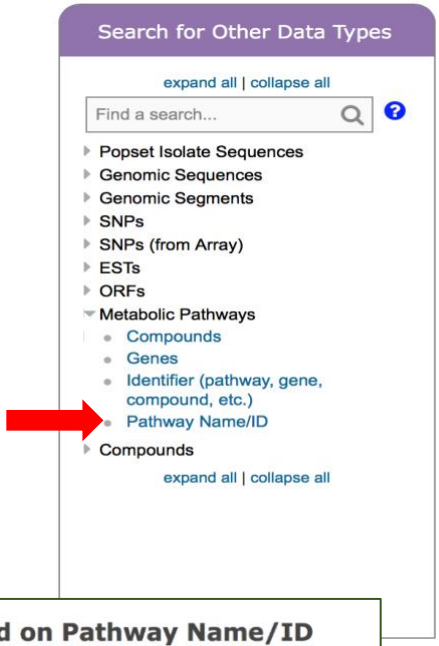
Metabolic Pathways
Exploring pathways and compounds

1. Find and explore the metabolic pathway for glycolysis.

For this exercise use http://plasmodb.org

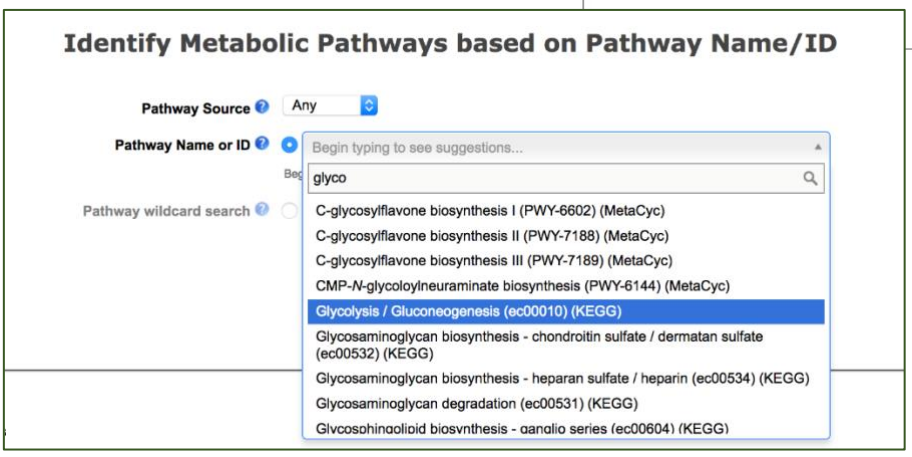
Navigate to the search page for Identify Metabolic Pathways based on Pathway Name/ID.

– Metabolic pathway and compound searches are available under the "Identify Other Data Types" head on the home page. You can find metabolic pathways based on the pathway name, genes involved in the pathway, or compounds involved in the pathway. Search for the **glycolysis** pathway using the Pathway Name/ID option.



