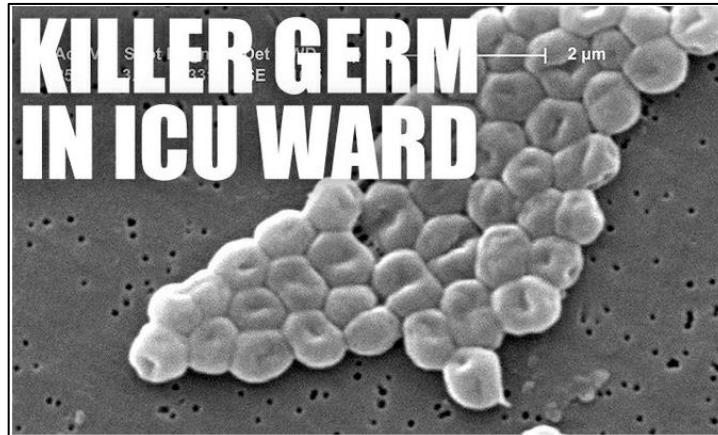


Exploring antibiotic resistance in *Acinetobacter baumannii*

Antibiotic resistance of bacterial pathogens is becoming an area of increasing concern, and this is especially true of *Acinetobacter baumannii*. This organism has emerged as one of the most troublesome bacteria for health institutions globally[1-3]. A recent study examined *A. baumannii*[4] and found that horizontal transfer plays an important part in the development of antibiotic resistance in this pathogen.



We will use a variety of the PATRIC tools to explore genomic islands and the genes associated with resistance and gene neighborhoods. This exercise has five parts.

I. Creating genome groups

In this part of the exercise, we will examine two particular strains of *A. baumannii* that are known to have benefited from horizontal transfer[4], and compare these two strains to other *A. baumannii* genomes to see how widely spread these genomic islands are.

1. Login to the PATRIC website (patricbrc.org) so that you can use your workspace in the downstream analysis.

The image shows the PATRIC login interface. It features a blue header bar with the PATRIC logo and the text "Pathosystems Resource Integration Center". Below the header is a search bar containing the text "AYE". To the right of the search bar is a magnifying glass icon. At the bottom of the interface, there are three buttons: "Login" (in a blue box), "Not Registered? Sign Up >" (in blue text), and "Learn About Registering" (in blue text).

2. Two *A. baumannii* genomes (strain AYE and SDF) were used to examine horizontal transfer and antibiotic resistance. In the global search box, enter the word AYE and hit return.

The image shows the PATRIC search results interface. It features a blue header bar with the PATRIC logo and the text "Pathosystems Resource Integration Center". Below the header is a search bar containing the text "AYE". To the right of the search bar is a magnifying glass icon. The main content area displays the search results for the term "AYE".

3. This will take you to the Search Results page that is divided into Features, Genomes, Taxonomy and Experiments. Fortuitously enough, under Genomes you

will see both the AYE and SDF genomes. You see SDF because AYE is mentioned in the comment field for the genome.

The screenshot shows the PATRIC search interface with the query 'AYE' entered. The results page displays two entries under 'Genomes (2)'. The first entry is 'Acinetobacter baumannii AYE', which has 1 Chromosome and 4 Plasmids. Its DISEASE is listed as 'Nosocomial infections' and its COMMENT as 'Acinetobacter baumannii VEB. This bacteria is representative of nosocomial strains. It is responsible for a high-mortality hospital epidemic that broke out in northern France. This strain is resistant to a variety of antibiotics.' The second entry is 'Acinetobacter baumannii SDF', which has 1 Chromosome and 3 Plasmids. Its SEQUENCED date is 2008-02-29 (Genoscope). Its HOST is 'Body louse, Pediculus humanus' and its DISEASE is 'Nosocomial infections'. Its COMMENT is 'Acinetobacter baumannii SDF. This strain is responsible for community-acquired infections, and is sensitive to antibiotics. It was isolated from body lice collected from homeless people living in France. Since the louse's internal body is usually sterile, the presence of this bacteria can only be due to cryptic bacteremic episodes. This strain is susceptible to antibiotics while strain AYE is highly resistant.'

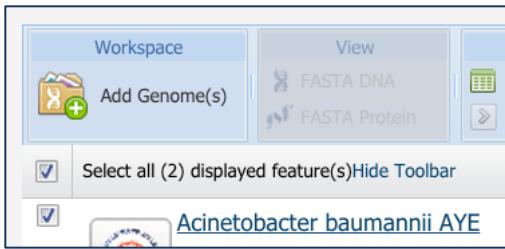
4. Click on the number 2 that follows the word Genomes.

Genomes (2)

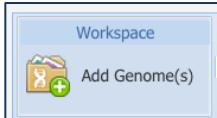
5. This opens up a page where you can filter results, or save selections to your workspace. The filter on the left side allows you to narrow down the results based on the metadata that is linked to them. You will need this later on in this exercise.

This screenshot shows the 'Show Results in:' dropdown menu open, with 'Genomes (2)' selected. The main panel displays the same two genome entries as the previous screenshot, but the sidebar on the left is now focused on genome metadata. It includes sections for 'Summary', 'Features (8786)', 'Genomes (2)', 'Taxa (1)', 'Experiments (0)', 'Genome Status (2)', 'Reference Genome (0)', 'Antimicrobial Resistance (0)', 'Antimicrobial Resistance Evidence', 'Isolation Country (1)', 'Host Name (1)', and 'Body louse, Pediculus humanus (1)'. The 'Genomes (2)' section is expanded, showing the detailed information for each genome, including their respective chromosome and plasmid counts, diseases, and comments.

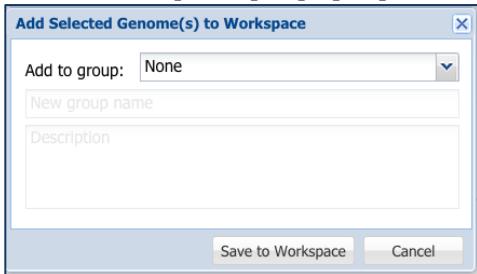
6. Click the check box in front of the words "Select all (2) displayed feature(s)." Doing this will click both of the genomes, and also open up specific tools above the genome list.



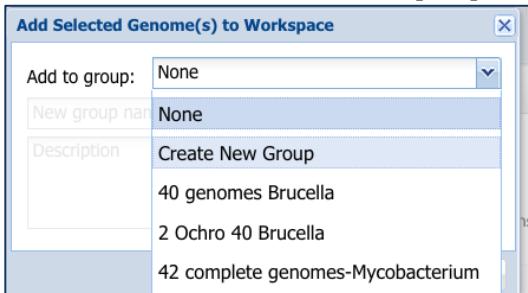
7. Click on the “Add Genomes” next to the folder icon in the Workspace header.



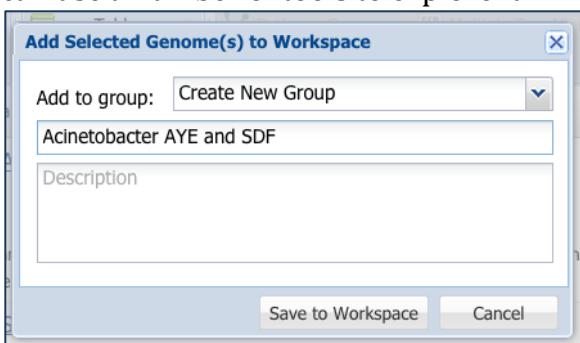
8. This will open up a pop-up window that allows you to save the group.



9. Select the “Create New Group” option.



10. Name the group and click “Save to Workspace”. Now that data is saved and you can use a number of tools to explore it.



Assignment

Create genome groups for the three categories below. Use the global search text box and the dynamic filter on the Search Results page. When you complete your assignment, you will have four different groups that include the one we just created.

- Create a genome group that contains all the complete *A. baumannii* genomes.
- Create a genome group that contains all the *A. baumannii* genomes that were isolated in China.
- Create a genome group that contains all the *A. baumannii* genomes that were isolated from hospitals (nosocomial).

Hint-To complete some of these tasks, you will need to resize the results table (its set to show 20 genomes) to match the number of genomes that are the result of the filtering parameters. You'll need to scroll to the bottom of the table to resize it before selecting all the genomes.

