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Pathway Tools “How-to” Guides

How to overlay proteomics data for a single pathway using Pathway tools

This guide provides step-by-step instructions using Pathway Tools to overlay proteomic expression data on a **known pathway** of interest (example shown in Figure 1).

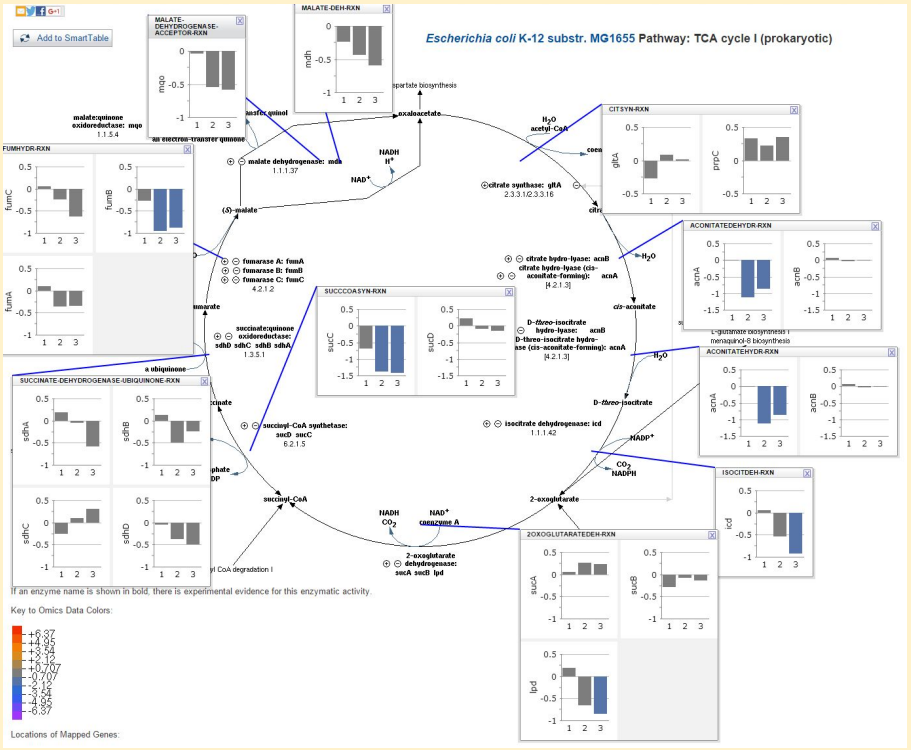


Figure 1 Overlay of experimental transcriptomic data on Metabolic Pathways. A close up of the TCA cycle in E.coli K-12, *with expression levels of constituent proteins shown in bar graphs across three experiments.*

This tutorial is for Pathway Tools version 19.5.

1 Introduction

Pathway Tools is a software suite from BioCyc database collection. Amongst its many functionalities (see http://bioinformatics.ai.sri.com/ptools/) , it supports the visual analysis of gene or protein expression and metabolomics datasets**,** such as overlaying omics data (eg transcriptomic expression values) onto diagrams of an organism's metabolic network, as shown in Figure 1.

By default, Pathway Tools contains four organism databases: (1) Homo sapiens, (2) Mus musculus, (3) *E.coli K-12* and (4) MetaCyc.

Pathway Tools also allow users to create their own organism-specific Pathway/Genome Database (PGDB) using the Pathologic tool. Creation of a customised database is covered in the online tutorial module "Pathway prediction and Annotations for new organisms".

2 Aim

This how-to guide will provide step-by-step instructions on how to use the overlay function of Pathway Tools using the web-browser.