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

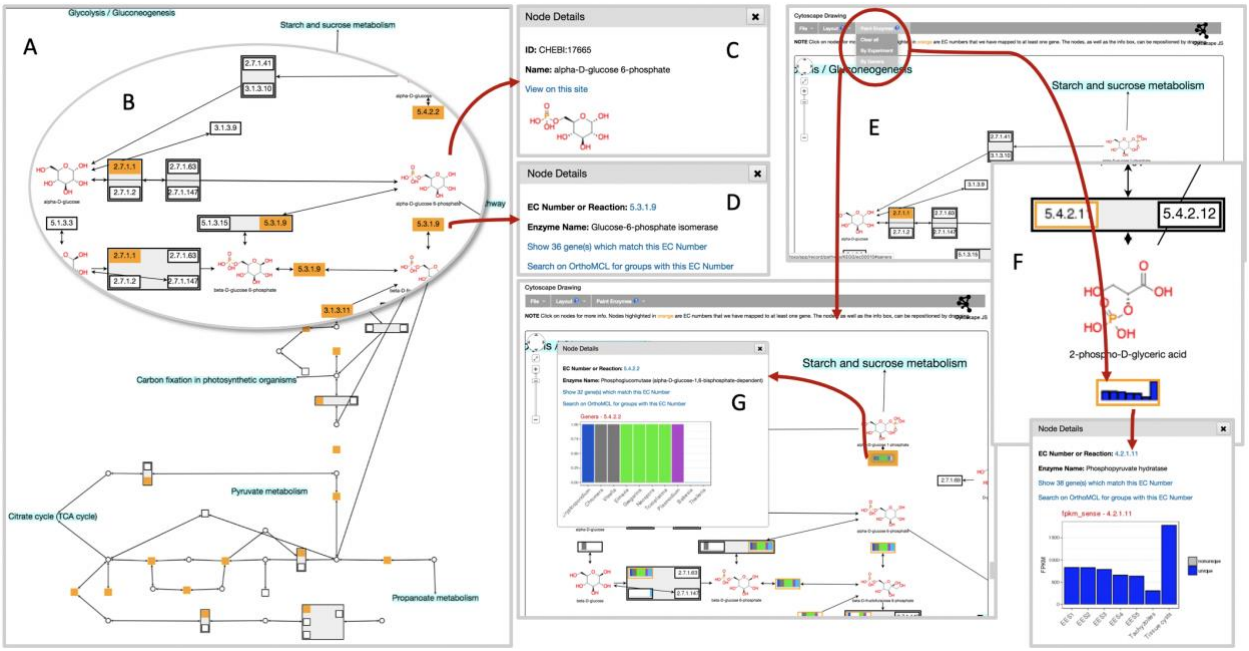
a. Examine the Glycolysis / Gluconeogenesis pathway.



– 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.

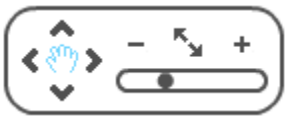


E. The drop-down menu under the heading "Paint Enzymes" allows you paint the pathway based on experiments or based on phyletic pattern.

F. Painting pathway by experiment provides a graphical representation of experimental results. Click on the graph to see more details.

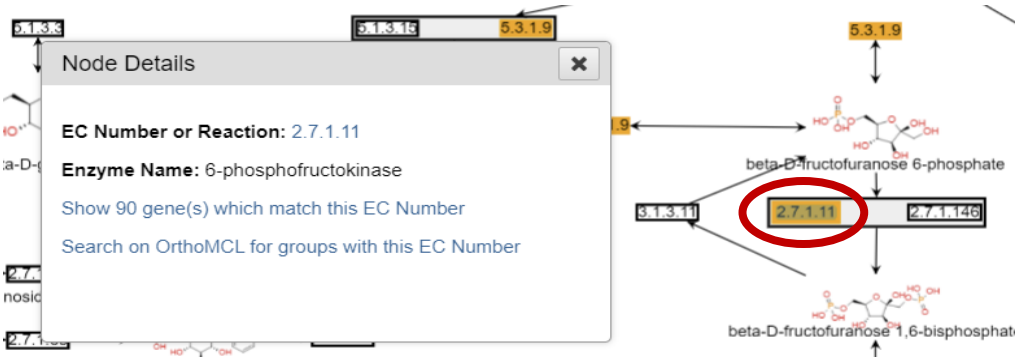
G. Painting pathway based on phyletic pattern provides a graphical representation of phyletic distribution. Clicking on the phyletic pattern graphic provides additional information.

- Use the Tool Box to move (drag) the map and individual nodes. Zoom in and out to help explore the map.



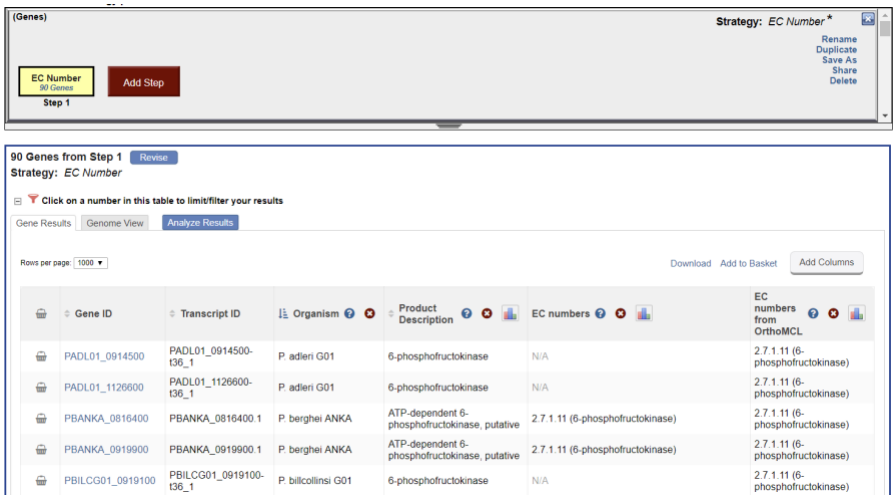
- What do the rectangles with numbers like 2.7.1.11 represent?
- What is the difference between the rectangular nodes that are orange and those that are not?
- Why are some enzymes grouped?
- Find the node representing 6-phosphofructokinase (EC number = 2.7.1.11). You may need to zoom and reposition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

