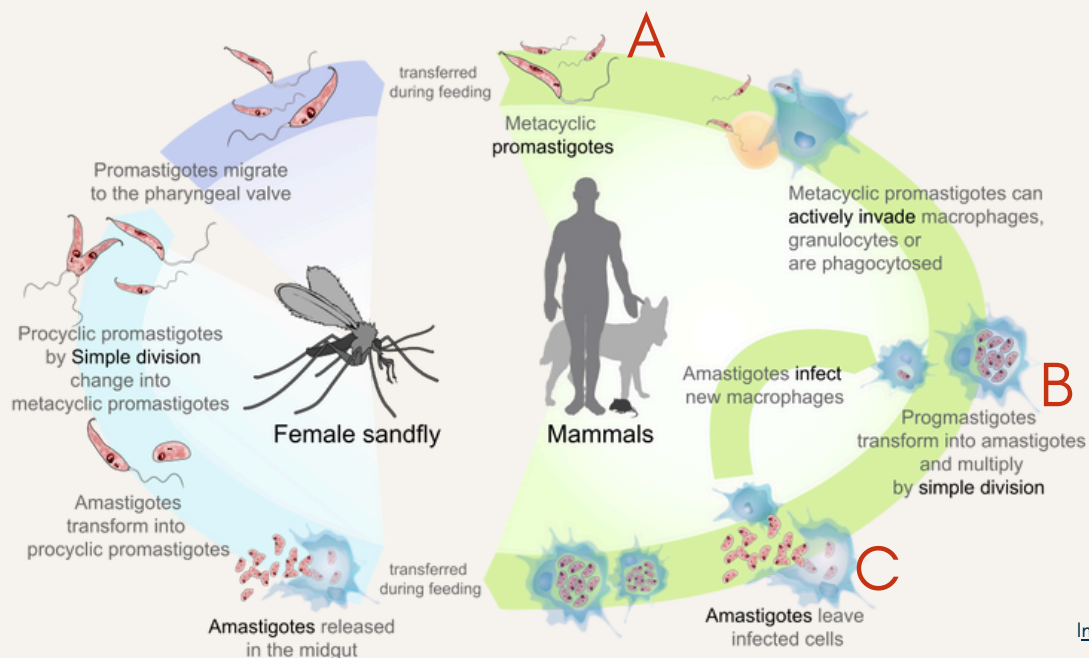


## The search question

*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)?**



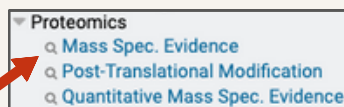
[Image credit](#)

**What platform is best suited for this question?**



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.

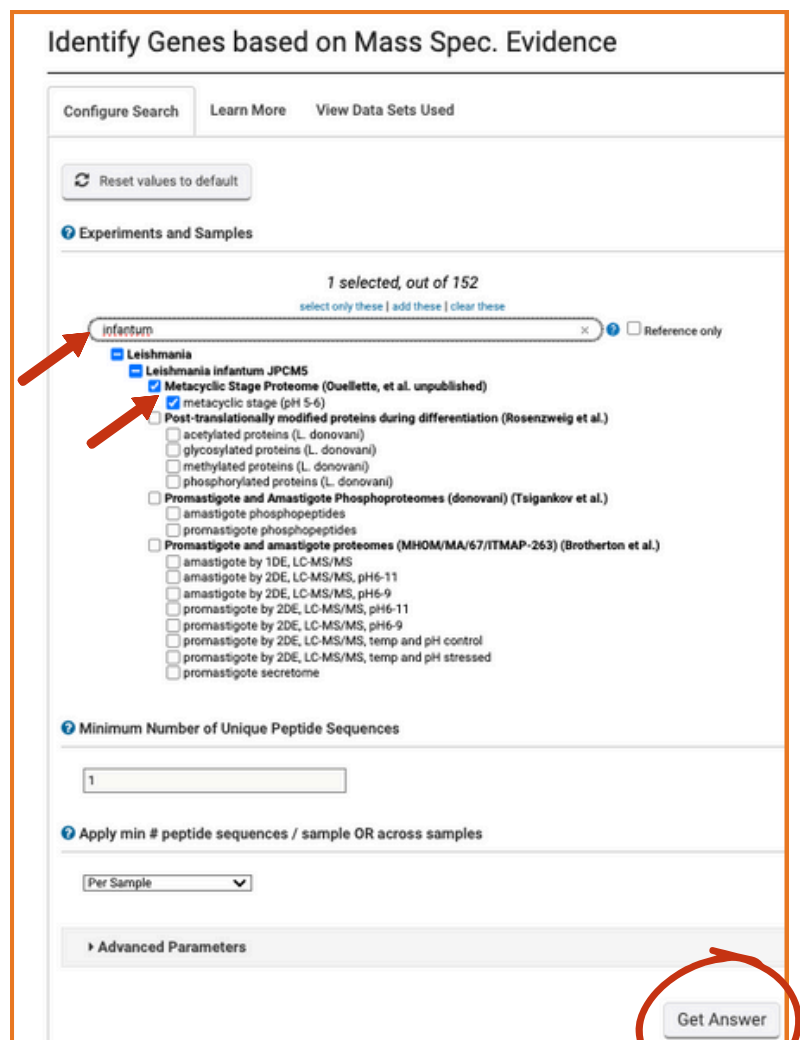
## 1. Navigate to the appropriate search

