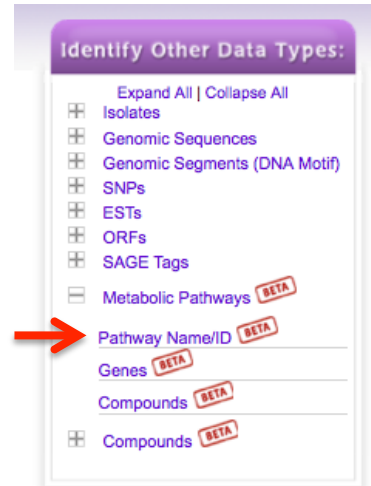


Exploring Metabolic Pathways and Compounds

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

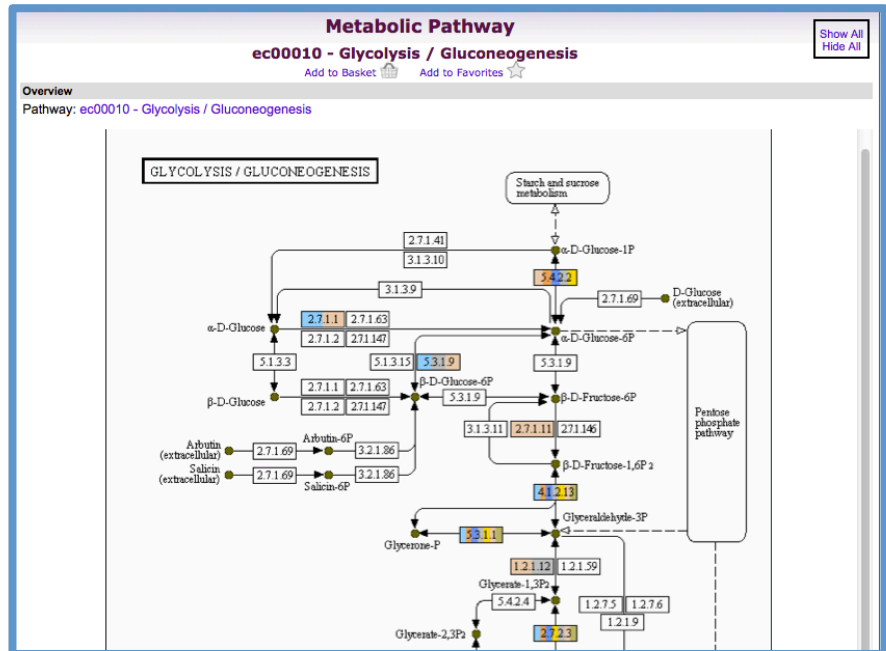
- Glycosaminoglycan biosynthesis - chondroitin sulfate (ec00532)
- Glycosphingolipid biosynthesis - globo series (ec00603)
- Glycosphingolipid biosynthesis - lacto and neolacto series (ec00601)
- Glycosylphosphatidylinositol(GPI)-anchor biosynthesis (ec00563)
- Glycosaminoglycan degradation (ec00531)
- 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