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



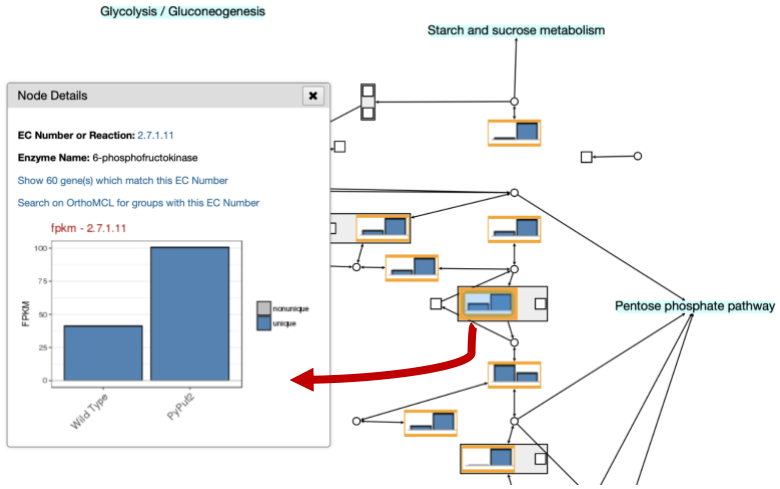
– How many genes in the database matched this EC number?

– Try the link 'Search for Gene(s) by EC Number'. Where did you end up? What do the 90 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?

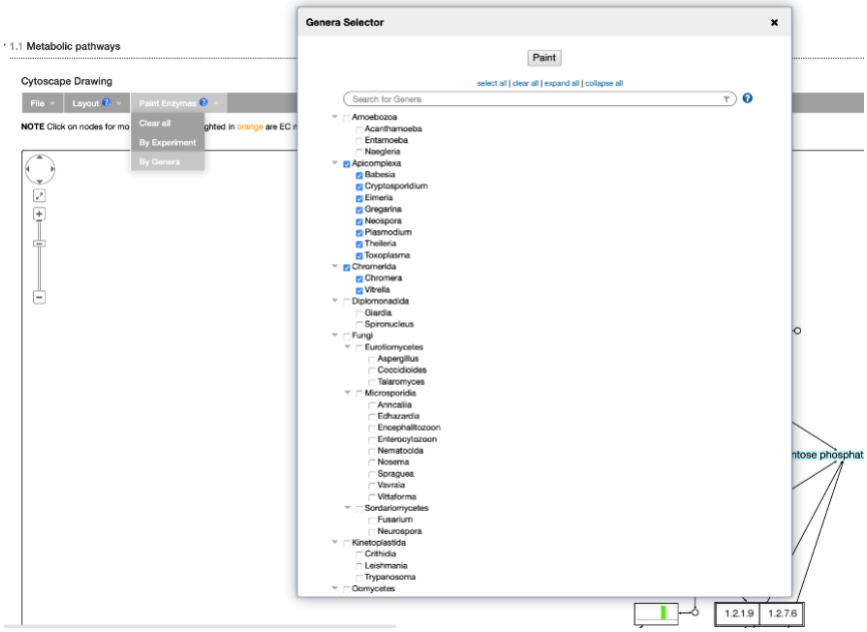


– Use your Browser's back button to return to the Glycolysis pathway record page and open the Paint Experiment menu. Choose the experiment "Salivary gland sporozoite transcriptomes: WT vs Pfu2-KO". Be patient while the graphs appear in place of the EC numbers.

