

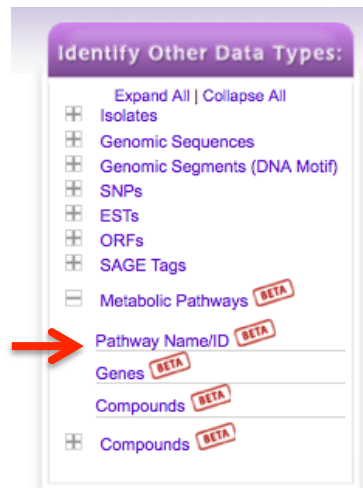
Metabolic Pathways and Compounds

Exercise 8

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

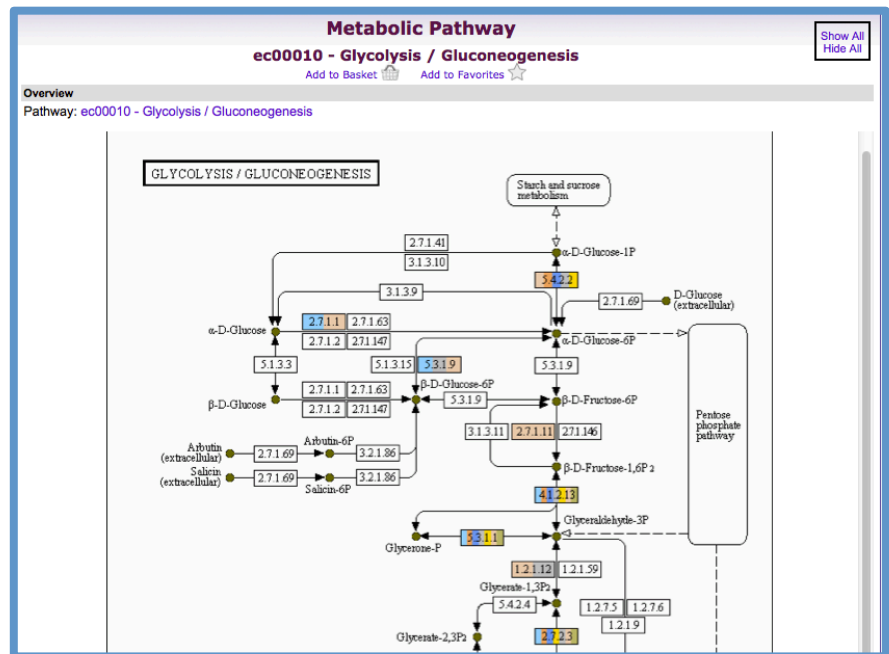
Find Pathways by Pathway Name.

Data Sets used by this search

- [KEGG Metabolic Pathways](#)

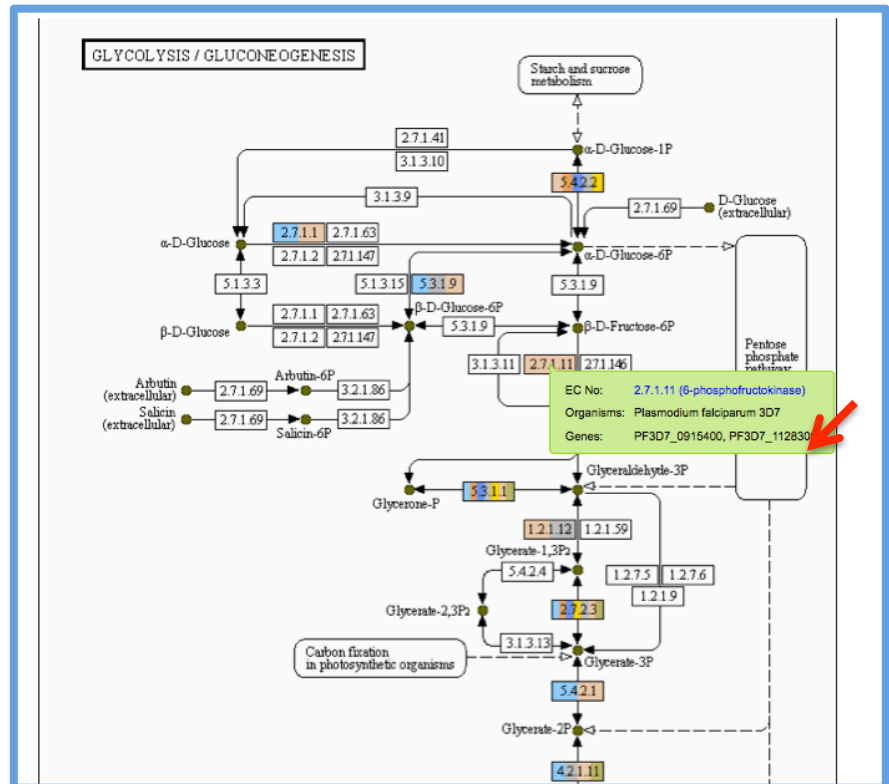
Metabolic Pathways and associations to Compounds and EC Numbers

