Metabolic Pathways
Exploring pathways and compounds

Note: *this exercise uses PlasmoDB.org as an example database, but the same functionality is available on all VEuPathDB resources.*

Learning objectives:

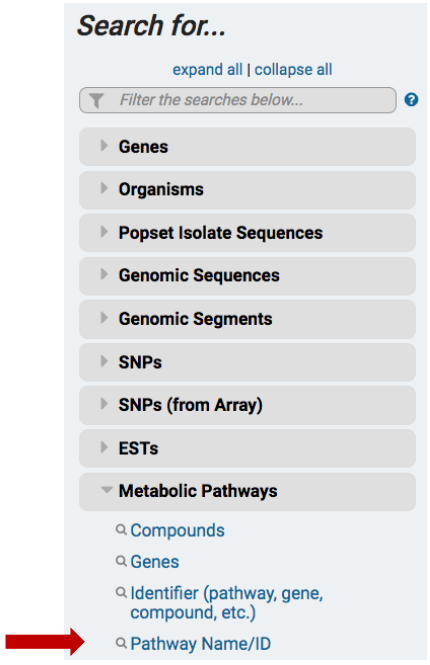
- Explore the metabolic pathways searches and visualization tools
- Search for a pathway using the name or pathway identifier
- Paint data onto pathway maps to explore:
    a. Which enzymes in a pathway are present in different genera
    b. How transcriptional abundance of enzymes in a pathway differs under experimental conditions
- Explore the compound search options

1. Find and explore the metabolic pathway for glycolysis.
For this exercise use http://plasmodb.org

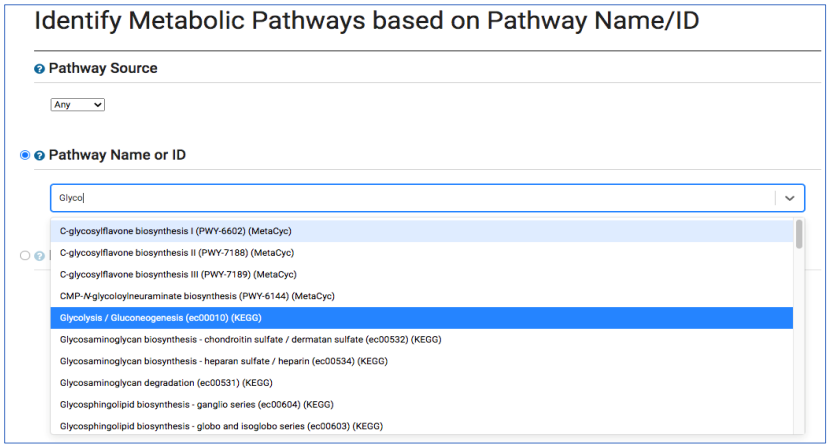
Navigate to the search page for Identify Metabolic Pathways based on Pathway Name/ID.

– Find the metabolic pathway searches on the home page. You can look under "Metabolic Pathways" or use the search filter. You can find metabolic pathways based on the pathway name or identifier, or using genes or compoundsinvolved in the pathway. Search for the **glycolysis** pathway using the Pathway Name/ID option.

– This search is equipped with a type-ahead function for finding the metabolic pathway name. Begin typing glycolysis and then choose the pathway name from the list that appears.