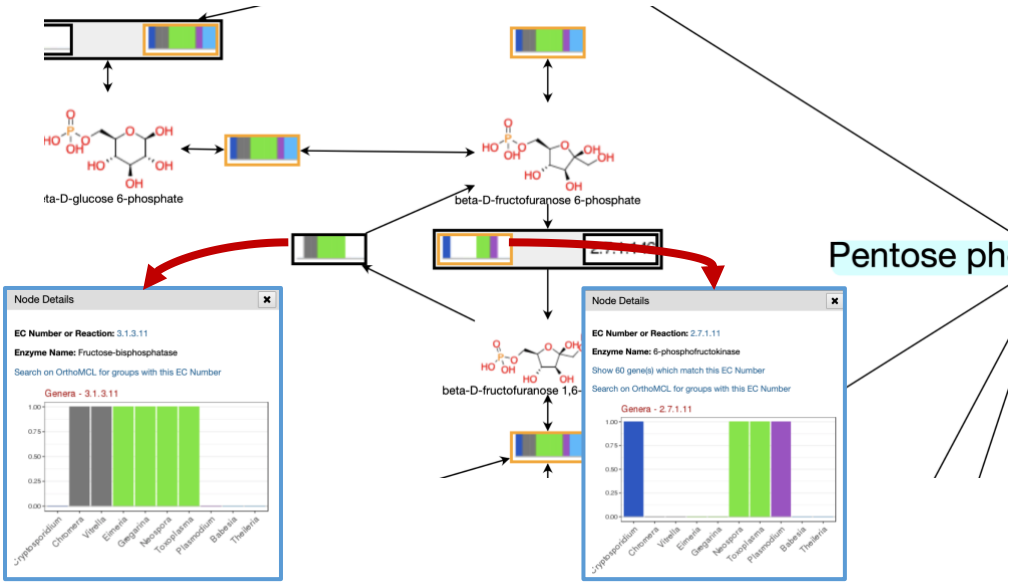
- Does 6-phosphofructokinase appear to be expressed in salivary gland sporozoites? What enzymes in this pathway are affected in knockouts of Pfu2?



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

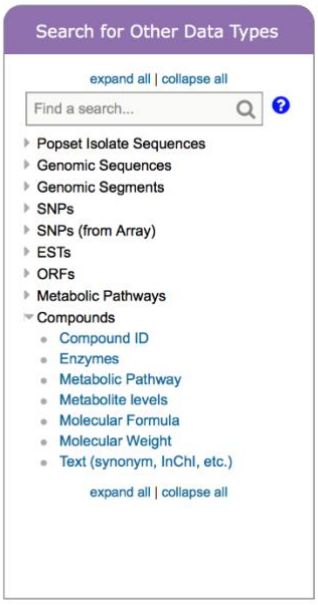


- What about the enzyme that catalyzes the reverse reaction (Fructose-bisphosphatase)?



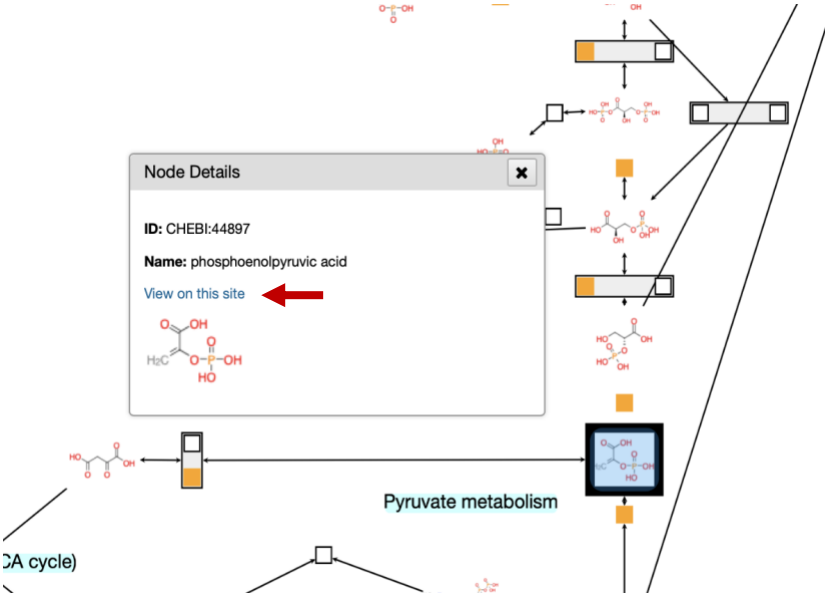
2. Find and explore the compound record page for phosphoenolpyruvate (phosphoenolpyruvic acid or PEP).

