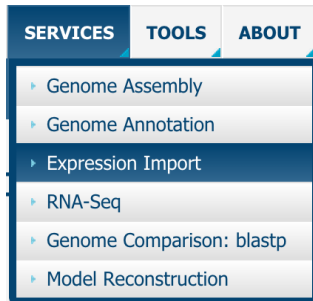# Differential Expression Analysis at PATRIC

The following step-by-step workflow is intended to help users learn how to upload their differential gene expression data to their private workspace using Expression Import Service and use dynamic gene list to detect differentially expressed genes.

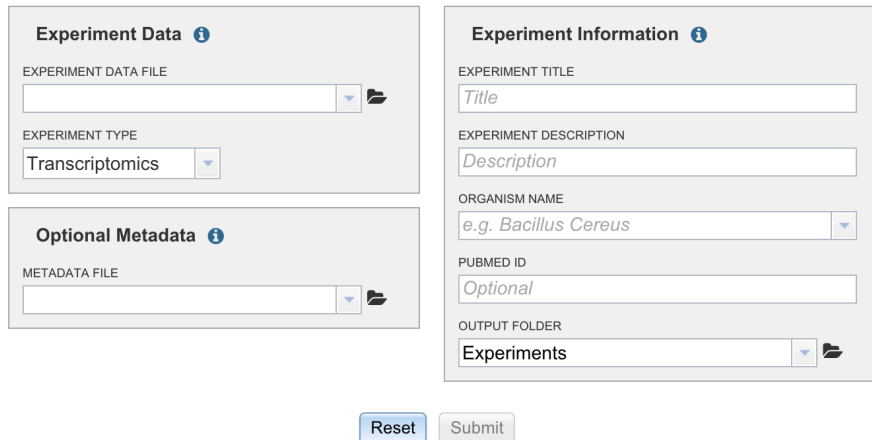1. **Import your differential expression data using Expression Import Service.**

   - From the top navigation bar, select Expression Import, under Services.

   - It will take you to the submission form for the Expression Import Service.

   - First, upload your differential gene/protein expression data files on one of the supported formats, gene matrix or gene list. Or, select the gene matrix file generated by the RNA-Seq Analysis service. Also, select appropriate experiment type using the dropdown menu.

- Provide necessary information about the experiment, such as experiment title, description, organism name, PubMed ID, and output folder.

- Click Submit. The green message above the submit button is an indication that the job has been submitted successfully.

2. **View Expression Import Results.**
   - Now, click on the Jobs panel in the bottom right corner to check the status of the job.

- It will take you to the job list page. The Expression Import job you just submitted should be listed at the top. Check the status of the job to see if it is completed.

● completed	6/15/15, 8:43 PM	DifferentialExpression

- Once the job is complete, click on it to go to the job results page, where you will see the summary of the experimental conditions and genes in the dataset you uploaded.

mshukla / home / RNA-Seq / Acinetobacter baumannii AR Treatments

Platform Organism: Acinetobacter baumannii AYE Pubmed ID: Undefined
Genes Mapped/Genes Total: 3796/3796 Samples: 3

Title	Genes	Significant Genes (Log Ratio)	Significant Genes (Z Score)	St
COL|MHB	3796	244	939	
MERO|COL	3796	212	735	
MERO|MHB	3796	256	759	

- Now, select all three conditions displayed in the table clicking on the table rows.

mshukla / home / RNA-Seq / Acinetobacter baumannii AR Treatments

Platform Organism: Acinetobacter baumannii AYE Pubmed ID: Undefined
Genes Mapped/Genes Total: 3796/3796 Samples: 3

Title	Genes	Significant Genes (Log Ratio)	Significant Genes (Z Score)	Strain	Gene Modification	Experiment Condition	Time Point
COL|MHB	3796	244	939				
MERO|COL	3796	212	735				
MERO|MHB	3796	256	759				

- Then, click on the Genes icon in the green action bar on the right, which will show your expression data as an interactive gene list.

3. Analyze gene expression data as an Interactive Gene List.

- Now, you should see your gene differential expression data displayed as an interactive gene list. For each gene, the table shows corresponding organism name, gene identifiers, gene, gene symbol, product, total comparisons a gene is present in, and number of comparisons in which the gene is up/down regulated. The gene list can be filtered using the options provided in the left panel.

- To filter the gene list using Log Ratio and/or Z-score, select appropriate values from the respective dropdown menus and click Filter button. In this example, we select |Log Ratio| >= 1 and |Z-score| >= 2.

