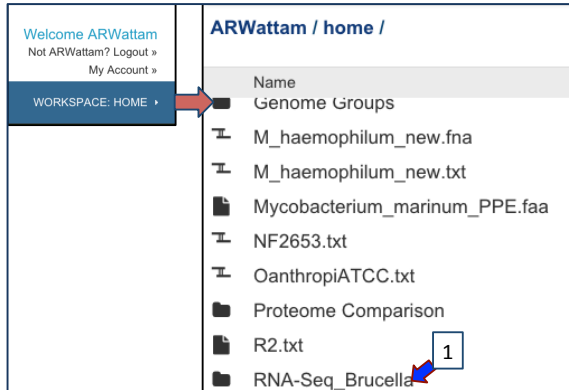
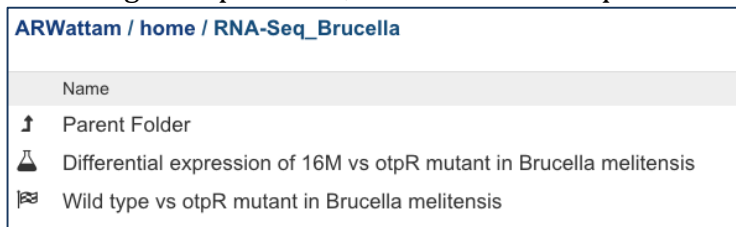


Transcriptomics Analysis: Examining RNA-Seq data in PATRIC

1. At the top of any PATRIC page, click on Workspace. This will open up your private workspace. Scroll down to find the folder where you stored your RNA-Seq job (Blue arrow 1 below).



2. This will open the folder to show you the jobs that you have there. A flask icon, indicating an experiment, indicates RNA-Seq results.



3. Click on the experiment (Blue arrow 1) and this will open up a new view that shows you the title of the experiment, the total genes, and the genes that are significant by log ratio and by Z score. Click on the row that contains the information. It will turn blue, and you will see an icon appear in the green band to the right. Click on the Genes icon (Blue arrow 3)

ARWattam / home / RNA-Seq_Brucella

- Name
- Parent Folder
- Differential expression of 16M vs otpR mutant in Brucella melitensis
- Wild type vs otpR mutant in Brucella melitensis

ARWattam / home / RNA-Seq_Brucella / Differential expression of 16M vs otpR

Platform Organism: Brucella melitensis bv. 1 str. 16M Pubmed ID: Undefined
Genes Mapped/Genes Total: 3562/3562 Samples: 1

Title	Genes	Significant Genes (Log Ratio)	Significant Genes (Z Score)
16M optR	3562	890	115

ARWattam / home / RNA-Seq_Brucella / Differential expression of 16M vs otpR mutant in Brucella melitensis

Platform Organism: Brucella melitensis bv. 1 str. 16M Pubmed ID: Undefined
Genes Mapped/Genes Total: 3562/3562 Samples: 1

File	Genes	Significant Genes (Log Ratio)	Significant Genes (Z Score)	Strain	Gene Modification	Experiment Condition	Time Point
16M optR	3562	890	115				

4. This will open the landing page for your experiment. On the left you will see a filter on the left and a table showing all the genes on the right.

