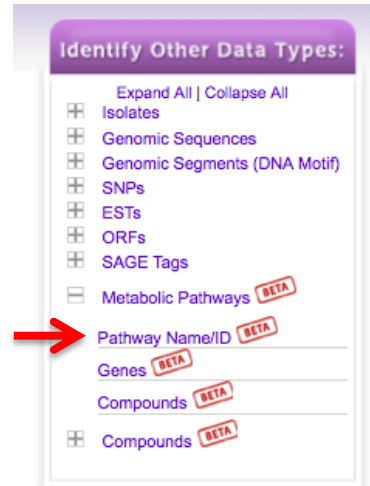


Exploring Metabolic Pathways and Compounds

Exercise 5

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

- Metabolic pathway and compound searches are available under the “Identify Other Data Types” heading on the home page. To find metabolic pathways by name, click on the “Pathway/Name/ID” option under the heading “Metabolic Pathways”.



- This search provides type-ahead options.

Identify Metabolic Pathways based on Pathway Name/ID BETA

Pathway Name or ID

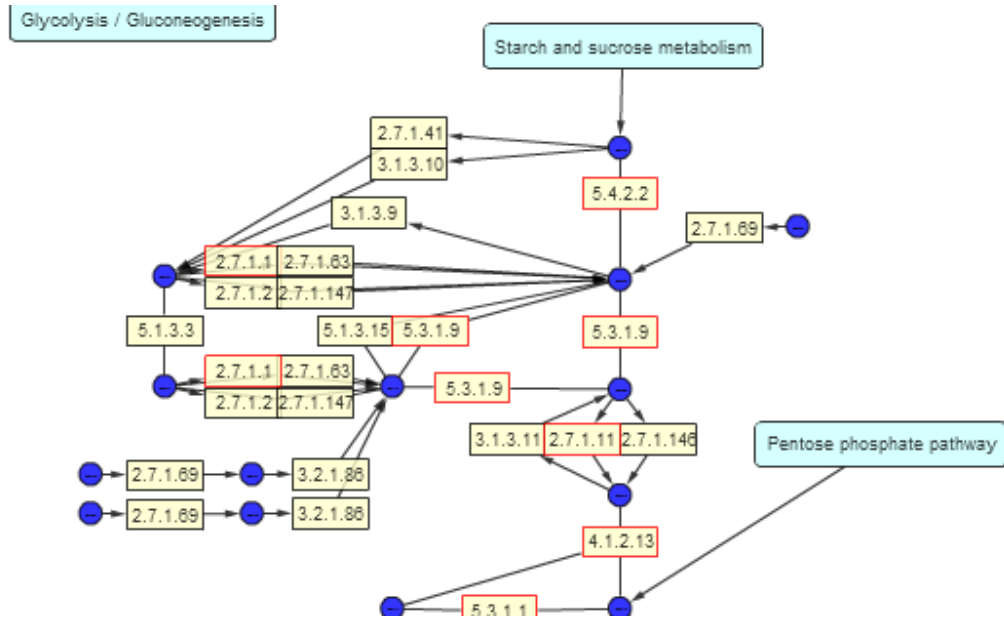
- Glycosaminoglycan biosynthesis - chondroitin sulfate (ec00532)
- Glycosphingolipid biosynthesis - globo series (ec00603)
- Glycosphingolipid biosynthesis - lacto and neolacto series (ec00601)
- Glycosylphosphatidylinositol(GPI)-anchor biosynthesis (ec00563)
- Glycosaminoglycan degradation (ec00531)
- Glycosphingolipid biosynthesis - ganglio series (ec00604)
- Glycosaminoglycan biosynthesis - keratan sulfate (ec00533)
- Glycosaminoglycan biosynthesis - heparan sulfate (ec00534)
- Glycolysis / Gluconeogenesis (ec00010)

Description
Find Pathways by Pathway Name.

