

## Protein Family Analysis: Comparative Pathway Viewer

- To look for similarities and differences in a particular pathway across different genomes in PATRIC, you can use the Comparative Pathway Viewer. Go to the Tools tab across the top of any PATRIC page and click on it. This will open up a list of tools (A below). Click on the Protein Family Sorter. This will take you to the landing page for that tool. If you are logged in, you will see the groups you have created in the box under “1. Select organism(s).”

The screenshot shows the PATRIC Comparative Pathway Viewer landing page. The left sidebar (A) contains a list of tools, including the 'Protein Family Sorter'. The main area (B) has a 'Select organism(s)' section with a search bar and a list of genome groups. A red arrow points to the '40 genomes Brucella' group in the list.

- The groups that you have created should be visible. You can select one, or many to compare. If you click on the + sign (Blue arrow 1) in front of any group, it will open up the list of all the genomes contained in that group and you can select, or deselect individual ones.

The screenshot shows the 'Search within: selected Workspace genomes' interface. A blue arrow points to the '+ 40 genomes Brucella' button in the first panel. A red arrow points to the expanded list of Brucella strains in the second panel. The list includes: Brucella melitensis bv. 3 str. Ether, Brucella neotomae 5K33, Brucella canis ATCC 23365, Brucella melitensis bv. 2 str. 63/9, Brucella sp. NF 2653, Brucella suis ATCC 23445, Brucella ceti M13/05/1, Brucella suis bv. 5 str. 513, Brucella abortus bv. 3 str. Tulya, Brucella abortus str. 2308 A, and Brucella abortus bv. 2 str. 86/8/59.

3. Once you have made your selection, you can see a list of all the KEGG pathways from the genomes in your selection that have genes in that pathway. Just click on the Search button (Red arrow 1) and the pathway table will load.

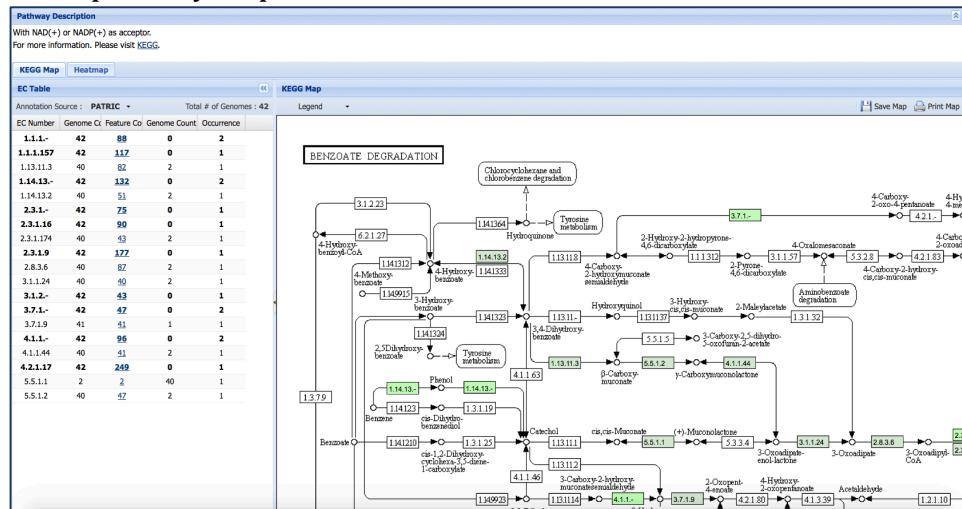
The screenshot shows the PATRIC feature table search interface. On the left, under 'Select organism(s)', 'selected Workspace genomes' is selected. On the right, the 'Enter keyword' section has 'Metabolism' selected in the dropdown. A red arrow labeled '1' points to the 'Search' button. Another red arrow labeled '2' points to the 'Annotation' dropdown menu. Below the search bar, the results table shows 135 unique pathway(s) found, with columns for Pathway ID, Pathway Name, Pathway Class, Annotation, Unique Genome, Unique Gene Cc, Unique EC Count, EC Conservation, and Gene Conservation. The first few rows include 00053 (Ascorbate and aldarate metabolism), 00190 (Oxidative phosphorylation), 00051 (Fructose and mannose metabolism), 01056 (Biosynthesis of type II polyketides), 00052 (Galactose metabolism), 01057 (Biosynthesis of type II polyketides), 00195 (Photosynthesis), and 00253 (Tetracycline biosynthesis).