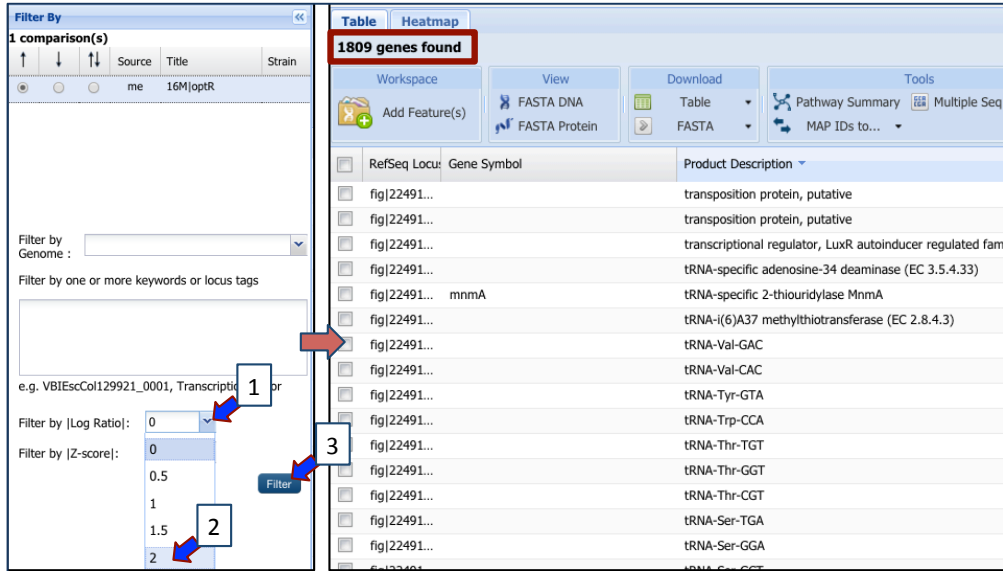
The screenshot shows the Genes and Strains tool interface. On the left, there is a 'Filter By' section with options for 'Genome' and 'Log Ratio'. The main area displays a table of 3562 genes found. The table has columns for 'RefSeq Locus', 'Gene Symbol', 'Product Description', 'Comparisons', 'Up', and 'Down'. The first few rows are:

RefSeq Locus	Gene Symbol	Product Description	Comparisons	Up	Down
fgj22491...		virulence-associated protein E	1	0	1
fgj22491...		transposition protein, putative	1	1	0
fgj22491...		transposition protein, putative	1	1	0
fgj22491...		transcriptional regulator, LuxR autoinducer regulated fam...	1	1	0
fgj22491...	smpB	tRNA-binding protein SmpB	1	0	1

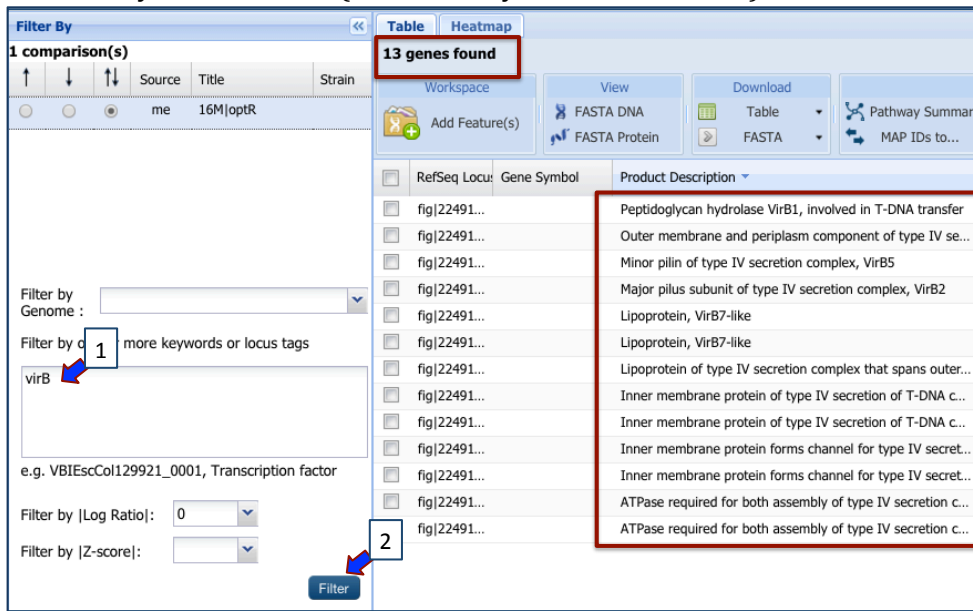
5. The default shows all genes. You can select genes that have a positive fold change by clicking on the up arrow (Blue arrow 1). This will resort the table to show the number of genes in that condition that meets that requirement.

The diagram illustrates the process of filtering genes. It shows two screenshots of the tool interface. In the top screenshot, the 'Filter By' section shows '1 comparison(s)' and '3562 genes found'. A blue arrow labeled '1' points to the up arrow icon in the 'Filter By' section. A red arrow points from this icon to the bottom screenshot. In the bottom screenshot, the 'Filter By' section shows '1 comparison(s)' and '1809 genes found', indicating that the table has been filtered to show only genes with a positive fold change.