II. Getting a freshly annotated genome ready for comparison in PATRIC

1. We have assembled and annotated two new *A. baumannii* genomes from NCBI's Short Reads Archive (SRA) that have not previously been assembled or annotated. You should have already started the process to annotate these genomes. Once they are annotated, you will first need to make them into their own individual genome group (Note: You can also add them to an existing group, but we'll start with this. Also note that this is MY workspace. You have your own unique workspace and names, and should use those in the following steps. You can find these genomes by using the global search box. Enter the strain number for the *Acinetobacter* genome you annotated into the search box (1000160) and hit return.



2. This will take you to the Search Results page.

A screenshot of the PATRIC search results page. At the top, it says "Showing results for 1000160". Below this, there are two main sections: "Features (3996)" and "Genomes (2)".

Features (3996)
The features section lists several entries:

- hypothetical protein (RefSeq) Yersinia pestis PY-100 CDS | YPPY100_0160 | YPPY100_0160
- serine protease (RefSeq) Mycobacterium tuberculosis NRITLDS1 CDS | BA10_00160 | BA10_00160
- hypothetical protein (RefSeq) Mycobacterium tuberculosis MD16765 CDS | AJ10_00160 | AJ10_00160

Genomes (2)
The genomes section lists:

- Acinetobacter baumannii 1000160 (23 Contigs)
- Candidatus Photodesmus katoptron (18 Contigs)
SEQUENCED: 2014-07-18T00:00:00Z (University of Michigan)
COLLECTED: 2011 (Vanuatu) | HOST: Anomalops katoptron
COMMENT: Sequencing of the Candidatus Photodesmus katoptron genome for comparison to relatives.

3. Under the “Genome” division click on the name of the genome you annotated (Acinetobacter baumannii 1000160).

The screenshot shows a list titled "Genomes (2)" with one item: "Acinetobacter baumannii 1000160". Below the title, there is a small circular icon with a red and orange design, followed by the text "23 Contigs".

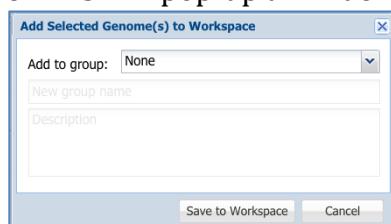
4. This will take you to the landing page for your private genome (the 1000160 genome).

The screenshot shows the genome landing page for "Acinetobacter baumannii 1000160". The top navigation bar includes links for Overview, Phylogeny, Genome Browser, Circular Viewer, Feature Table, Specialty Genes, Pathways, Protein Families, and Literature. On the left, there are search tools like Genome Finder, Feature Finder, Comparative Pathway Tool, and Protein Family Sorter, along with a link to Recent PubMed Articles. The main content area displays genome summary information, including a table with details like Genome ID (1310800.4), Genome Status (WGS), and Contigs (23). A blue button labeled "Add Genome to Workspace" is located in the top right corner of the summary table.

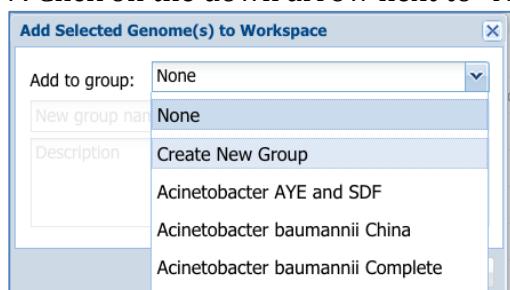
5. To make a genome group using this genome, you will need to click the blue button that says “Add Genome to Workspace.”



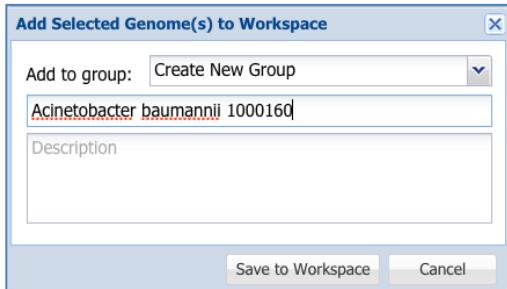
6. This will pop-up a window that allows you to save a group.



7. Click on the down arrow next to “None” and then click on “Create New Group.”



8. In the text box below “Add to group” put in a unique name for the genome. Then click the “Save to Workspace” button at the bottom of the window.



Assignment: Create a new genome group for the second Acinetobacter genome that you have annotated (strain 1592857).

III. Comparing genome groups in PATRIC using the Protein Family Sorter tool

1. To look for presence or absence of the protein families within a genome group that you have created, click on the Tools tab and under Comparative Genomics, select the Protein Family Sorter tool

2. This will take you to the landing page for that tool.

3. Scroll down in the Select Organism box until you see the genome groups you created. Select the boxes in front of the three following *Acinetobacter* groups you created:

- *Acinetobacter AYE and SDF*
- *Acinetobacter baumannii 1592897*
- *Acinetobacter baumannii 1000160*

① Select organism(s)

My Groups **Taxonomy Tree** **A-Z List**

Jump to:

Search within:
selected Workspace genomes

Acinetobacter AYE and SDF
Acinetobacter baumannii China
Acinetobacter baumannii Complete
Acinetobacter baumannii nosocomial
Acinetobacter baumannii 1592897
Acinetobacter baumannii 1000160

4. Hit the Search button under the keyword search box.

② Enter keyword

Keyword

Search

5. This takes you to the Protein Family Sorter landing page. On the left you will see a dynamic filter, and on the right a table that lists all the protein families.

Filter By

4 genomes (Change Genome Selection)

Present in all families

Absent from all families

Either/Mixed

Genome Name

Workspace

Add Feature(s)

View

FASTA DNA

Download

Table

Fasta

Tools

Pathway Summary

Multiple Seq Alignment

MAP IDs to...

Columns

Show/Hide

3388 families found

ID	Proteins	Genomes	Product Description	Min AA Length
FIG00000001	3	3	Cysteine desulfurase (EC 2.8.1.7)	653
FIG00000004	12	4	3-ketoadyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase	390
FIG00000011	8	4	Multimodular transpeptidase-transglycosylase (EC 2.4.1.129) @	798
FIG00000013	4	4	Hydroxyacylglutathione hydrolase (EC 3.1.2.6)	243
FIG00000015	4	4	Signal peptidase I (EC 3.4.21.89)	275
FIG00000017	7	4	Peptide deformylase (EC 3.5.1.88)	158
FIG00000019	5	3	Octaprenyl-diphosphate synthase (EC 2.5.1.-) / Dimethylallyl...	303
FIG00000022	4	4	Shikimate 5-dehydrogenase I alpha (EC 1.1.1.25)	262
FIG00000023	5	4	Transaldolase (EC 2.2.1.2)	119

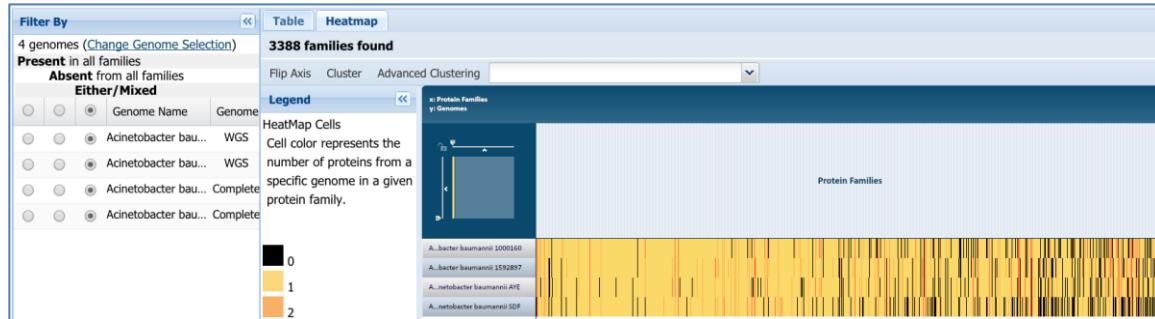
6. One way you can examine differences in your genome groups is to visualize the data. To do this, click on the Heatmap at the top of the table (next to the Table tab).