4. You can also use this page to narrow down your search by entering a keyword for a particular pathway in the text box (Red arrow 1) and then click on the Search button (Red arrow 2). In this example, I used the key word “Benzoate” and this narrowed the returns to the “Benzoate Degradation via Hydroxylation” pathway.

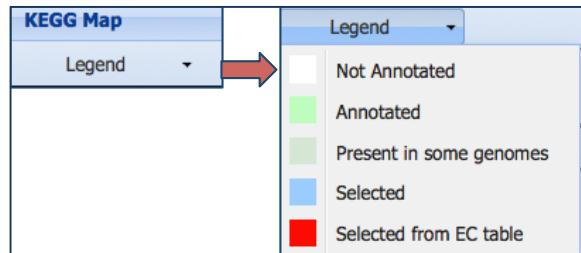
The screenshot shows the PATRIC feature table search interface. On the left, under 'Select organism(s)', 'selected Workspace genomes' is selected. On the right, the 'Enter keyword' section has 'Benzoate' entered in the text box. A red arrow labeled '1' points to the text box. Another red arrow labeled '2' points to the 'Search' button. Below the search bar, the results table shows 1 unique pathway(s) found, with columns for Pathway ID, Pathway Name, Pathway Class, Annotation, Unique Genome, Unique Gene Cc, Unique EC Count, EC Conservation, and Gene Conservation. The single row is 00362 (Benzoate degradation via hydroxylation), which is bolded to indicate it is present in all selected genomes.

5. **KEGG Map.** This will open up the pathway view. On the right you will see a table that lists the EC numbers and their presence and absence across the genomes from your selection. Bold numbers indicate that all of the genomes in the selection have that particular gene annotated, and non bold indicate that only some of the genomes

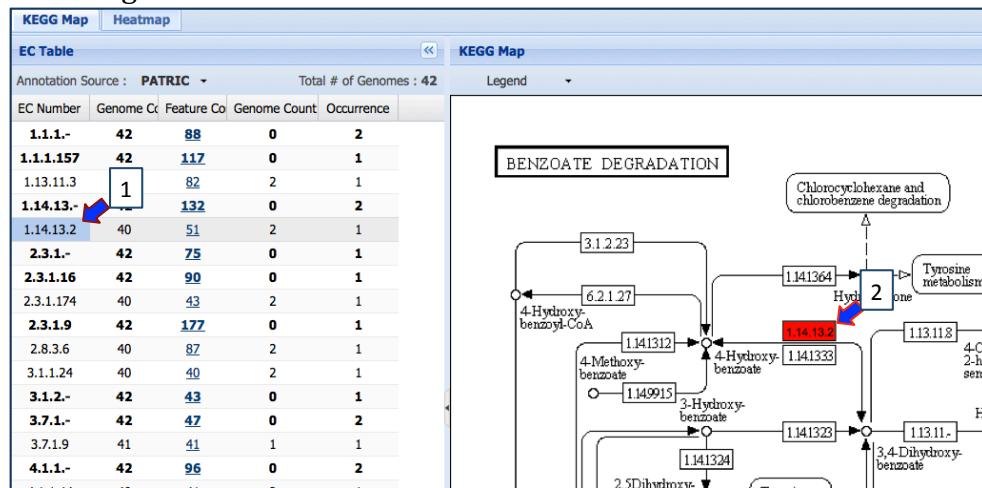
have a gene with that particular EC number annotated. On the left you can see the KEGG pathway map with the different EC numbers colored, or not.



