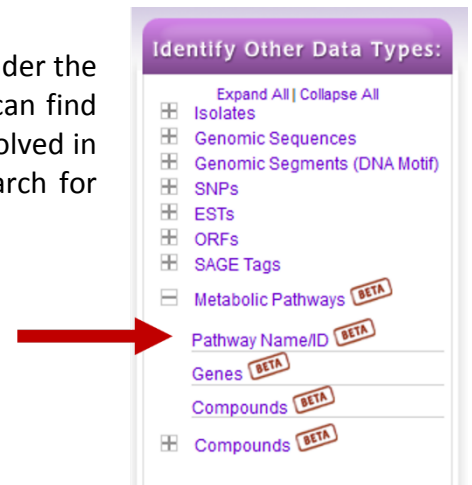


Metabolic Pathways and Compounds: Exercise 5

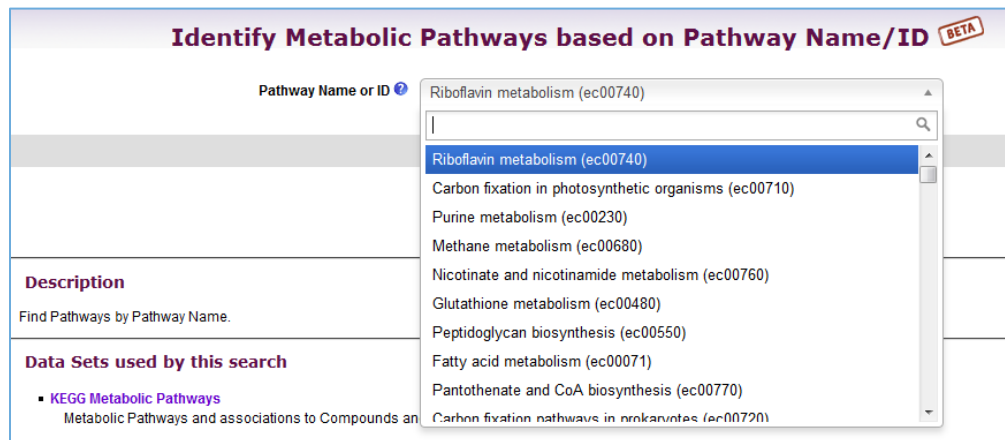
1. Find the metabolic pathway for glycolysis.

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 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.



- 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.

Metabolic Pathway
ec00010 - Glycolysis / Gluconeogenesis

Overview
Pathway: ec00010 - Glycolysis / Gluconeogenesis

File ? Layout ? Paint Experiment ? Paint General ?

File menu with options for saving images, changing the format/layout and layering data at the nodes.

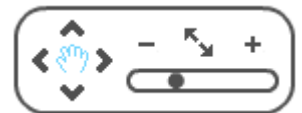
Interactive pathway map that will display information about the gene or compound node that click.

Repositioning: Drag the entire pathway map, individual nodes, or the legend to reposition.

Interactive legend that will display information about the last node you clicked on.

Click on nodes for more info. Nodes highlighted in red are EC numbers that we have mapped to at least one gene. The nodes, as well as this info box, can be repositioned by dragging.

- 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.
 - o FILE – Zoom around and reposition some of the nodes; then use the file menu to save an image or xml file of the map.
 - o 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 RNA Se 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.

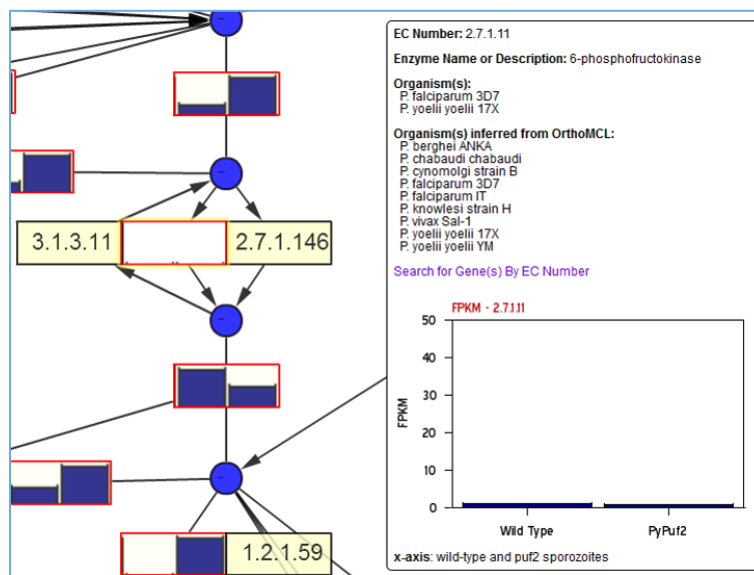
- Find the node representing 6-phosphofructokinase. (EC number = 2.7.1.11) You may need to zoom and reposition the map to find the node.
- Click on the 2.7.1.11 node to populate the legend with information about genes that are annotated with the EC number 2.7.1.11.
- What genomes contain genes that are 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*?

