
How to Explore the ESKAPE Pathogens Using MORF Genomes

A step-by-step tutorial for researchers, educators, and students

MORF Genomes | morf-db.org | A MORF Demos Project

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Introduction

MORF Genomes is a web-based platform that allows you to explore, compare, and analyse microbial genome sequences without any coding or specialist bioinformatics knowledge. This tutorial walks you through the key features of MORF using the ESKAPE pathogens as an example. The ESKAPE pathogens are six antibiotic-resistant bacteria responsible for the majority of life-threatening hospital-acquired infections worldwide.

By the end of this tutorial you will be able to

- Navigate the ESKAPE Pathogens project in MORF
- Browse individual genome pages and search for specific genes

- Use the Genome Features tool to see what AMR genes are present in the different ESKAPE pathogens
- Interpret ortholog analysis results to compare genomes
- Use what you have learned from MORF to work through the exercises in section 5.

Section 1: Getting started

Accessing public projects in MORF

Go to <https://morf-db.org/projects/home> you will see All Projects. Click on the **ESKAPE Pathogens** tile to open the project, or use morf-db.org/projects/MGR00010/MORF000158

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Project Home

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Genome Browser

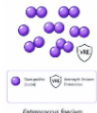
MORF / Projects / MORF Demos / ESKAPE Pathogens / Project Home

ESKAPE Pathogens

The ESKAPE pathogens - *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. - are responsible for the majority of life-threatening hospital-acquired infections worldwide. Their name reflects both their identities and their ability to 'escape' the effects of antibiotics, making them among the most dangerous organisms in modern medicine.

This project contains the complete genome sequences of example strains all six ESKAPE pathogens. You can browse each genome individually to explore annotated genes, virulence factors, and antibiotic resistance determinants, or compare all six genomes simultaneously using the ortholog analysis tool to identify shared and unique gene families. The BLAST Search tool can be used to search for specific genes of interest across any of the six genomes.

Project Highlights



Enterococcus faecium
Enterococcus faecium Aus0004



Staphylococcus aureus
Staphylococcus aureus subsp. *aureus* NCTC 8325



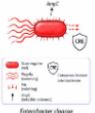
Klebsiella pneumoniae
Klebsiella pneumoniae subsp. *pneumoniae* MGH 78578



Acinetobacter baumannii
Acinetobacter baumannii AB5075-UW



Pseudomonas aeruginosa
Pseudomonas aeruginosa PAO1



Enterobacter cloacae
Enterobacter cloacae subsp. *cloacae* ATCC 13047

Browse All Data

Genomes	Orthologs	BLAST
<i>Enterococcus faecium</i> Aus0004 <i>Staphylococcus aureus</i> subsp. <i>aureus</i> NCTC 8325 <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> MGH 78578 <i>Acinetobacter baumannii</i> AB5075-UW <i>Pseudomonas aeruginosa</i> PAO1 <i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047	<i>A. baumannii</i> vs <i>P. aeruginosa</i> vs <i>E. faec...</i>	- Searching <i>S. aureus</i> NCTC 8325 with <i>S. au...</i> - Searching <i>E. faecium</i> Aus0004 with <i>S. aur...</i> - Searching <i>K. pneumoniae</i> MGH 78578 with <i>S...</i> - Searching <i>A. baumannii</i> with <i>S. aureus</i> al... - Searching <i>P. aeruginosa</i> PAO1 with <i>S. aur...</i> - Searching <i>E. cloacae</i> ATCC 13047 with <i>S....</i> +16 more (show all)

 **Screenshot:** The ESKAPE Pathogens project homepage showing the six genome entries and navigation options

Tip: The MORF Demos are publicly available through **All Projects** at the top of the side bar. You do not need a subscription to browse these projects, which includes all our Public and Demo projects.

Section 2: Browsing Individual Genomes

Navigating to a Genome Page

For each of the ESKAPE organism, a graphic shows their main features, including their morphology, motility, key antibiotic resistance property and if they are Gram positive or negative. Each pathogen has its own genome page where you can browse all annotated genes, view the circular genome map, and explore individual gene entries.

Step 1 From the ESKAPE Pathogens project homepage, click on any of the six summary images to see a larger view of the features of this bacterium, and on the name below this to link through to the Genome page. For example, click [Staphylococcus aureus subsp. aureus NCTC 8325](#).

Step 2 You will see the genome page showing the circular genome map and a linear view below it. Scroll down to find the Genome Details section showing the accession number, taxonomy, genome size and total feature count.

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ESKAPE Pathogens

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Genomes

Enterococcus faecium Aus0004

Staphylococcus aureus subsp. aureus NCTC 8325

Klebsiella pneumoniae subsp. pneumoniae MGH 78578

Acinetobacter baumannii AB5075-UW

Pseudomonas aeruginosa PAO1

Enterobacter cloacae subsp. cloacae ATCC 13047

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MORF / Projects / MORF Demos / ESKAPE Pathogens / Genomes / Staphylococcus aureus subsp. aureus NCTC 8325 / View

Staphylococcus aureus subsp. aureus NCTC 8325

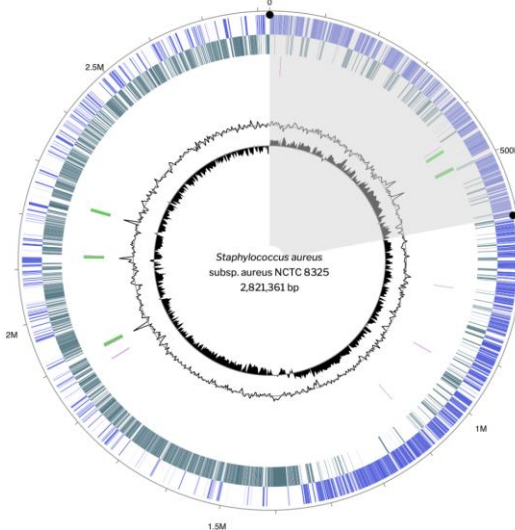
Search by: Keyword Search for a gene or feature