Table

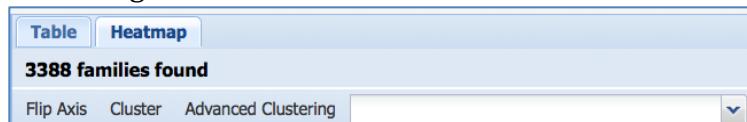
Heatmap

3388 families found

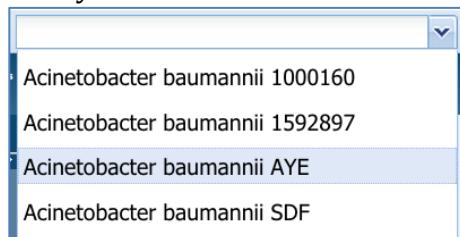
7. This will take you the heatmap view, where absence (black cells) and presence (yellow, mustard and orange cells) can be seen across all genomes. The genomes are on the y-axis, and the protein families on the x-axis.



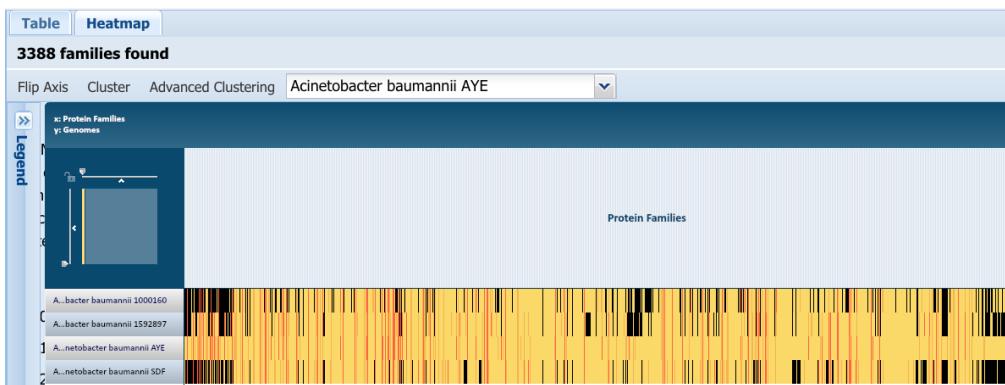
11. You can order the protein families by the way the genes occur in a given genome. This is a good way to check for something called genomic islands, which are parts of a genome that were not directly inherited, but are obtained from different bacteria in what is described as horizontal transfer. To do this using the Protein Family Sorter, click on the down arrow in the text box next to the words “Advanced Clustering.”



12. This will open up a list of genomes that are included in the groups. Scroll down until you find AYE strain of *Acinetobacter baumannii*. Click on that name.



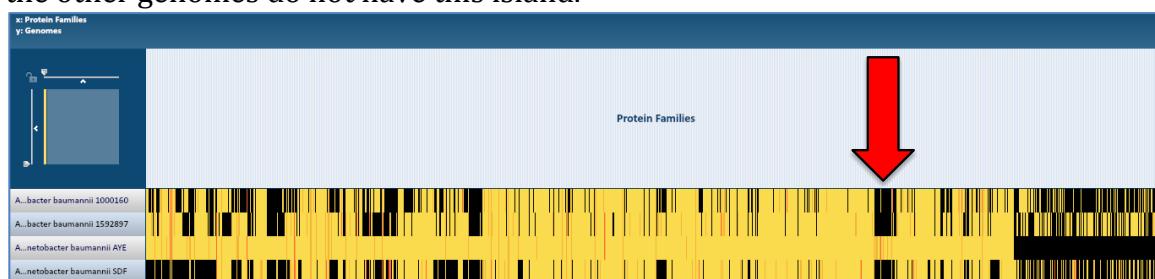
13. This will order all the protein families along the order that the genes occur in the AYE strain. You'll notice that the SDF, 1000160 and 1592897 genomes appear to have long black boxes associated with them. This indicates that these genomes are missing a long section of the genome that is present in the AYE strain. This is an indication of a genomic island.



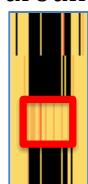
14. Fournier[4] identified a genomic island associated with resistance to antibiotics at the end of the AYE genome. Later on in this exercise, we will find the genes at each end of the island, but for now we will look for the resistance island at the end of the AYE genome. To do this, we'll need to scroll down the heatmap view by moving the slider at the bottom of the heatmap all the way to the right.



15. The island described by Fournier is found at the far right end. You can see that the other genomes do not have this island.



16. To see the genes that are in this area, you should use your mouse to draw a box around the area of the genome that is next to a black box.



17. This generates a pop-up window that gives the user choices on what they want to do with the selected data. Click the Show Proteins button at the bottom of the pop-up window.



18. This will open a new window that shows the genes found in that section of the heatmap view that you selected.

36 features found in 26 protein families							
	ID	Genome Name	PATRIC ID	RefSeq Locus Tag	Alt Locus Tag	Gene Symbol	Product
<input type="checkbox"/>	FIG01311579	Acinetobacter baumannii AYE	fig 509173.8.peg.3408	ABAYE3552	YBtAcBau09881_3408		FIG003
<input type="checkbox"/>	FIG01328895	Acinetobacter baumannii AYE	fig 509173.8.peg.3410	ABAYE3554	YBtAcBau09881_3410		Univer
<input type="checkbox"/>	FIG01624646	Acinetobacter baumannii AYE	fig 509173.8.peg.3412		YBtAcBau09881_3412		FIG003
<input type="checkbox"/>	FIG01304221	Acinetobacter baumannii AYE	fig 509173.8.peg.3413	ABAYE3560	YBtAcBau09881_3413		CdtII
<input type="checkbox"/>	FIG01545402	Acinetobacter baumannii AYE	fig 509173.8.peg.3414		YBtAcBau09881_3414		FIG003
<input type="checkbox"/>	FIG01843859	Acinetobacter baumannii AYE	fig 509173.8.peg.3417		YBtAcBau09881_3417		FIG003
<input type="checkbox"/>	FIG01558676	Acinetobacter baumannii AYE	fig 509173.8.peg.3418		YBtAcBau09881_3418		similar
<input type="checkbox"/>	FIG00067777	Acinetobacter baumannii AYE	fig 509173.8.peg.3419	ABAYE3570	YBtAcBau09881_3421	aadA1	Strepto
<input type="checkbox"/>	FIG01307316	Acinetobacter baumannii AYE	fig 509173.8.peg.3420	ABAYE3575	YBtAcBau09881_3424	int	Integr
<input type="checkbox"/>	FIG01058610	Acinetobacter baumannii AYE	fig 509173.8.peg.3425		YBtAcBau09881_3425		BsuBI
<input type="checkbox"/>	FIG00034170	Acinetobacter baumannii AYE	fig 509173.8.peg.3427	ABAYE3578	YBtAcBau09881_3427		Aminoy
<input type="checkbox"/>	FIG01311602	Acinetobacter baumannii AYE	fig 509173.8.peg.3429	ABAYE3581	YBtAcBau09881_3429	trpR	Phage
<input type="checkbox"/>	FIG01316038	Acinetobacter baumannii AYE	fig 509173.8.peg.3432	ABAYE3584	YBtAcBau09881_3432		transp
<input type="checkbox"/>	FIG01391915	Acinetobacter baumannii AYE	fig 509173.8.peg.3433		YBtAcBau09881_3433		Insert
<input type="checkbox"/>	FIG000002773	Acinetobacter baumannii AYE	fig 509173.8.peg.3442	ABAYE3605	YBtAcBau09881_3442	merA	Mercur
<input type="checkbox"/>	FIG00141620	Acinetobacter baumannii AYE	fig 509173.8.peg.3445		YBtAcBau09881_3445		Tns N
<input type="checkbox"/>	FIG01558676	Acinetobacter baumannii AYE	fig 509173.8.peg.3446		YBtAcBau09881_3446		similar
<input type="checkbox"/>	FIG00067777	Acinetobacter baumannii AYE	fig 509173.8.peg.3453	ABAYE3618	YBtAcBau09881_3453	aadA1	Strepto
<input type="checkbox"/>	FIG01319118	Acinetobacter baumannii AYE	fig 509173.8.peg.3455	ABAYE3620	YBtAcBau09881_3455	cmlA	Chlor
<input type="checkbox"/>	FIG01739024	Acinetobacter baumannii AYE	fig 509173.8.peg.3460		YBtAcBau09881_3460		transp
<input type="checkbox"/>	FIG00452786	Acinetobacter baumannii AYE	fig 509173.8.peg.3473	ABAYE3640	YBtAcBau09881_3473		Bicyclo
<input type="checkbox"/>	FIG00033623	Acinetobacter baumannii AYE	fig 509173.8.peg.3477	ABAYE3647	YBtAcBau09881_3477	strB	Aminoy
<input type="checkbox"/>	FIG00034170	Acinetobacter baumannii AYE	fig 509173.8.peg.3478	ABAYE3648	YBtAcBau09881_3478	aphE	Aminoy

19. To see the order the genes occur in, first resize the table by changing the number at the bottom of it to include all the genes and hit return.

