Metabolic Pathways and Compounds: 
Exercise 5

1. Find the metabolic pathway for glycolosis. 
For this exercise use PlasmoDB.org

- 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 glycolosis pathway using the Pathway Name/ID option.

- This search is equipped with a type-ahead function for choosing the metabolic pathway name. Begin typing glycolosis and then choose the pathway name from the list that appears.
- The search takes you straight to the record page for the Glycolysis / Gluconeogenesis (ec00010) metabolic pathway. Examine the pathway. The overview section of the record page contains an interactive representation of the pathway. The pathway map and the legend can be repositioned. Located under the map is a table of Metabolic Pathway Reactions.

- Use the Tool Box to move (drag) the map and individual nodes. Zoom in and out to help explore the map.
- What do the circles represent?
- What do the rectangles with numbers like 2.7.1.11 represent?
- Hover over the pathway nodes.
- What is the difference between the rectangular nodes that are outlined in red and those that are outlined in black?
- Click on the nodes. Do you see new information appear in the legend? Try some of the new links in the legend.
- Notice the gray dropdown menus above the map.
  - FILE – Zoom around and reposition some of the nodes; then use the file menu to save an image or xml file of the map.
  - Layout – change the look of the map to something more familiar to you.
- Paint Experiment – change the EC number in the nodes to a graph representing that gene’s expression in RNASe or microarray experiments. We will explore this function later.
- Paint Genera – change the EC number in the nodes to a graph representing the presence of orthologs in other genera.

- 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 populate the legend with information about genes that are annotated with the EC number 2.7.1.11.
- What genomes contain genes that are annotated with the EC number 2.7.1.11?
- What organisms have orthologs of these genes?

- Try the link ‘Search for Gene(s) by EC Number’. Where did you end up? What do the 10 genes in the result list represent? Is 6-phosphofructokinase unique to P. falciparum?
- 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: wild type vs Pfu2 knockout. Be patient while the graphs appear in place of the EC numbers.
- Does 6-phosphofructokinase appear to be expressed in salivary gland sporozoites? What other enzymes in this pathway are affected in knockouts of Pfu2?
- Use the Paint Genera option to determine whether 6-phosphofructokinase has orthologs in Toxoplasma.

2. Find Phosphoenolpyruvate (PEP) and visit its record page.

- 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 blue circle representing

Examine the PEP record page.
- What data sections do you see?
- Under which conditions is PEP present at highest concentrations? (Open the Mass Profiles for Compounds data section)
3. Identify metabolites (compounds) that are 20-fold enriched at pH7.4 in saponin lysed infected red blood cell (IRBCs) pellets compared to the pH7.4 percoll pellet.

- This requires running a metabolite levels search (20-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 20-fold in the pH7.4 saponin media fraction?
- To which metabolic pathways do these compounds belong? Click Add Step and transform the results to metabolic pathways.