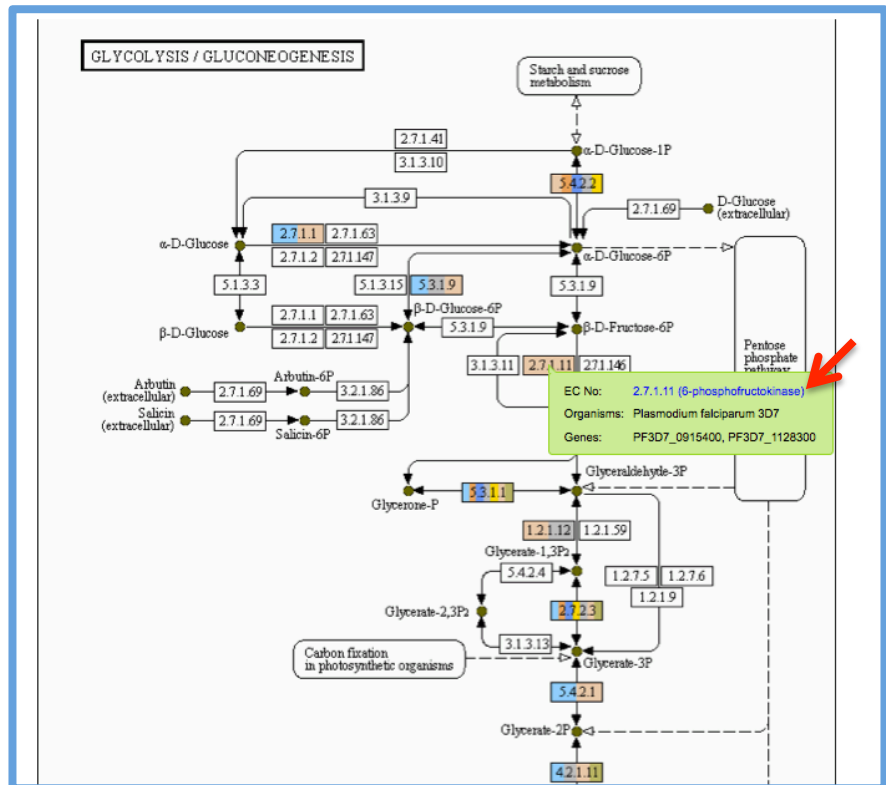
- Once you find glycolysis, the result page will display a graphical KEGG representation of the pathway. Examine the pathway – What do the rectangles with numbers like 2.7.1.11 represent? What do the circles represent? What do the colors mean? (Note that you can mouse over the various elements in the pathway to reveal popups with additional information).



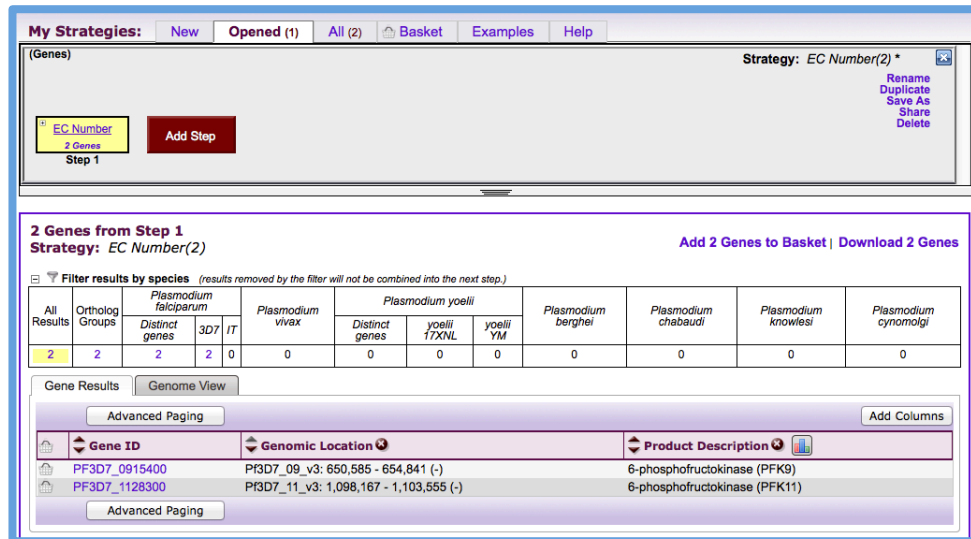
LEGEND

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-phosphofruktokinase (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) *
 EC Number 2 Genes Step 1 Add Step
 Rename Duplicate Save As Share Delete

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.)

