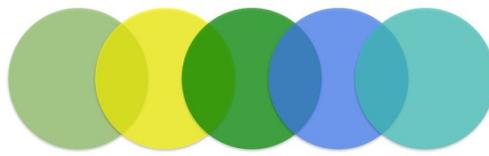# How to overlay comparative gene or protein expression data on a metabolic pathway

STEP	Task
1	Go to http://omics.data.edu.au/use/
2	Launch the platform and log in
4	Add the "ABPRI-Data" and "ABPRI-Pathway Tools" apps to the toolbar.
5	Open the "ABPRI-Data" app.
6	Log-into the "ABPRI-Data" app using the username and password given to you (you will need to enter **omics** as the domain).

Steps 7-10 are to locate a file containing differential gene expression level data for a number of different E. coli mutants.

Note the data used for this testing exercise is not from the ABPRI consortium but has been obtained from GEO and is related to the following 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 the study, transcriptomic and proteomic levels were analysed in three E. coli K12 mutants (*Δppk1*, *Δppx*, and *ΔpolyP*). The test will use transcriptomic differential expression data.

7	Search for "intermediate" "transcriptomic" data from "Escherichia coli". NOTE: because the data for this exercise is not from the ABPRI effort, you will need to change the search interface from the default **SEPSIS-ALL Basic Query** to the **OMICS (Training) Query** to select the appropriate underlying database to query.
8	Send the resultant data file (`all_mutants_transcriptomic_modified.txt`) to the GenomeSpace part of the OMICs platform.
9	Locate the data you just sent to GenomeSpace.
10	Download the file onto your desktop.

The file you have downloaded is a simple tab delimited files with 4 columns:

`Gene_symbol`
`log2_WT_PPK1`- relative expression levels wild type vs *Δppk1*
`log2_WT_PPX` - relative expression levels wild type vs *Δppx*

omics.data.edu.au

log2_WT_PPK1_PPX - relative expression levels wild type vs *ΔpolyP*		
Varas et al found that components of the TCA cycle were highly differentially expressed between the 3 mutants. The next steps are to overlay the differential gene expression data onto a graphical representation of the TCA cycle.		
11	Launch "APBRI-Pathway Tools" by clicking on the app you added to the GenomeSpace toolbar in step 4. Log-in is not necessary.	
12	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.	
13	Click on "**Pathways**" (in table #3), and navigate to the TCA cycle by following steps 14-16 below	
14	Click on "**Generation of Precursor Metabolites and Energy (42 instances)**"	
15	Click on "**TCA cycle(2)**"	
16	Click on "**TCA cycle I (prokaryotic)**"	
The TCA cycle should be loaded into your browser. Now overlay the differential gene expression data onto the TCA cycle image:		
17	In the Operations Panel that appears on the right hand side of the window, click on "**Customize or Overlay Omics Data on Pathway Diagram**"	
A new, pop-up window entitled "**Pathway Diagram Customization Options**" should appear. Enter the following values:		
18	**Superimpose Omics Data on Pathway**: check this box	
19	**Specify File Containing Omics Data**: select the file that you previously downloaded which contains the gene symbols and gene expression level data across the 3 mutants (all_mutants_transcriptomic_modified.txt)	
20	**Items in the First Column of the File are:** Gene names and/or Identifiers	
21	**Data Column(s) to Use**: 1-3 NOTE: Pathways Tools uses a zero-based numbering convention where the first column is denoted as 0, and the next 3 columns are denoted as 1-3.	
22	**Data Values Use a:** 0-centered scale	
23	**Display Omics Data in Popups:** checked	
24	**Omics Display style:** Vertical Bar Chart	

25	Leave all other fields as their default settings.
26	Click on **Apply**

The following should be loaded which shows the TCA cycle and graphs illustrating the expression differences for each constituent enzyme (in each of the graphs, column one shows wild type vs *Δppk1* levels; column two: wild type vs *Δppx*; and column three: wild type vs *ΔpolyP*.

Further tutorials about Pathway tools can be found by following the Training link at omics.data.edu.au and then selecting **Proteomics > Pathway annotation and prediction**

Additional Pathway Tool guides can be found on the http://omics.data.edu.au/platform/guides/

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