Show	36	per page
------	-----------	----------

20. A close examination of all the results shows that I have captured data genes that appear to be ordered sequentially (ABAYE3552 to ABAYE3667), and then two distally located genes (ABAYE3739 and ABAYE3853).

Assignment

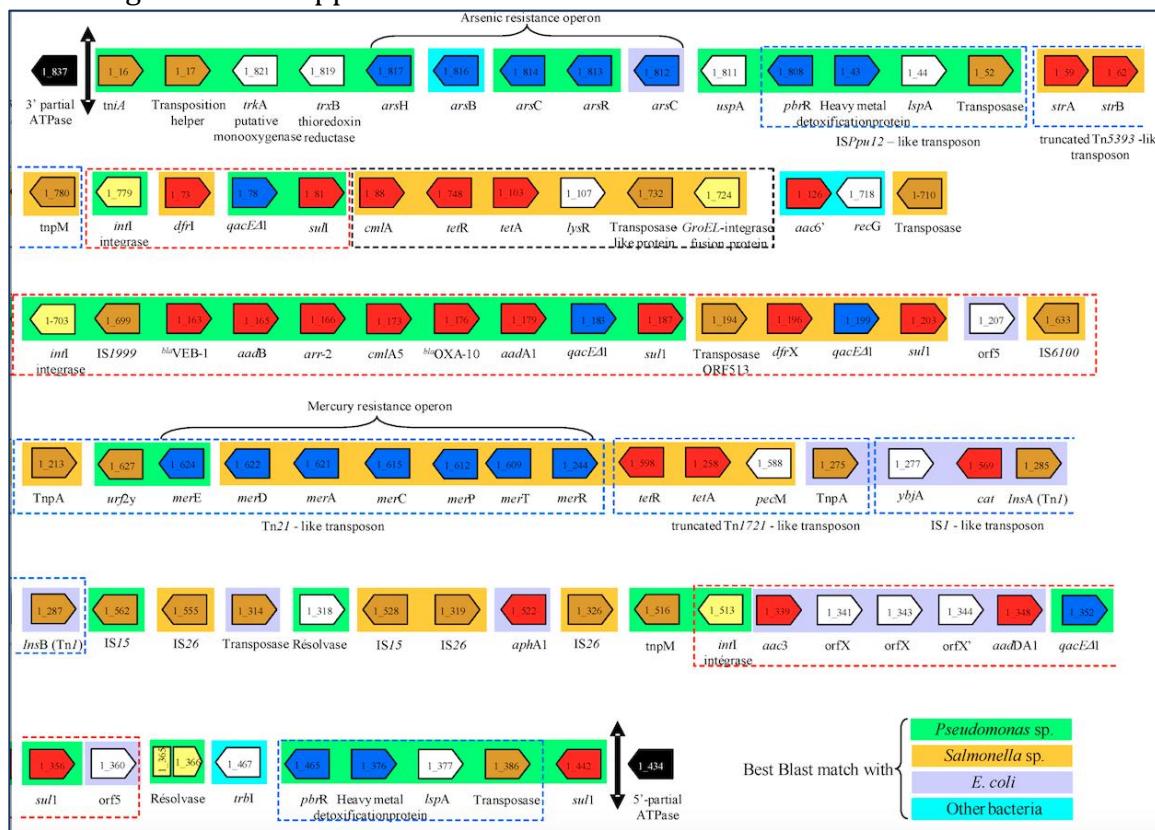
You have created other genome groups in Part I (Creating genome groups) of this exercise. Using the Protein Family Sorter tool as you have learned in this exercise, answer the following questions:

- Do you see any islands in the SDF genome that are not shared with AYE?
- How many of the genomes in each of the groups you created have the resistance island that is specific to the AYE genome?

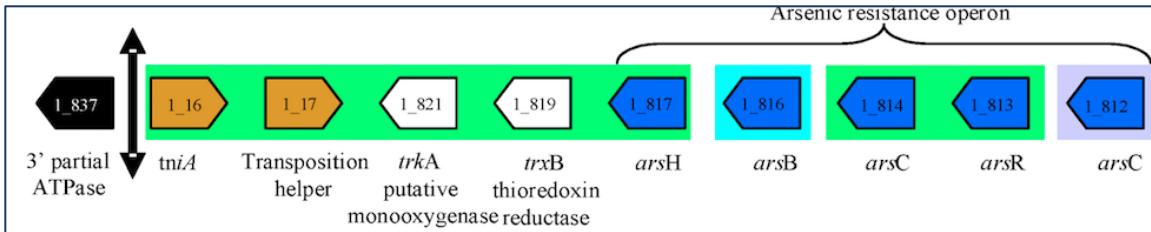
- How many of the genomes in each of the three groups you created share some of the islands that you can see that are specific to the SDF genome?

IV. Finding genes of interest from a published article

Scientists often have a difficult time taking published information and finding it in the public data repositories. The article published by Fournier and others[4] on the two *A. baumannii* genomes is a good example of this difficulty. In their article they show a detailed map of the genes available on an 86-kb resistance island that they identify in the AYE strain, providing the gene names and functional descriptions for some as well as some other information including best BLAST hits (see figure below). In this exercise, we will try to find some of those particular genes in PATRIC. At times this will be a bit frustrating, but it is supposed to demonstrate how individual researchers would need to do to find this. The end goal of this exercise is to find the genes at the beginning and end of the resistance island so that we can figure out the approximate 5' and 3' ends.



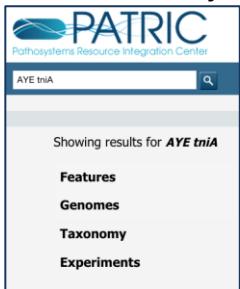
1. Fournier et al. show that the resistance island begins with a number of transposable elements and some other genes of interest, but does not show the exact chromosomal location. These genes include an arsenic resistance operon.



You may want to start by choosing the first gene that from the figure. Enter the genome strain and the gene name in the search box (AYE tniA)



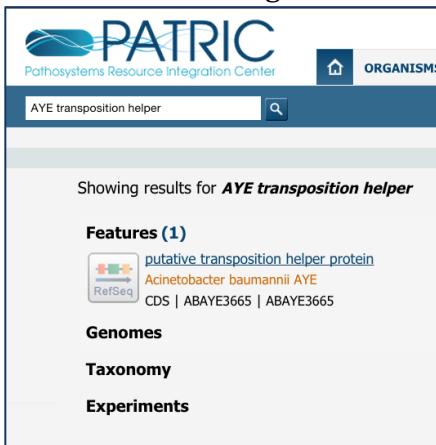
2. Unfortunately, this does not return a result.



3. Take the next gene, which is called Transposition helper, and use that to search for a corresponding gene in the AYE genome



4. This returns one gene.



5. We need to make sure that this is the right gene. To do this, you will need to click on the blue text that gives the functional description of the gene.

[putative transposition helper protein](#)

6. This takes you to the landing page for that gene. You can see the PATRIC locus tag (fig|509173.8.peg.3495), the RefSeq locus tag (ABAYE3665) and location of the start site (3692574) and the end of the gene (3693497).