Compound records are accessed by running a compound search available under the "Identify Other Data Types" heading on the home page. For example, 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.

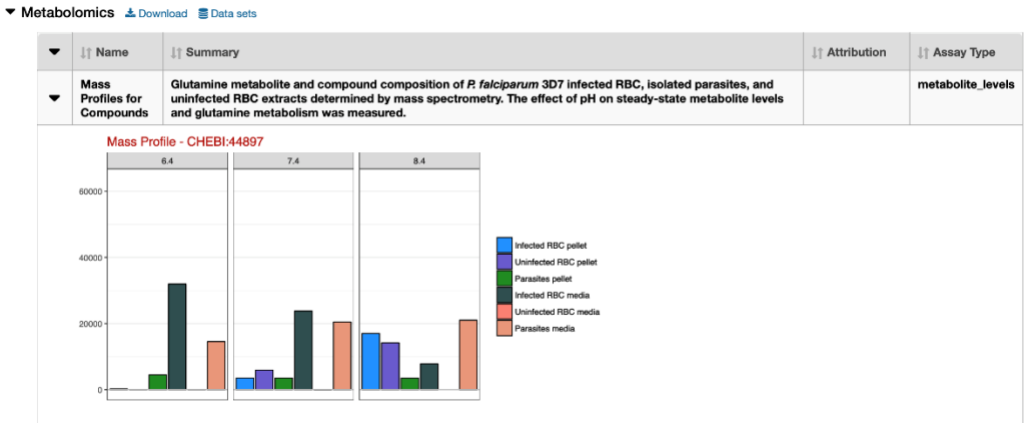


- Choose one of these searches and retrieve the PEP record page.

- 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

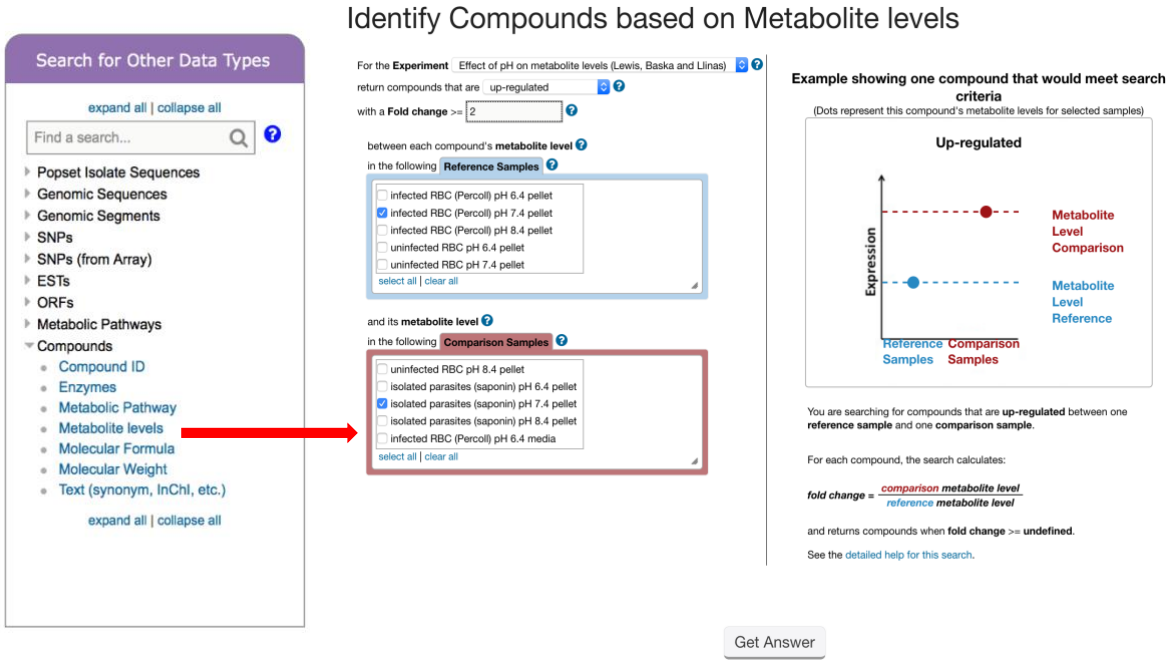


- Which method did you use to get to the PEP record page? What compound name worked the best?
- Examine the PEP record page.
- What data sections do you see?
- Under which conditions is PEP present at highest concentrations? (Hint: navigate to the Metabolomics section)