- This will filter the gene list to show only subset of genes that pass the selected log ratio and Z-score threshold. Note the change in the number of genes displayed in the table, which goes down from 3796 to 681.

	Genome Name	PATRIC ID	RefSeq Locus Tag	Alt Locus Tag	Gene Symbol	Product Description	Comparisons	Up	Down
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		YaeQ protein	3	1	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...	dctA	C4-dicarboxylate transport protein	3	0	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		hypothetical protein	3	2	0
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Dihydrodipicolinate synthase (EC 4.2...	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		OsmC/Ohr family protein	3	0	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		putative hemerythrin-like protein	3	2	0
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		FIG00350997: hypothetical protein	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		FIG00350000: hypothetical protein	3	2	0
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Flavodoxin reductases (ferredoxin-N...	3	2	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...	gabD	Succinate-semialdehyde dehydrogen...	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Benzoate 1,2-dioxygenase (EC 1.14...	3	2	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Benzoate 1,2-dioxygenase (EC 1.14...	3	0	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Tartrate dehydrogenase (EC 1.1.1.9...	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		FIG00350547: hypothetical protein	3	2	0
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...	bioD	Dethiobiotin synthetase (EC 6.3.3.3)	3	0	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		Biotin synthesis protein bioH	3	3	0
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...	otsB	Trehalose-6-phosphate phosphatase...	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		putative signal peptide	3	1	1
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		FIG00350244: hypothetical protein	3	0	2
☐	Acinetobacter baumannii AYE	fig|509173.8.pe...	fig|509173.8.pe...	VBIAciBau6988...		FIG00350235: hypothetical protein	3	1	1

- You can use up/down filters on the left to identify genes, which are up or down regulated in one or more comparisons using the radio buttons. For example, to see the genes that are up regulated in COL|MHB and MERO|MHB, make the selection as shown below. Note that the number of genes displayed in the table has changed to 146.

- Similarly, to see the genes that are down regulated in COL|MHB and MERO|MHB comparisons, make the selection as shown below. Note the number of genes displayed in the table, which has now changed to 76 genes.
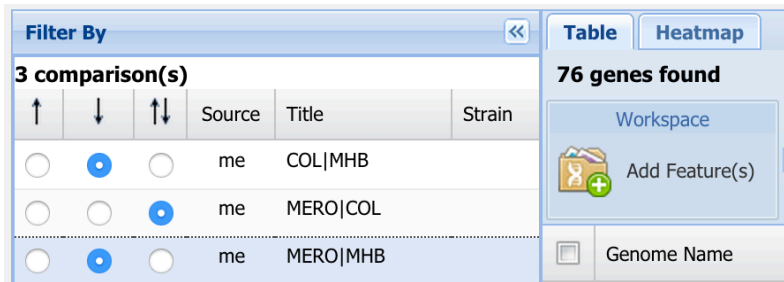
4. Download filtered genes as tab-delimited or Excel files.

- Once you have filtered the gene list to show the genes you are interested in, use various download options available in the table tool bar to download them as tab-delimited or Excel file. You can also select genes using the check boxes and download corresponding fasta DNA/protein sequences, or save genes as a feature group in your workspace.

5. Generate Pathway Summary for select genes.

- Increase the number of genes displayed in the table to show all 76 genes and then click on the check box next to "Genome Name" to select all 76 genes. Then, click on Pathway Summary in the tool bar.
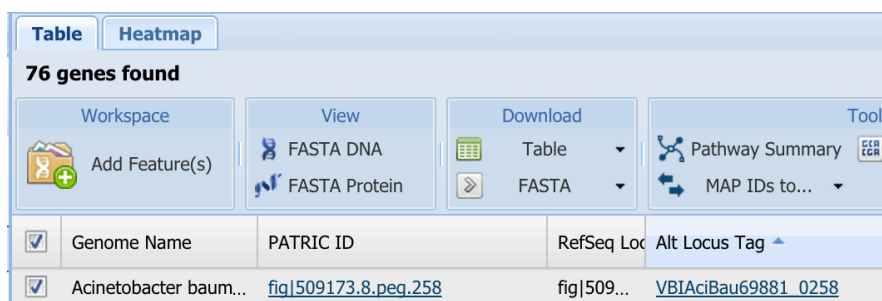

- It will take you to a page that lists all the pathways corresponding to the selected genes. For each pathway, it summarizes the number of genes present in the selection, total number of genes annotated in that pathway in respective genome, and %coverage.