Bacteria > Proteobacteria > Gammaproteobacteria > Pseudomonadales > Moraxellaceae > Acinetobacter > Acinetobacter baumannii AYE >
fig|509173.8.peg.3495 | ABAYE3665 | VBIAcBau69881_3495 | TnIB NTP-binding protein ⓘ

Overview Genome Browser Compare Region Viewer Pathways Transcriptomics Interactions Correlated Genes Literature

Add PATRIC Feature to Workspace

Gene ID	PATRIC ID: fig 509173.8.peg.3495	RefSeq: ABAYE3665	Alt Locus Tag: VBIAcBau69881_3495
Protein ID	RefSeq: YP_001715391.1	UnitProt: B0VCG0	23 IDs are mapped

View NT Sequence
View AA Sequence

External Tools

The SEED Viewer
NCBI CDD Search
STRING: Protein-Protein Interactions
STITCH: Chemical-Protein Interactions

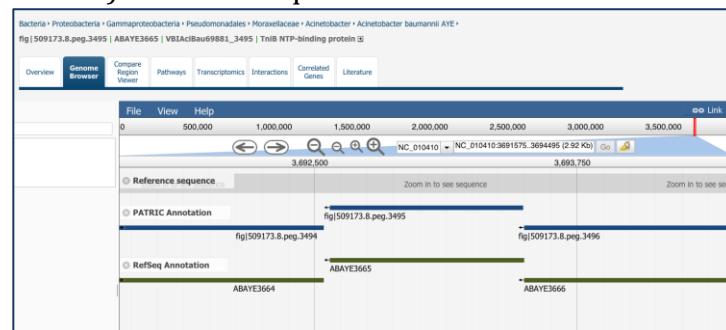
Annotation Locus Tag Start End NT Length AA Length Product

PATRIC	VBIAcBau69881_3495	3692574	3693494	921	306	TnIB NTP-binding protein
RefSeq	ABAYE3665	3692574	3693497	924	307	putative transposition helper protein

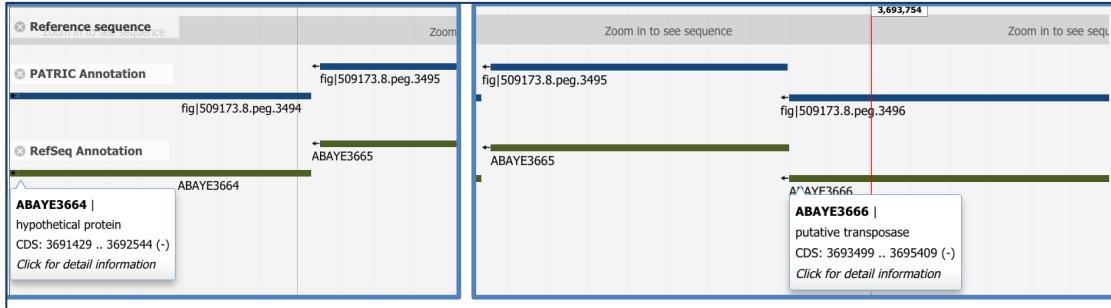
7. In order to see if we have the right gene, we will need to look at the genes that surround it to see if they match the genes described in the large figure at the top of page 12. To do this, you need to click on the second tab (Genome Browser) at the top of the page.



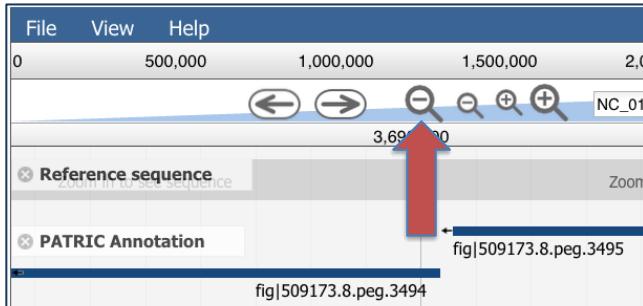
8. This will load the genome browser that shows two different annotations in the same view. The top (blue arrows) is the PATRIC annotation. The bottom (green arrows) is the RefSeq annotation.



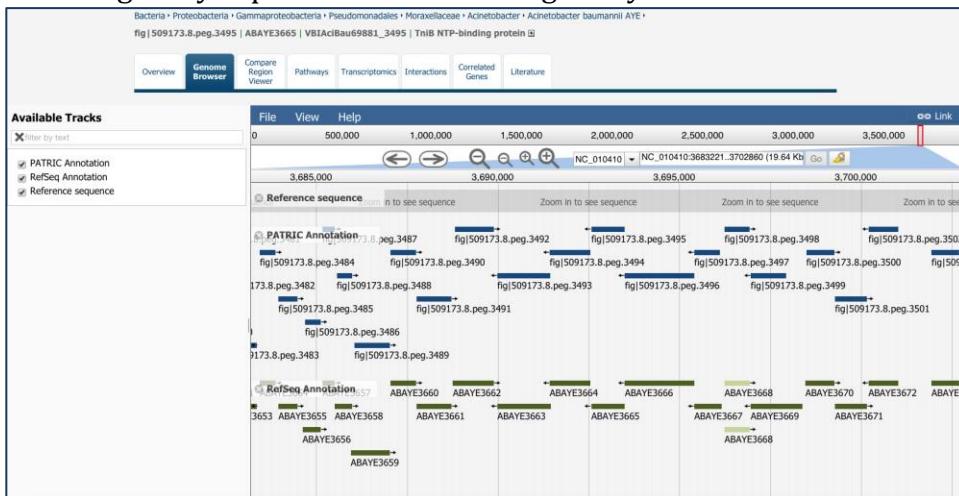
9. Mousing over the genes on either side will show their names. Although the transposase at the 3' end makes sense, the hypothetical protein at the 3' end still does not tell us that we are looking at the right gene.



10. We need to expand the view of the browser. To do this, click on the magnifying glass with the large minus sign in it.

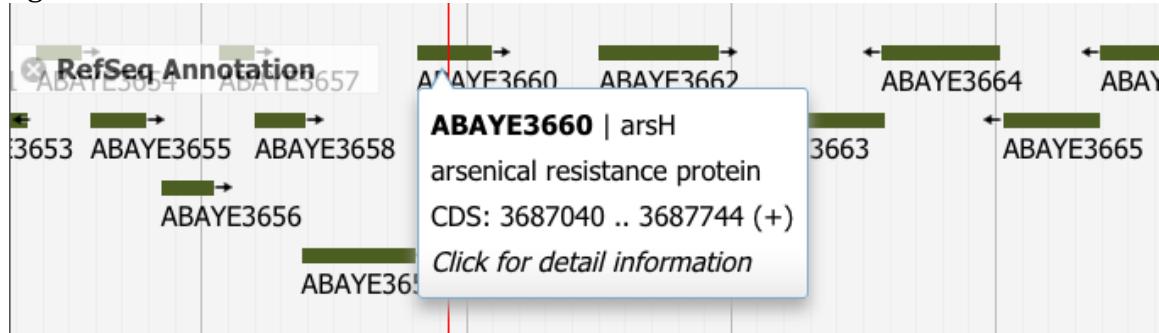


11. This greatly expands the number of genes you see.



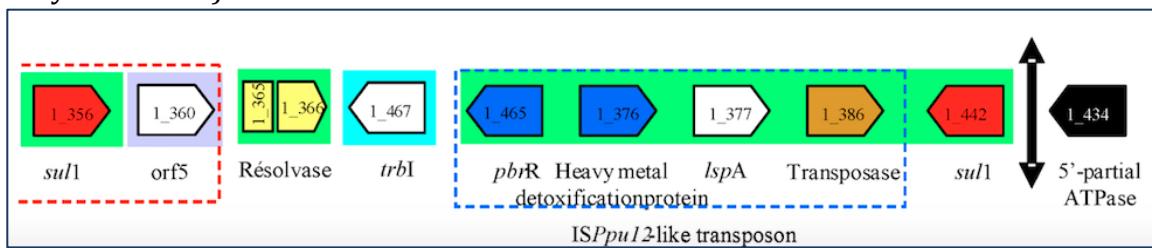
12. Mousing over the genes shows the names. ABAYE3660 corresponds to the arsenic resistance protein (*arsH*) that is mentioned in the figure on page 12 of this workflow. We have identified that this corresponds to the same *arsH* gene that Fournier et al shows in the diagram, and now we know that one end of the resistance island is right around the gene identified by the locus tag fig|509173.8.peg.3495 (PATRIC) and ABAYE3665 (RefSeq). You'll notice that the

genome browser show the genes in the reverse orientation that you see in the figure.



Assignment

- Find the other end of the resistance island in the AYE strain based on the part of the diagram shown below (Hint! You can use the information we found in III.20 above. I also recommend using the work “detoxification” as part of your search).



IV. Examining gene neighborhoods

The Fournier paper detailed a careful BLAST analysis that they performed to determine where the genes in the resistance islands came from. PATRIC provides a tool called the Compare Region View that can help to determine if a region has been laterally transferred. You can find any gene of interest using the global search, but I also want to introduce you to a powerful function that PATRIC has in allowing you to filter the feature table. Keep in mind that the global search is a more direct approach, but try to learn how to use the feature table to get specific information you are interested in.

- In the global search box, enter the word AYE and hit return.

