Functional traces of antibiotic resistance in metagenomes

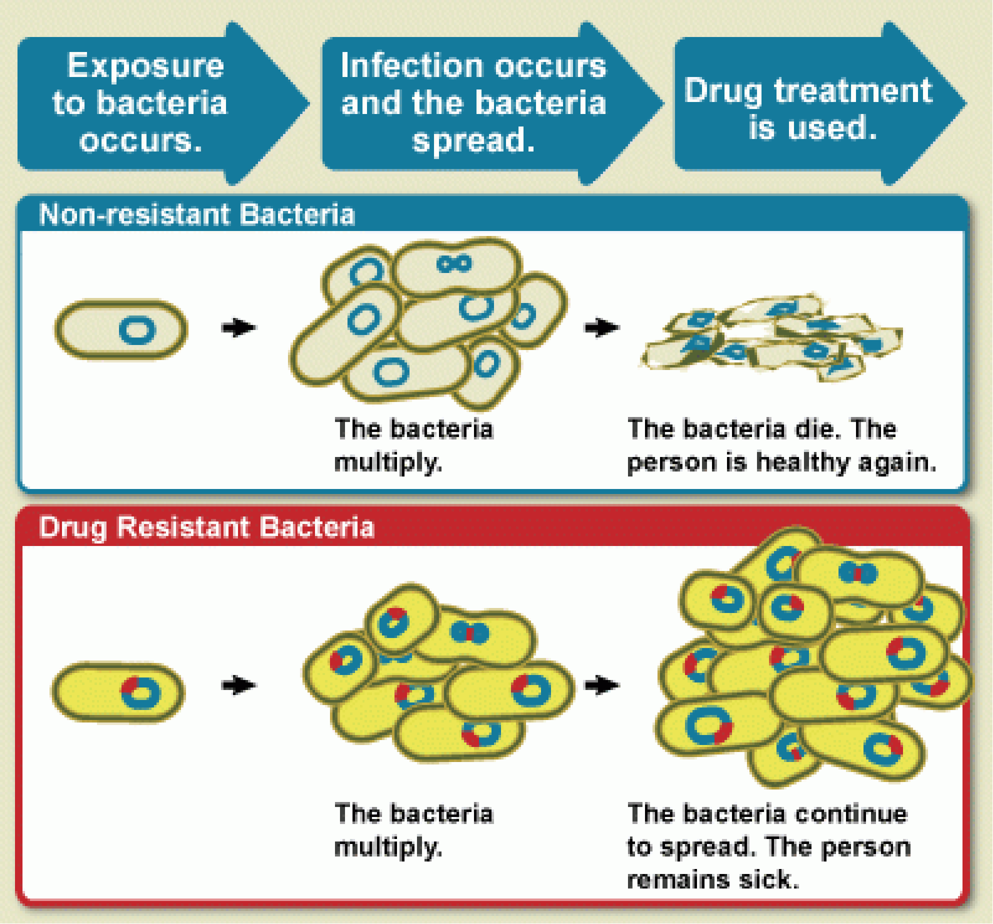
Meeting Toscano di Bioinformatica e Systems Biology

Bioinformatiha 11

14 Ottobre 2024 – Plesso Didattico Morgagni

Matteo Ramazzotti – matteo.ramazzotti@unifi.it

Understanding antibiotic resistance



Exposure to bacteria occurs.

Infection occurs and the bacteria spread.

Drug treatment is used.

The bacteria multiply.

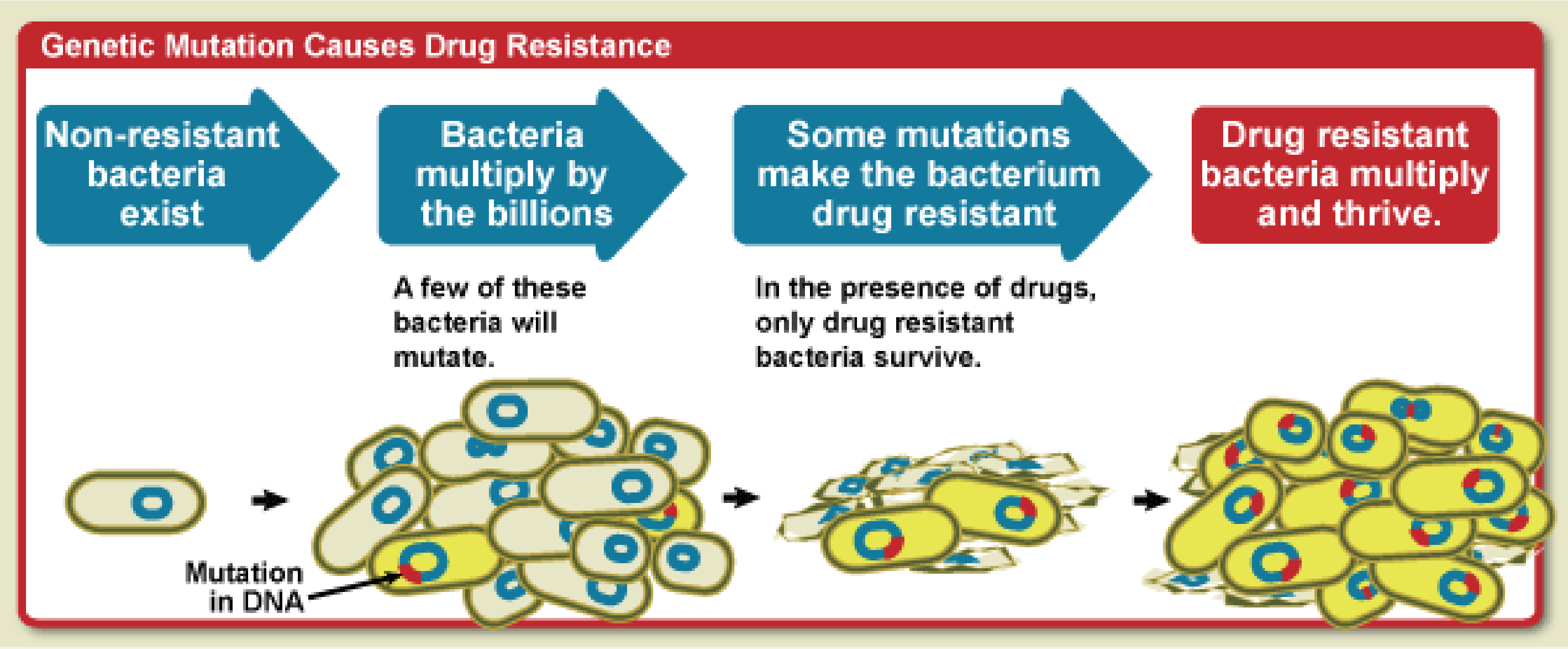
The bacteria die. The person is healthy again.