- 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 2.7.1.11 represent? What do the circles represent? What do the colors mean? (Note that you can mouse over the various elements in the pathway to reveal popups with additional information).



Plasmodium vivax SaI-1	Plasmodium chabaudi chabaudi
Plasmodium yoelii yoelii 17XNL	Plasmodium falciparum IT
Plasmodium berghei ANKA	Plasmodium yoelii yoelii YM
Plasmodium knowlesi strain H	Plasmodium cynomolgi strain B
Plasmodium falciparum 3D7	

- 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? (hint: you can click on the enzyme name in the popup).

- Once you click on the enzyme name/EC no. What results did you get? How can find orthologs of this gene in other *Plasmodium* species?

My Strategies: New Opened (1) All (2) Basket Examples Help

(Genes) Strategy: EC Number(2) * [Rename](#) [Duplicate](#) [Save As](#) [Share](#) [Delete](#)

EC Number 2 Genes Step 1 [Add Step](#)

2 Genes from Step 1 Strategy: EC Number(2) [Add 2 Genes to Basket](#) | [Download 2 Genes](#)

Filter results by species (results removed by the filter will not be combined into the next step.)

All Results	Ortholog Groups	Plasmodium falciparum		Plasmodium vivax	Plasmodium yoelii			Plasmodium berghei	Plasmodium chabaudi	Plasmodium knowlesi	Plasmodium cynomolgi
		Distinct genes	3D7 IT		Distinct genes	yoelii 17XNL	yoelii YM				
2	2	2	2	0	0	0	0	0	0	0	0

Gene Results Genome View

Advanced Paging [Add Columns](#)

Gene ID	Genomic Location	Product Description
PF3D7_0915400	PF3D7_09_v3: 650,585 - 654,841 (-)	6-phosphofructokinase (PFK9)
PF3D7_1128300	PF3D7_11_v3: 1,098,167 - 1,103,555 (-)	6-phosphofructokinase (PFK11)

Advanced Paging

- 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. Next select the organisms you want to transform to and click on get answer).

My Strategies: New Opened (1) All (2) Basket Examples Help

(Genes) Strategy: EC Number(2) * [Rename](#) [Duplicate](#) [Save As](#) [Share](#) [Delete](#)

EC Number 2 Genes Step 1 [Orthologs](#) 18 Genes Step 2 [Add Step](#)

18 Genes from Step 2 Strategy: EC Number(2) [Add 18 Genes to Basket](#) | [Download 18 Genes](#)

Filter results by species (results removed by the filter will not be combined into the next step.)

All Results	Ortholog Groups	Plasmodium falciparum		Plasmodium vivax	Plasmodium yoelii			Plasmodium berghei	Plasmodium chabaudi	Plasmodium knowlesi	Plasmodium cynomolgi
		Distinct genes	3D7 IT		Distinct genes	yoelii 17XNL	yoelii YM				
18	2	2	2	2	2	2	2	2	2	2	

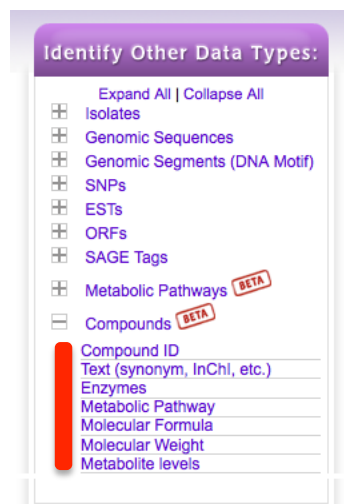
Gene Results Genome View

Advanced Paging [Add Columns](#)