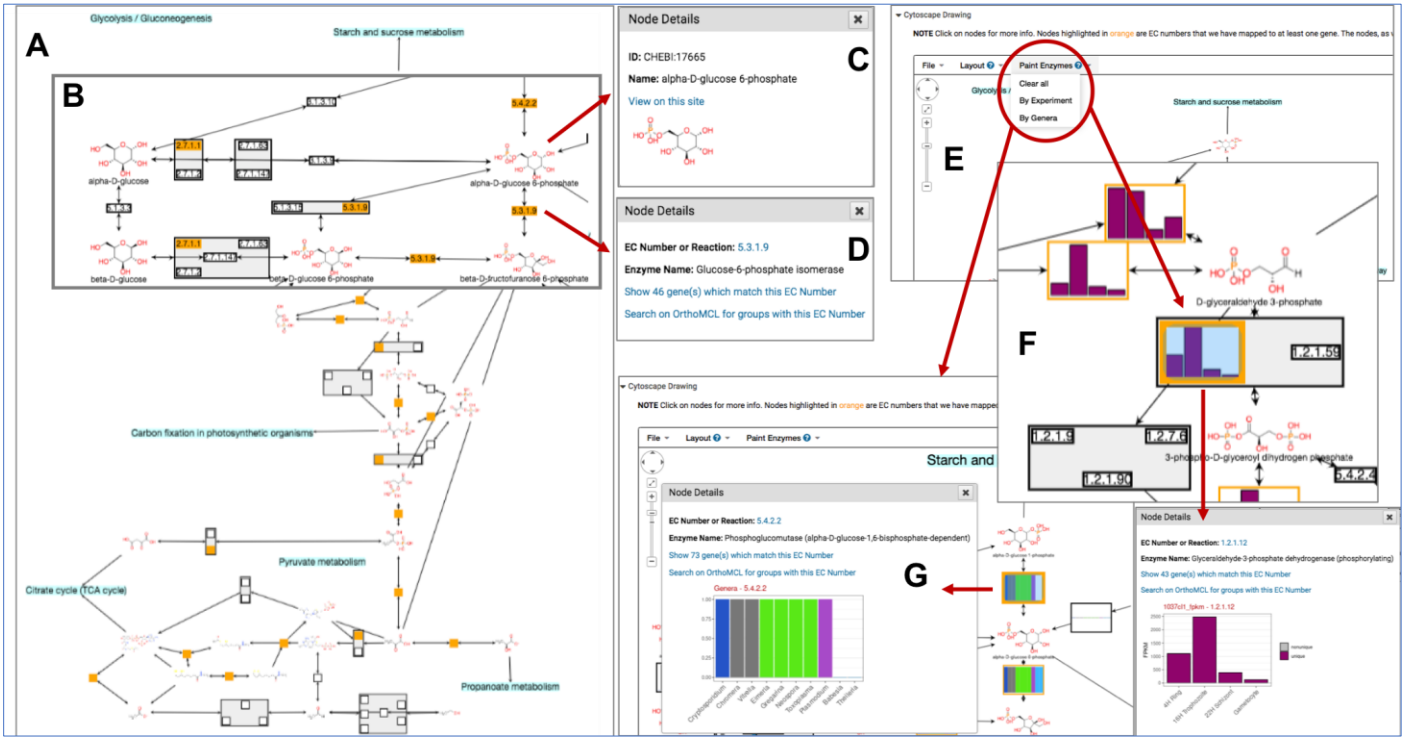


a. Examine the Glycolysis / Gluconeogenesis pathway.

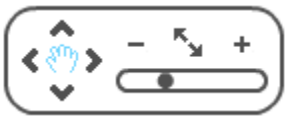


– The search takes you straight to the record page for the Glycolysis / Gluconeogenesis (ec00010) metabolic pathway from KEGG. The overview section of the record page contains an interactive graphical representation of the pathway. The pathway map and the legend can be repositioned.

A. Initial pathway view is zoomed out.
B. Zoom in to see more details including EC numbers and metabolite structures.
C. Click on a metabolite structure to get additional information.
D. Click on the EC number to get more info about the enzyme including links to retrieve all genes in the database assigned to this EC number.