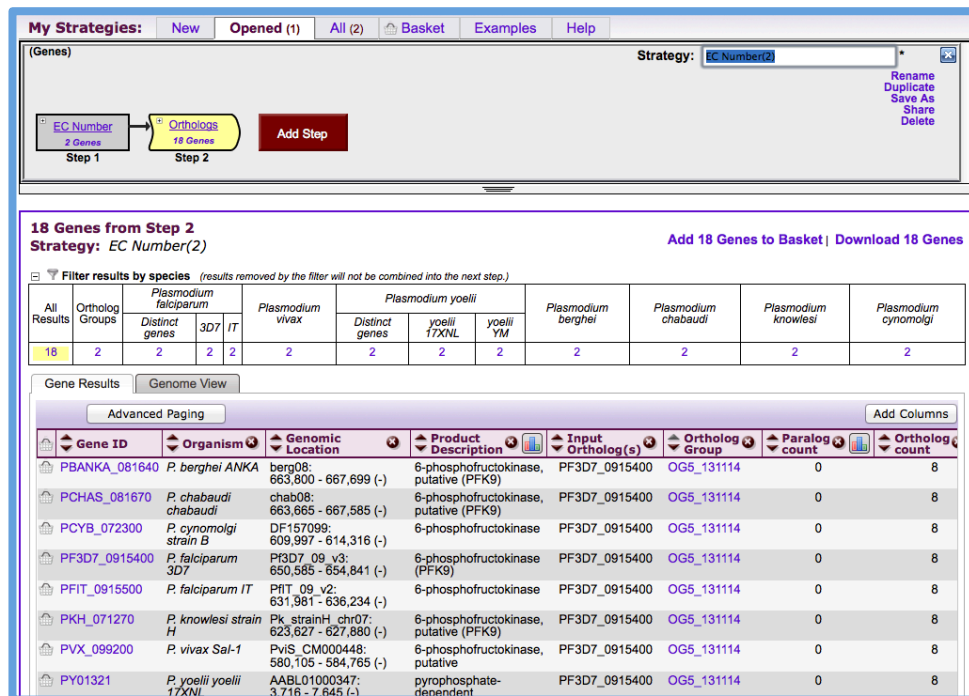
All Results	Ortholog Groups	Plasmodium falciparum			Plasmodium vivax	Plasmodium yoelii			Plasmodium berghei	Plasmodium chabaudi	Plasmodium knowlesi	Plasmodium cynomolgi
		Distinct genes	3D7	IT		Distinct genes	yoelii 17XNL	yoelii YM				
2	2	2	2	0	0	0	0	0	0	0	0	0

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 Add Columns

- 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) *
 EC Number 2 Genes Step 1 Orthologs 18 Genes Step 2 Add Step
 Rename Duplicate Save As Share Delete

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	2	2

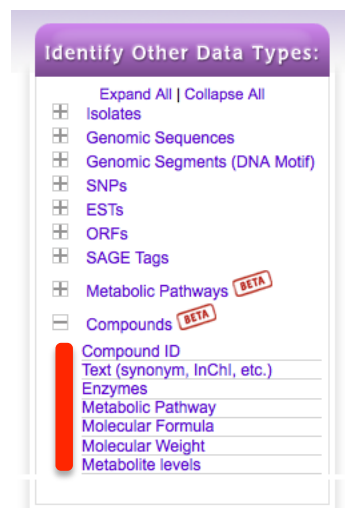
Gene Results Genome View
 Advanced Paging Add Columns

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

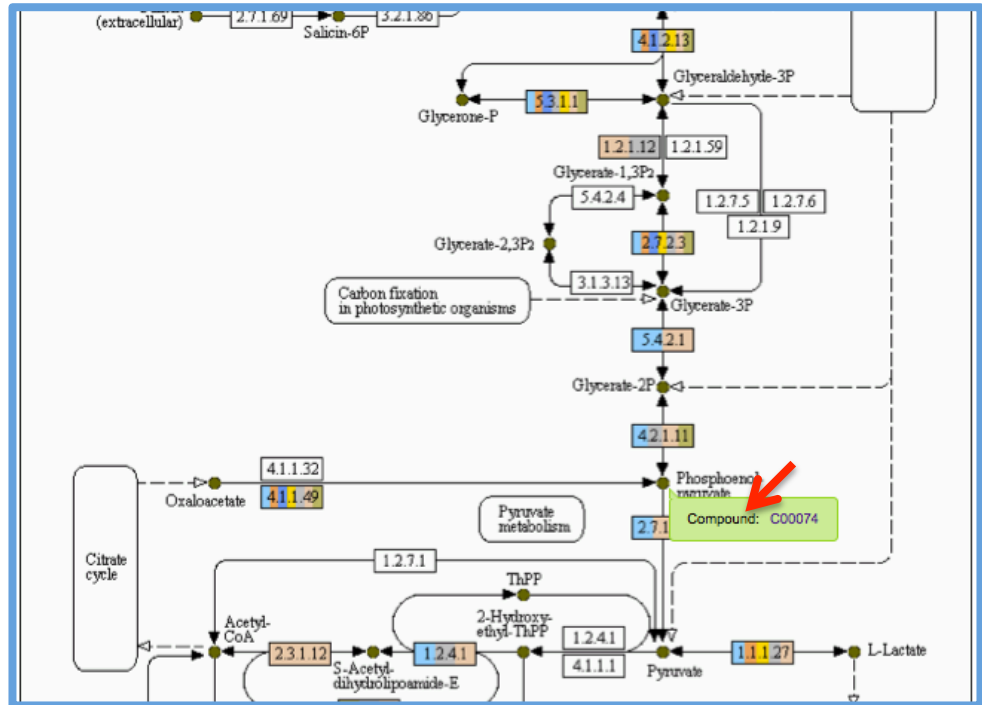
2. Compound records can be accessed by running a specific compound search available under “Identify Other Data Types” heading on the home page. Compound records can also be accessed from the mouse over popups in a metabolic pathway.
- Find Phosphoenolpyruvate (PEP) and visit its record page.