The bacteria multiply.

The bacteria continue to spread. The person remains sick.

www.niaid.nih.gov

Understanding antibiotic resistance



Genetic Mutation Causes Drug Resistance

Non-resistant bacteria exist

Bacteria multiply by the billions

Some mutations make the bacterium drug resistant

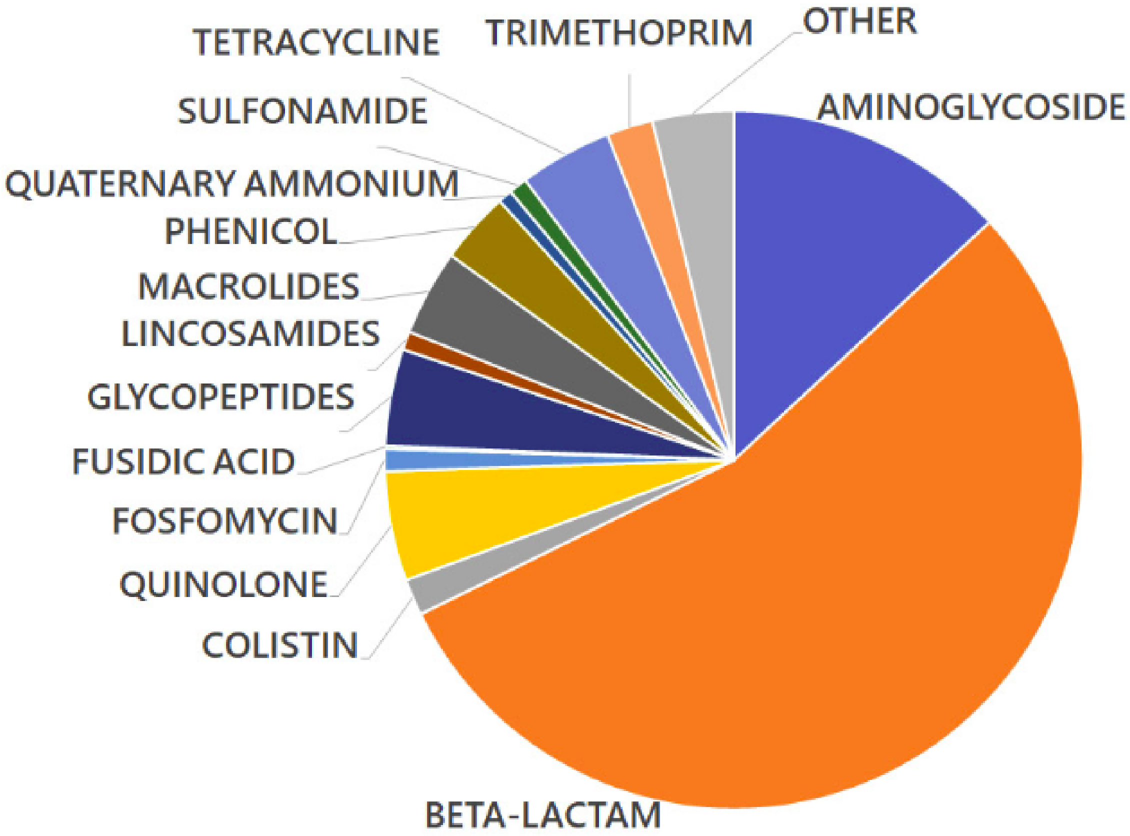
Drug resistant bacteria multiply and thrive.

A few of these bacteria will mutate.

In the presence of drugs, only drug resistant bacteria survive.