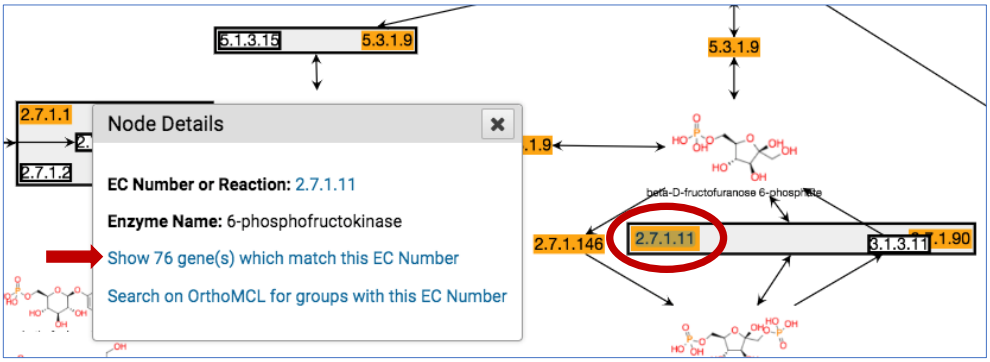


E. The drop-down menu under the heading “Paint Enzymes” allows you paint the pathway based on experimental data or phyletic pattern.
F. Painting pathway by experiment provides a graphical representation of experimental results. Click on the graph to see more details.
G. Painting pathway based on phyletic pattern provides a graphical representation of phyletic distribution. Clicking on the phyletic pattern graphic provides additional information.



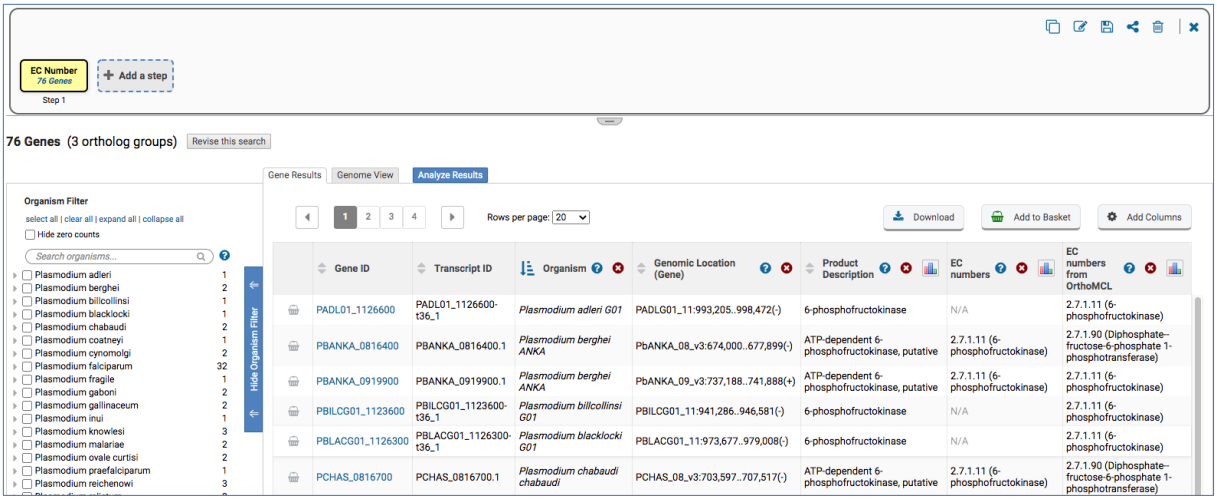
- Use the Tool Box to move (drag) the map and individual nodes. Zoom in and out to help explore the map.
- What do the rectangles with numbers like 2.7.1.11 represent?
- What is the difference between the rectangular nodes that are orange and those that are not?
- Why are some enzymes grouped?
- Find the node representing 6-phosphofructokinase (EC number = 2.7.1.11). You may need to zoom and reposition the map to find the node.

- Click on the 2.7.1.11 node to open a popup with information about this enzyme.