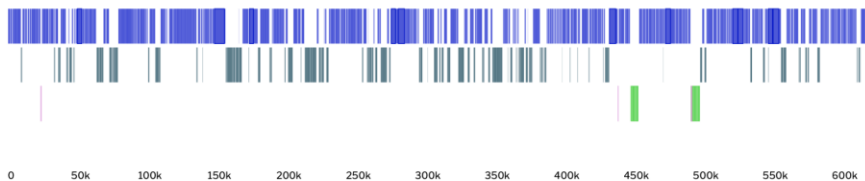
Show CDS

Show GC%

Show GC Skew

Show Stable RNAs





Genome Details [Edit](#)

Accession: GCF_000013425.1

Organism: Staphylococcus aureus subsp. aureus NCTC 8325

Taxonomy: Bacteria > Firmicutes > Bacilli > Bacillales > Staphylococcaceae > Staphylococcus

Chromosomes (1): 28,21,361 bp

Total Size: 28,21,361 bp

Features: 2872 genes, 2767 CDSs, 16 rRNAs, 61 tRNAs

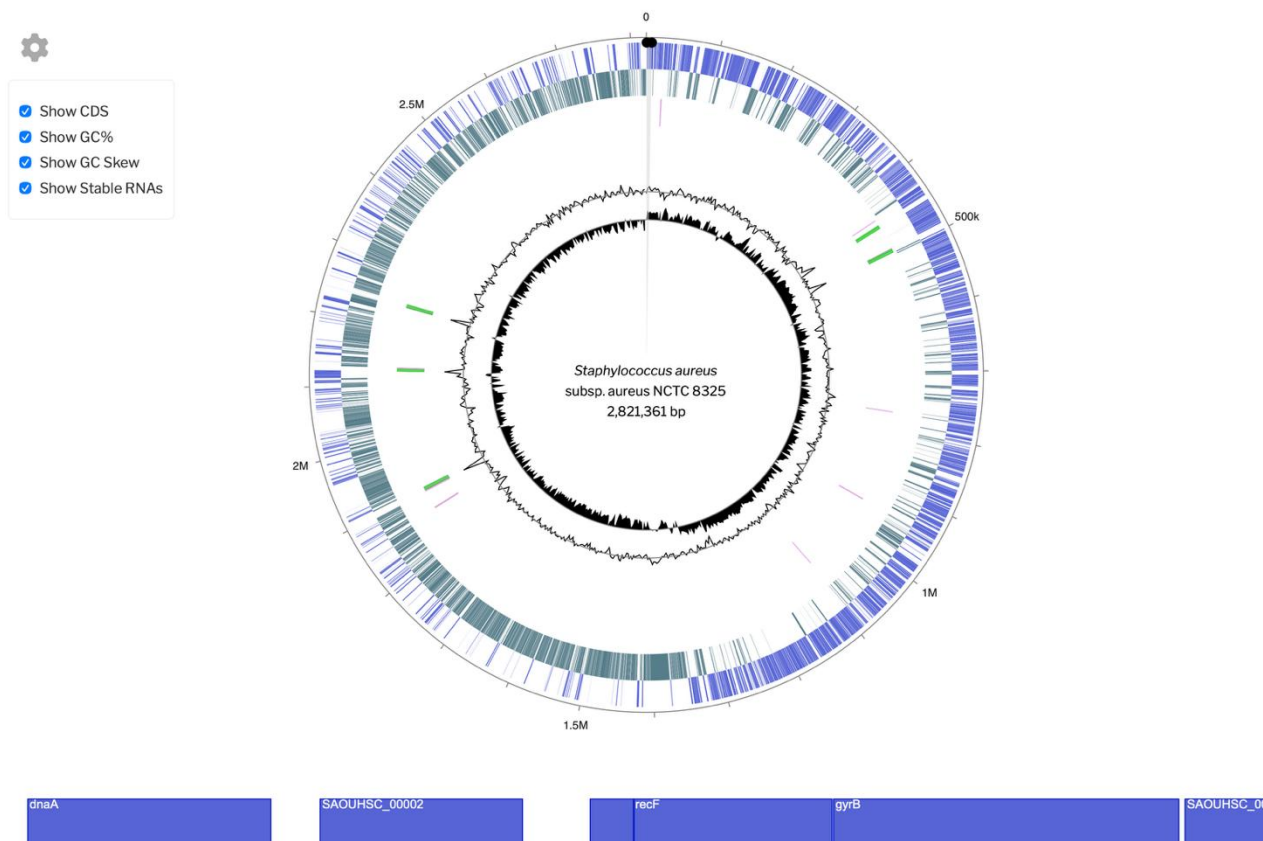
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Page 4

Screenshot: The *S. aureus* genome page showing the circular genome map with search options, and the Genome Details section below showing accession, taxonomy and feature count

What can we learn from viewing the circular *Staphylococcus aureus* genome?