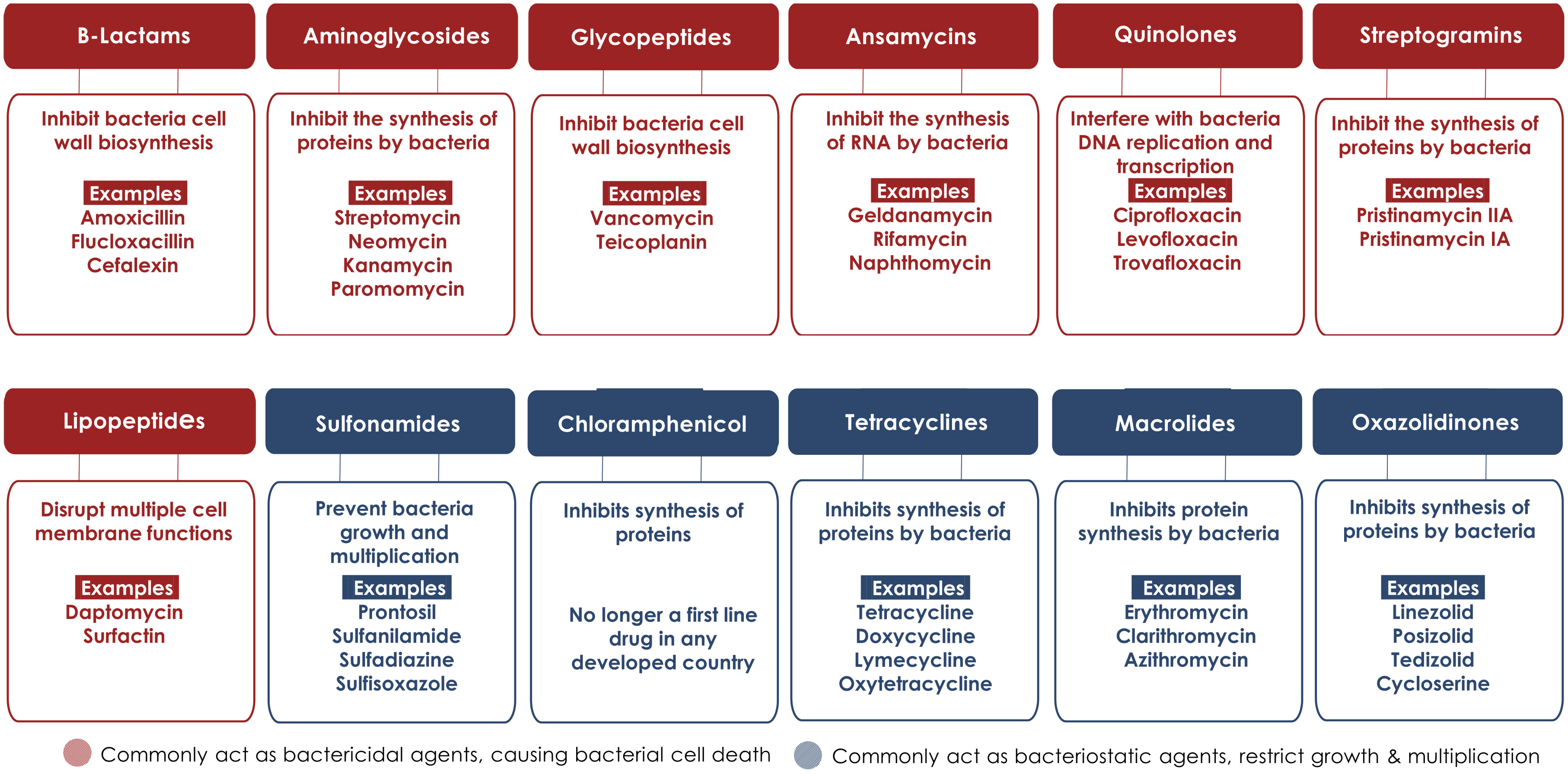
www.niaid.nih.gov

Major classes of antibiotics



https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/

Major classes of antibiotics



B-Lactams
Inhibit bacteria cell wall biosynthesis
Examples
Amoxicillin
Flucloxacillin
Cefalexin

Aminoglycosides
Inhibit the synthesis of proteins by bacteria
Examples
Streptomycin
Neomycin
Kanamycin
Paromomycin

Glycopeptides
Inhibit bacteria cell wall biosynthesis
Examples
Vancomycin
Teicoplanin

Ansamycins
Inhibit the synthesis of RNA by bacteria
Examples
Geldanamycin
Rifamycin
Naphthomycin

Quinolones
Interfere with bacteria DNA replication and transcription
Examples
Ciprofloxacin
Levofloxacin
Trovafloxacin

Streptogramins
Inhibit the synthesis of proteins by bacteria
Examples
Pristinamycin IIA
Pristinamycin IA

Lipopeptides
Disrupt multiple cell membrane functions
Examples
Daptomycin
Surfactin

Sulfonamides
Prevent bacteria growth and multiplication
Examples
Prontosil
Sulfanilamide
Sulfadiazine
Sulfisoxazole

Chloramphenicol
Inhibits synthesis of proteins

No longer a first line drug in any developed country

Tetracyclines
Inhibits synthesis of proteins by bacteria
Examples
Tetracycline
Doxycycline
Lymecycline
Oxytetracycline

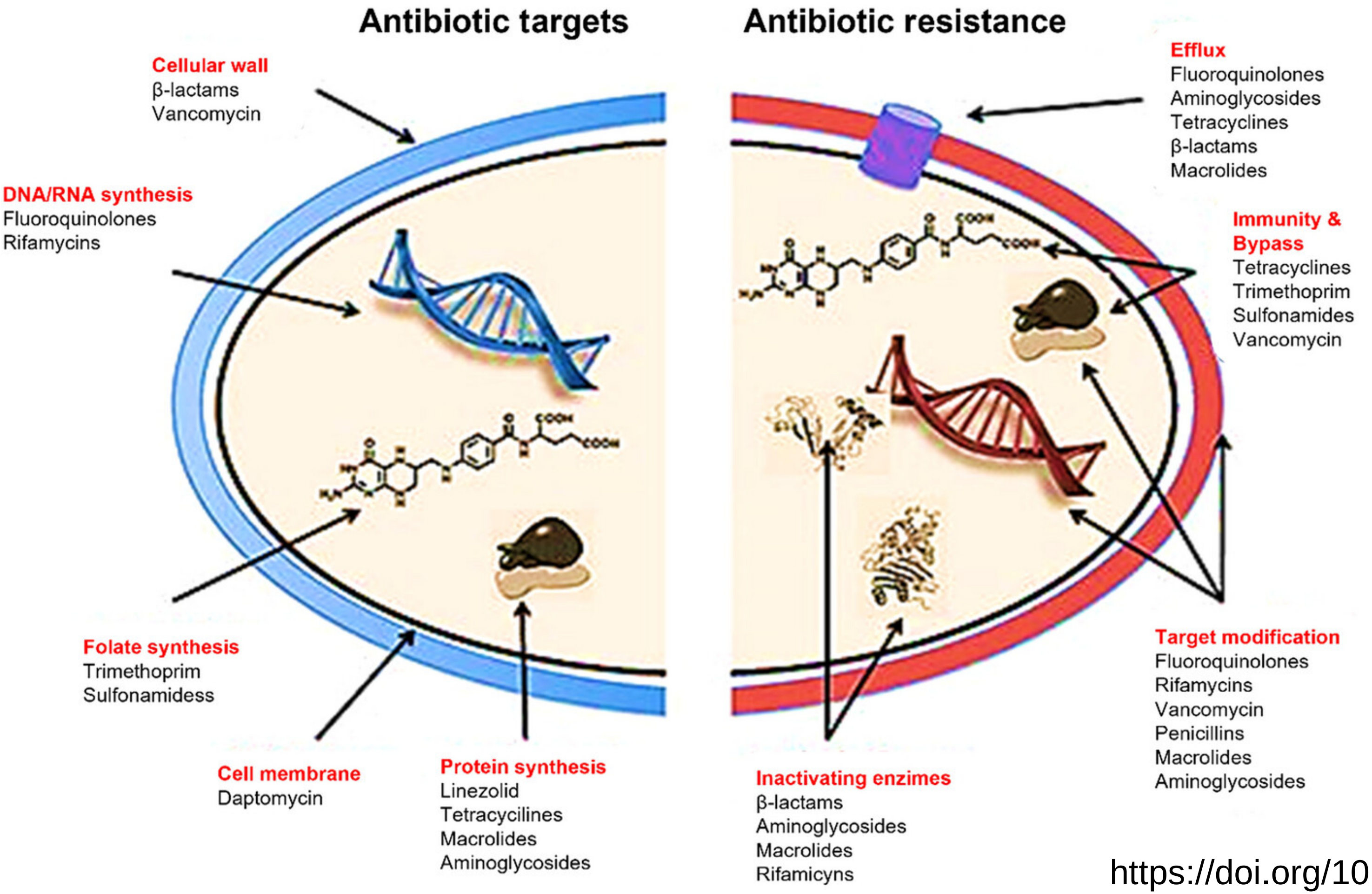
Macrolides
Inhibits protein synthesis by bacteria
Examples
Erythromycin
Clarithromycin
Azithromycin

Oxazolidinones
Inhibits synthesis of proteins by bacteria
Examples
Linezolid
Posizolid
Tedizolid
Cycloserine