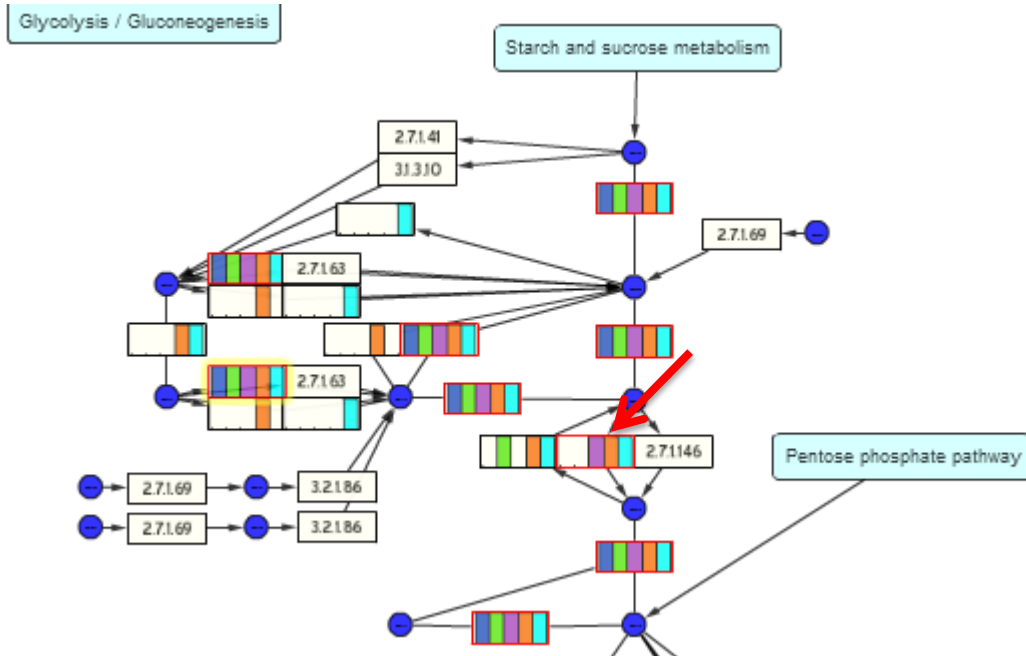
Data Sets used by this search

- KEGG Metabolic Pathways
Metabolic Pathways and associations to Compounds and EC Numbers

- Once you find glycolysis, the result page will display a graphical KEGG representation of the pathway. Examine the pathway – What do the rectangles with numbers like 2.7.1.41 represent? What do the circles represent?



- Turn on 'Paint Genera', 'Cryp, Toxo, Plas, Tryp, Host'. What do the colors mean? (Note that you can mouse over the various elements in the pathway to reveal popups with additional information).



- Find the rectangle representing 6-phosphofructokinase. (hint: its EC number is 2.7.1.11).
- Do you believe that this enzyme is only present in *P. falciparum*? What are some possibilities? How can you determine if this enzyme has orthologs in other *Plasmodium* species?
- Click on enzyme name/EC no taking you to a EuPathDB strategy. You get 10 genes but this is not necessarily all the orthologs identified by OrthoMCL. How can find orthologs of this gene in other *Plasmodium falciparum*. How many in other species?

(Genes)

EC Number
10 Genes
Step 1

Add Step

10 Genes from Step 1
Strategy: EC Number(2)

Click on a number in this table to limit/filter your results

All Results	Ortholog Groups	Plasmodium									
		<i>P.berghei</i> ANKA	<i>P.chabaudi</i> chabaudi	<i>P.cynomolgi</i> strain B	<i>P.falciparum</i> (nr Genes: 2)		<i>P.gallinaceum</i> 8A	<i>P.knowlesi</i> strain H	<i>P.reichenowii</i> Dennis	<i>P.vivax</i> Sal-1	<i>P.y...</i> yoelii 17)
10	2	1	1	0	2	1	0	1	0	1	0

- Orthologs can be identified by add an “ortholog transform” step to the search strategy. (hint: click on add step, then select ortholog transform from the popup window. In this case allow all the organism).

(Genes)

EC Number
10 Genes
Step 1

Orthologs
20 Genes
Step 2

Add Step

20 Genes from Step 2
Strategy: EC Number(3)

Click on a number in this table to limit/filter your results

All Results	Ortholog Groups	Plasmodium												
		<i>P.berghei</i> ANKA	<i>P.chabaudi</i> chabaudi	<i>P.cynomolgi</i> strain B	<i>P.falciparum</i> (nr Genes: 2)		<i>P.gallinaceum</i> 8A	<i>P.knowlesi</i> strain H	<i>P.reichenowii</i> Dennis	<i>P.vivax</i> Sal-1	<i>P.yoelii</i> (nr Genes: 2)			
20	2	2	2	2	2	2	0	2	0	2	2	2	2	

Gene Results Genome View

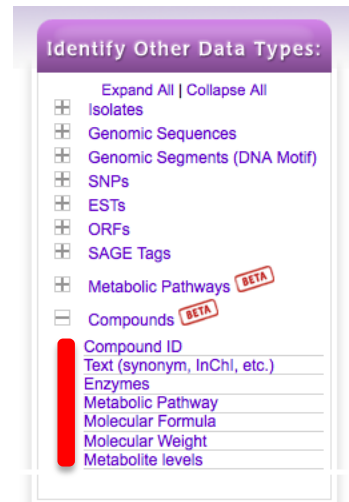
Advanced Paging

- What do your results show? Is 6-phosphofructokinase unique to *P. falciparum*?

- Compound records can be accessed by running a specific compound search available under “Identify Other Data Types” heading on the home page. Compound records can also be accessed from the mouse over popups in a metabolic pathway.