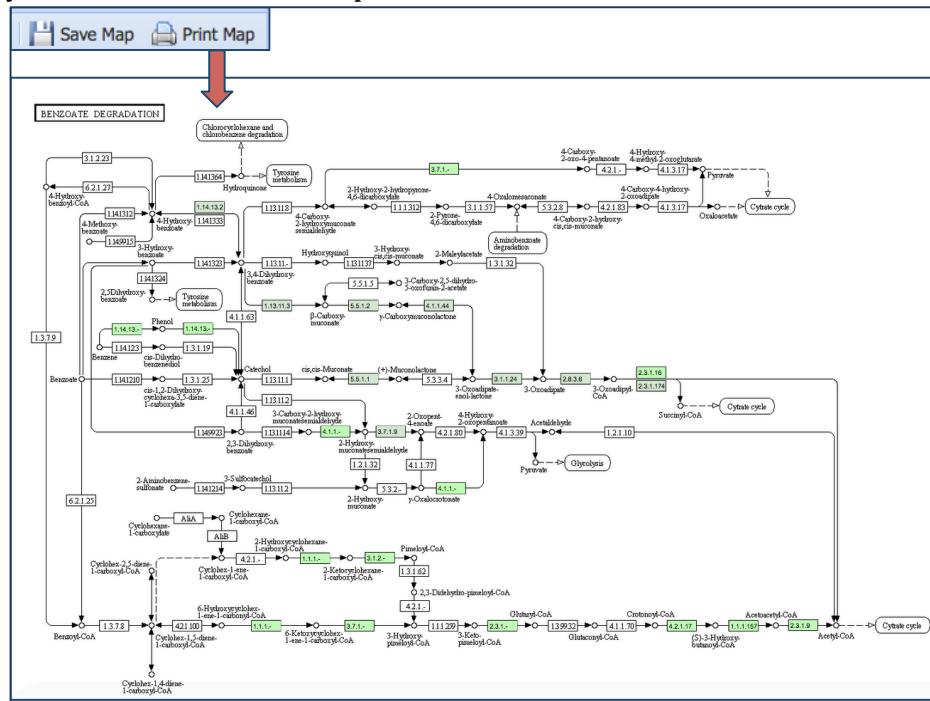
6. Clicking on the legend opens it. When you are viewing multiple genomes, green boxes indicate that ALL genomes have at least one gene annotated with that EC number. The olive color boxes indicate that some, but not all genomes have that EC number annotated, and white boxes indicate that none of the genomes have a gene annotated with that EC number.



7. Clicking on any EC number on the left will highlight its position in the KEGG map on the right.



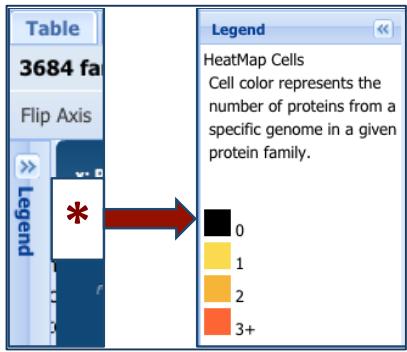
8. Clicking on the Print Map button at the top of the page will open a window where you can see the entire map.



9. **Heatmap.** Clicking on the heatmap tab will show you the presence or absence of the genes involved in the pathway across all the genomes.



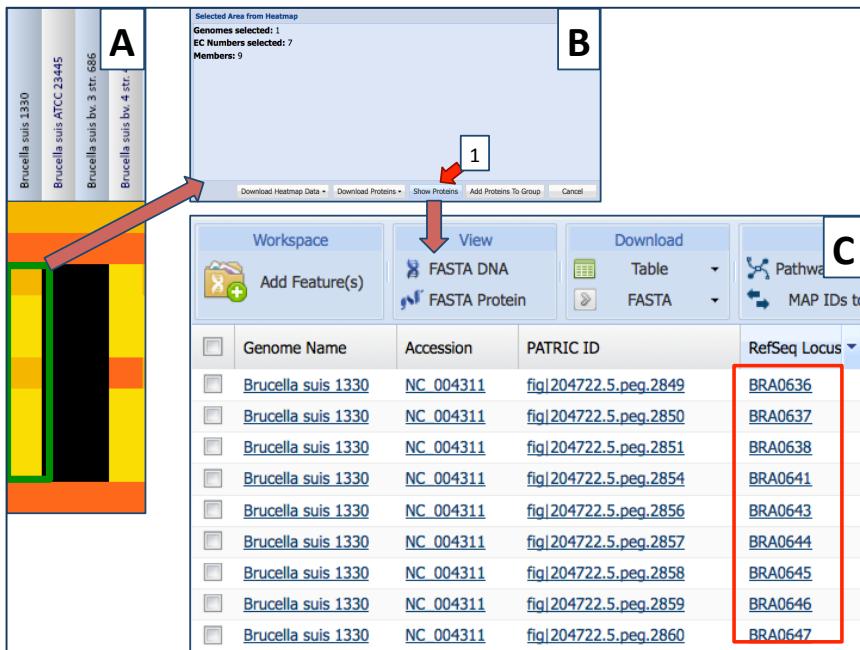
10. You can click on the legend (Red asterisk) to see what the different colors of the cells mean, but basically, black cells indicate a gene absence and all other colors indicate gene presence.