- Navigate to [TriTrypDB.org](http://TriTrypDB.org)
- From the sidebar or header, search or scroll for “proteomics” and click on **Mass Spec. Evidence**

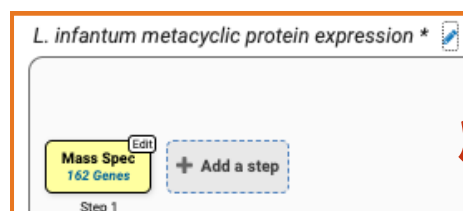


## 2. Choose appropriate experiments/ samples & parameters within the search

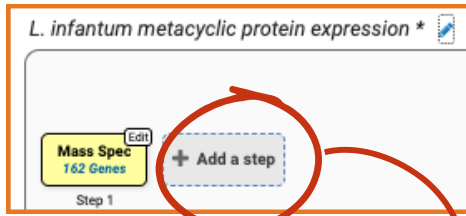
- Filter the experiment and sample tree by typing a word in the filter box: **“infantum”**.
- The first step in our strategy is to identify genes that show protein expression in the metacyclic stages.
- Select all *L. infantum* samples that come from the metacyclic stage proteome.
- Keep the default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



**How many genes did you get?**

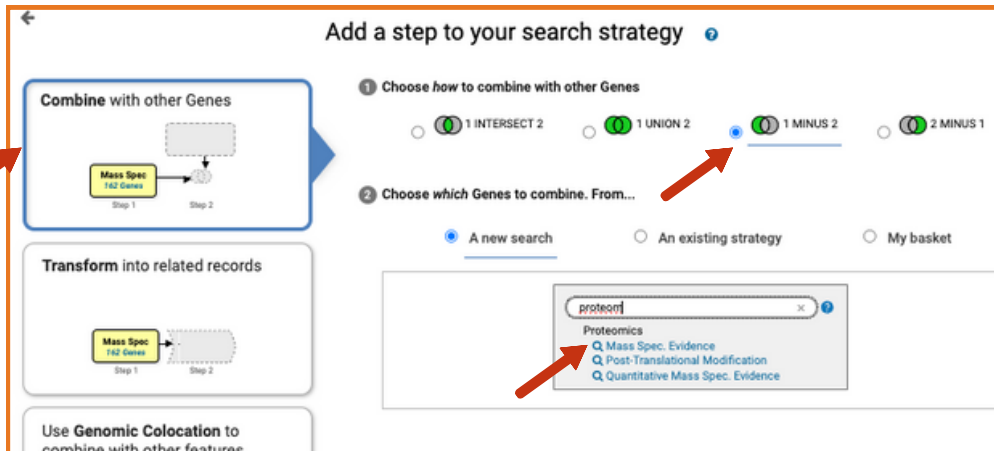


### 3. Add a step to your search strategy

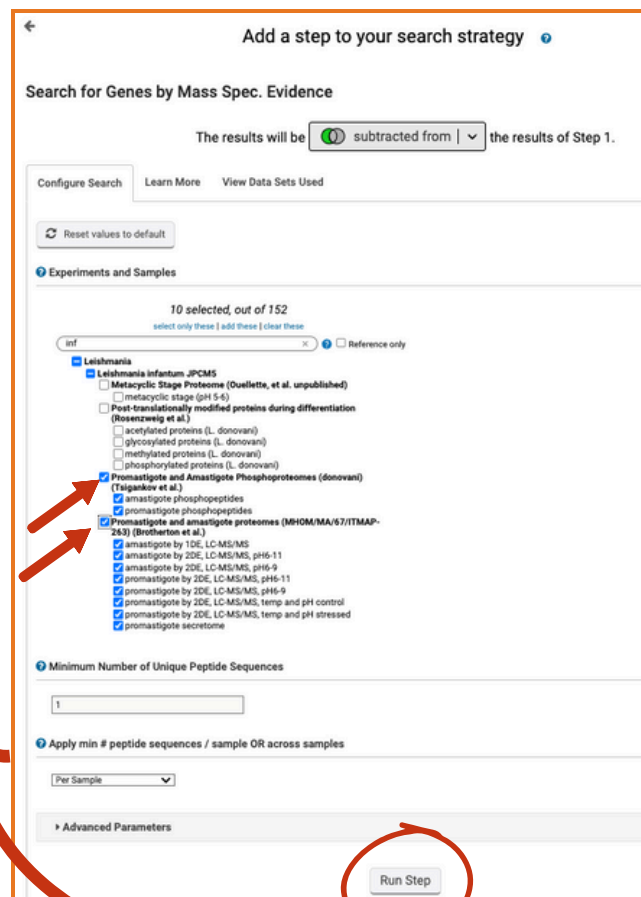
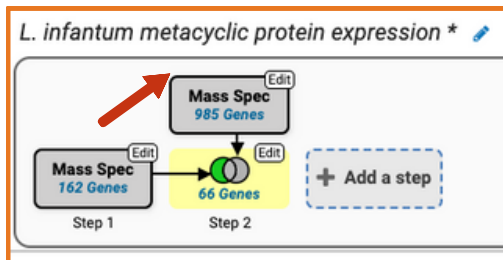


- Now subtract the genes that have protein expression in the promastigote and amastigote stages.
- To do this, **add a step** to your strategy that combines the first step with other genes using a **1 minus 2 operator**.
- To choose which genes to combine, click on **Mass Spec**.

#### 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!

*L. infantum metacyclic protein expression* \*

Mass Spec 162 Genes (Step 1) → Mass Spec 985 Genes (Step 2) → 66 Genes