All Results	Ortholog Groups	<i>P.berghei</i> ANKA	<i>P.chabaudi</i> chabaudi	<i>P.cynomolgi</i> strain B	<i>P.falciparum</i> 3D7	<i>P.falciparum</i> IT	<i>P.gallinaceum</i> 8A	<i>P.knowlesi</i> strain H	<i>P.preichenowi</i> Dennis	<i>P.vivax</i> Sal-1	<i>P.yoelii</i> yoelii 17XNL	<i>P.yoelii</i> yoelii 17X	<i>P.yoelii</i> yoelii YM
10	2	1	1	0	2	1	0	1	0	1	0	2	1

Gene ID	Genomic Location	Product Description
PBANKA_091990	berg09: 772,653 - 777,353 (+)	ATP-dependent phosphofructokinase, putative
PCHAS_092450	chab09: 848,095 - 852,842 (-)	ATP-dependent phosphofructokinase, putative
PF3D7_0915400	PF3D7_09_v3: 650,585 - 654,841 (-)	6-phosphofructokinase (PFK9)

- 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.

Identify Other Data Types:

- Expand All | Collapse All
- Isolates
- Genomic Sequences
- Genomic Segments (DNA Motif)
- SNPs
- ESTs
- ORFs
- SAGE Tags
- Metabolic Pathways **BETA**
- Compounds **BETA**
- Compound ID **BETA**
- Text (synonym, inChI, etc.) **BETA**
- Enzymes **BETA**
- Metabolic Pathway **BETA**
- Molecular Formula **BETA**
- Molecular Weight **BETA**
- Metabolite levels **BETA**

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

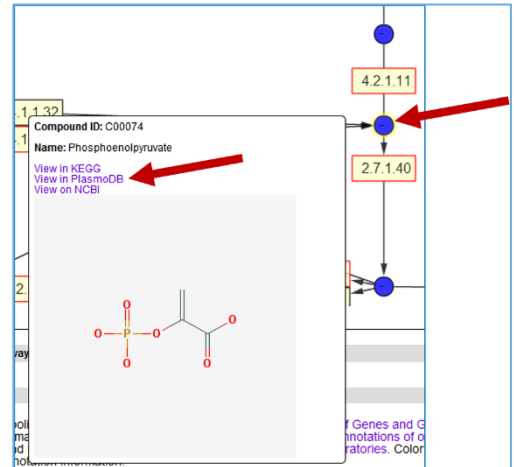
Identify Compounds based on Text (synonym, InChI, etc.) BETA

Text term (use * as wildcard)

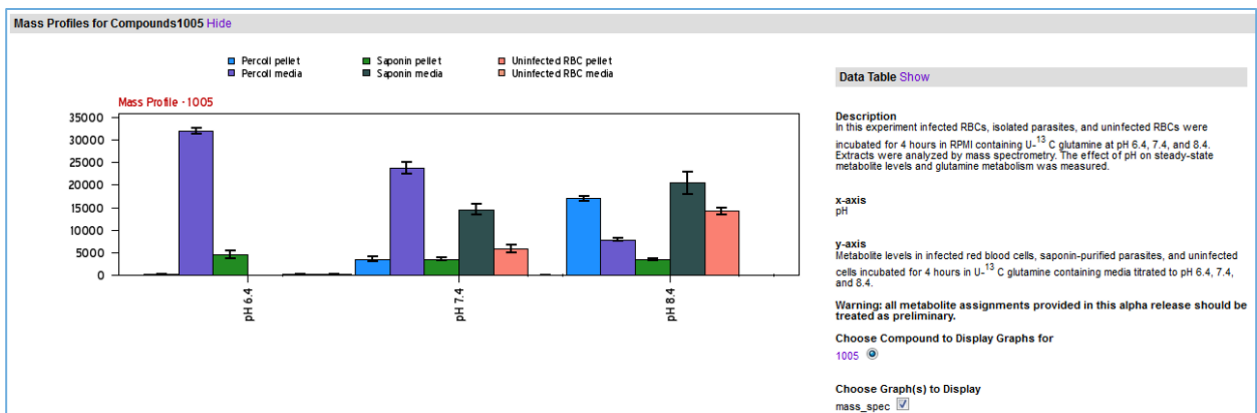
Fields Name, Property (InChI, IUPAC Name, SMILES, Molecular Weight)
 Synonym
 Substance Properties
 Reaction/Pathway/Enzyme
[select all](#) | [clear all](#)