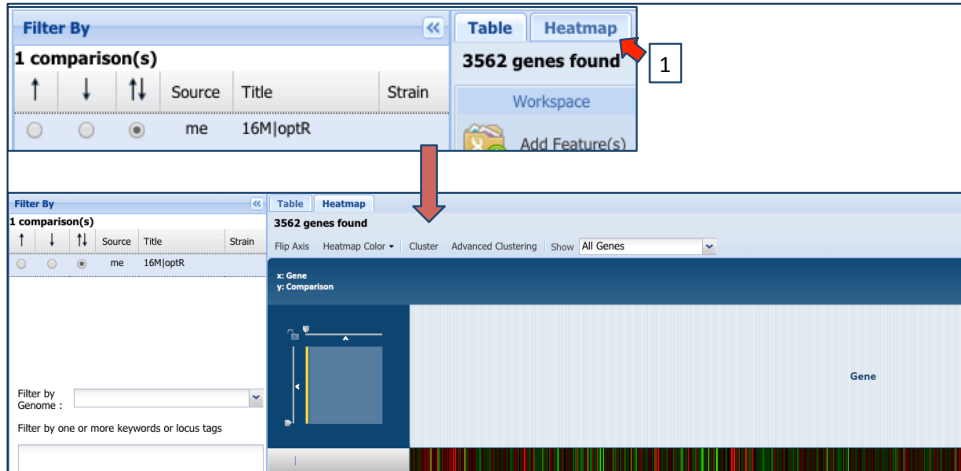
6. You can also filter on the fold change. Click on the down arrow next to Filter by log ratio (Blue arrow 1 below). This will open up a drop down box that shows you the fold change values you can select. Click on one of them (Blue arrow 2) and then click on the filter button (Blue arrow 3). This will resort the table to show the number of genes that meet that condition.



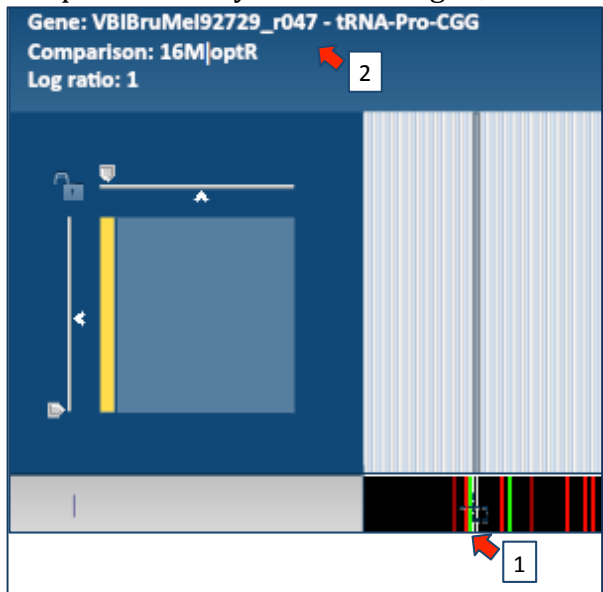
7. You can also enter names of genes (like virB) in the text box (Blue arrow 1) and then click on the Filter button (Blue arrow 2). This will resort the table to show the results of your selection (indicated by red boxes below).



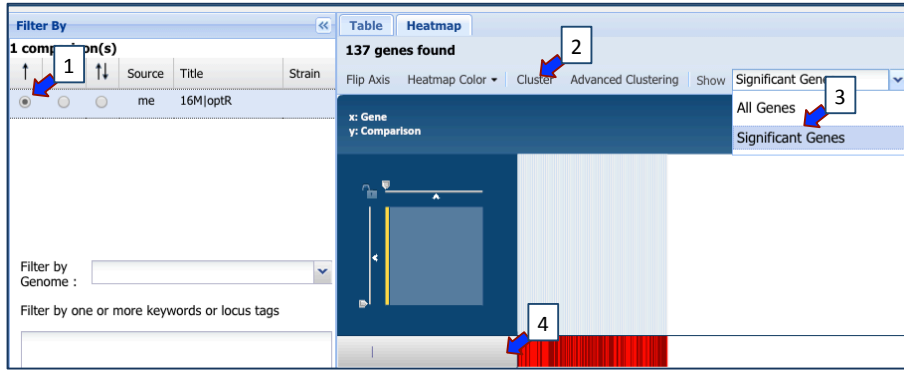
8. **Heatmap view of protein families.** You can see the expression pattern of genes in your experiment by looking at the Heatmap view. To see this, click on the Heatmap tab (Blue arrow 1 below) and this will open the heatmap view page. Genes are listed on the x-axis across the top of this view, and genomes along the y-axis.