This guide uses the metabolic pathways from the model organism *Escherichia coli K-12* and the data from the published study Varas *et al*, 2017, *Multi-level evaluation of Escherichia coli polyphosphate related mutants using global transcriptomic, proteomic and phenomic analyses***,** Biochimica et Biophysica Acta 1861:871-883. In this study, the expression of all genes and proteins of *E.coli K-12* was measured using microarray and LC-ESI-MS/MS methods. The expressed were measured across three mutant strains (*Δ ppk1, Δ ppx, and Δ polyP),* which lack enzymes related to inorganic polyphosphate metabolism.

2 Data

The proteomic data files are sourced from the Appendix section of the paper:

- Appendix B-1: Raw data from q-proteomic experiments (Δ ppk mutant).
- Appendix B-2: Raw data from q-proteomic experiments (Δ ppx mutant).
- Appendix B-3: Raw data from q-proteomic experiments (Δ ppk- Δ ppx mutant).

Pathway Tools simply takes in any data with 2 or more columns, where the first column contains a protein accession number and subsequent columns are the expression level values for different experimental conditions.

We have already performed the data transformation required to match the format required by pathway tools. The input file for this guide is **"all_proteomic_FDR_modified.txt"**. Box 3.1, below, describes the steps used for transforming and concatenating the datasets into one file.

3.1 Data transformation

As noted above, Pathway Tools takes in data with 2 or more columns, where the first column contains a protein accession number and subsequent columns are the expression values. Here we describe how the data transformation was done for this guide.

The proteomics data in study was generated by a LC-ESI-MS/MS approach (q-proteomic (Isotope-Coded Protein Labeling, ICPL) on a Ultimate 3000 nanoHPLC. Subsequent MSMS spectra was searched against a database using Mascot. Only proteins identified and quantified with at least two peptides were considered. Ratios were log2-transformed and normalised by subtracting the median value.

The proteomic data files used in this example have been sourced from corresponding entries in the appendix section of the paper. Only the columns "Accession" and "Avg" on the "FDR" tab in the excel spreadsheet was extracted and log2-transformed for this tutorial. The modified data was concatenated into a new file named **"all_proteomic_FDR_modified.txt"** with the following four columns:

1. protein accession number
2. log2(wt_ *Δppk1*) – relative expression level of wild type vs ppk1 mutant
3. log2(wt_ *Δppx*) - relative expression level of wild type vs ppx mutant
4. log2(wt_ *ΔpolyP*) – relative expression level of wild type vs (ppk1 + ppx) mutant

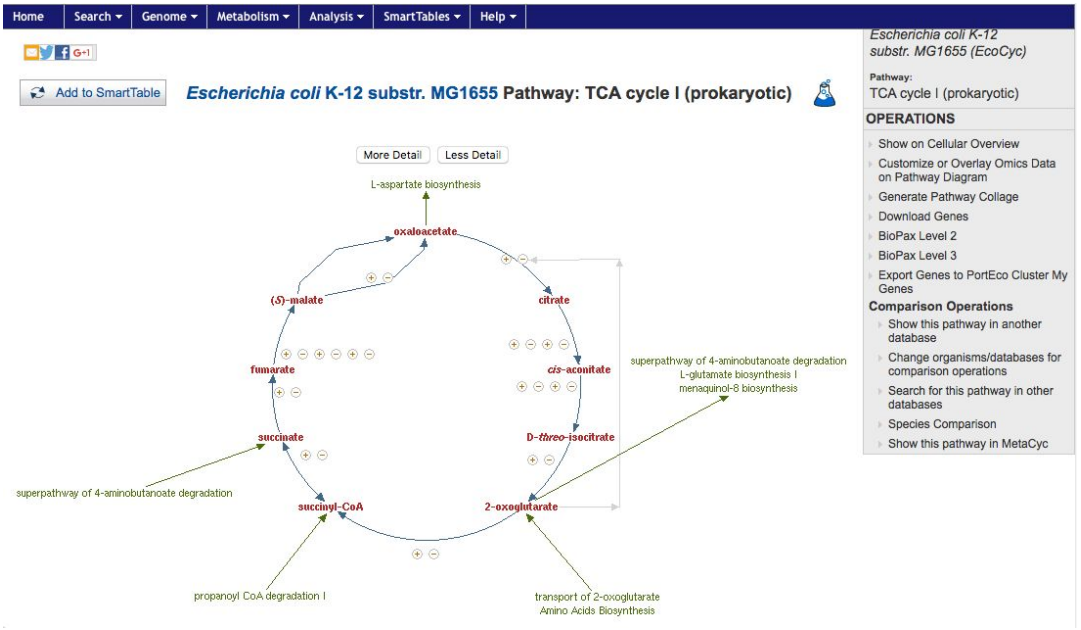
This file **"all_proteomic_FDR_modified.txt"** is used as input for this guide.