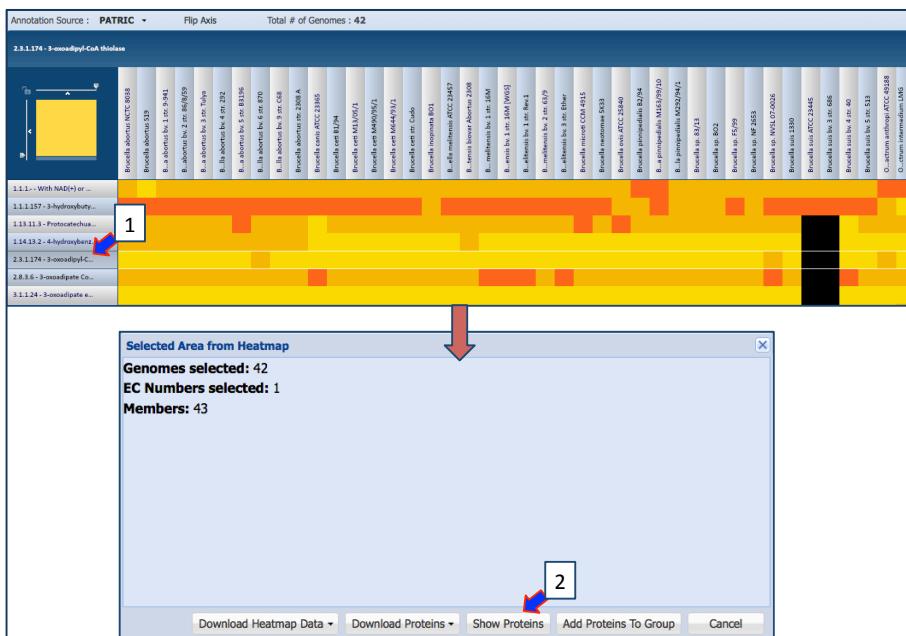
11. You can arrange each of the rows or columns to arrange the data any way you like. To change the order of the rows, click and hold on the head of the row and move it up or down. You can do this with several rows to get the data you are interested in next to each other (Panel B below).



12. **Selection from Heatmap.** To see what proteins are missing, you will need to select the proteins in a neighboring column by drawing a box around them using your mouse (Panel A below, green rectangle). This will open a pop-up window will appear that allows you to download the heatmap data, download the proteins from your selection, show the proteins from your selection, add the proteins to a group, or cancel (Panel B). To see the proteins, click on “Show Proteins” (Arrow 1). This will open up a new page that shows you all the proteins that were in the row you selected in the heatmap (Panel B). You can see that these proteins are all sequential (rectangle in Panel C).



13. You can also click on the heading to a single row (Arrow 1 below) to examine all the information about all the proteins in that row. Clicking on that header will open a pop-up window that allows you to download the heatmap data, download the proteins from your selection, show the proteins from your selection, add the proteins to a group, or cancel. To see the proteins, click on "Show Proteins" (Arrow 2 below).



14. This will open up a new page that shows you all the proteins that were in the row you selected in the heatmap.

Pathway Table  
43 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.