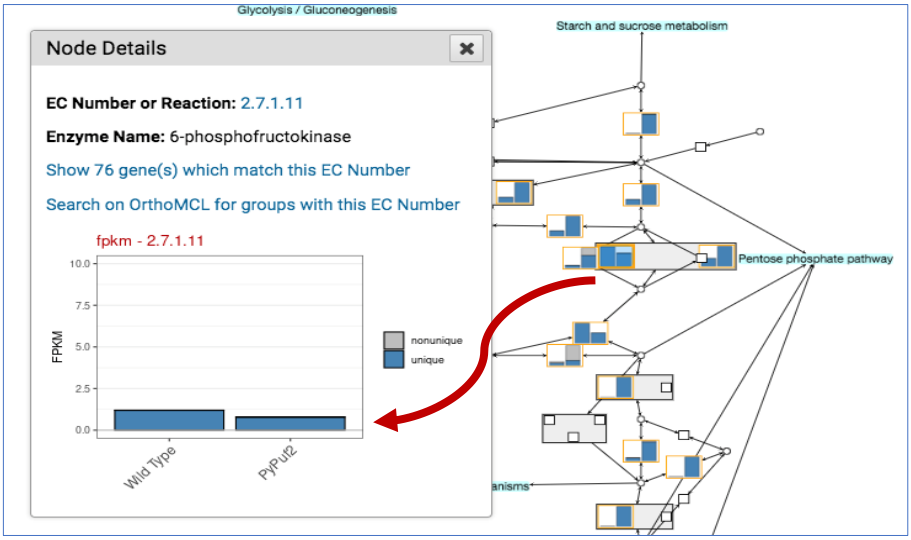
- How many genes in the database matched this EC number?

- Try the link ‘Search for Gene(s) by EC Number’. Where did you end up? What do the 76 genes in the result list represent? Is 6-phosphofructokinase unique to P. falciparum? Notice the two columns called “EC numbers” and “EC numbers from OrthoMCL”. What do these columns represent?

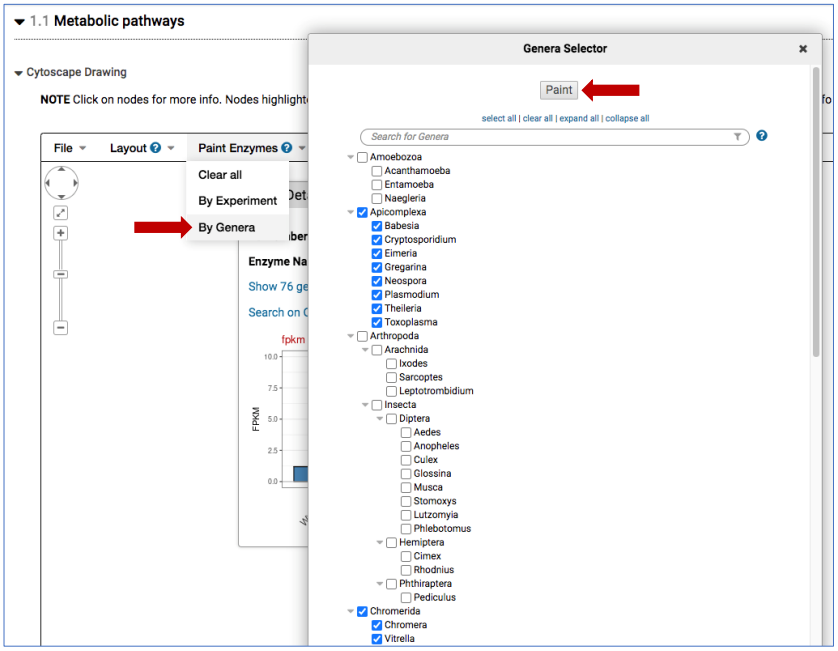


– Use your Browser's back button to return to the glycolysis pathway record page and open the Paint Experiment menu. Choose the experiment "Salivary gland sporozoite transcriptomes: WT vs Puf2-KO (Lindner et al)". Be patient while the graphs appear in place of the EC numbers.