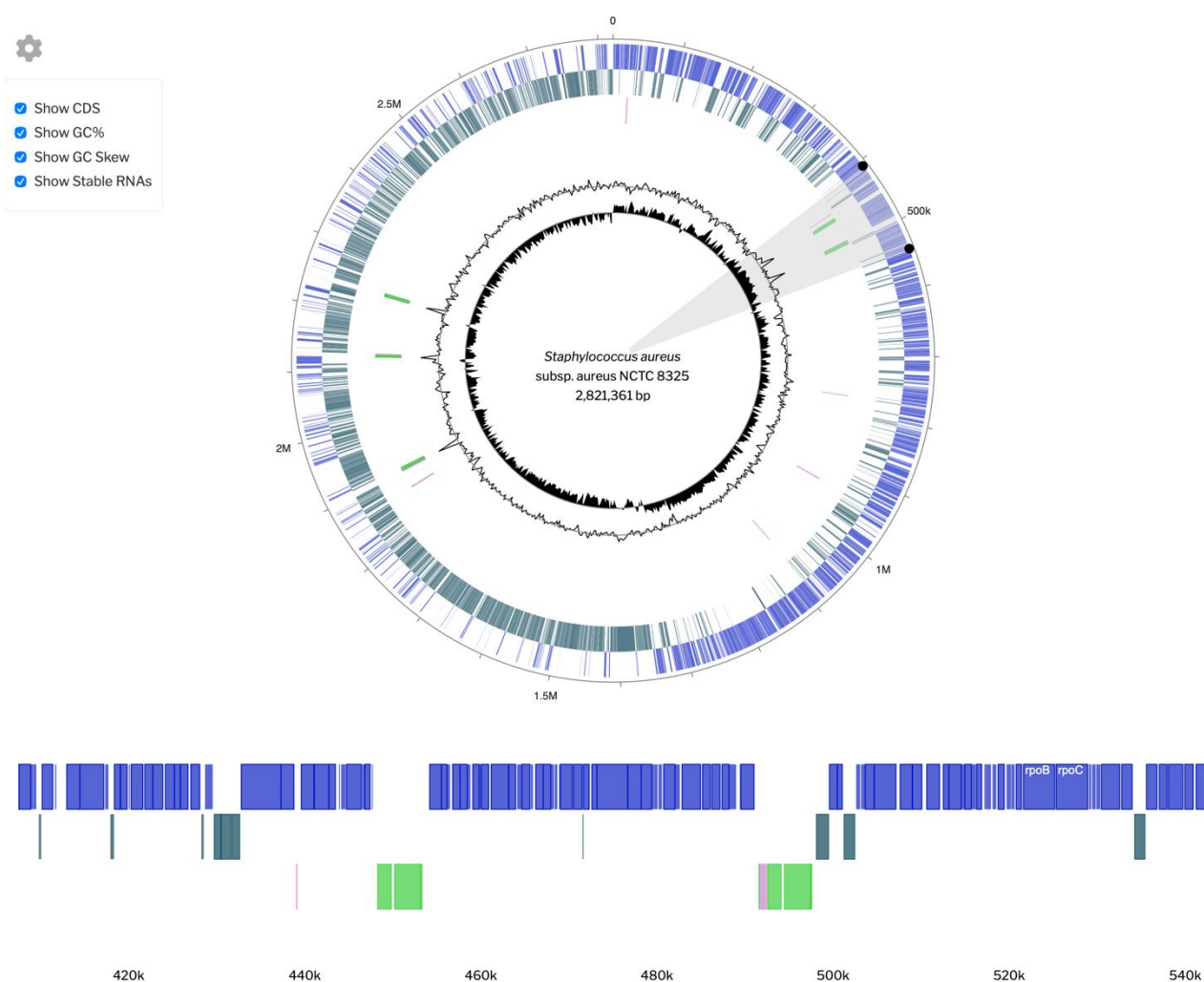
The genome overview tells you about the starting point of replication, coding regions and any potential acquisition of new DNA that the organism may have gained.



Screenshot: The *S. aureus* circular genome view. The *dnaA* gene is normally encoded adjacent to the *oriC* region that marks the origin of DNA replication and is depicted at the top of the circle. Most genome annotations of bacteria use *dnaA* to define the 'start' of the genome. Any region you have selected in the circular view is displayed in a zoomed linear representation below, from where you can link to individual gene pages.

Step 1 In the overview you can adjust the genes displayed in the zoomed linear view by moving the dots to expand or contract the selected region. Alternatively double click to move the highlight any region you want to look at. Here we have focussed on the starting point of replication which is close to a gene called *dnaA*.

You will notice that there are two colours in the genome, one represents the genes coded on the forward strand (blue), and those coded on the reverse strand (green). Some bacteria like *S. aureus* have a very strong strand bias such that the direction of transcription of a gene correlates with the direction of bidirectional DNA replication from the origin to the terminators (about 6 o'clock in the circle).



Screenshot: Ribosomal RNAs are depicted in bright green

Step 2 If we change our focus to the stable RNAs highlighted in bright green, which are shown on the next two tracks in the circular view and below the protein coding tracks in the linear view, we can see there are 5 regions encoding the ribosomal RNA (rRNA). Having multiple copies of rRNA encoding genes is common trick bacteria use to ensure that when they are growing quickly they have enough ribosomes to ensure high rates of protein synthesis. Because these regions of the chromosome are transcribed but not translated, their GC content is quite a bit different to the average of the whole chromosome, which you can see clearly by the spikes on the GC% content plot, which is the 5th track in the circular view.

Bacteria chromosomes have evolved over millions of years to have a certain average nucleotide content, which we usually report as the percentage of G and C bases in the DNA. For the model bacterium *Escherichia coli* this is about 50%, but this can vary quite considerably in other branches of the bacterial tree of life. Hence, if a bacterial genome contains a region with a GC content that is different to the rest of the genome it can tell us that it is 'new' DNA that has been recently acquired by the bacterium – a process that bacteria are experts at and which makes movement of antibiotic resistance genes a real problem.

Tip: Toggle the checkboxes on or off to add or remove the genome features.

HGT and antibiotic resistance in *Enterococcus faecium*

How do bacteria grab hold of DNA from other bacteria? There are several routes they have evolved to do this, which are known collectively as Horizontal Gene Transfer (HGT). In brief there are three main modes of HGT:

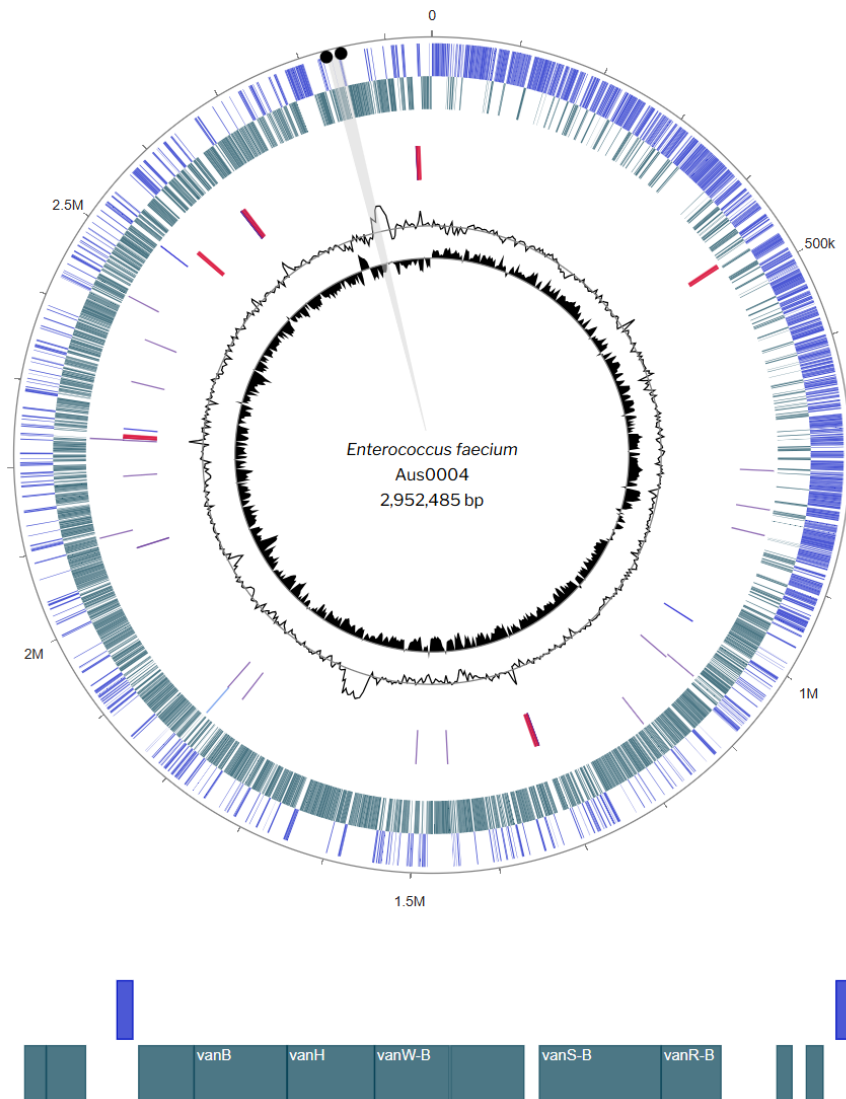
Transformation: Where the bacteria can simply take up free DNA from the environment, which it then moves into its own genome.

Transduction: Where a virus (bacteriophage) when escaping from a bacterium it has killed, packages some of that organisms DNA with its own DNA. Hence when it injects its DNA into another bacterium this DNA can sometimes end up integrated with the phage DNA into the chromosome.

Conjugation: Where two closely related bacteria can transfer DNA between them directly by creating a bridge (pili).

While the DNA that is transferred can really be any DNA, and most of the mobilised DNA is not kept, if the DNA encodes an antibiotic resistance gene, it could immediately provide a benefit to the cell and will be kept. So in this case the bacterium hasn't come up with a new way to resist the antibiotic's action, it 'acquires' a solution from some other bacterium which is already resistant. This mechanism is what we call acquired resistance, which is different in intrinsic resistance that we will look at later. The bottom line is that bacteria are very good at

sharing DNA, so that if one evolves resistance to an antibiotic, it is possible that that gene can end up in other more bacteria that cause disease in humans.



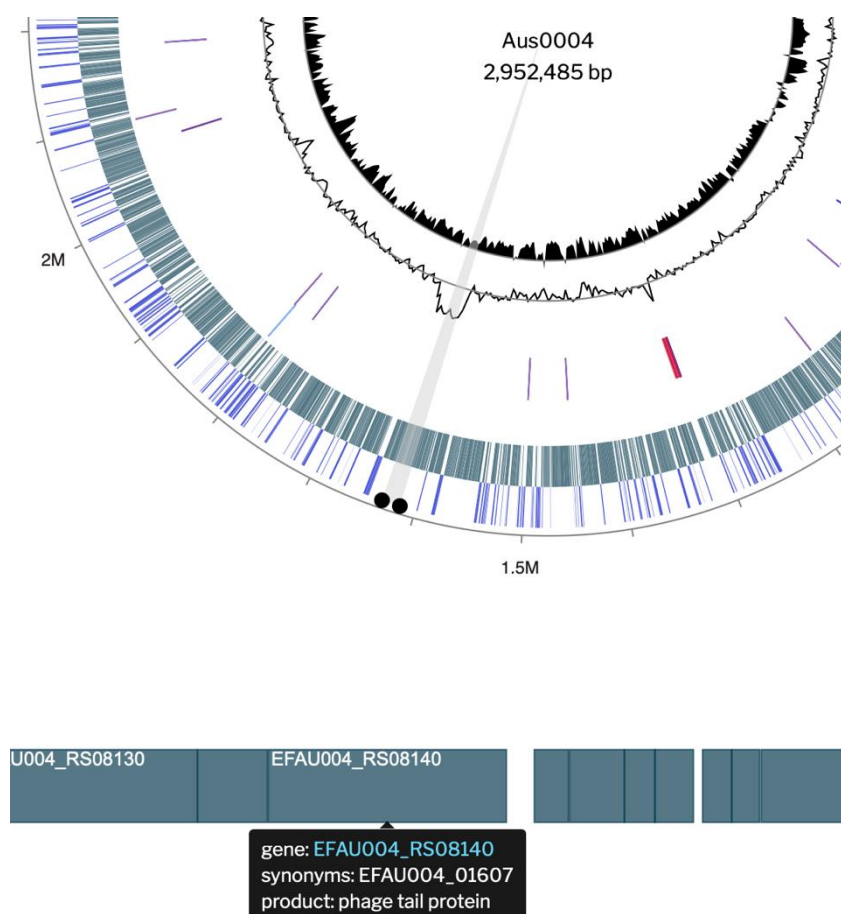
 **Screenshot** The change in GC% indicates where HGT may have occurred

Step 1 If we navigate to the sidebar and click on [Enterococcus faecium](#) you will see the gene overview.

Step 2 As with the *S. aureus* genome, focus on the bump in the GC% at about 11 o'clock, and on the linear zoom you will notice an array of genes all with the prefix 'van'. These are all involved in Vancomycin resistance. The antibiotic works by stopping the bacteria building a strong cell wall. The cell wall is made from long chains of sugars, but its torsional strength comes by bridging these chains together with cross-links formed by short peptides. The antibiotic stops the peptide cross links from forming so the wall is weak and the cells die. The *van* genes encode proteins that enable the cell to make a different peptide to link the sugar chains, which is not recognised by the antibiotic. This cunning protection mechanism may have come from a bacterium that makes vancomycin itself (remember most antibiotics we use are naturally made by other bacteria, particularly soil bacteria called *Streptomyces* spp.) and needs to protect itself from being killed. We can see from the location of these genes in region of abnormal GC content that it has been picked up recently by one of the

HGT mechanisms and ‘acquired’ an existing resistance mechanism to add to the capabilities of its own genome.