Gene ID	Organism	Genomic Location	Product Description	Input Ortholog(s)	Ortholog Group	Paralog count	Ortholog count
PBANKA_081640	P. berghei ANKA	berg08: 663,800 - 667,699 (-)	6-phosphofructokinase, putative (PFK9)	PF3D7_0915400	OG5_131114	0	8
PCHAS_081670	P. chabaudi chabaudi	chab08: 663,865 - 667,585 (-)	6-phosphofructokinase, putative (PFK9)	PF3D7_0915400	OG5_131114	0	8
PCYB_072300	P. cynomolgi strain B	DF157099: 609,997 - 614,316 (-)	6-phosphofructokinase	PF3D7_0915400	OG5_131114	0	8
PF3D7_0915400	P. falciparum 3D7	PF3D7_09_v3: 650,585 - 654,841 (-)	6-phosphofructokinase (PFK9)	PF3D7_0915400	OG5_131114	0	8
PFIT_0915500	P. falciparum IT	PfIT_09_v2: 631,981 - 636,234 (-)	6-phosphofructokinase	PF3D7_0915400	OG5_131114	0	8
PKH_071270	P. knowlesi strain H	Pk_strainH_chr07: 623,627 - 627,880 (-)	6-phosphofructokinase, putative (PFK9)	PF3D7_0915400	OG5_131114	0	8
PVX_099200	P. vivax Sal-1	PviS_CM000448: 580,105 - 584,765 (-)	6-phosphofructokinase, putative	PF3D7_0915400	OG5_131114	0	8
PY01321	P. yoelii yoelii 17XNL	AABL01000347: 3,716 - 7,645 (-)	pyrophosphate-dependent	PF3D7_0915400	OG5_131114	0	8

- What do your results show? Is 6-phosphofructokinase unique to *P. falciparum*?
- 2. 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.

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



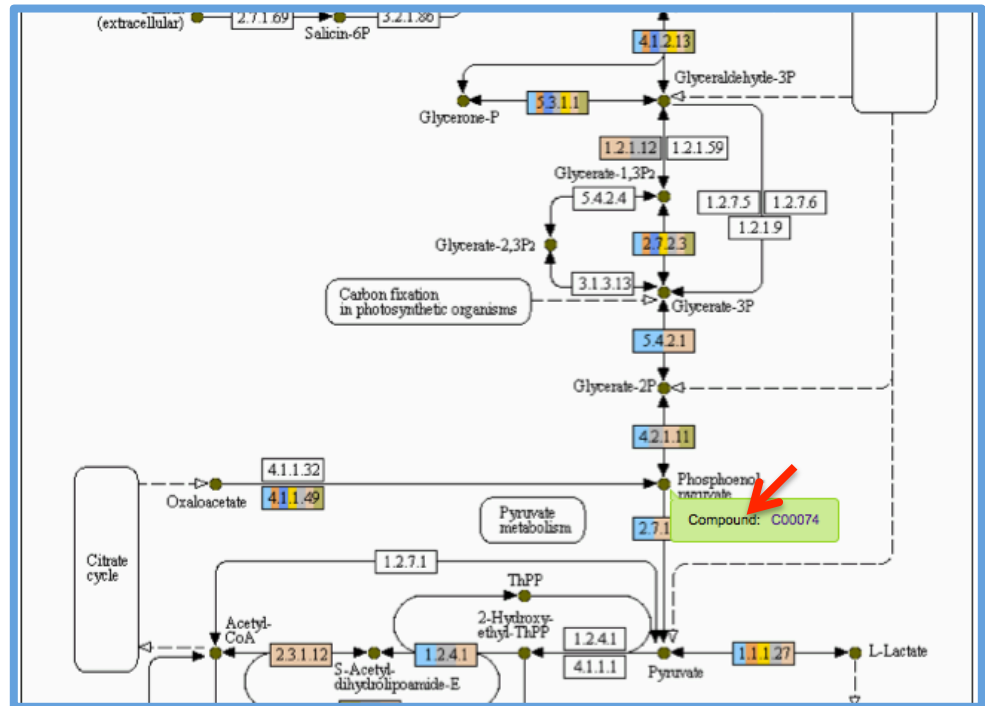
Identify Compounds based on Text (synonym, InChI, etc.) BETA

Text term (use * as wildcard)