🔴 Commonly act as bactericidal agents, causing bacterial cell death 🔵 Commonly act as bacteriostatic agents, restrict growth & multiplication

https://www.reactgroup.org/toolbox/understand/antibiotics/how-do-antibiotics-work/

Mechanisms of antibiotic resistance



Antibiotic targets

Cellular wall
β-lactams
Vancomycin

DNA/RNA synthesis
Fluoroquinolones
Rifamycins

Folate synthesis
Trimethoprim
Sulfonamidess

Cell membrane
Daptomycin

Protein synthesis
Linezolid
Tetracycilines
Macrolides
Aminoglycosides

Antibiotic resistance

Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides

Immunity & Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin

Target modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides

Inactivating enzimes
β-lactams
Aminoglycosides
Macrolides
Rifamicyns

https://doi.org/10.1002/fft2.458

Mechanisms of antibiotic resistance

- Mutations
- Gene acquisition
- Membrane impermeability
- Alteration of the binding site
- Enzymatic transformation - vph of of S. vinaceus - Viomycin kinase - **P18623**
- Efflux pumps - NorA of S. aureus - Quinolone resistance protein - **P0A0J7**

Searching for antibiotic resistance in metagenomes

- **Identify** the **candidate** protein – reference
- **Search in Uniprot** for similar proteins
  - by name?
  - by sequence?
  - uniprot defined?
- **Align** all candidates using a multiple sequence aligner (e.g. Clustal)
- Is the alignment reasonable? If not try **removing disturbing proteins**
- **Repeat** until the **alignment** is **optimized**
- **Build** an **Hidden Markov Model** of the alignment

Now we have **a mathematical model** to search in genomes - or reads !!!

Studying resistance to viomycin

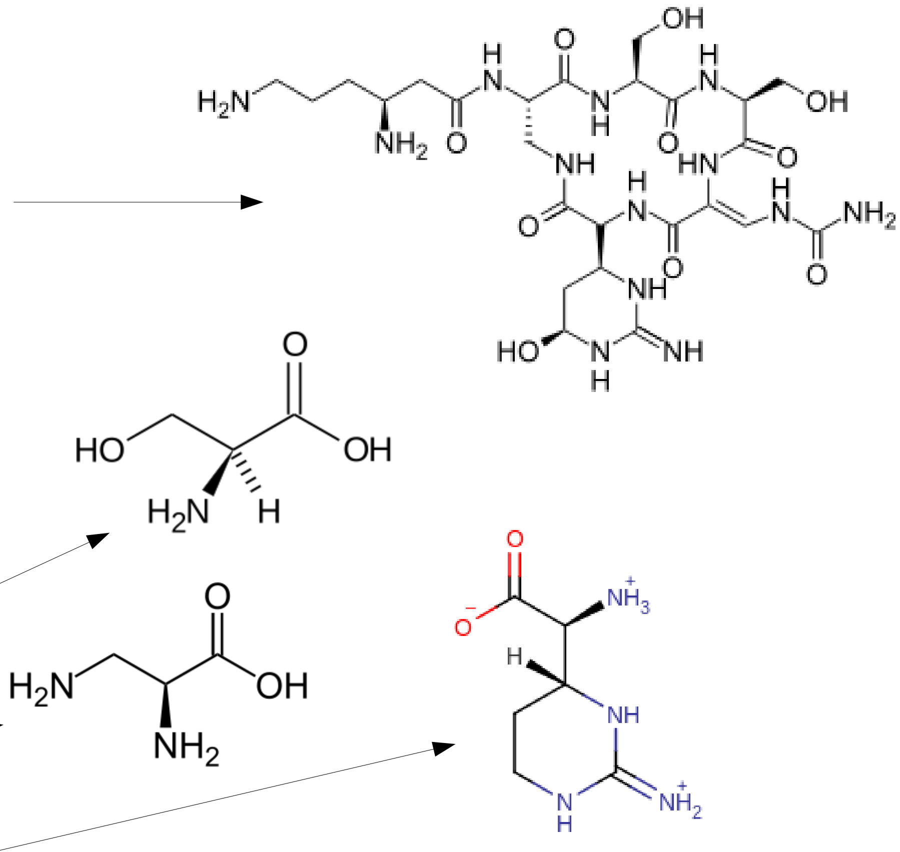
Viomycin is a member of the tuberactinomycin family, a group of nonribosomal **peptide antibiotics** exhibiting **anti-tuberculosis** activity

It consists of a central cyclic pentapeptide produced by a **nonribosomal peptide synthetase** composed by VioA, VioF, VioI and VioG genes that condense and cyclize

2 x L-2,3-diaminopropionate (L-Dap)

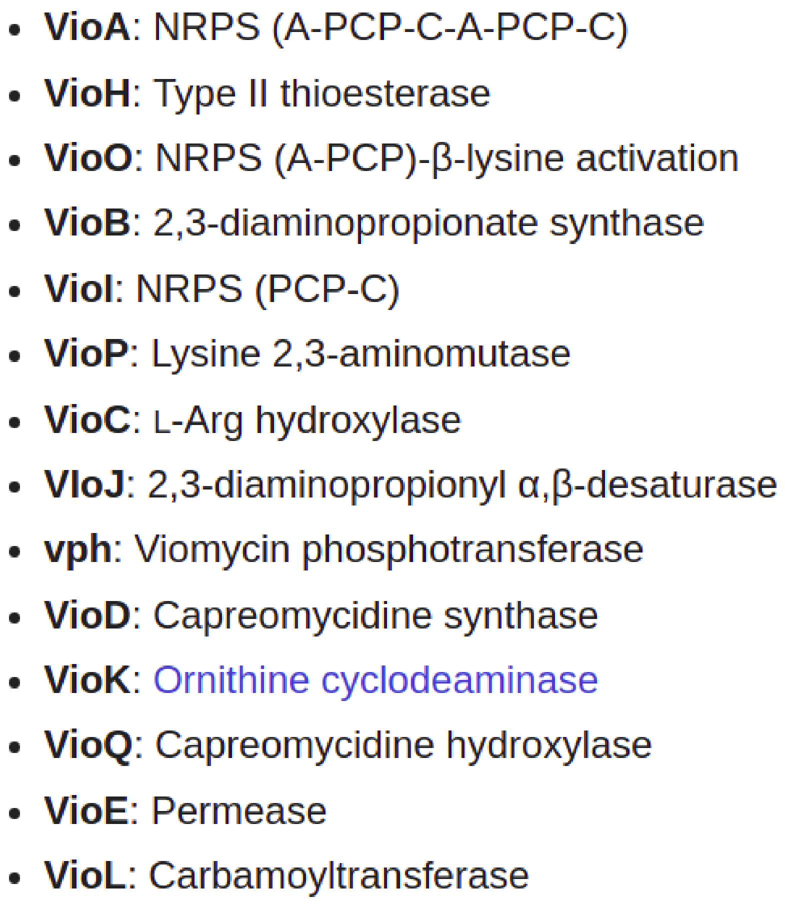
2 x L-serine (L-Ser)