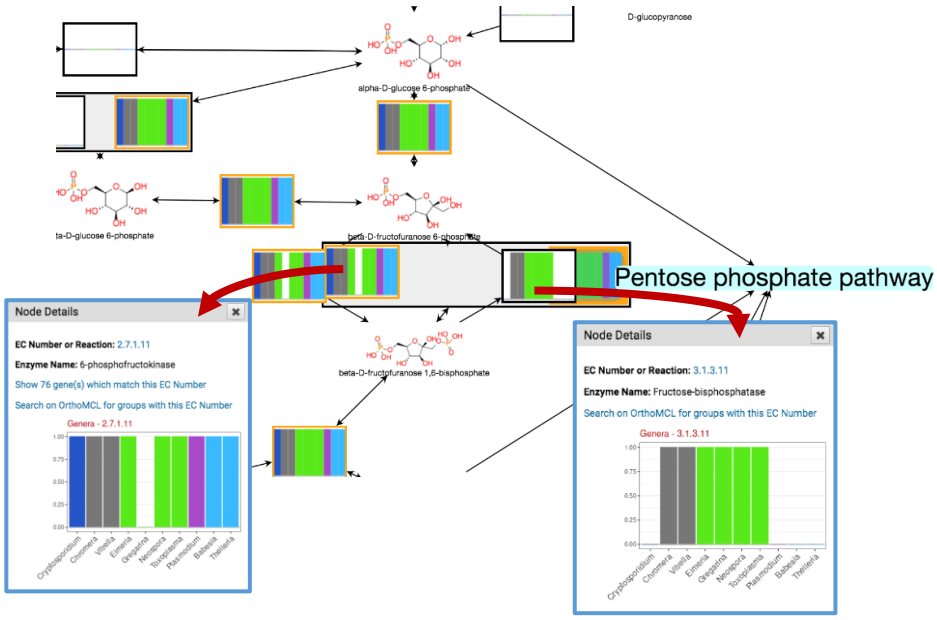
- Does 6-phosphofructokinase appear to be expressed in salivary gland sporozoites? What enzymes in this pathway are affected in knockouts of Puf2?



- Use the Paint Genera option to determine whether 6-phosphofructokinase has orthologs across Apicomplexa and Chromerida.

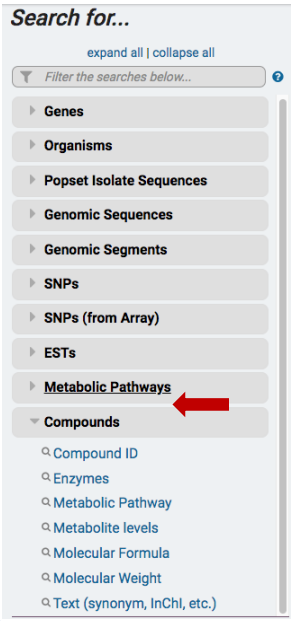


- What about the enzyme that catalyzes the reverse reaction (Fructose-bisphosphatase)?



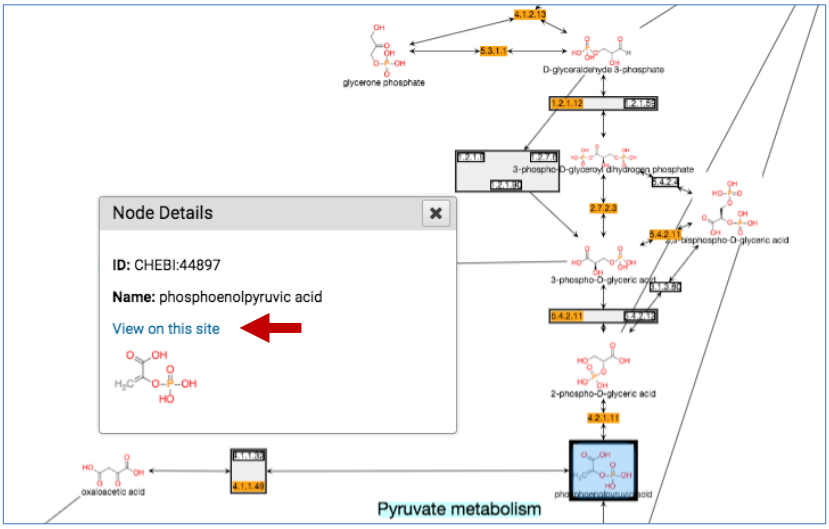
2. Find and explore the compound record page for phosphoenolpyruvate (phosphoenolpyruvic acid or PEP).