Step 3 *E. faecium* has another bump in the GC content. If you focus on this area you will see that one of the genes encode for a phage tail protein and those around it bare the hallmarks of this being a bacteriophage that has integrated into the chromosome of the bacteria. We call this a prophage, and in theory this could reactivate at any time, form phage particles and kill the cell. Hence, carrying these prophage is a ‘double edged sword’, they could bring antibiotic resistance genes, or other useful genes with them, but are a capable of killing their ‘host’ at any time. Interestingly if they persist for long enough in the chromosome they will acquire mutants themselves are not be able to reactivate, so are permanatly fixed in the chromosome. We call these cryptic prophage, and sometime it is hard to tell from the sequence alone if a prophage can active or cryptic.

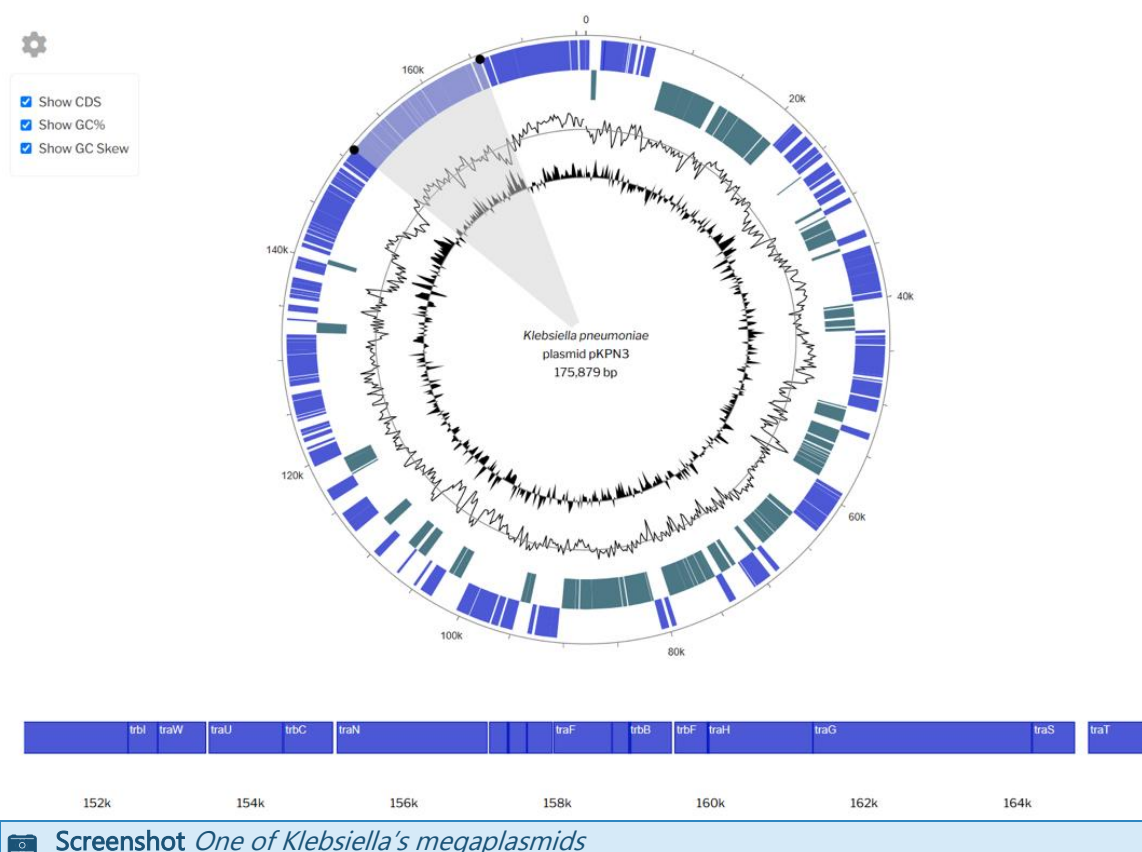


Screenshot Changes in GC% reveal HGT

We have looked at two Gram positive organisms where the focus of HGT has been on the chromosome, lets look at the Gram negatives and mobile genetic elements involved in HGT.

Looking at plasmids in *Klebsiella pneumoniae* (and *Enterobacter cloacae*)

If we open up the genome for [K. pneumoniae](#) you may notice at the top there are extra icons for the plasmids. These are extra-chromosomal pieces of DNA that are usually circular and can replicate independently from the chromosome. You can click on any of these elements to view the plasmids in the same way as we've viewed the chromosome before.



Step 1 You may notice there are three plasmids, two of which are extremely large. These are classified as megaplasmids. Click on the icon for pKPN3.

Step 2 Highlight the area of the chromosome where there is a large cluster of genes on the forward strand (blue colour). You can see from the rest of the plasmid that this is unusual for there to be such a long string of genes on only one strand. We know that bacteria arrange sets of genes they want to express at the same time in operons, which are on the same strand of the DNA as transcription of all these genes is driven from a single promoter. Hence, long strings of genes on the same strand suggests that these are arranged as operons, which often also suggests they are involved in the same cellular process.

Step 3 If you look at the linear representation of the genes in this big cluster, you will see these are all labelled as 'tra' genes. These genes are all involved in the same process and all need to be transcribed at the same time. Together they encode all the complex machinery that allows the plasmid to move from one bacteria to another via conjugation (they were originally named as tra genes, as they were needed for gene transfer from one bacterium to another).

Section 3: Search Genome Features

So we have established that our strain of *K. pneumoniae* contains a big plasmid, pKPN4, which looks like it can move about by conjugation. What else is encoded on this plasmid? One other quick way to look at this is to browse the features of this megaplasmid using the Browse Genome Features tool.

Step 1 On the genome overview page, scroll down to the Genome details. Click on the 'Features' pop-out box. This lists all the gene features present in the genome.