- Click on a pathway name, Fluorobenzoate degradation, to see the pathway map. The enzymes corresponding to the selected genes are highlighted in blue, whereas all other enzymes annotated in the respective genome are highlighted in green.
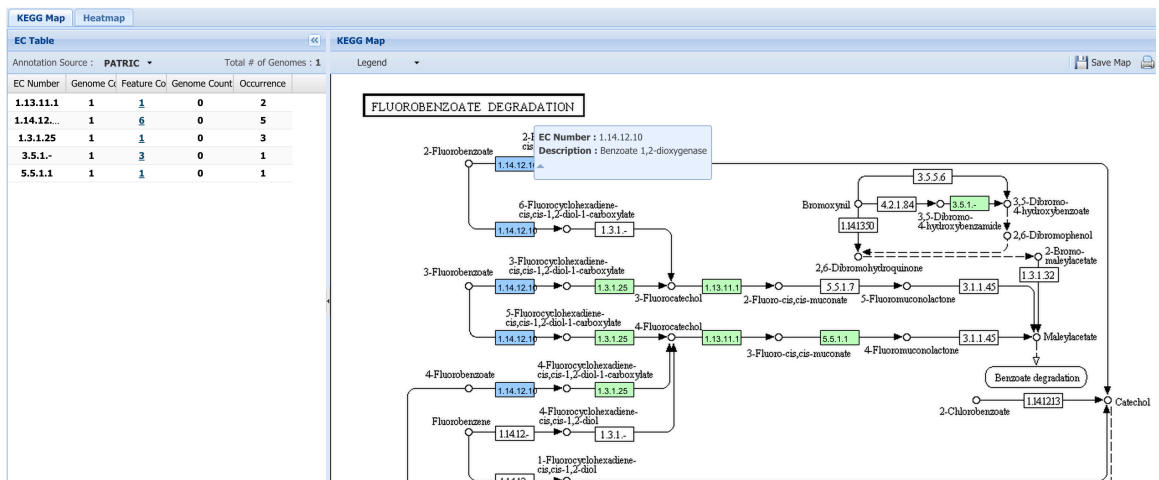
6. **Analyze genes using interactive heatmap.**

- First, set the up/down filters to neutral as shown below, which shows 681 genes in the gene list. Now, click on the heatmap tab above the table.

- It will take you to in interactive heatmap view showing expression of the select genes across various conditions as a heatmap. By default, heatmap displays conditions on the x-axis and genes on the y-axis. It uses red-black-

green color scheme, where up regulated genes are colored red and down regulated genes are colored green.
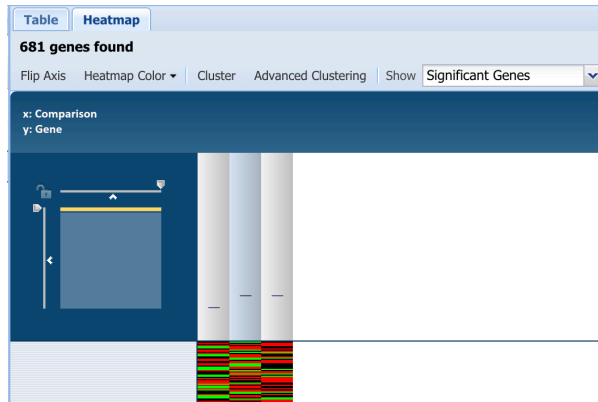
- Click on the Flip Axis button to display genes on the x-axis and conditions on the y-axis.