1 x (2S,3R)-capreomycidine (L-Cam)



https://en.wikipedia.org/wiki/Viomycin

Studying viomycin production



- **VioA**: NRPS (A-PCP-C-A-PCP-C)
- **VioH**: Type II thioesterase
- **VioO**: NRPS (A-PCP)-β-lysine activation
- **VioB**: 2,3-diaminopropionate synthase
- **VioI**: NRPS (PCP-C)
- **VioP**: Lysine 2,3-aminomutase
- **VioC**: L-Arg hydroxylase
- **VIoJ**: 2,3-diaminopropionyl α,β-desaturase
- **vph**: Viomycin phosphotransferase
- **VioD**: Capreomycidine synthase
- **VioK**: Ornithine cyclodeaminase
- **VioQ**: Capreomycidine hydroxylase
- **VioE**: Permease
- **VioL**: Carbamoyltransferase

In ***Streptomyces puniceus***

the viomycin gene cluster includes

36.3 kb of contiguous DNA that encodes

20 open reading frames (ORFs) that

are involved in the

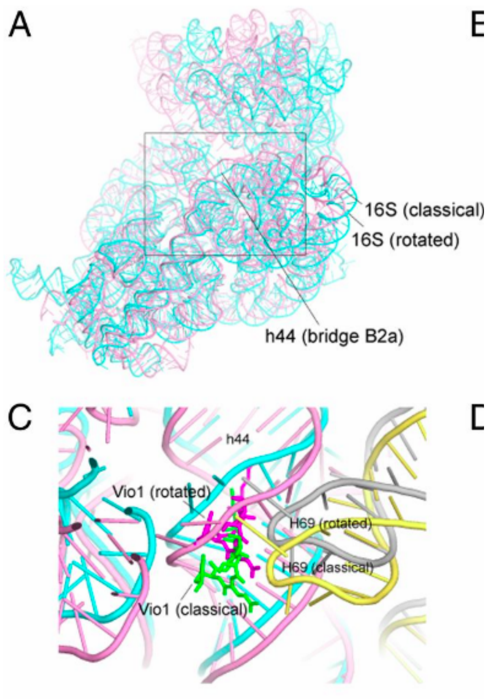
biosynthesis, regulation and activation

of viomycin

https://en.wikipedia.org/wiki/Viomycin

Viomycin blocks protein synthesis

Viomycin blocks the translocation step of protein synthesis, stabilizing the ribosome in a state of intersubunit rotation, resembling the hybrid-state intermediate.

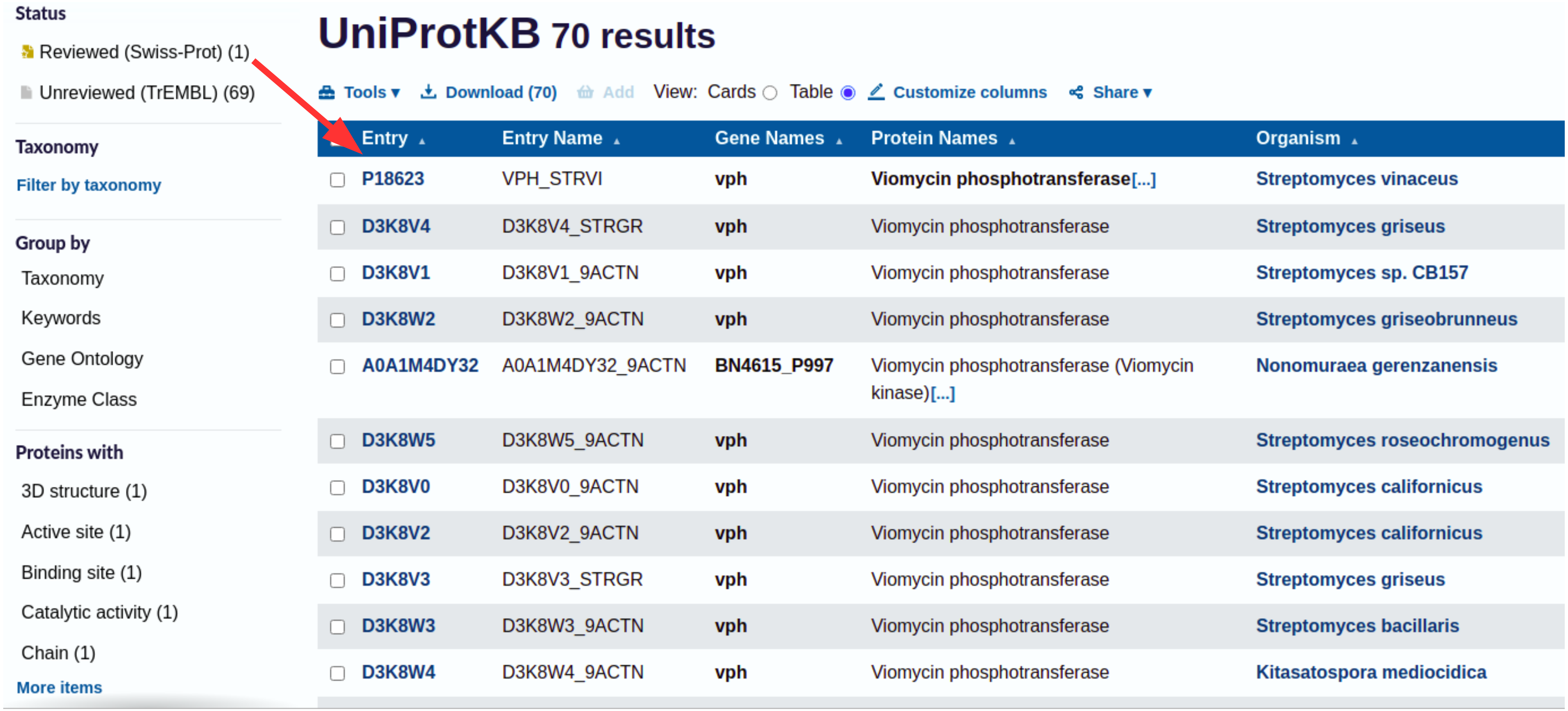


Viomycin is toxic for Streptomyces ribosomes, so it is:

1. phosphorylated (Vph) - **inactivation**
2. exported (VioE)
3. dephosphorylated (VioS) – reactivation

The presence of **Viomycin phosphotransferase homologues** in bacteria may indicate potential drug resistance

Protein sequences: viomycin phosphotrasferase



Status

Reviewed (Swiss-Prot) (1)

Unreviewed (TrEMBL) (69)

Taxonomy

Filter by taxonomy

Group by

Taxonomy

Keywords

Gene Ontology

Enzyme Class

Proteins with

3D structure (1)

Active site (1)

Binding site (1)

Catalytic activity (1)

Chain (1)

More items

UniProtKB 70 results

☐	Entry ▴	Entry Name ▴	Gene Names ▴	Protein Names ▴	Organism ▴
☐	P18623	VPH_STRVI	vph	**Viomycin phosphotransferase**[...]	Streptomyces vinaceus
☐	D3K8V4	D3K8V4_STRGR	vph	Viomycin phosphotransferase	Streptomyces griseus
☐	D3K8V1	D3K8V1_9ACTN	vph	Viomycin phosphotransferase	Streptomyces sp. CB157
☐	D3K8W2	D3K8W2_9ACTN	vph	Viomycin phosphotransferase	Streptomyces griseobrunneus
☐	A0A1M4DY32	A0A1M4DY32_9ACTN	BN4615_P997	Viomycin phosphotransferase (Viomycin kinase)[...]	Nonomuraea gerenzanensis
☐	D3K8W5	D3K8W5_9ACTN	vph	Viomycin phosphotransferase	Streptomyces roseochromogenus
☐	D3K8V0	D3K8V0_9ACTN	vph	Viomycin phosphotransferase	Streptomyces californicus
☐	D3K8V2	D3K8V2_9ACTN	vph	Viomycin phosphotransferase	Streptomyces californicus
☐	D3K8V3	D3K8V3_STRGR	vph	Viomycin phosphotransferase	Streptomyces griseus
☐	D3K8W3	D3K8W3_9ACTN	vph	Viomycin phosphotransferase	Streptomyces bacillaris
☐	D3K8W4	D3K8W4_9ACTN	vph	Viomycin phosphotransferase	Kitasatospora mediocidica

matteo.ramazzotti@unifi.it - dSBSC - UniFi

The uniprot reference is in Streptomyces vinaceus

P18623 · VPH_STRVI

Protein: Viomycin phosphotransferase

Gene: vph

Status: UniProtKB reviewed (Swiss-Prot)

Organism: Streptomyces vinaceus

Amino acids: 287 (go to sequence)

Protein existence: Inferred from homology

Annotation score: 2/5

Function

The aminoglycoside phosphotransferases achieve inactivation of their antibiotic substrates by phosphorylation.

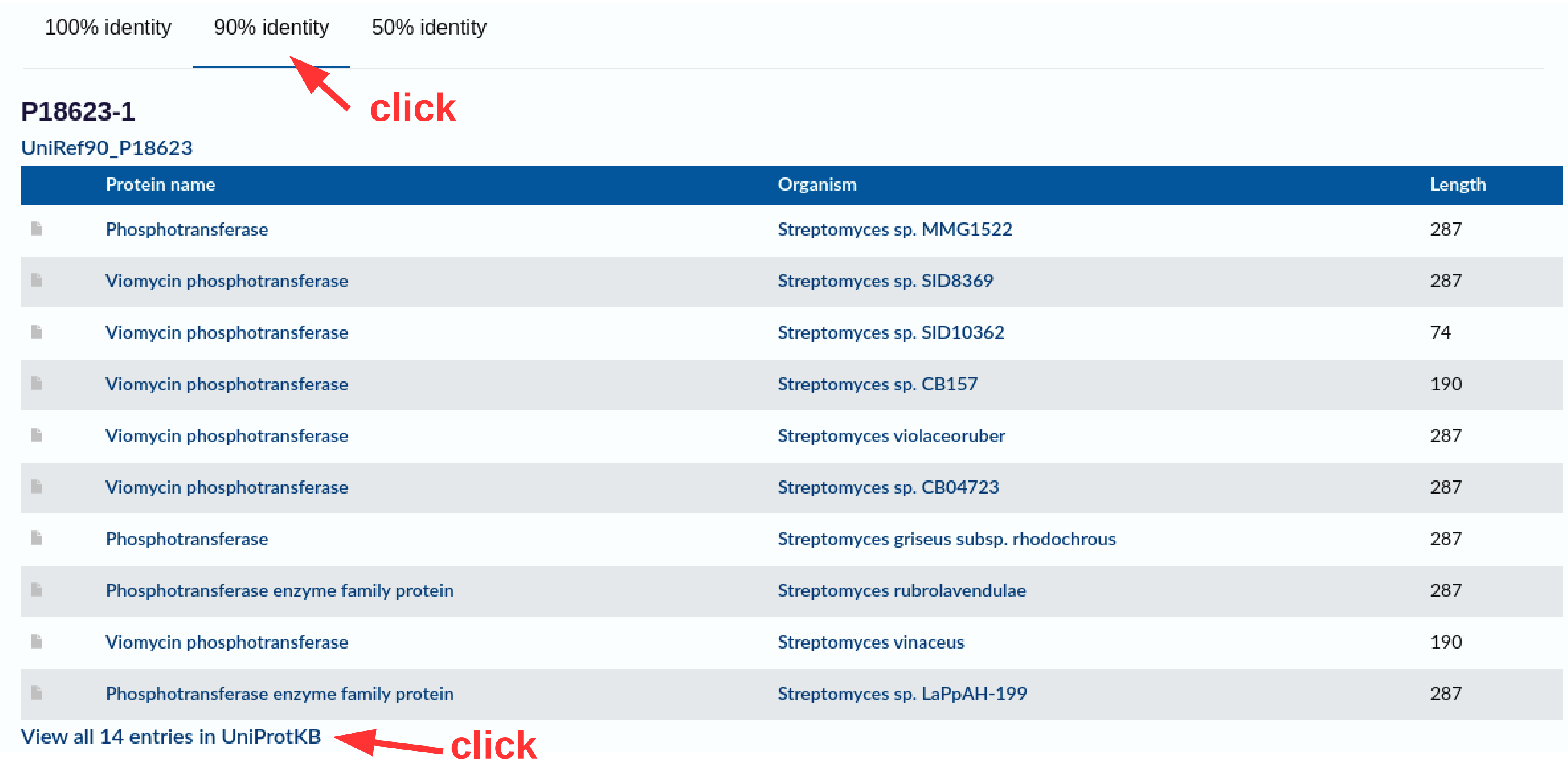
Catalytic activity

Rhea 20509: ATP + viomycin = ADP + H^+ + O-phosphoviomycin

EC:2.7.1.103 (UniProtKB | ENZYME | Rhea)