Advanced Parameters

- 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 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).

Identify Other Data Types:

- Expand All | Collapse All
- Isolates
- Genomic Sequences
- Genomic Segments (DNA Motif)
- SNPs
- ESTs
- ORFs
- SAGE Tags
- Metabolic Pathways **BETA**
- Compounds **BETA**
- Compound ID **BETA**
- Text (synonym, InChI, etc.) **BETA**
- Enzymes **BETA**
- Metabolic Pathway **BETA**
- Molecular Formula **BETA**
- Molecular Weight **BETA**
- Metabolite Levels **BETA**

Identify Compounds based on Metabolite level

Experiment Effect of pH on metabolite levels (Lewis, Baska and Linas)

Reference Samples Percoll pH 7.4 pellet

Comparison Samples Saponin pH 7.4 pellet

Fold change >= 20

Direction up-regulated

Advanced Parameters

Get Answer

- 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?

My Strategies: New Opened (5) All (5) Basket Public Strategies (8) Help

(Compounds) **fold change** 12 Compounds Step 1 **Add Step**

Add Step

Run a new Search for
Add contents of Basket
Add existing Strategy
Transform to Genes
Transform to Pathways

Compounds

Compound ID **BETA**
Text (synonym, InChI, etc.) **BETA**
Enzymes **BETA**
Metabolic Pathway **BETA**
Molecular Formula **BETA**
Molecular Weight **BETA**
Metabolite levels **BETA**

Strategy: fold change *

Rename Duplicate Save As Share Delete

(Compounds) **fold change** 12 Compounds Step 1 **fold change** 2 Compounds Step 2 **Add Step**

Add Step 2 : Metabolite levels

Experiment Effect of pH on metabolite levels (Lewis, Baska and Linas)

Reference Samples Percoll pH 7.4 pellet

Comparison Samples Saponin pH 7.4 media

Fold change >= 20

Direction up-regulated

Advanced Parameters

Combine Compounds in Step 1 with Compounds in Step 2:

1 Intersect 2 1 Minus 2
 1 Union 2 2 Minus 1

Run Step

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

My Strategies: [New](#) [Opened \(5\)](#) [All \(5\)](#) [Basket](#) [Public Strategies \(8\)](#) [Help](#)

(Pathways) Strategy: Rename Duplicate Save As Share Delete

fold change 12 Compounds Step 1 → fold change 2 Compounds Step 2 → cpd->pthwy 15 Pathways Step 3

[Add Step](#)

15 Metabolic Pathways from Step 3 Strategy: *fold change* [Add 15 Metabolic Pathways to Basket](#) | [Download 15 Metabolic Pathways](#)

Metabolic Pathway Results

Advanced Paging [Add Columns](#)

Pathway Id	Pathway	Source	No. of Compounds	Total Pathway Enzymes	Total Pathway Compounds	Map - Painted With Transformed Compounds (new window)
ec00230	Purine metabolism	ec00230	2	174	100	Pathway Map
ec00030	Pentose phosphate pathway	ec00030	1	42	34	Pathway Map
ec00052	Galactose metabolism	ec00052	1	49	43	Pathway Map
ec00053	Ascorbate and aldarate metabolism	ec00053	1	55	45	Pathway Map
ec00270	Cysteine and methionine metabolism	ec00270	1	82	58	Pathway Map
ec00290	Valine, leucine and isoleucine biosynthesis	ec00290	1	31	27	Pathway Map