Workspace		View	Download	Tools	Columns
	Add Feature(s)	Fasta DNA	Table	Pathway Summary	Multiple Seq Alignment
		Fasta Protein	FASTA	MAP IDs to...	Show/Hide Default
<input type="checkbox"/>	Genome Name	Accession	PATRIC ID	RefSeq Locus Tag	Gene Symbol
<input type="checkbox"/>	Ochrobactrum int...	NZ_ACQA01...	fig 641118.3.peg.3367	QINT_20008...	VBIochInt64...
<input type="checkbox"/>	Ochrobactrum ant...	NC_009668	fig 439375.7.peg.4196	Qant_4020	VBIOchAnt7...
<input type="checkbox"/>	Ochrobactrum ant...	NC_009668	fig 439375.7.peg.3882	Qant_3718	VBIOchAnt7...
<input type="checkbox"/>	Brucella suis bv. 5...	NZ_DS999712	fig 520489.3.peg.391	Bsub55_010...	VBIBruSui73...
<input type="checkbox"/>	Brucella sp. F5/99	NZ_GG663472	fig 437701.3.peg.340	Bruf5_0101...	VBIBruSp659...
<input type="checkbox"/>	Brucella sp. 83/13	NZ_DS999659	fig 520449.3.peg.2258	Bru83_0101...	VBIBruSp753...
<input type="checkbox"/>	Brucella sp. NF2653	NZ_ADFB010...	fig 693748.4.peg.279	BRCD_0276	VBIBruSp153...
<input type="checkbox"/>	Brucella suis 130	NC_004311	fig 204722.5.peg.2849	BRA0636	VBIBruSui10...
<input type="checkbox"/>	Brucella pinnipedia...	NZ_EQ999534	fig 520462.3.peg.1152	BpinM2_010...	VBIBruPin74...
<input type="checkbox"/>	Brucella ovis ATCC...	NC_009504	fig 444178.3.peg.681	BOV_A0598	VBIBruOvi13...
<input type="checkbox"/>	Brucella microt CC...	NC_013118	fig 568815.3.peg.617	BMI_I1633	VBIBruMic92...
<input type="checkbox"/>	Brucella melitensis...	NZ_EQ999588	fig 520466.3.peg.1188	Bmelb3E_01...	VBIBruMel11...
<input type="checkbox"/>	Brucella melitensis...	NZ_ACEM01...	fig 520465.3.peg.683	Bmelb26_01...	VBIBruMel34...
<input type="checkbox"/>	Brucella melitensis...	NZ_EQ999555	fig 520464.3.peg.538	Bmelb1R_01...	VBIBruMel10...
<input type="checkbox"/>	Brucella melitensis...	NC_003318	fig 224914.11.peg.2970	BMEI01646	VBIBruMel92...
<input type="checkbox"/>	Brucella melitensis...	NC_012442	fig 46272.3.peg.2895	BMEA_B0609	VBIBruMel14...
<input type="checkbox"/>	Brucella sp. BO2	NZ_ADFB010...	fig 693750.4.peg.574	BIBO2_0515	VBIBruSp14...
<input type="checkbox"/>	Brucella inopinata...	NZ_ADEZ010...	fig 470735.4.peg.2558	BIBO1_2424	VBIBruSp109...
<input type="checkbox"/>	Brucella ceti M490...	NZ_EQ999599	fig 520458.3.peg.231	BctM4_010...	VBIBruCet49...
<input type="checkbox"/>	Brucella ceti str. C...	NZ_ACJD010...	fig 595497.3.peg.3102	BCETI_7000...	VBIBruCet28...

Show 20 per page Apply Apply to ALL tables

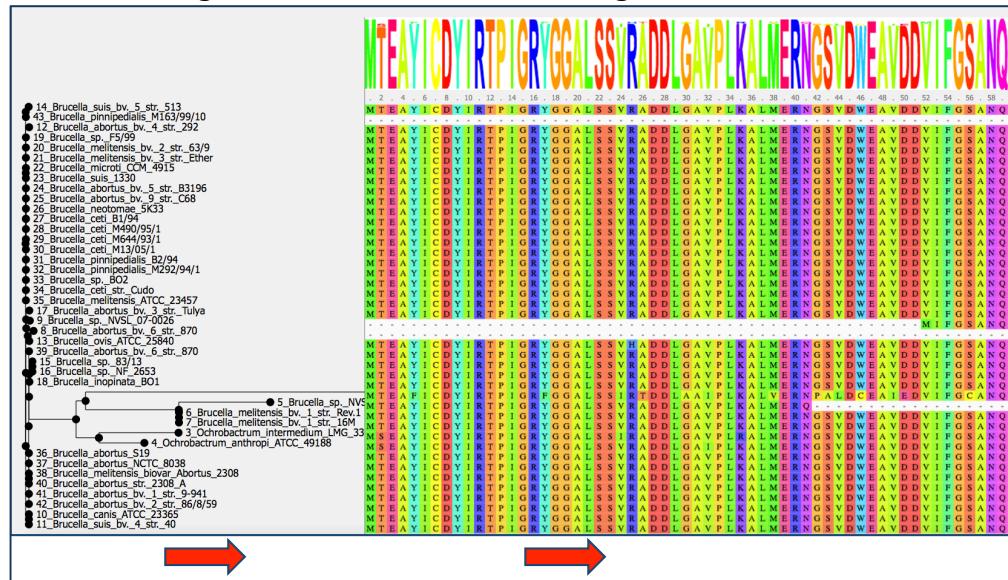
15. You will need to resize the table to see all the entries. To do this, change the default (20) at the bottom of the table to the total number.