- If you wish, you can select an alternate red-white-blue color scheme using Heatmap Color dropdown menu.
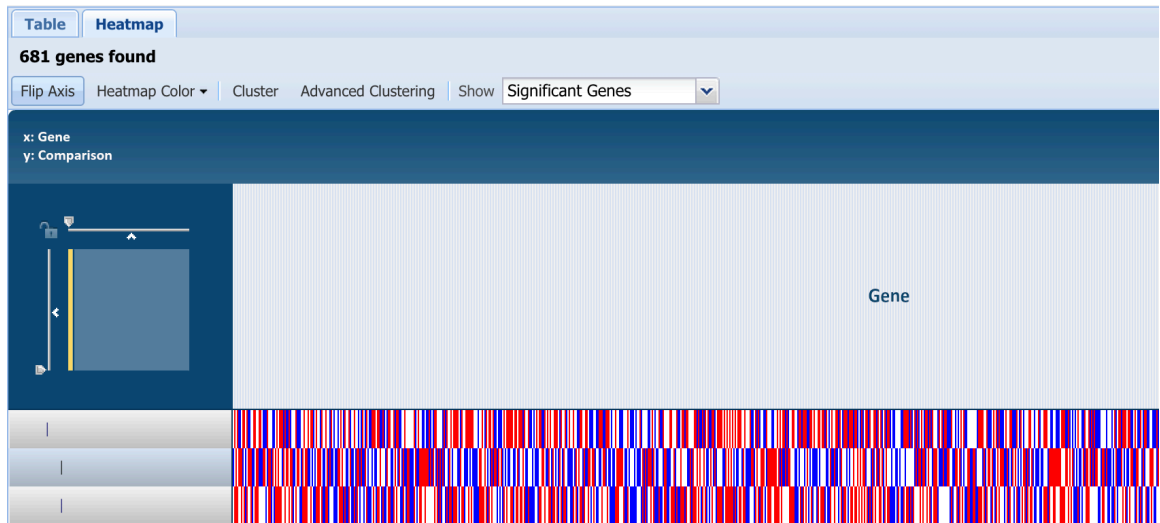
- Click on Cluster button to perform hierarchical clustering, which rearranges all the genes and conditions. The default clustering option uses Pearson correlation and pairwise average linkage distance matrix.
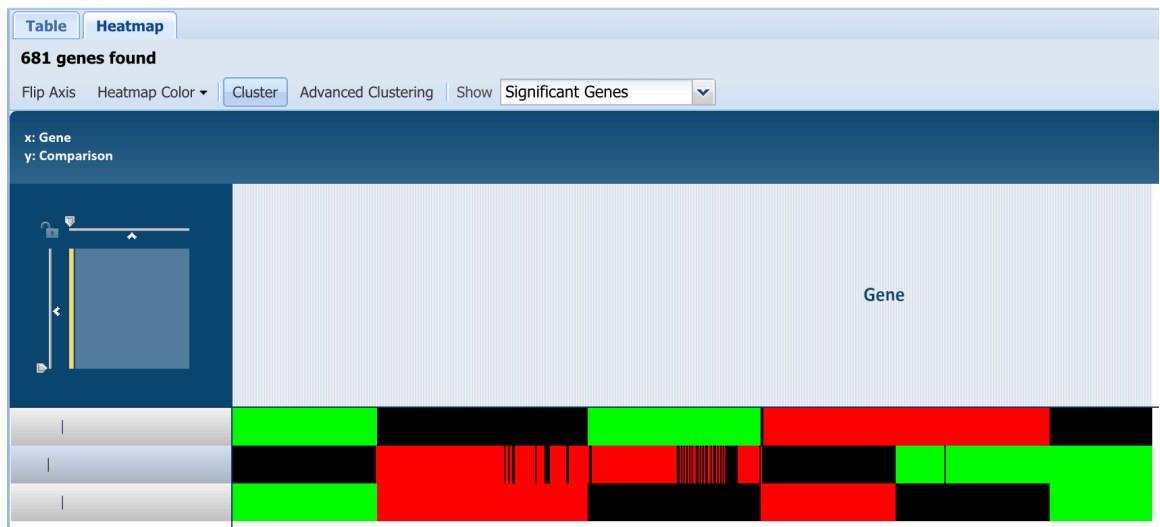
- Now, from the heatmap, select genes that are up regulated in COL|MHB and MERO|MHB conditions, by drawing a box around genes displayed in red.
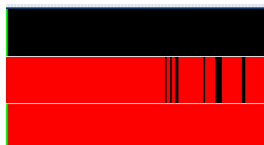
- It will show a popup window, which allows you to download data for the selected heatmap as tab delimited or Excel file. Or, you can chick on "Show Genes" to see genes as a table.