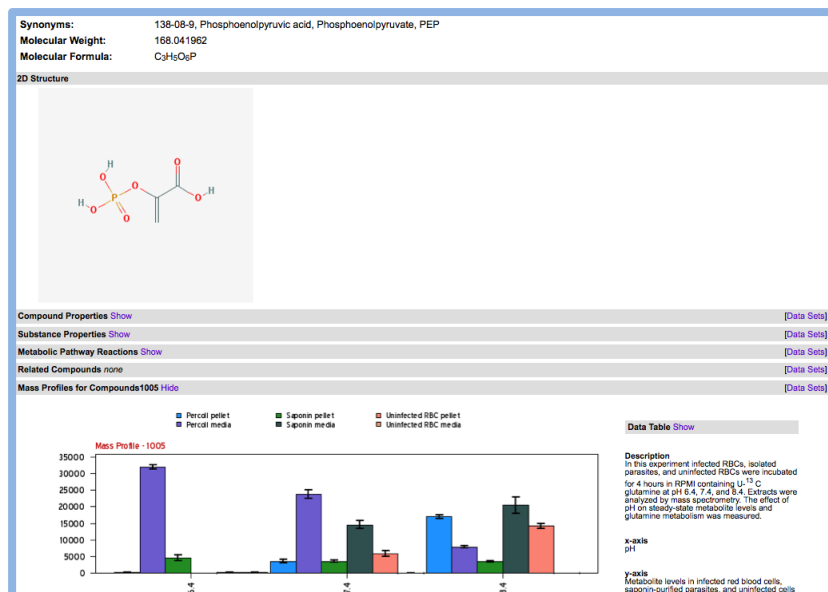
- Find Phosphoenolpyruvate (PEP) and visit its record page.

- o PEP can be identified using a specific compound search. For example, compounds may be identified by ID, text search, metabolic pathway, Molecular formula, molecular weight and metabolite levels.



- o Choose one of these options to identify PEP. For example, you can type phosphoenolpyruvate in the compound text search:

- Examine the PEP record page. Note that sections (ie. Metabolic Pathway Reactions) may be expanded by clicking on the “show” link.



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 media.

- This requires running a metabolite levels search (20-fold enriched in saponin pellet compared to the percoll media 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

Text (synonym, InChI, etc.)

Enzymes

Metabolic Pathway

Molecular Formula

Molecular Weight

Metabolite levels ←

Identify Compounds based on Metabolite levels BETA

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

Reference Samples ? Percoll pH 7.4 media

Comparison Samples ? Saponin pH 7.4 pellet

Fold change >=

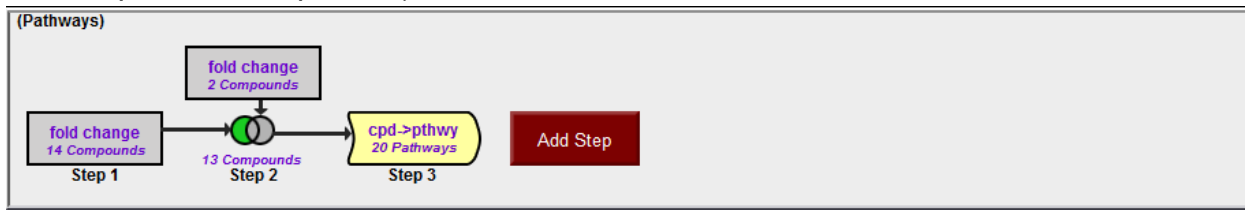
Direction ? up or down regulated

- How many compounds did you get?
- How many of these metabolites are not enriched (by 20-fold) in pH7.4 saponin media fraction compared to the Percoll media fraction? (hint: this will require adding a second step and using a subtraction operation).

The image shows a workflow interface for identifying compounds. It consists of three main parts:

- Top Left:** A window titled "(Compounds)" showing a box for "fold change 14 Compounds Step 1" and a red "Add Step" button.
- Top Right:** An "Add Step" dialog box with a list of data types. "Metabolite levels" is selected and highlighted with a red arrow.
- Bottom Left:** The main workflow window now shows two steps: "fold change 14 Compounds Step 1" and "fold change 2 Compounds Step 2". A red arrow points from the "Add Step" button to the "Step 2" box.
- Bottom Right:** A "Revise Step 2 : Metabolite levels" dialog box. It contains the same search parameters as the top window. Below the parameters, there are options for combining results: "Intersect 2", "Union 2", "Minus 1", and "Minus 2". The "Minus 2" option is selected, indicating a subtraction operation. A red arrow points from the "Metabolite levels" selection in the "Add Step" dialog to this dialog.

- To which metabolic pathways do these compounds belong? (hint: click on add step and transform the results to metabolic pathways, any of the specified compounds).



20 Metabolic Pathways from Step 3

Strategy: *fold change(4)*

Metabolic Pathway Results

Advanced Paging

Pathway Id	Pathway	Source	No. of Compounds	Total Pathway Enzymes	T
ec00240	Pyrimidine metabolism	ec00240	3	107	66
ec00230	Purine metabolism	ec00230	2	174	100
ec00480	Glutathione metabolism	ec00480	2	61	42
ec00520	Amino sugar and nucleotide sugar metabolism	ec00520	2	120	94
ec00680	Methane metabolism	ec00680	2	97	94
ec00760	Nicotinate and nicotinamide metabolism	ec00760	2	56	39
ec00908	Zeatin biosynthesis	ec00908	2	10	30
ec00040	Pentose and glucuronate interconversions	ec00040	1	65	53