- In the Search results page, click on the genome name *Acinetobacter baumannii* AYE

3. This will take you to the landing page for the AYE genome.

4. Click on the 5th tab over, the Features Tab.

5. This will show you all the genes in the genome.

RefSeq ID	Alt Locus Tag	Gene Symbol	Product Description
fj 509173.8.peg.3712	ABAYE3903	VBlAcBau69881_3712	rpmlH
fj 509173.8.peg.3711	ABAYE3902	VBlAcBau69881_3711	rmpA
fj 509173.8.peg.3710	ABAYE3901	VBlAcBau69881_3710	Protein YidC
fj 509173.8.peg.3709	ABAYE3900	VBlAcBau69881_3709	oxaA
fj 509173.8.peg.3708	ABAYE3899	VBlAcBau69881_3708	trmE
fj 509173.8.peg.3707	ABAYE3898	VBlAcBau69881_3707	Uncharacterized protein YalN in formaldehyde detoxification operon
fj 509173.8.peg.3706	ABAYE3897	VBlAcBau69881_3706	Cobalt-zinc-cadmium resistance protein CzcD
fj 509173.8.peg.3705	ABAYE3895	VBlAcBau69881_3705	Mobile element protein
fj 509173.8.peg.3704	ABAYE3894	VBlAcBau69881_3704	Cardiolipin synthetase (EC 2.7.8.-)
fj 509173.8.peg.3703	ABAYE3893	VBlAcBau69881_3703	Cardiolipin synthetase (EC 2.7.8.-)

6. Look at the filter mechanism across the top of this table.

Feature Type:	CDS	Annotation:	PATRIC	Keyword:	<input type="button" value="Filter Table"/>
---------------	-----	-------------	--------	----------	---

7. Enter your gene of interest into the keyword search (I used “aphE”) and click on the Filter Table button

Keyword:	aphE	<input type="button" value="Filter Table"/>
----------	------	---

8. This will filter the table to show the locus tags and information associated with aphE for this genome.

1 features found					
Workspace	View	Download	Tools	Columns	
Add Feature(s)	Fasta DNA Fasta Protein	Table FASTA	Pathway Summary Multiple Seq Alignment MAP IDs to...	Show/Hide Default	
<input type="checkbox"/> PATRIC ID	RefSeq Locus Tag	Alt Locus Tag	Gene Symbol	FIGfam ID	Product Description
fig 509173.8.peg.3478	ABAYE3648	VBIaciBau69881_3478	aphE	FIG00034170	Aminoglycoside 3'-phosphotransferase (EC 2.7.1.95)

9. Any of the three locus tags can be clicked to take you to the information on that gene. Click on any one of these (they are blue and underlined).

PATRIC ID	RefSeq Locus Tag	Alt Locus Tag
fig 509173.8.peg.3478	ABAYE3648	VBIaciBau69881_3478

10. This will open up the gene-landing page for the aphE gene.

Bacteria • Proteobacteria • Gammaproteobacteria • Pseudomonadales • Moraxellaceae • Acinetobacter • Acinetobacter baumannii AYE • fig|509173.8.peg.3478 | ABAYE3648 | VBIaciBau69881_3478 | aphE | Aminoglycoside 3'-phosphotransferase (EC 2.7.1.95) □

Overview	Genome Browser	Compare Region Viewer	Pathways	Transcriptomics	Interactions	Correlated Genes	Literature
Add PATRIC Feature to Workspace							
View NT Sequence							
View AA Sequence							
External Tools							
The SEED Viewer							
NCBI CDD Search							
STRING: Protein-Protein Interactions							
STITCH: Chemical-Protein Interactions							
Recent PubMed Articles							
No pubmed record is available. Please try PMC or Google Scholar							
Functional Properties							
GO Assignments							
EC Assignments							
FIGfam Assignments							

aphE

CDS

Annotation	Locus Tag	Start	End	NT Length	AA Length	Product
PATRIC	VBIaciBau69881_3478	3679656	3680489	834	277	Aminoglycoside 3'-phosphotransferase (EC 2.7.1.95)
RefSeq	ABAYE3648	3679656	3680492	837	278	streptomycin 3'-kinase (streptomycin 3'-phosphotransferase) (streptomycin 6-phosphotransferase) (streptomycin 6-kinase)

11. To examine the gene neighborhood, click on the 3rd tab at the top of the page (Compare Region Viewer).

Overview	Genome Browser	Compare Region Viewer
--------------------------	--------------------------------	---------------------------------------

12. This will show you the gene neighborhood around your gene of interest, and also show you the genomes that have similar neighborhoods. Look at the screenshot below. The two top hits are for *Acinetobacter baumannii* genomes, but the next three are for *Salmonella* genomes. As there are currently 1025 *A. baumannii* genomes in PATRIC, you would expect that if this were common to this species, the image would contain only genomes from this species. AYE3648 corresponds to the gene identified as *strA* in the Fournier paper. That gene is one that they identified as having the best BLAST match with *Salmonella* sp.



Assignment

- Open up the feature tables for both the AYE and SDF genomes.
- Identify the genes that correspond to the two islands described by Fournier et al. (see the figures above in the description).
- Using Command Click, go to the gene-landing page for each of the genes and look at the Compare Region View. Which genes appear to match the best BLAST hits that Fournier et al described?

V. Discovering antibiotic resistance genes from other genomes using homology

1. Enter the name *Acinetobacter baumannii* into the global search box and hit return.



2. This will take you to the search results page. Scroll down until you see the Taxonomy section. Click on *Acinetobacter baumannii* (species).

Showing results for ***Acinetobacter baumannii***

Features (7351853)

- 49124..49331
RefSeq
Acinetobacter baumannii
misc_binding
- 63665..64035
RefSeq
Acinetobacter baumannii
misc_feature
- 42046..42133
RefSeq
Acinetobacter baumannii
misc_binding

Genomes (1066)

- Acinetobacter baumannii W6976*
459 Contigs
SEQUENCED: 2012-02-21 (University of Birmingham)
COMMENT: -
- Acinetobacter baumannii W7282*
118 Contigs
SEQUENCED: 2012-02-21 (University of Birmingham)
COMMENT: -
- Acinetobacter baumannii 1605*
127 Contigs
SEQUENCED: 2013-07-26 (JCVI)
COMMENT: Isolate 1605

Taxonomy (1056)

- Acinetobacter baumannii* (species)
1025 Genome(s)
- Acinetobacter baumannii VEB* (no rank)
0 Genome(s)



3. This will take you to the landing page for the *A. baumannii* species.

Bacteria > Proteobacteria > Gammaproteobacteria > Pseudomonadales > Moraxellaceae > *Acinetobacter* > *Acinetobacter baumannii*

Data Overview Tutorial »
Download genome data »

Overview **Taxonomy** **Phylogeny** **Genome List** **Feature Table** **Specialty Genes** **Protein Families** **Pathways** **Transcriptomics** **Interactions** **Diseases** **Literature**

Search Tools

- GF Genome Finder
- FF Feature Finder
- CP Comparative Pathway Tool
- PFS Protein Family Sorter

Taxonomy Summary

Taxonomy ID	470
Lineage	cellular organisms > Bacteria > Proteobacteria > Gammaproteobacteria > Pseudomonadales > Moraxellaceae > <i>Acinetobacter</i> > <i>Acinetobacter calcoaceticus/baumannii complex</i> > <i>Acinetobacter baumannii</i>
External Links	Immune Epitope Database and Analysis Resource

Summary Terms - Click on number to view genomes associated with term ([see PATRIC FAQS](#))

Genome Status	WGS (1004), Complete (21)
Reference Genome	Representative (1)
Antimicrobial Resistance	Susceptible (13), Resistant (5), show all 19 genomes
Antimicrobial Resistance Evidence	Comment (14), AMR Panel (2)
Isolation Country	USA (741), China (63), show all 895 genomes
Host Name	Human, Homo sapiens (979), Body louse, Pediculus humanus (1), show all 982 genomes
Disease	Nosocomial infections (23), Pneumonia (10), show all 34 genomes
Collection Date	2007 (51), 2008 (37), show all 411 genomes
Completion Date	2014 (771), 2013 (118), show all 1018 genomes

[View all genomes and summary terms »](#)

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2015 Jun 15

4. Click on the 6th tab at the top of the page, Specialty Genes.

Overview **Taxonomy** **Phylogeny** **Genome List** **Feature Table** **Specialty Genes**

5. This will take you to the Specialty Gene landing page for that species. There is a filter on the left-hand side, and a table showing the results on the right.