View | Analyze | **Revise** | Insert step before | Orthologs | Delete

**Details for step Mass Spec** 162 Genes

Experiments and Samples metacyclic stage (pH 5-6)

Minimum Number of Unique Peptide Sequences 1

sample OR across samples Per Sample

gene (applied per sample) 1

**Identify Genes based on Mass Spec. Evidence**

Configure Search | Learn More | View Data Sets Used

Reset values to default

Experiments and Samples

1 selected, out of 152

Filter list below...

☒ Leishmania

- ☐ Leishmania braziliensis MHOM/BR/75/M2904
- ☐ Leishmania donovani BPK282A1
- ☒ Leishmania infantum JPCMS
- ☒ Metacyclic Stage Proteome (Ouellette, et al. unpublished)
- ☐ Post-translationally modified proteins during differentiation (Rosenzweig et al.)
- ☐ Promastigote and Amastigote Phosphoproteomes (donovani) (Tsigankov et al.)
- ☐ Promastigote and amastigote proteomes (MHOM/MA/67/ITMAP-263) (Brotherton et al.)
- ☐ Leishmania major strain Friedlin
- ☐ Leishmania mexicana MHOM/GT/2001/U1103
- ☐ Trypanosoma

Minimum Number of Unique Peptide Sequences

5

Apply min # peptide sequences / sample OR across samples

Per Sample

Advanced Parameters

**Revise**

*L. infantum metacyclic protein expression* \*

Mass Spec 248 Genes

Mass Spec 85 Genes (Step 1) → 44 Genes (Step 2) → + Add a step

How did this change your results?  
Would you consider these results more stringent?