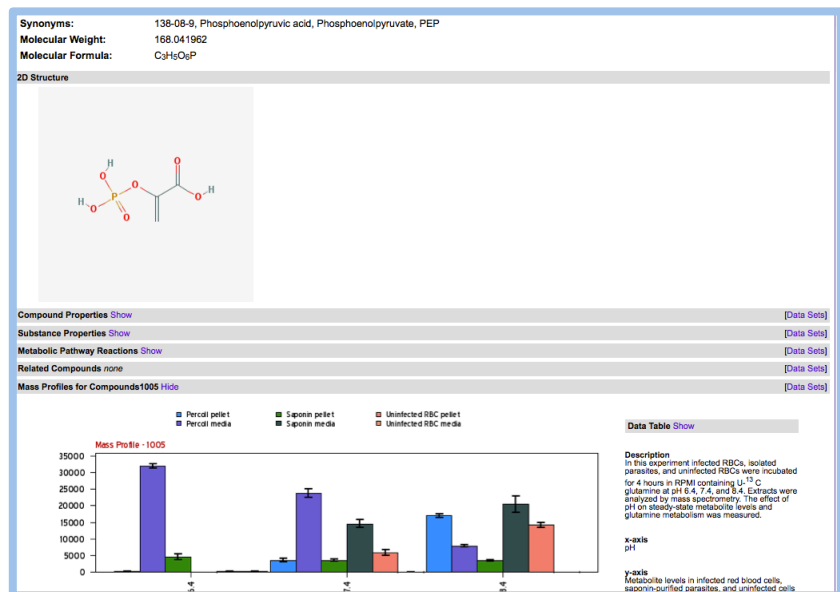
Fields ☐ Property (IUPAC Name, InChI, SMILES, Mass) ☒ Synonym ☐ Reaction/Pathway/Enzyme

[select all](#) | [clear all](#)

- PEP can be found in a metabolic pathway where it is present as a substrate or a product of an enzymatic reaction (ie. glycolysis). (hint: click on the compound ID in the popup).

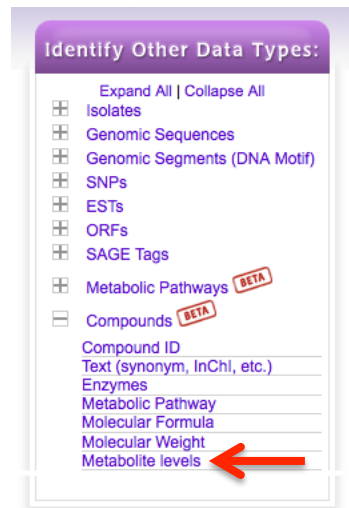


- Examine the PEP record page. Note that sections (ie. Metabolic Pathway Reactions) may be expanded by clicking on the “show” link.
- Under which conditions is PEP most highly? (hint: examine the “Mass Profiles for Compounds” section).



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 Compounds based on Metabolite levels **BETA**

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

Reference Samples ?

Comparison Samples ?

Fold change >= ?

Advanced Parameters

- 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 screenshot shows the 'Add Step' dialog box with 'Metabolite levels' selected. Below it, the 'Combine Compounds in Step 1 with Compounds in Step 2' section shows '1 Minus 2' selected. The main window shows 'Step 1' with 'fold change' and '12 Compounds', and 'Step 2' with 'fold change' and '2 Compounds'.

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

The screenshot shows the 'Add Step' dialog box with 'Metabolic Pathways' selected. Below it, the '15 Metabolic Pathways from Step 3' section shows a table of pathways.

Pathway Id	Pathway	Source	No. of Compounds	Total Pathway Enzymes	Total Pathway Compounds	Map - Painted With Transformed Compounds (new window)
ec00230	Purine metabolism	Metabolic Pathways - KEGG	2	106	92	Pathway Map
ec00030	Pentose phosphate pathway	Metabolic Pathways - KEGG	1	37	32	Pathway Map
ec00052	Galactose metabolism	Metabolic Pathways - KEGG	1	37	41	Pathway Map
ec00053	Ascorbate and aldarate metabolism	Metabolic Pathways - KEGG	1	45	45	Pathway Map
ec00270	Cysteine and methionine metabolism	Metabolic Pathways - KEGG	1	64	56	Pathway Map
ec00290	Valine, leucine and isoleucine biosynthesis	Metabolic Pathways - KEGG	1	18	27	Pathway Map
ec00480	Glutathione metabolism	Metabolic Pathways - KEGG	1	40	38	Pathway Map