Step 2 Type in the filter results 'pKPN4' and expand the rows to 100 rows per page (at the bottom of the list). You should get the table below:

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Project Home

▼ Genomes

Enterococcus faecium Aus0004

Staphylococcus aureus subsp. *aureus* NCTC 8325

Klebsiella pneumoniae subsp. *pneumoniae* MGH 78578

Acinetobacter baumannii AB5075-UW

Pseudomonas aeruginosa PAO1

Enterobacter cloacae subsp. *cloacae* ATCC 13047

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Thomas Lab

Fascione Lab

MORF / Projects / MORF Demos / ESKAPE Pathogens / Genomes / *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578

/ Browse

Browse Genome Features

Search by:

There are **5491** genes within this genome: **5368** CDSs, **25** rRNAs, **86** tRNAs, **11** ncRNAs.

Name	Synonyms	Product	Type	Sequence	Start (bp)	Length (bp)	Length (aa)	Size (kDa)
KPN_RS27155	KPN_pKPN4p07038	DUF6404 family protein	CDS	plasmid pKPN4	1	348	115	13.37
KPN_RS27160	KPN_pKPN4p07039	hypothetical protein	CDS	plasmid pKPN4	563	546	181	21.08
KPN_RS32665		hypothetical protein	CDS	plasmid pKPN4	1469	387	128	14.17
KPN_RS27170	KPN_pKPN4p07040	S-type pyocin domain-containing protein	CDS	plasmid pKPN4	1768	2019	672	70.26
KPN_RS27175	KPN_pKPN4p07041	bacteriocin immunity protein	CDS	plasmid pKPN4	3788	279	92	10.32
KPN_RS32520		hypothetical protein	CDS	plasmid pKPN4	4150	81	27	2.97
KPN_RS27180	KPN_pKPN4p07042	phospholipase D family nuclease	CDS	plasmid pKPN4	4335	552	183	20.26
KPN_RS27185	KPN_pKPN4p07043	replication regulatory protein RepA	CDS	plasmid pKPN4	5207	279	92	10.4
tap	KPN_RS29750	RepA leader peptide Tap	CDS	plasmid pKPN4	5702	78	25	2.75
repA	KPN_RS27190; KPN_pKPN4p07044	incFII family plasmid replication initiator RepA	CDS	plasmid pKPN4	5772	858	285	33.2
KPN_RS27200	KPN_pKPN4p07045	C2H2-type zinc finger protein	CDS	plasmid pKPN4	7673	327	108	12.49
KPN_RS27205	KPN_pKPN4p07046	hypothetical protein	CDS	plasmid pKPN4	8181	282	93	10.41

 **Screenshots** The genome features can be viewed from the link in Genome Details.

Step 3 You can just browse this gene list by scrolling down and you will soon see a gene encoding a beta-lactamase, which is an enzyme bacteria use to inactivate beta-lactam antibiotics like penicillin. If you scroll further you find other AMR genes like *aac* and *aadA1* gene which allow bacteria to modify aminoglycoside antibiotics to stop them working. Hence, if this plasmid moves around between different strains of *K. pneumoniae* via conjugation, it is also moving AMR genes at the same time.

How many beta-lactamases are encoded in this genome? If you change the filter to look for 'beta-lactamase' you will now see there are 5 encoded on the genome, 3 of which are on the pKPN4 megaplasmid. Cripes!

Step 4 Now click on the link for beta lactamase [SHV-12](#). This will take you to the gene page for the gene encoding this protein. As you can see it is named as KPN_RS27220. This naming convention tells you that this specific gene is found in the genome of *Klebsiella*

pneumoniae, in the NCBI database with a unique identifier. If you searched that locus tag in the NCBI database you will find that specific gene.

MORF / Projects / MORF Demos / ESKAPE Pathogens / Genomes / *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 / Gene

Gene Details: KPN_RS27220

Search by:

Keyword Search for a gene or feature



Name:	KPN_RS27220	Locus Tag:	KPN_RS27220
Nucleotide Position:	10117 - 10977 (861 bp)	Synonyms:	KPN_pKPN4p07050
Product:	extended-spectrum class A beta-lactamase SHV-12	Strand:	Forward

External Links

GenPept:	WP_002904004.1	UniProt:	A6TIK7
----------	----------------	----------	--------

Pathways

GO Term:	GO:0008800: beta-lactamase activity GO:0046677: response to antibiotic GO:0030655: beta-lactam antibiotic catabolic process
----------	---

Protein Properties

Start codon:	ATG	Isoelectric point:	8.84
Molecular weight (kDa):	31.27	Number of amino acids:	286

 Screenshot The gene page for KPN_RS27220

Reading a Gene Page

Each gene page contains detailed information about that gene including:

- Gene name and product description
- Location on the chromosome (start and end position, length and strand)
- External links to UniProt
- GO term annotations and InterPro family entries

Go Terms

There is a lot of information in this one page. The **GO Terms** (Gene Ontology) are a standardised naming convention to describe the properties of the resulting protein and describe three types of biological features.

Molecular function: A description of the activity that the synthesised protein will do; in the above example it is beta-lactamase activity. Note: this only describes the function, it doesn't specify the mode of action or what process it is involved in.

Cellular Component: The bacteria export this protein to the periplasm, which is where beta-lactams antibiotics work, so it needs to be in this place to break them down.

Biological process: This is biological process the protein is involved in, in this case the protein is made in response to an antibiotic, and it specifically breaks down the beta-lactam antibiotic.

Section 4: Ortholog Analysis

What is Ortholog Analysis?

Ortholog analysis compares the genes across multiple genomes simultaneously to identify which are shared and which are unique to individual organisms. MORF uses OrthoFinder v3.1.3 to group genes into orthologous sets of proteins (orthogroups), which means that they are all encoded by the same single gene present in the ancestor of the organism used in the analysis.

There are four categories of orthogroups:

Single copy: One copy in every genome - these are the core essential genes shared by all organisms.

Orthogroup: Present in more than one genome but not necessarily all and may include duplicates (paralogues).

Strain specific: Multiple examples of a orthogroup, but only found in one genome – these are gene families that have expanded in each lineage.