The screenshot shows the PATRIC interface for the 'Specialty Genes' landing page. At the top, there's a navigation bar with tabs: Overview, Taxonomy, Phylogeny, Genome List, Feature Table, Specialty Genes (which is the active tab), Protein Families, Pathways, Transcriptomics, Interactions, Diseases, and Literature. Below the navigation bar, a message states: "For this taxonomy/genome level, the list below provides Specialty Genes, i.e. genes that are of particular interest to the infectious disease researches, such as virulence factors, antibiotic resistance genes, drug targets, and human homologs. Genes with the designation 'Literature' in the Evidence field have been experimentally verified. Those with Evidence designated as 'BLASTP' are identified based on sequence homology. For more details, see Specialty Genes FAQs." On the left, there's a 'Filter By' sidebar with sections for Property (Human Homolog, Antibiotic Resistance, Drug Target, Virulence Factor), Source (Human, CARD, ARDB, Vectors, VFDB, TTD, PATRIC_VF), and Evidence (BLASTP, Literature). Below these are filters for Query Coverage (>= 0), Subject Coverage (>= 0), and Identity (>= 0). A 'Filter' button is at the bottom of the sidebar. The main area displays a table titled 'Specialty Genes' with 138352 records found. The table has columns: Evidence, Property, Source, Genome Name, PATRIC ID, RefSeq Loc, Alt Locus Tag, Source ID, Gene, Product, Pubmed, and Identity. The table lists various genes from Acinetobacter baumannii, including their source (Human, CARD, VFDB, TTD, etc.), evidence type (BLASTP, Vectors, VFDB, CARD, TTD), and various gene products like S-formylglutathione synthetase, Glutaryl-CoA dehydratase, NADH-ubiquinone oxidoreductase, SSU ribosomal protein, Translation elongation factor, Betaine aldehyde dehydrogenase, FKBp-type peptidyl-prolyl isomerase, Membrane fusion component, Inner membrane component, 5-methyltetrahydrofolate reductase, Transcriptional regulator, Argininosuccinate synthetase, and Mobile element protein. Some entries have a 'PubMed' link next to them.

6. To find the genes that have homology to antibiotic resistance genes that have been identified, click on the text box in the filter that is in front of “Antibiotic Resistance.”

This screenshot shows the same PATRIC interface as above, but with a different filter applied. In the 'Filter By' sidebar, under the 'Evidence' section, the 'BLASTP' checkbox is checked. This narrows the search results to 57745 records found. The table on the right now only lists genes with BLASTP evidence, all of which are associated with 'Antibiotic Resistance'. The columns shown are Evidence, Property, Source, and Gene.

7. PATRIC has several sources of data for specialty genes. For antibiotic resistance genes we include genes from the Comprehensive Antibiotic Resistance Database (CARD)[5] and from the Antibiotic Resistance Database (ARDB)[6]. Click on the text box in the filter that is in front of word “CARD.”

Filter By

keyword Clear All

Property (49681)
 clear
 Antibiotic Resistance (49681)

Source (57745)
 clear
 CARD (49681)
 ARDB (8064)

Evidence (49681)
 BLASTP (45516)
 Literature (4165)

Specialty Genes
49681 records found

Workspace View
Add Feature(s) FASTA DNA FASTA Protein

	Evidence	Property	Source
<input type="checkbox"/>	BLASTP	Antibiotic Resistance	CARD
<input type="checkbox"/>	BLASTP	Antibiotic Resistance	CARD
<input type="checkbox"/>	BLASTP	Antibiotic Resistance	CARD
<input type="checkbox"/>	BLASTP	Antibiotic Resistance	CARD

8. You can also filter on genes that have experimental evidence that has been published, as opposed to BLAST-only homology. Click on the text box in front of the word “Literature” to see genes that have experimental evidence.

Filter By

keyword Clear All

Property (1410)
 clear
 Antibiotic Resistance (1410)

Source (1832)
 clear
 CARD (1410)
 ARDB (422)

Evidence (15137)
 clear
 BLASTP (13727)
 Literature (1410)

8. You can also filter genes by the BLAST scores. Scroll down to the bottom of the filter box where you will see additional filters

Filter BLAST Hits by:

Source organism
 Same Genome
 Same Species
 Same Genus
 All

% Query Coverage >=

% Subject Coverage >=

% Identity >=

Filter

9. Using the arrows behind the numbers, change the values for % Query, % Subject and % Identity to 100%.

Filter BLAST Hits by:

Source organism

- Same Genome
- Same Species
- Same Genus
- All

% Query Coverage >=

% Subject Coverage >=

% Identity >=

Filter

10. Each of these filters will be reflected in the changing number at the top of the table on the right.

Specialty Genes

1410 records found

11. The genes are reflected in the table.

Specialty Genes														
1410 records found														
Workspace		View		Download		Tools		Columns				Help		
<input type="button" value="Add Feature(s)"/>		<input type="button" value="FASTA DNA"/>	<input type="button" value="FASTA Protein"/>	<input type="button" value="Table"/>	<input type="button" value="FASTA"/>	<input type="button" value="Pathway Summary"/>	<input type="button" value="Multiple Seq Alignment"/>	<input type="button" value="Show/Hide"/>	<input type="button" value="Default"/>			<input type="button" value="PATRIC FAQs"/>		
	Evidence	Property	Source	Genome Name ▲		PATRIC ID	RefSeq Loc	Alt Locus Tag	Source ID	Gene	Product	Pubmed	Identity	E-value
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.135			NC_011...		Beta-lactamase	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.577			AF0246...		Aminoglycoside 3'-ph...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.731			NC_011...		putative DNA binding...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.1181			NC_011...		Transcriptional regul...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.1530			NC_011...		Maltose O-acetyltran...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.1819			NC_011...		RND efflux system, ...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.2094			NC_011...		Topoisomerase IV su...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.2239			NC_010...		Membrane fusion co...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.2240			NC_010...		Inner membrane co...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.2341			NC_011...		Transcriptional regul...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.2372			NC_011...		Chloramphenicol ace...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.3024			AY7509...		Beta-lactamase	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.3487			NC_010...		Ethidium bromide-m...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_593.peg.3879			NC_011...		DNA-directed RNA p...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.74			NC_011...		Beta-lactamase	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.82			NC_010...		Spectinomycin 9-O-a...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.85			NC_011...		Gentamicin 3'-N-acet...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.147			HQ4510...		ribosomal RNA meth...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.216			NC_011...		Transcriptional regul...	100	0	
	Literature	Antibiotic Resistance	CARD	Acinetobacter bauma...		fig 470_576.peg.297			NC_010...		Inner membrane co...	100	0	

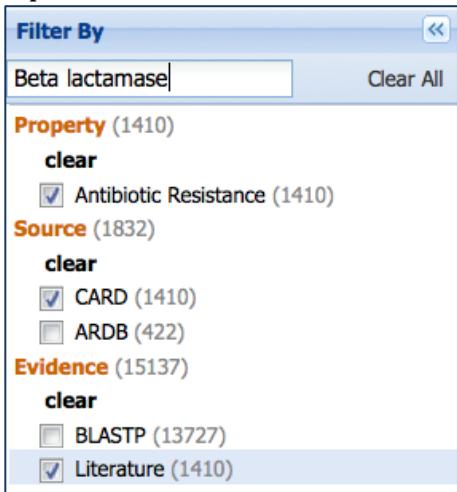
12. To sort the genes by name, click once on the Product column head.

Product ▲
Beta-lactamase

13. This will sort the gene alphabetically by their product description.

	Evidence	Property	Source	Genome Name	PATRIC ID	RefSeq Loc	Alt Locus Tag	Source ID	Gene	Product ▾
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 470.593.peg.577			AF02460...		Aminoglycoside 3'-ph...
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 470.771.peg.3386			X13542....		Aminoglycoside N(3')...
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1449755.3.peg.31...			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1449755.3.peg.37...			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458921.3.peg.175			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458921.3.peg.35...			AY75090...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458926.3.peg.982			AY75090...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458926.3.peg.23...			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458922.3.peg.23...			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458922.3.peg.26...			AY75090...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458925.3.peg.278			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458923.3.peg.132			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458920.3.peg.12...			NC_011...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458920.3.peg.28...			AY75090...		Beta-lactamase
	Literature	Antibiotic Resistance	CARD	Acinetobacter b...	fig 1458924.3.peg.121			NC_011...		Beta-lactamase

14. You can use the filter on the left to sort for specific names. In the text box at the top of the filter, enter the words “Beta lactamase” and hit return.