3 Step-by-step guide

Step	Instruction	Note(s)
1	Go to http://abrpi.genome.edu.au:9655/	Note: this is a private hosted instance of Pathway Tools that is part of the larger OMICs platform (http://omics.data.edu.au/use/).
2	Select **"Escherichia coli K-12 substr. MG1655 (EcoCyc)"** as the organism database by clicking the **"change organism database"** under the search column at the top right corner of the webpage.	The E.coli K12 MG1655 database contains the gene identifier and metabolic pathway information for E.coli Strain K12. This has been prepared previously by EcoCyc Staff.
3	Click on **"Pathways"** (in table #3)	Navigation to and selection of the (prokaryotic) TCA cycle pathway.
4	Click on **"Generation of Precursor Metabolites and Energy (42 instances)"**	
5	Click on **"TCA cycle(2)"**	

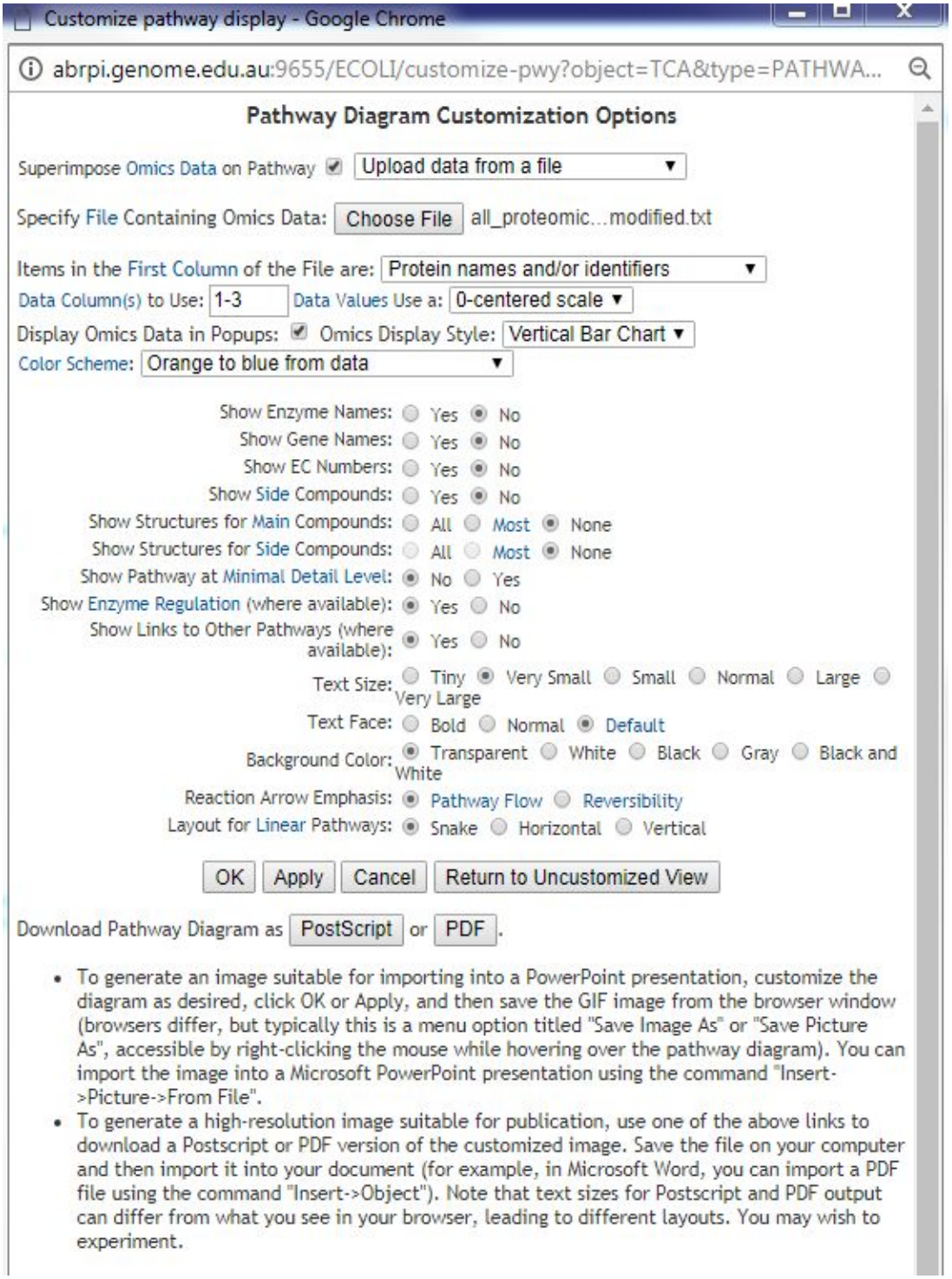
6	Click on **"TCA cycle I (prokaryotic)"**	