Unassigned: Unique genes encoding proteins with no detectable relationship to any other genome in the comparison.

Viewing the Ortholog Analysis Results for acquired resistance

Step 1 From the ESKAPE Pathogens project homepage click Orthologs in the left sidebar or in 'Browse all data'.

Step 2 Dropdown gives you Results - click that to get the Ortholog Analysis Results.

MORF / Projects / MORF Demos / ESKAPE Pathogens / Orthologs / Results / View Result

A. baumannii vs P. aeruginosa vs E. faecium vs K. pneumoniae vs E. cloacae vs S. aureus

Filter Results

x

▼

Orthologous groups of genes (**orthogroups**) were identified from protein sequences using **OrthoFinder v3.1.3**. An **orthogroup** is defined as a set of genes that all descend from a single gene in the last common ancestor of the species (or strains) being compared. Orthogroups may include **paralogs**, which arise when gene duplication has occurred in one or more genomes.

In total, **10070 orthogroups** were identified across the 6 genomes. These can be divided into several categories:

- **Single copy:** Groups in which each genome contributes exactly one gene (strict one-to-one orthologs). We identified **317** such groups.
- **Orthogroup:** Groups containing orthologs that are present in more than one genome, but not necessarily all genomes, and which may include paralogs. We identified **3758** such groups.
- **Strain specific:** Groups where all members come from only one genome, reflecting lineage-specific gene family amplification. We identified **598** such groups.
- **Unassigned:** Unique genes not placed into any orthogroup. These represent sequences with no detectable orthology within any other genomes analysed. We identified **5397** unique genes in total with **803** in genome *Pseudomonas aeruginosa* PAO1 and **913** in genome *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 and **1209** in genome *Enterobacter cloacae* subsp. *cloacae* ATCC 13047 and **999** in genome *Staphylococcus aureus* subsp. *aureus* NCTC 8325 and **694** in genome *Enterococcus faecium* *Enterococcus faecium* Aus0004 and **779** in genome *Acinetobacter baumannii*

Group ID	Category	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> MGH 78578	<i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> N
OG0001047	single copy	rpmH: 50S ribosomal protein L34	ECL_05166: 50S ribosomal protein L34	rpmH: 50S ribosomal protein L34
OG0001046	single copy	rnpA: ribonuclease P protein component	ECL_05165: ribonuclease P	rnpA: ribonuclease P
OG0001045	single copy	mmnE: tRNA uridine-5-carboxymethylaminomethyl(34) synthesis GTPase MnmE	ECL_05163: tRNA modification GTPase TrmE	trmE: tRNA modification GTPase TrmE

 Screenshot *The ESKAPE pathogen ortholog analysis*

Step 3 The summary at the top shows the key numbers. For the six ESKAPE pathogens: 317 genes are shared as a single copy across all six, while 598 orthogroups are strain specific.

Step 4 Scroll down to browse the full gene table. Each row shows the orthogroup ID, its category, and the corresponding gene in each of the six genomes.

Step 5 As we are interested in AMR genes let's search for a gene that is identical across all 6 species. Broad spectrum antibiotics are aimed at being effective across a wide range of bacteria, so they usually have one specific target. The *rpsL* gene encodes the S12 subunit of the ribosome and is a known target for binding of the aminoglycoside antibiotic streptomycin.

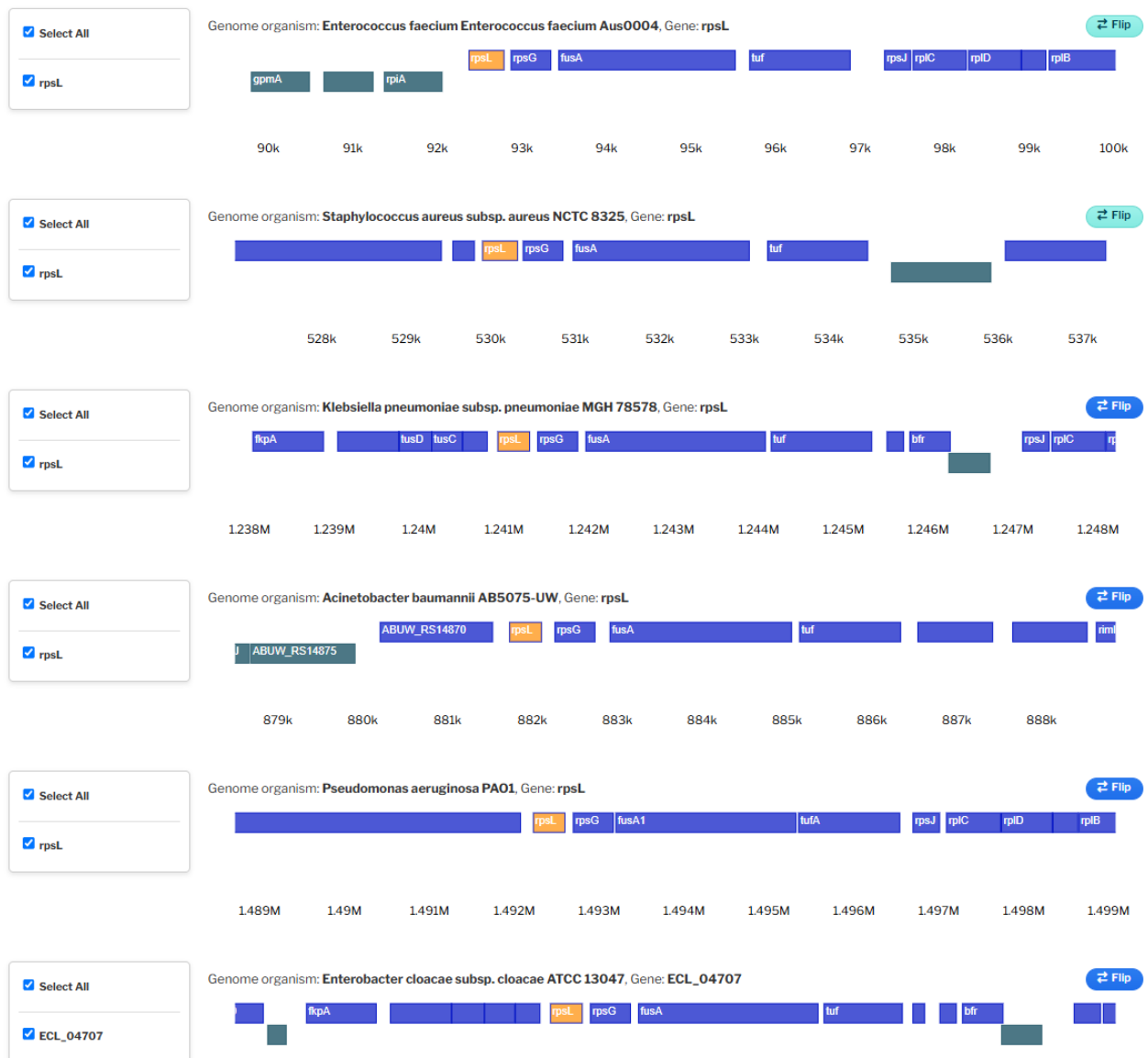
Step 6 if we type *rpsL* in the search box we get one hit. Click on the Orthogroup ID ([OG0000715](#)).