Compound records are accessed by running one of the compound searches available under the “Compounds” heading. Compounds may be retrieved by ID, text, metabolic pathway, molecular formula, molecular weight and metabolite levels. Compound records can also be accessed from the metabolic pathway legend after clicking on a compound (blue circle) in the map.



- Choose one of these searches and retrieve the PEP record page.

- Alternatively, you can reach the PEP record page via a metabolic pathway where it is present as a substrate or a product of an enzymatic reaction (ie. glycolysis). Click on the node representing a compound

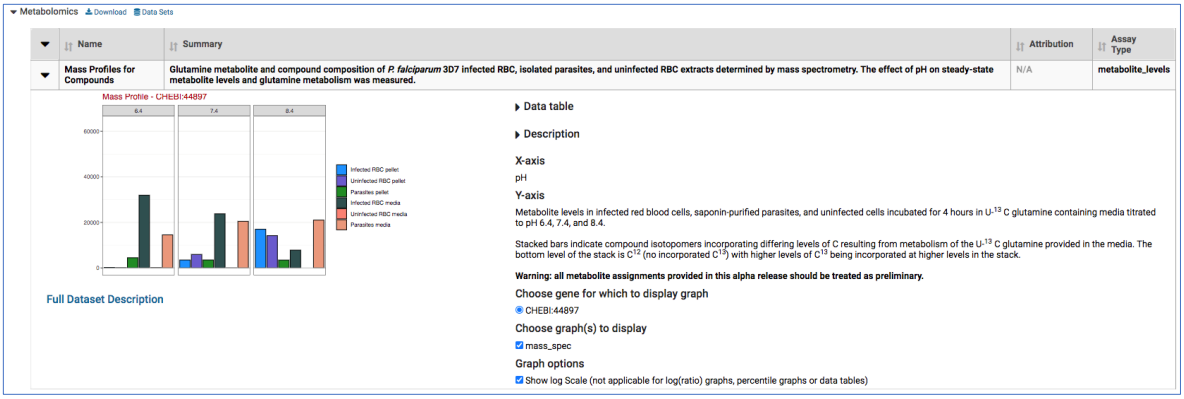


- Which method did you use to get to the PEP record page? What compound name worked the best?

- Examine the PEP record page.

- What data sections do you see?

- Under which conditions is PEP present at highest concentrations? (Hint: navigate to the Metabolomics section)



10) The metabolite abundance experiment in PlasmoDB compares the following conditions at 3 pH levels:

a) Parasites isolated from infected red blood cells using saponin lysis

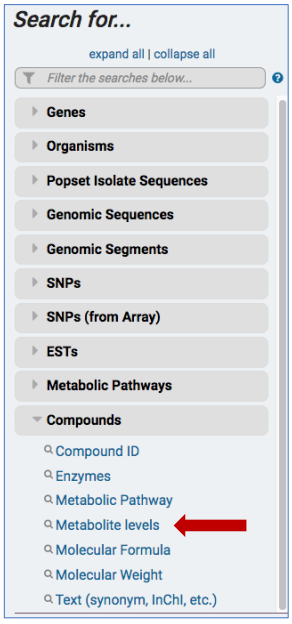
b) Whole infected red blood cells isolated with Percoll