click

The most similar proteins can be used to build a model



click

P18623-1

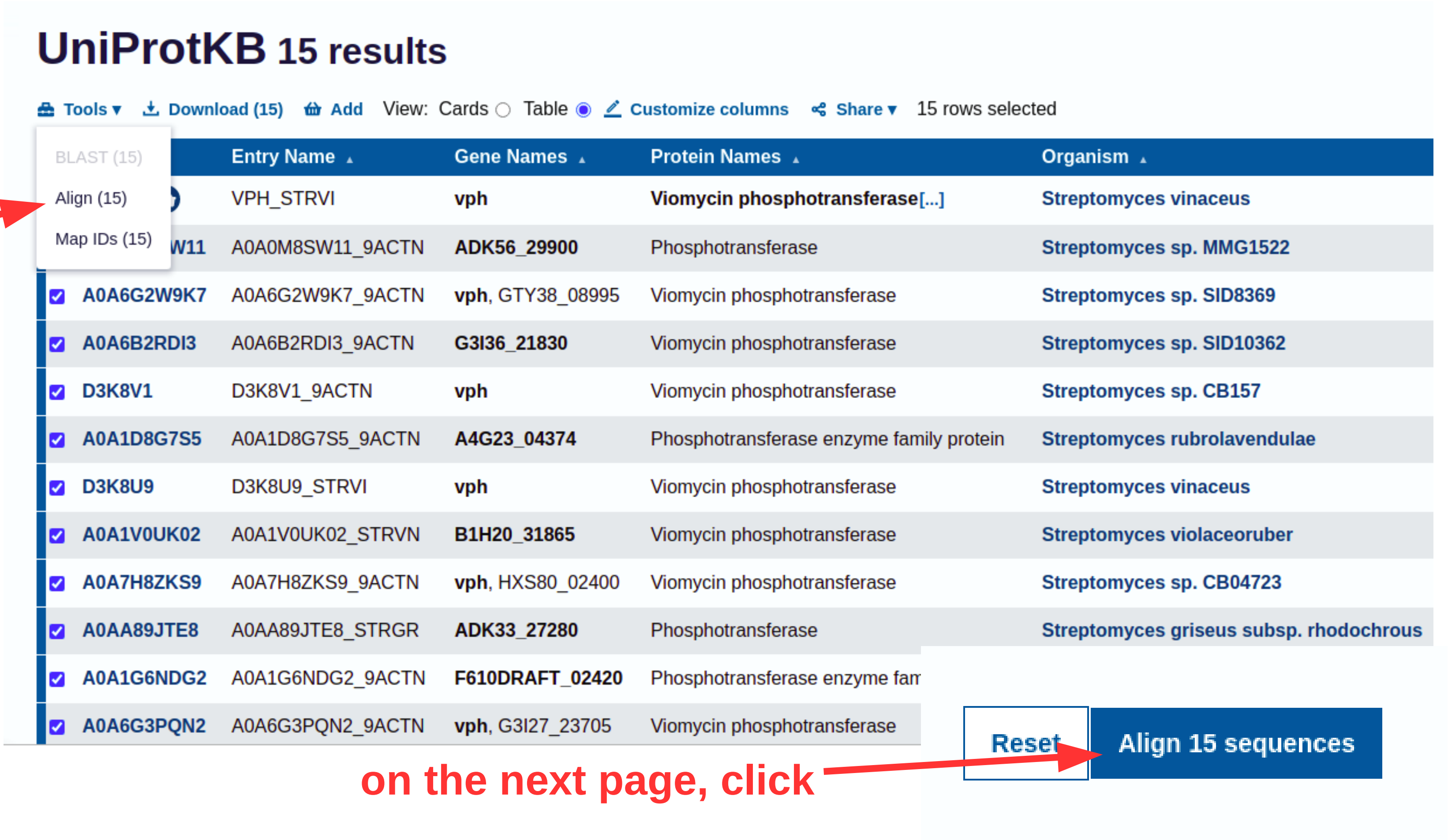
UniRef90_P18623

Protein name	Organism	Length
Phosphotransferase	Streptomyces sp. MMG1522	287
Viomycin phosphotransferase	Streptomyces sp. SID8369	287
Viomycin phosphotransferase	Streptomyces sp. SID10362	74
Viomycin phosphotransferase	Streptomyces sp. CB157	190
Viomycin phosphotransferase	Streptomyces violaceoruber	287
Viomycin phosphotransferase	Streptomyces sp. CB04723	287
Phosphotransferase	Streptomyces griseus subsp. rhodochrous	287
Phosphotransferase enzyme family protein	Streptomyces rubrolavendulae	287
Viomycin phosphotransferase	Streptomyces vinaceus	190
Phosphotransferase enzyme family protein	Streptomyces sp. LaPpAH-199	287