15. The experimental evidence that validates Beta lactamase as being important in antibiotic resistance could come from experiments that researchers have done on different genomes, which would mean that the source of the experimental data could come from different genes. To see the source of the evidence you will need to expand the Source column head by grabbing the right side of the header and then moving it to the right.

Source ID	Source ID
NC_011...	NC_011586.7044972.p01
AY7509...	AY750909.1.gene1.p01
NC_011...	NC_011586.7044972.p01
AY7509...	AY750909.1.gene1.p01
	NC_011586.7044972...01

16. Click on the Source ID column head to sort the results.

17. Resize the page to see all the genes with the Beta lactamase description. Enter 150 in the text box at the bottom of the table and hit return.

Show	150	per page
------	-----	----------

16. You can see that there are several sources of the experimental evidence that define Beta lactamase as being important in antibiotic resistance.

136 records found													
	Evidence	Property	Source	Genome Name	PATRIC ID	RefSeq Lc	Alt Locus Tag	Source ID	Gene	Product	Pubmed	Identity	E-value
<input type="checkbox"/>	Literat...	Antibiotic Resista...	CARD	Acinetobacter ba...	fig 1236532.3.pe...			AM279652.1.gene1.p01		Beta-lactamase	100	0	
<input type="checkbox"/>	Literat...	Antibiotic Resista...	CARD	Acinetobacter ba...	fig 470.771.peg...			AY750908.1.gene1.p01		Beta-lactamase	100	0	
<input type="checkbox"/>	Literat...	Antibiotic Resista...	CARD	Acinetobacter ba...	fig 470.553.peg...			AY750908.1.gene1.p01		Beta-lactamase	100	0	
<input type="checkbox"/>	Literat...	Antibiotic Resista...	CARD	Acinetobacter ba...	fig 1458921.3.pe...			AY750909.1.gene1.p01		Beta-lactamase	100	0	
<input type="checkbox"/>	Literat...	Antibiotic Resista...	CARD	Acinetobacter ba...	fig 1458926.3.pe...			AY750909.1.gene1.p01		Beta-lactamase	100	0	

17. Many of these genes have “AY750909.1.gene1.p01” as the source. In the text box, enter “AY750909.1.gene1.p01” and hit return

Filter By	<input type="text" value="AY750909.1.gene1.p01"/>	<input type="button" value="Clear All"/>
Properties (53)		

18. This will show the genes that had AY750909.1.gene1.p01 as the gene that had the original evidence linking it to antibiotic resistance.

53 records found													
	Specialty Genes			Tools									
	Evidence	Property	Source	Genome Name	PATRIC ID	View	Download	Tools	Pathway Summary	Multiple Seq	MAP IDs to...	Help	PATRIC FAQs
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.587.peg.1135								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.601.peg.3580								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.600.peg.2651								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.594.peg.2680								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.596.peg.2456								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.597.peg.2895								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.604.peg.1458								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.599.peg.3624								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.602.peg.3184								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.581.peg.3119								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.583.peg.1465								
<input type="checkbox"/>	Literature	Antibiotic Resistance	CARD	Acinetobacter baumannii	fig 470.582.peg.1404								

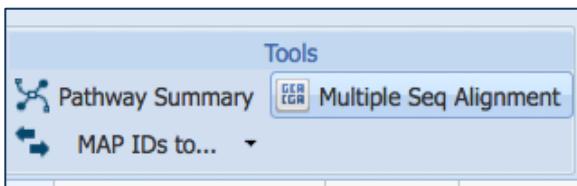
19. Resize the table to see all the genes

Show	53	per page
------	----	----------

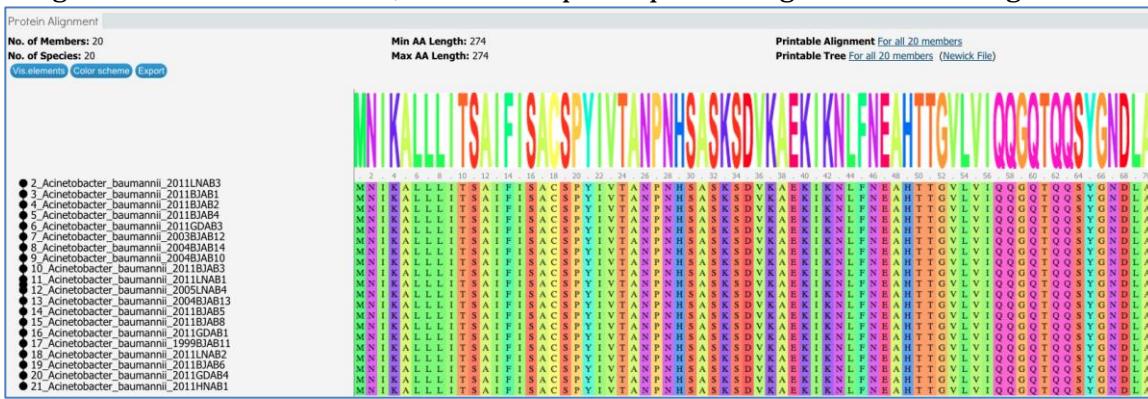
20. Let's look at a multiple sequence alignment for these genes. To do this you must first select all of them. Click in the box in the column head in front of the word “Evidence.” This will select all the genes.



21. Above the table in the box labeled "Tools" click "Multiple Sequence Alignment."



22. This will open a new window with the multiple sequence alignment from all the genes that were selected. Included is a gene tree showing the relationships among the genes selected on the left, and a multiple sequence alignment on the right.



Assignment

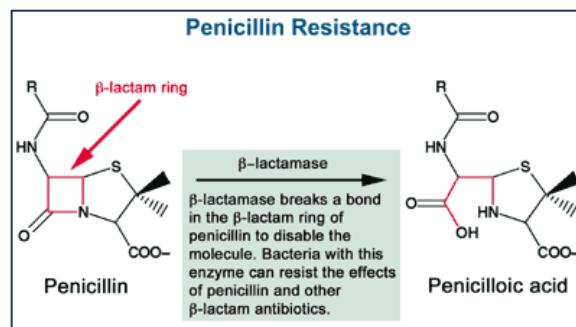
- Use the filter on the Specialty Gene landing page for *Acinetobacter baumannii* to find genes with lower Query coverage and check the multiple sequence alignment for some that have the same product description.
- Use the filter on the Specialty Gene landing page for *Acinetobacter baumannii* to find genes with lower Subject coverage and check the multiple sequence alignment for some that have the same product description.
- Use the filter on the Specialty Gene landing page for *Acinetobacter baumannii* to find genes with lower % Identity and check the multiple sequence alignment for some that have the same product description
- What happens when you compare genes with the same product description, but come from different sources?

Extra Assignment: Exploring the Beta lactamase gene family.

Beta-lactam antibiotics are structurally related to penicillin, which inhibits synthesis of the bacterial cell wall. Beta lactamase enzymes cleave the four-atom lactam ring, rendering these antibiotics harmless to the bacteria. Researchers found that the beta-lactamase OXA-23 gene in *A. baumannii* was flanked by insertion sequences, suggesting acquisition by horizontal transfer[7].

Using what you have learned above, please explore the Beta lactamases from *A. baumannii* in the following ways:

- How many different genes act as the source for the Beta lactamase genes you see in the Specialty Genes page for *A. baumannii*?
- Using the multiple sequence alignment and the gene tree tools, explore which alignments map to the specific Source ID genes.
- Using the genes in the Source ID, can you tell many Beta lactamase genes each of the genomes have?
- When you look at the genomes that result from filtering the Specialty Gene page data as described above, you will not see the *A. baumannii* AYE genome. Does the AYE genome have any genes that have the words “Beta lactamase” in the functional description? How many?
- If you are able to find Beta lactamase genes in the AYE genomes, generate an alignment with those genes and the genes you saw on the Specialty Gene page (Hint: You will have to add genes to different groups when you are in your Workspace). Do any of the AYE genes have strong homology to any of the genes we saw and built alignments for previously?
- Using the Compare Region Viewer and/or the Genome Browser, choose some of these Beta lactamase genes and see if they are flanked by insertion sequences (transposases or mobile elements).



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