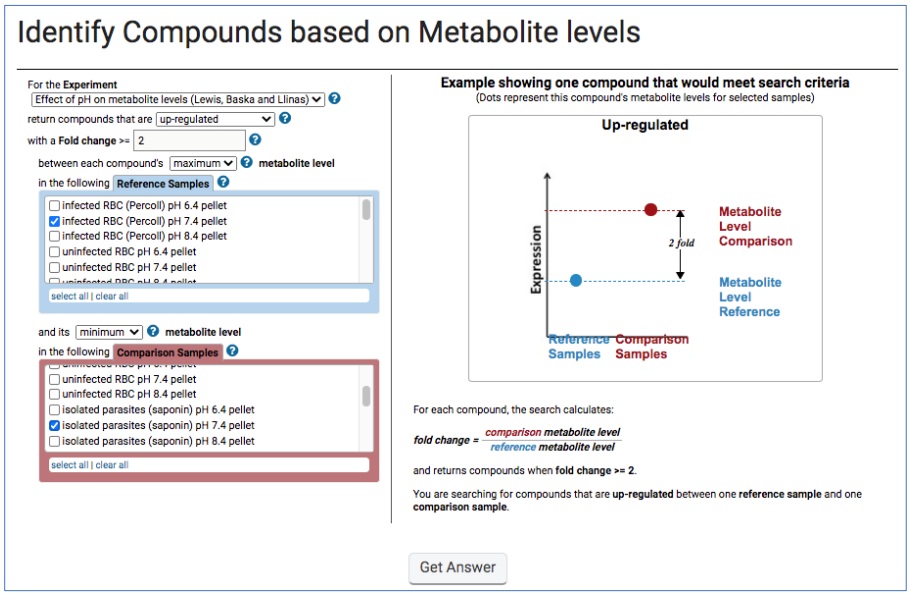
c) Whole uninfected red blood cells

For both conditions, data was collected from the cell pellet and the media supernatant.

Find metabolites that are enriched in the isolated parasites (saponin) compared to infected red blood cells (Percoll) in the cell pellet at pH 7.4.

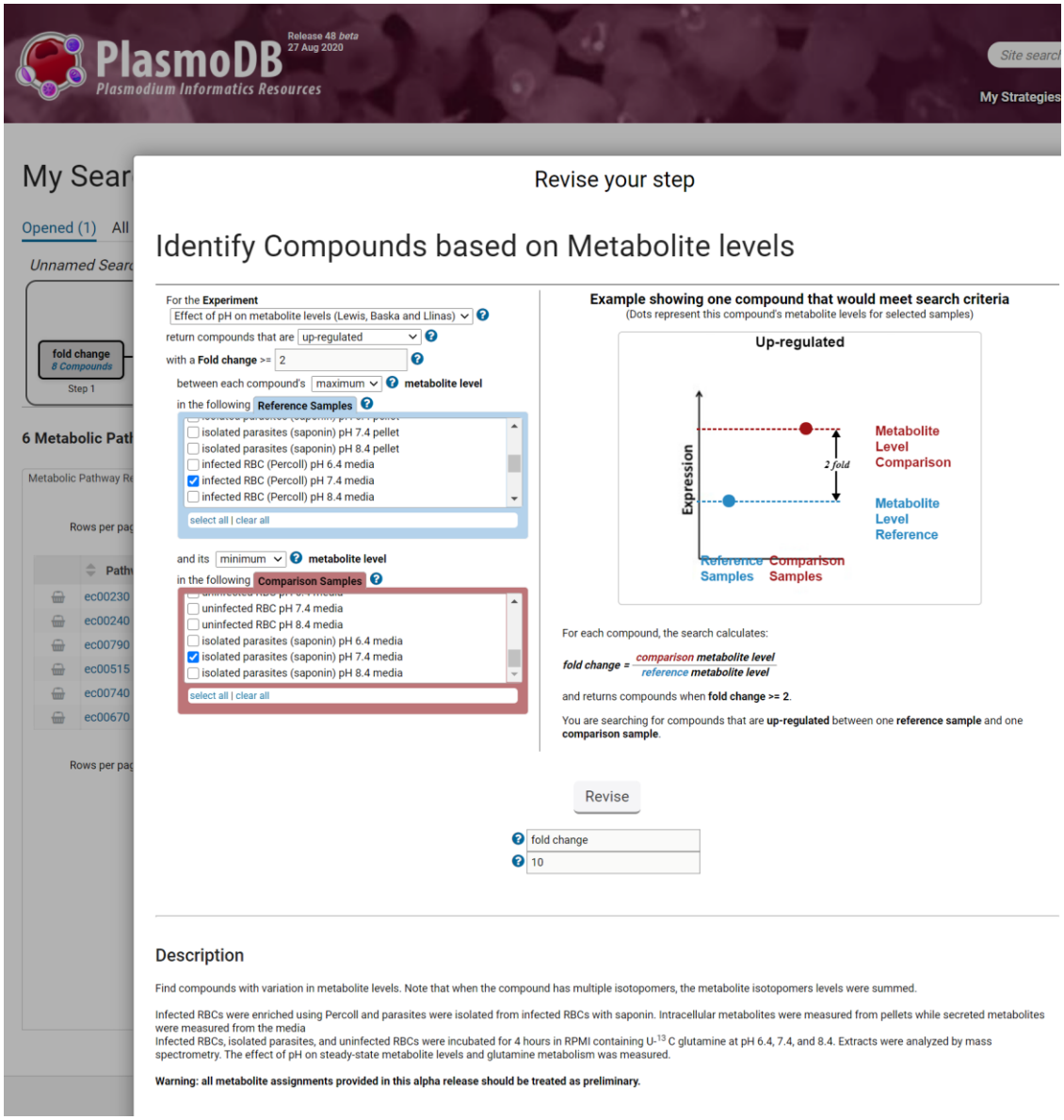
This can be done using the metabolite levels search, which looks a lot like the fold-change searches you have previously seen for transcriptomics data.