Ortholog Group: OG0000715

[Lock](#)
[Reset](#)


Step 7 You will see that there is an alignment of the genome with the *rpsL* gene highlighted in yellow. We can see that the two Gram positive bacteria encode the gene on the forward strand, however if you click flip for the Gram negatives, then the genes now align. You may also notice that there are identical genes in the same location (*rpsG*, *fusA* and *tuf*). These are known as superclusters – highly conserved regions that encode genes critical for the cell's survival.

Ortholog Group: OG0000715

[Lock](#) [Reset](#)


Ortholog Analysis for intrinsic resistance

We have seen that there are a number of **acquired** resistance genes from bacteriophage or other mobile genetic elements, now we are going to look at some of the **intrinsic** resistances in [Pseudomonas aeruginosa](#). While *P. aeruginosa* does have some acquired resistance genes, it also carries many intrinsic resistances, more than most bacteria.

P. aeruginosa intrinsic resistance mechanisms are mainly to do with reducing the rate at which an antibiotic can enter the cell; through narrowing of the channels on the cell surface, as well as having many efflux pumps; actively pushing the antibiotic out of the cell. We would expect to see a large number of these transporter genes within the *P. aeruginosa* genome compared to the other ESKAPE pathogens.

Step 1 Bring up the Ortholog Analysis Results for the ESKAPE pathogens and type in '**RND Transporter**' in the Filter Results box. You will see there are 7 orthologous groups listed. If you scroll down you will see apart from one hypothetical protein, there are no genes listed in the Gram positive columns.

Step 2 Type in OG0000065 – you will now see how many more Transporter genes *P. aeruginosa* has compared to the other bacteria, including other Gram -ve ESKAPE pathogens.

Group ID	Category	Klebsiella pneumoniae subsp. pneumoniae MGH 78578	Enterobacter cloacae subsp. cloacae ATCC 13047
OG0000065	orthologous group	KPN_RS04250: efflux RND transporter periplasmic adaptor subunit; KPN_RS11565: efflux RND transporter periplasmic adaptor subunit; KPN_RS13590: MdtA/MuxA family multidrug efflux RND transporter periplasmic adaptor subunit; oqxA: multidrug efflux RND transporter periplasmic adaptor subunit OqxA	oqxA: RND family multidrug efflux membrane fusion protein OqxA; ECL_02243: RND family efflux transporter MFP subunit; ECL_03401: multidrug efflux system subunit MdtA
Staphylococcus aureus subsp. aureus NCTC 8325 Acinetobacter baumannii AB5075-UW Pseudomonas aeruginosa PAO1 Enterococcus faecium Aus0004			
		ABUW_RS00165: efflux RND transporter periplasmic adaptor subunit; ABUW_RS03325: efflux RND transporter periplasmic adaptor subunit; adeF: multidrug efflux RND transporter periplasmic adaptor subunit AdeF	PA0156: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA0157: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA1435: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA2493: resistance-nodulation-cell division (RND) multidrug efflux membrane fusion protein MexE; PA2528: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA3523: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA3677: resistance-nodulation-cell division (RND) efflux membrane fusion protein; mexH: resistance-nodulation-cell division (RND) efflux membrane fusion protein; PA4374: resistance-nodulation-cell division (RND) efflux membrane fusion protein

Gram negative bacteria use RND pumps which have an inner and outer membrane and the RND pumps spans both membranes with an inner membrane transporter protein, and periplasmic fusion protein and an outer membrane channel. Gram positive cell walls lack the outer membrane components which are needed for the tripartite structure of most RND pumps, therefore these bacteria cannot form regular RNA systems.

This brings us to the end of our demo. We hope you have enjoyed it and learnt something about these bacteria and how they have evolved resistance to different antibiotics.

Section 5: Try It Yourself

Suggested Exercises

Now that you have completed this tutorial, try these exercises to explore the ESKAPE genomes further:

Exercise 1: We found a conjugative plasmid in *K. pneumoniae* which we discovered contained some antimicrobial resistance (AMR) genes.

- a) Can you find another ESKAPE pathogen in the project which has a similar conjugatable megaplasmid?
- b) When you browse the other genes in this plasmid, can you find any that are likely to be involved in AMR? How many beta-lactamases are encoded in this genome and on what genome elements are they transcribed.

Exercise 2: Look at the 317 single copy genes shared by all six pathogens in the ortholog analysis. Find *rpoB* - the RNA polymerase gene targeted by rifampicin. Open this orthologous group. Try and see if you can flip the genomes so they all have the same layout.

- a) Can you define the supercluster of genes within which *rpoB* appears to sit?

Exercise 3: There is a GO term for 'antibiotic catabolic process', which can sometimes be linked to beta-lactamases. Search the 6 genomes for the GO Term GO:0017001 using the 'Search by:' tool on the top right.

- a) In which organisms is the hit and what is the gene called?
- b) You can see that this enzyme has some other GO terms associated with it. Find out how many other proteins in *E. cloacae* are predicted to be located in the periplasm (periplasmic space).