The TCA cycle will be loaded:



7	In the Operations Panel, click on **"Customize or Overlay Omics Data on Pathway Diagram"**	This option is found in the grey menu on the right hand side of the window.

A new window will appear:



The following are **mandatory** settings:

8	**Superimpose Omics Data on Pathway:** check this box	
9	**Specify File Containing Omics Data:** select the file containing the protein accession number and expression data	In this example, use the **all_proteomic_FDR_modified.txt** file. Navigate to a local copy and upload it.

10	**Items in the First Column of the File are**: Protein names and/or identifiers	The first column (column "0") of this file contain protein accession number whereas columns 1-3 contain expression levels of the 3 mutants relative to wildtype E.coli K12.
11	**Data Column(s) to Use**: 1-3	
12	**Data Values Use a**: 0-centered scale	Implies that the numerical data of your file can contain positive and negative values. The value 0 is considered to be the centre of the numerical values provided in your data file.
13	**Display Omics Data in Popups**: checked	
14	**Omics Display style**: Vertical Bar Chart	
15	Leave all other fields as their default settings.	
16	Click on **Apply**	

When the file has been uploaded, you should see something similar this Figure 2:

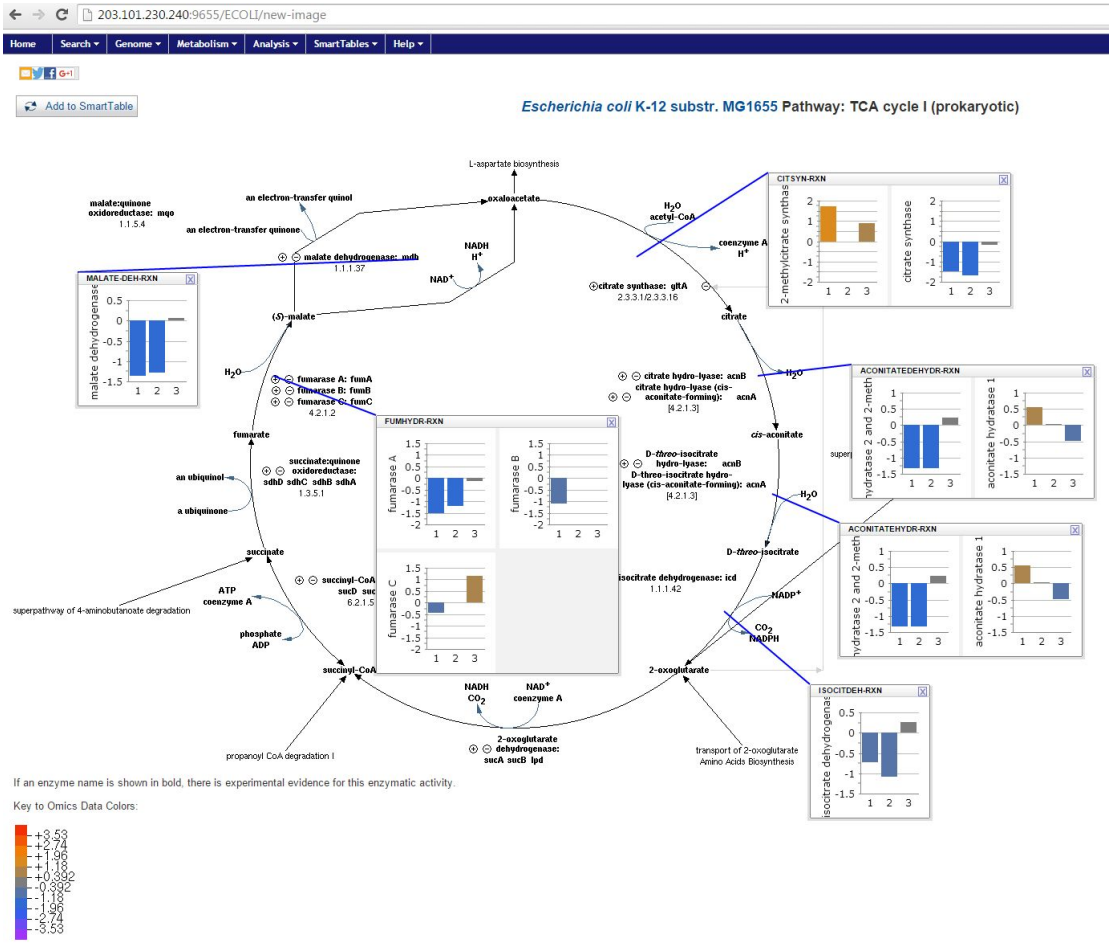
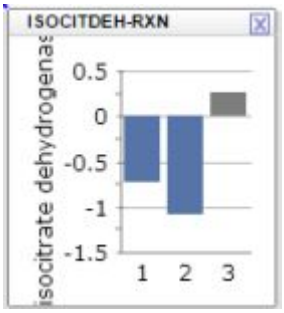


Figure 2 TCA cycle I (prokaryotic) with expression barplot.

Figure 2 shows the overlay of the proteomic data (all_proteomic_FDR_modified.txt) on the TCA cycle I pathway is shown. Each bar plot shows the expression value on the log2 scale (as was provided by teh input dataset) for the corresponding gene(s). Each vertical bar is the expression of the protein from the different experimental condition. For example, ISOCITDEH-RXN reaction.

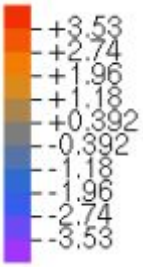


- Column one: wild type vs ppk1 mutant
- Column two: wild type vs ppx mutant
- Column three: wild type vs ppk1 + ppx mutant

The number of bars that are visible depends on the number of columns in the data file being uploaded.

You can also change which columns in the data to show by modifying the "**Data Column(s) to Use**" field in step 11.

The colour of the bars represents the expression range as shown by the colour legend at the bottom. Grey bars indicate values in the range between +0.392and -0.392 and blue bars indicate values in the range between -1.18 and -2.74.



If you need further assistance please contact us at omicsdataservices@lists.unimelb.edu.au