Selected Area from Heatmap

Number of comparisons selected: 2
Number of features selected: 159

Download Heatmap Data ▾ | Download Genes ▾ | Show Genes | Add Genes To Group | Cancel
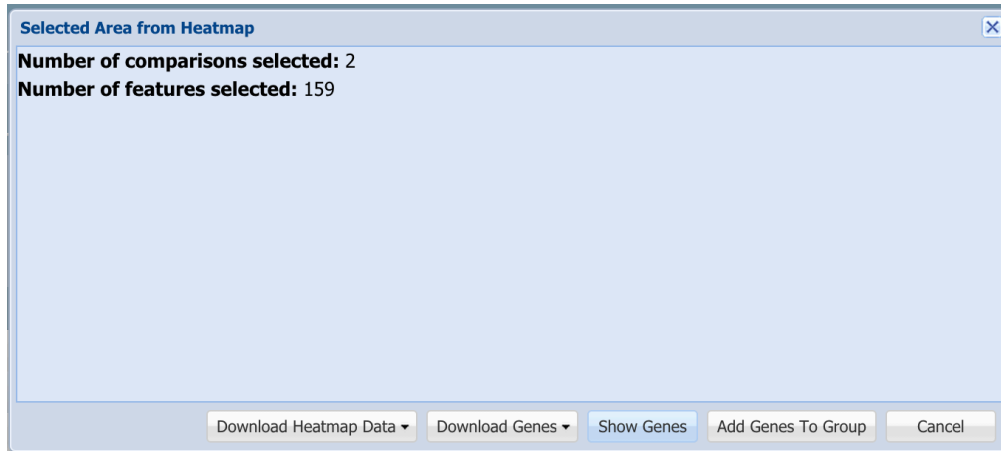
- Clicking on Show Genes displays selected genes as a dynamic table, where you can see more information about the genes.

159 features found
Feature tables contain all of the identified features for all of the genomes in a particular genus. Tables may be refined to show subsets of features via various user controls, as described in Feature Table FAQs.

Genome Name ▲	PATRIC ID	RefSeq Locus Tag	Alt Locus Tag	Gene Symbol	Product Description
Acinetobacter baumannii AYE	fig|509173.8.peg.2816	ABAYE2924	VBIAciBau69881_2816		putative hemerythrin-like protein
Acinetobacter baumannii AYE	fig|509173.8.peg.2837	ABAYE2945	VBIAciBau69881_2837		FIG00350000: hypothetical protein
Acinetobacter baumannii AYE	fig|509173.8.peg.2896	ABAYE3005	VBIAciBau69881_2896		Biotin synthesis protein bioH
Acinetobacter baumannii AYE	fig|509173.8.peg.2930		VBIAciBau69881_2930		FIG00351272: hypothetical protein
Acinetobacter baumannii AYE	fig|509173.8.peg.2988	ABAYE3106	VBIAciBau69881_2988	ogt	Methylated-DNA--protein-cysteine methyltransferase (EC 2.1.1.63)
Acinetobacter baumannii AYE	fig|509173.8.peg.3130	ABAYE3258	VBIAciBau69881_3130		4'-phosphopantetheinyl transferase (EC 2.7.8.-)
Acinetobacter baumannii AYE	fig|509173.8.peg.3141	ABAYE3269	VBIAciBau69881_3141		Membrane-associated phospholipid phosphatase
Acinetobacter baumannii AYE	fig|509173.8.peg.3157	ABAYE3286	VBIAciBau69881_3157		FIG00350862: hypothetical protein
Acinetobacter baumannii AYE	fig|509173.8.peg.3246	ABAYE3382	VBIAciBau69881_3246		Acyl-CoA dehydrogenase family protein
Acinetobacter baumannii AYE	fig|509173.8.peg.3247	ABAYE3383	VBIAciBau69881_3247		Acyl-CoA dehydrogenase; probable dibenzothiophene desulfurization enzyme
Acinetobacter baumannii AYE	fig|509173.8.peg.3249	ABAYE3385	VBIAciBau69881_3249	rpmE2	LSU ribosomal protein L31p @ LSU ribosomal protein L31p, zinc-independent
Acinetobacter baumannii AYE	fig|509173.8.peg.3265	ABAYE3402	VBIAciBau69881_3265	csaA	Protein secretion chaperonin CsaA
Acinetobacter baumannii AYE	fig|509173.8.peg.3278	ABAYE3417	VBIAciBau69881_3278		Beta-lactamase class C and other penicillin binding proteins
Acinetobacter baumannii AYE	fig|509173.8.peg.3330	ABAYE3473	VBIAciBau69881_3330		FIG00352559: hypothetical protein
Acinetobacter baumannii AYE	fig|509173.8.peg.3444		VBIAciBau69881_3444		hypothetical protein
Acinetobacter baumannii AYE	fig|509173.8.peg.3588	ABAYE3764	VBIAciBau69881_3588		Enoyl-CoA hydratase (EC 4.2.1.17)
Acinetobacter baumannii AYE	fig|509173.8.peg.3605	ABAYE3781	VBIAciBau69881_3605		Acid-resistant locus arI7 (Fragment)