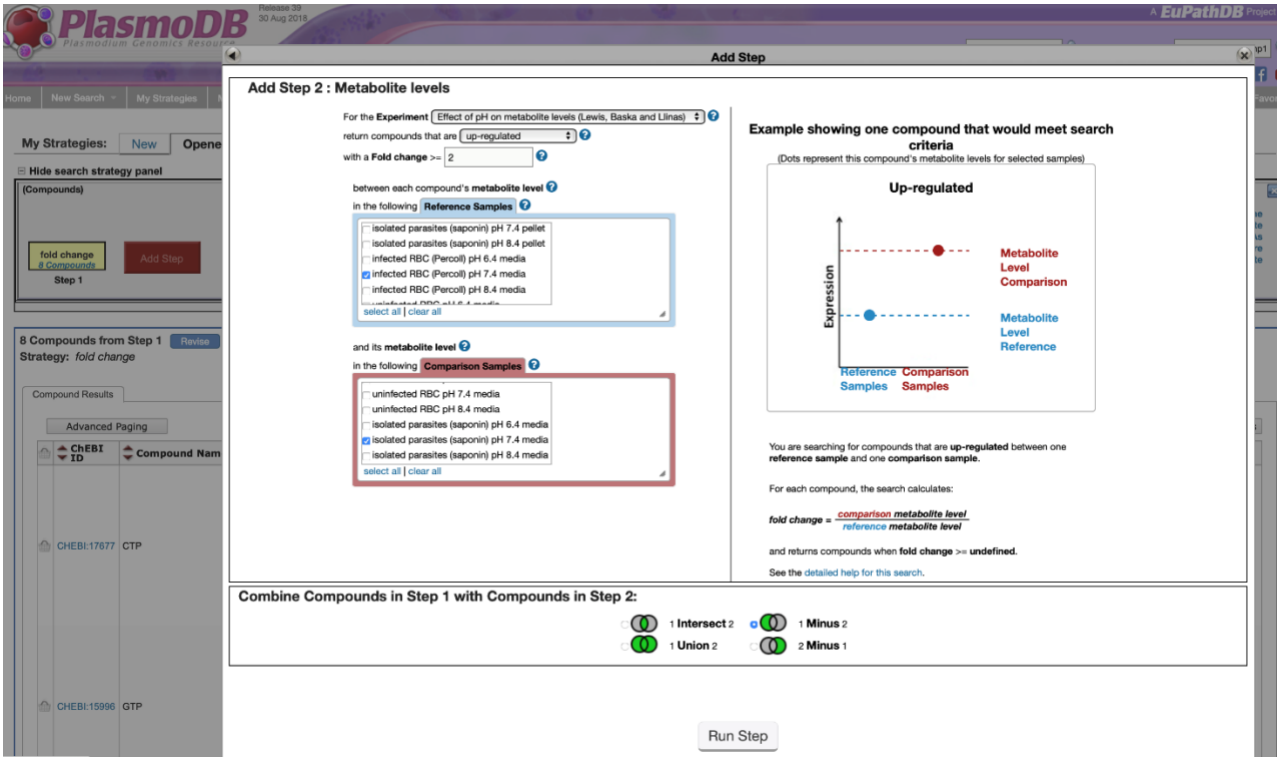


10. Identify metabolites (compounds) that are 20-fold enriched at pH7.4 in saponin lysed infected red blood cell (iRBCs) pellets compared the pH7.4 percoll pellet.

This requires running a metabolite levels search (2-fold enriched in saponin pellet compared to the percoll pellet as the reference).



- How many compounds did you get?
- How many of these compounds (metabolites) are NOT enriched by 2-fold in the pH7.4 saponin media fraction compared to the percoll media as reference?



To which metabolic pathways do these compounds belong? Click Add Step and transform the results to metabolic pathways.