- 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:



The image shows a search form titled "Identify Compounds based on Text (synonym, InChI, etc.)" with a red "BETA" badge. The form includes a text input field with the value "phosphoenolpyruvate" and a help icon. Below the input field, there is a "Fields" section with three checkboxes: "Property (IUPAC Name, InChI, SMILES, Mass)", "Synonym" (checked), and "Reaction/Pathway/Enzyme". There are also links for "select all" and "clear all". At the bottom of the form, there is an "Advanced Parameters" section and a "Get Answer" button.

- 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).

Synonyms: 138-08-9, Phosphoenolpyruvic acid, Phosphoenolpyruvate, PEP
Molecular Weight: 168.041962
Molecular Formula: C₃H₅O₆P

2D Structure

Compound Properties Show [Data Sets]
Substance Properties Show [Data Sets]
Metabolic Pathway Reactions Show [Data Sets]
Related Compounds none [Data Sets]
Mass Profiles for Compounds1005 Hide [Data Sets]

Mass Profile - 1005

pH	Percal pellet	Percal media	Saponin pellet	Saponin media	Uninfected RBC pellet	Uninfected RBC media
6.4	~30000	~5000	~2000	~1000	~15000	~5000
7.4	~25000	~10000	~15000	~10000	~18000	~8000
8.4	~20000	~12000	~22000	~15000	~18000	~12000

Description
 In this experiment infected RBCs, isolated parasites, and uninfected RBCs were incubated for 4 hours in RPMI containing U-¹³C glutamine at pH 6.4, 7.4, and 8.4. Extracts were analyzed by mass spectrometry. The effect of pH on steady-state metabolite levels and glutamine metabolism was measured.

x-axis
pH

y-axis
Metabolite levels in infected red blood cells, saponin-cultured parasites, and uninfected cells

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
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 **?**

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 for 'Metabolite levels'. The 'Experiment' is set to 'Effect of pH on metabolite levels (Lewis, Baska and Linas)'. The 'Reference Samples' is 'Percoll pH 7.4 media' and the 'Comparison Samples' is 'Saponin pH 7.4 media'. The 'Fold change >=' is set to 20. The 'Combine Compounds in Step 1 with Compounds in Step 2' section shows the '1 Minus 2' option selected. Below the dialog, a workflow diagram shows 'Step 1' (fold change, 12 Compounds) leading to 'Step 2' (fold change, 2 Compounds) via a subtraction operation, resulting in 11 Compounds. A red arrow points from the 'Add Step' button in the workflow to the 'Add Step' dialog.

- 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 for 'Metabolic Pathways'. The 'Strategy' is 'fold change(2)'. The workflow diagram shows 'Step 1' (fold change, 12 Compounds) leading to 'Step 2' (fold change, 11 Compounds) via a subtraction operation, which then leads to 'Step 3' (Metabolic Pathways, 15 Pathways) via a transformation operation. Below the dialog, a table titled '15 Metabolic Pathways from Step 3' is displayed. The table has columns for Pathway Id, Pathway, Source, No. of Compounds, Total Pathway Enzymes, Total Pathway Compounds, and Map - Painted With Transformed Compounds (new window).

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