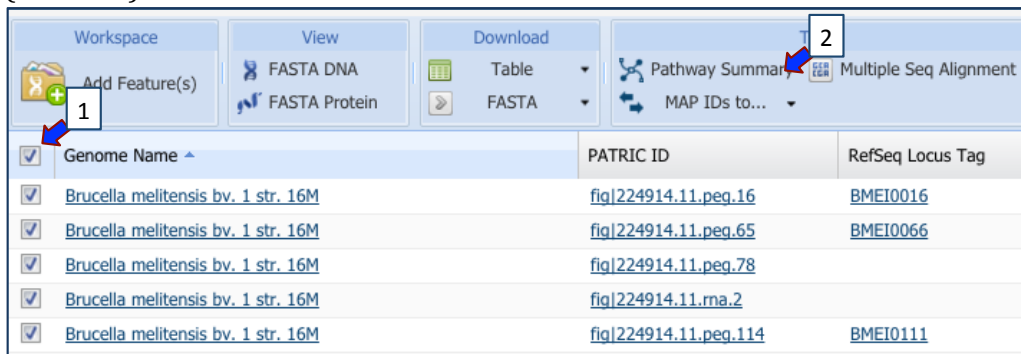
9. Mousing over a particular cell will show you the gene, the gene name, the comparison that you are looking at, and the fold change.



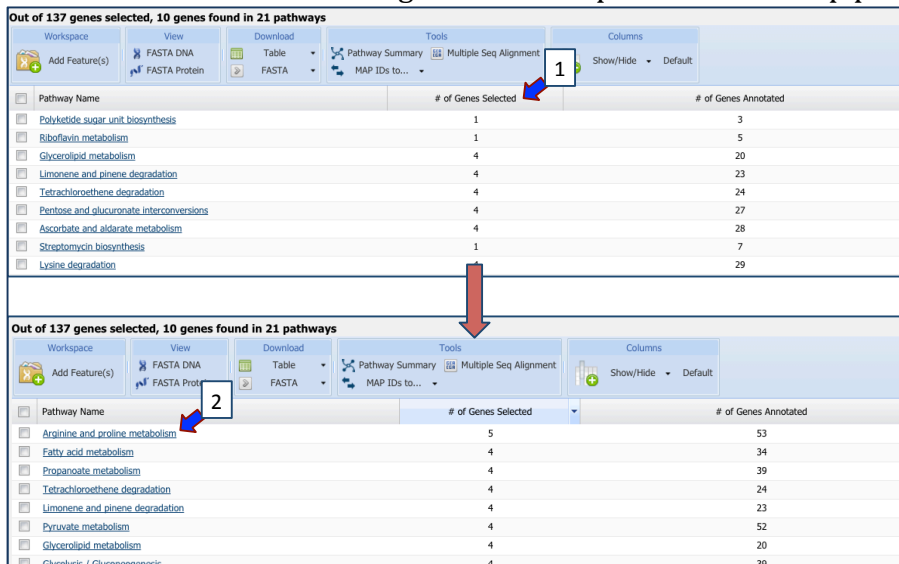
10. You can use a variety of filtering devices to narrow down to specific genes. You could click on all the genes that are up regulated (Blue arrow 1), you could cluster the genes (Arrow 2), or you could use the drop down box to select all the significant genes (Arrow 3). You can download the results of any combination of these by clicking on the header for the row (Arrow 4).



11. This will download a table with a list of all the genes from your selection. If you wanted, for example, to see if any of these genes were involved in any pathways, you could select them all (Blue arrow 1) and then click on the Pathway Summary button (Arrow 2).



12. This will download a table that shows all the pathways that have at least one gene from your selection. To find the pathway that contains the most genes from those that were up-regulated in your experiment, double click on # of Genes Selected (Blue arrow 1). This will resort the pathway list to show the pathways with the most number of selected genes at the top. Click on the top pathway (Arrow 2).



13. This will open a page that shows you a list of all the EC numbers in this pathway for your particular genome on the left, and a KEGG pathway map on the right. The genes from your selection will be colored blue on this map, as seen in Arrow 1 below.