1 → Show 42

16. **Multiple Sequence Alignment.** You can do a number of things with these genes. You could save them to a group, get their nucleotide or amino acid sequences, download the information, see if they were important in any metabolic pathways, or generate a multiple sequence alignment for them. To generate an alignment, click on the box in front of "Genome Name" (Arrow 1 below). This will select all the genes in the table. Then you need to click on the Multiple Sequence Alignment button at the top of the table (Arrow 2).

Workspace		View	Download	Tools	
	Add Feature(s)	Fasta DNA	Table	Pathway Summary	Multiple Seq Alignment
		Fasta Protein	FASTA	MAP IDs to...	Show/Hide Default
<input checked="" type="checkbox"/>	Genome Name	Accession	PATRIC ID	RefSeq Locus Tag	Gene Symbol
<input checked="" type="checkbox"/>	Brucella ceti str. C...	NZ_ACJD010...	fig 595497.3.peg.3102	BCETI_7000...	VBIBruCet28...
<input checked="" type="checkbox"/>	Brucella microt CC...	NC_013118	fig 568815.3.peg.617	BMI_I1633	VBIBruMic92...
<input checked="" type="checkbox"/>	Brucella abortus N...	NZ_GG703762	fig 575591.3.peg.713	BAUG_0627	VBIBruAbo9...
<input checked="" type="checkbox"/>	Brucella melitensis...	NC_007624	fig 359391.4.peg.2899	BAB_0606	VBIBruMel86...
<input checked="" type="checkbox"/>	Brucella ovis ATCC...	NC_009504	fig 444178.3.peg.681	BOV_A0598	VBIBruOvi13...
<input checked="" type="checkbox"/>	Brucella canis ATC...	NC_010104	fig 483179.4.peg.2852	BCAN_B0636	VBIBruCan2...
<input checked="" type="checkbox"/>	Brucella inopinata ...	NZ_ADEZ010...	fig 470735.4.peg.2558	BIBO1_2424	VBIBruSp109...
<input checked="" type="checkbox"/>	Brucella suis 130	NC_004311	fig 204722.5.peg.2849	BRA0636	VBIBruSui10...
<input checked="" type="checkbox"/>	Brucella melitensis...	NC_012442	fig 546272.3.peg.2895	BMEA_B0609	VBIBruMel14...
<input checked="" type="checkbox"/>	Brucella pinnipedia...	NZ_DS999750	fig 520463.3.peg.657	BAGG_00462	VBIBruPin12...
<input checked="" type="checkbox"/>	Brucella melitensis...	NZ_EQ999555	fig 520464.3.peg.536	Bmelb1R_01...	VBIBruMel10...
<input checked="" type="checkbox"/>	Brucella abortus S19	NC_010740	fig 430066.4.peg.623	BAbS19_I105...	VBIBruAbo3...
<input checked="" type="checkbox"/>	Ochrobactrum ant...	NC_009668	fig 439375.7.peg.3882	Qant_3718	VBIOchAnt7...

17. The multiple sequence alignment tool has a gene tree on the left side, and a multiple sequence alignment with a weblogo summary across the top. You can scroll to the right on either the tree or the alignment to see more.



18. Scrolling to the right reveals a large deletion that you can see in all the *Brucella abortus* sequences.