## 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.

**L. infantum metacyclic protein expression \***

Mass Spec 248 Genes  
Mass Spec 85 Genes  
Step 1  
Step 2  
Add a step

44 Genes (30 ortholog groups)

Some Genes in your combined result have Transcripts that were not returned

Gene Results | Genome View | Analyze Results

Show only the Genes in my basket.

Rows per page: 20

Download

Gene ID	Transcript ID	Genomic Location (Gene)	Product Description
LINF_010008000	LINF_010008000-T1	LinJ.01:78,817..79,980(-)	acidocalcisomal exopolyphosphatase - putative
LINF_050005300	LINF_050005300-T1	LinJ.05:10,914..12,659(+)	paraflagellar rod component par4 - putative
LINF_060009000	LINF_060009000-T1	LinJ.06:129,917..131,056(+)	glutamine synthetase - putative
LINF_100010200	LINF_100010200-T1	LinJ.10:223,957..225,756(+)	GP63 - leishmanolysin
LINF_100010300	LINF_100010300-T1	LinJ.10:227,009..228,808(+)	GP63 - leishmanolysin
LINF_100010600	LINF_100010600-T1	LinJ.10:233,094..234,893(+)	GP63 - leishmanolysin
LINF_100010700	LINF_100010700-T1	LinJ.10:236,147..237,946(+)	GP63 - leishmanolysin
LINF_100010800	LINF_100010800-T1	LinJ.10:239,194..240,993(+)	GP63 - leishmanolysin
LINF_100010900	LINF_100010900-T1	LinJ.10:242,241..244,040(+)	GP63 - leishmanolysin
LINF_100011000	LINF_100011000-T1	LinJ.10:245,288..247,087(+)	GP63 - leishmanolysin
LINF_100011100	LINF_100011100-T1	LinJ.10:248,335..250,134(+)	GP63 - leishmanolysin
LINF_110005900	LINF_110005900-T1	LinJ.11:27,051..28,475(+)	seryl-tRNA synthetase - putative
LINF_130013600	LINF_130013600-T1	LinJ.13:297,855..299,441(+)	mitochondrial processing peptidase alpha subunit - putative
LINF_180005900	LINF_180005900-T1	LinJ.18:24,644..27,082(-)	alpha glucosidase II subunit - putative
LINF_180012600	LINF_180012600-T1	LinJ.18:304,871..306,271(+)	Elongation factor Tu - mitochondrial - putative
LINF_210013400	LINF_210013400-T1	LinJ.21:237,092..238,516(+)	proteasome regulatory non-ATP-ase subunit 5 - putative

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

**LINF\_100010200**

expand all | collapse all

Search section names...

- 1 Gene models
- 2 Annotation, curation and identifiers
- 3 Link outs
- 4 Genomic Location
- 5 Literature
- 6 Taxonomy
- 7 Orthology and system
- 8 Phenotype
- 9 Genetic variation
- 10 Transcriptomics
- 11 Sequences
- 12 Sequence analysis
- 13 Structure analysis
- 14 Protein features and properties
- 15 Function prediction
- 16 Pathways and interactions
- 17 Proteomics**
- 18 Immunology

17 Proteomics

Mass Spec-based Expression Evidence

Mass Spec-based expression evidence in Protein Browser

Proteomics data displayed as mapped peptides in the protein browser (/Browse). Hover over or click a peptide for more details.

Transcript ID	Total Sequence Count	Unique Sequence Count	Sum Spectrum Count	Number of Samples	Protein Browser
LINF_100010200-T1	10	8	26	2	Interactive

View in protein browser

Reset view

Reference sequence: 125 250 375 500

2. Promastigote and amastigote proteomes (MPOH/VAJ/ITMAP-263) MS/MS Peptides Brotherton et al.

2. Metacyclic Stage Proteome MS/MS Peptides Ouellette, et al. unpublished



Questions? Comments? Write to  
help@veupathdb.org