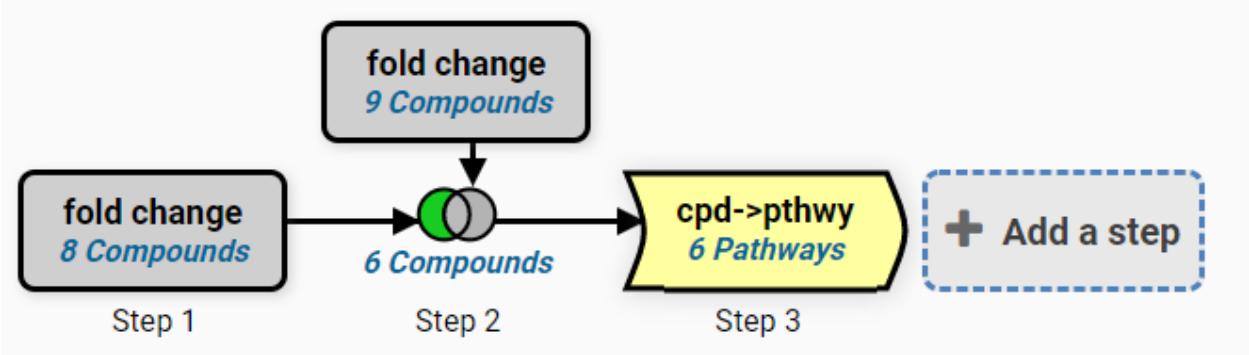


- How many compounds did you get?

- Add a step and use the same search to find out how many of these compounds (metabolites) are **NOT** enriched by 2-fold in isolated parasites (saponin) compared to the infected red blood cells (Percoll) in the media supernatant at pH 7.4. Make sure to use the correct operator!



How many compounds do you have now? Which metabolic pathways do these compounds belong to? Click Add a Step and transform the results to metabolic pathways.


fold change
9 Compounds
fold change
8 Compounds
6 Compounds
cpd->pthwy
6 Pathways
Add a step
Step 1
Step 2
Step 3