View all 14 entries in UniProtKB

click

The model must learn protein conservation



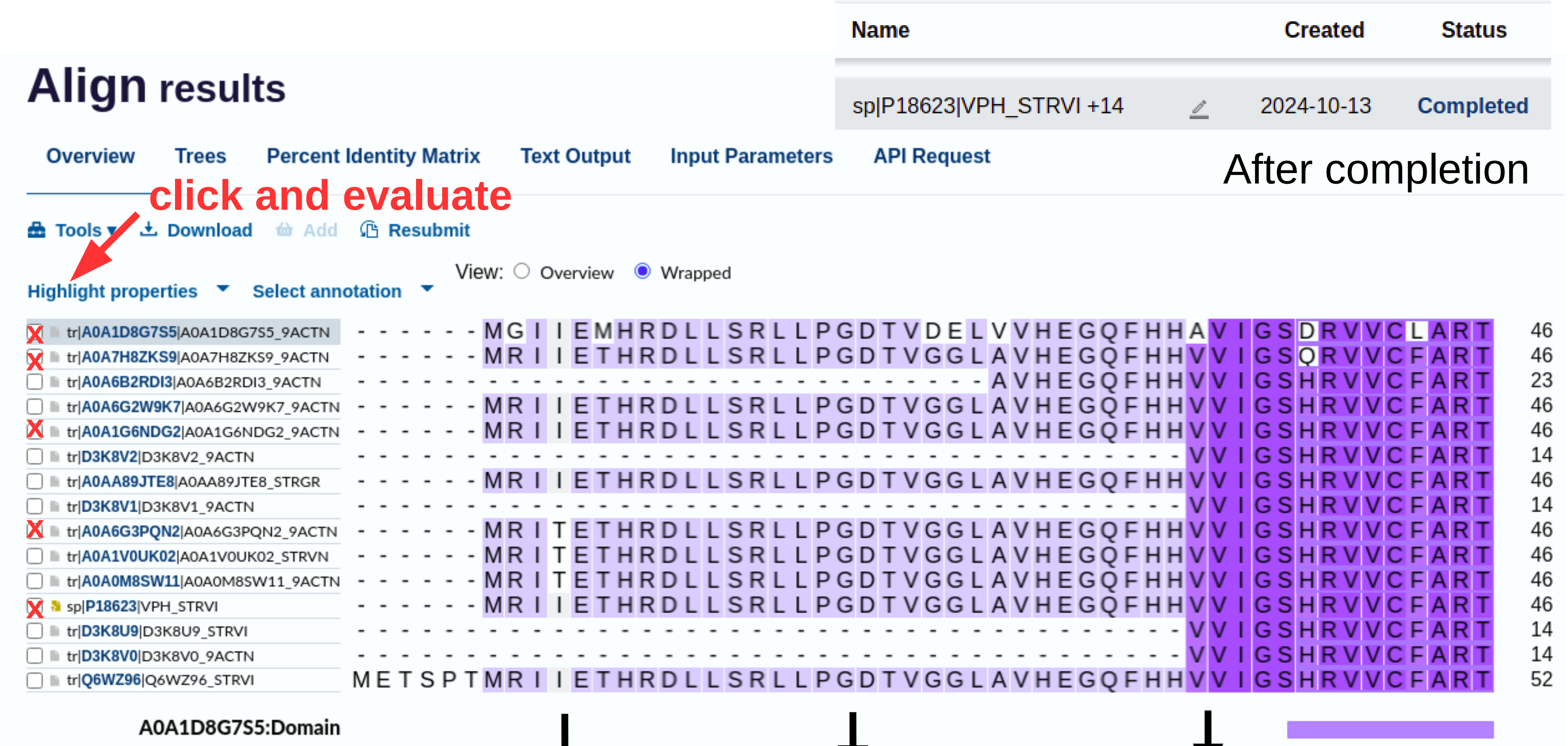
		Entry Name ▲	Gene Names ▲	Protein Names ▲	Organism ▲
		VPH_STRVI	**vph**	**Viomycin phosphotransferase**[...]	**Streptomyces vinaceus**
	W11	A0A0M8SW11_9ACTN	**ADK56_29900**	Phosphotransferase	**Streptomyces sp. MMG1522**
☑	**A0A6G2W9K7**	A0A6G2W9K7_9ACTN	**vph**, GTY38_08995	Viomycin phosphotransferase	**Streptomyces sp. SID8369**
☑	**A0A6B2RDI3**	A0A6B2RDI3_9ACTN	**G3I36_21830**	Viomycin phosphotransferase	**Streptomyces sp. SID10362**
☑	**D3K8V1**	D3K8V1_9ACTN	**vph**	Viomycin phosphotransferase	**Streptomyces sp. CB157**
☑	**A0A1D8G7S5**	A0A1D8G7S5_9ACTN	**A4G23_04374**	Phosphotransferase enzyme family protein	**Streptomyces rubrolavendulae**
☑	**D3K8U9**	D3K8U9_STRVI	**vph**	Viomycin phosphotransferase	**Streptomyces vinaceus**
☑	**A0A1V0UK02**	A0A1V0UK02_STRVN	**B1H20_31865**	Viomycin phosphotransferase	**Streptomyces violaceoruber**
☑	**A0A7H8ZKS9**	A0A7H8ZKS9_9ACTN	**vph**, HXS80_02400	Viomycin phosphotransferase	**Streptomyces sp. CB04723**
☑	**A0AA89JTE8**	A0AA89JTE8_STRGR	**ADK33_27280**	Phosphotransferase	**Streptomyces griseus subsp. rhodochrous**
☑	**A0A1G6NDG2**	A0A1G6NDG2_9ACTN	**F610DRAFT_02420**	Phosphotransferase enzyme far	
☑	**A0A6G3PQN2**	A0A6G3PQN2_9ACTN	**vph**, G3I27_23705	Viomycin phosphotransferase	

click

on the next page, click

The model must be carefully observed and refined

check to add



Name		Created	Status
sp|P18623|VPH_STRVI +14		2024-10-13	Completed

Align results

View: ○ Overview ● Wrapped

```
☒ tr|A0A1D8G7S5|A0A1D8G7S5_9ACTN   ------MGIIEMHRDLLSRLLPGDTVDELVVHEGQFHHAVIGSDRVVCLART  46
☒ tr|A0A7H8ZKS9|A0A7H8ZKS9_9ACTN   ------MRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSQRVVCFART  46
☐ tr|A0A6B2RDI3|A0A6B2RDI3_9ACTN   -----------------------------AVHEGQFHHVVIGSHRVVCFART  23
☐ tr|A0A6G2W9K7|A0A6G2W9K7_9ACTN   ------MRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☒ tr|A0A1G6NDG2|A0A1G6NDG2_9ACTN   ------MRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☐ tr|D3K8V2|D3K8V2_9ACTN           --------------------------------------VVIGSHRVVCFART  14
☐ tr|A0AA89JTE8|A0AA89JTE8_STRGR   ------MRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☐ tr|D3K8V1|D3K8V1_9ACTN           --------------------------------------VVIGSHRVVCFART  14
☒ tr|A0A6G3PQN2|A0A6G3PQN2_9ACTN   ------MRITETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☐ tr|A0A1V0UK02|A0A1V0UK02_STRVN   ------MRITETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☐ tr|A0A0M8SW11|A0A0M8SW11_9ACTN   ------MRITETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☒ sp|P18623|VPH_STRVI              ------MRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  46
☐ tr|D3K8U9|D3K8U9_STRVI           --------------------------------------VVIGSHRVVCFART  14
☐ tr|D3K8V0|D3K8V0_9ACTN           --------------------------------------VVIGSHRVVCFART  14
☐ tr|Q6WZ96|Q6WZ96_STRVI           METSPTMRIIETHRDLLSRLLPGDTVGGLAVHEGQFHHVVIGSHRVVCFART  52

A0A1D8G7S5:Domain
```

click and evaluate

After completion

they are all good: all in !!

the alignment continues down here

Ready to learn using Hidden Markov Models

Start with a multiple sequence alignment

⇩

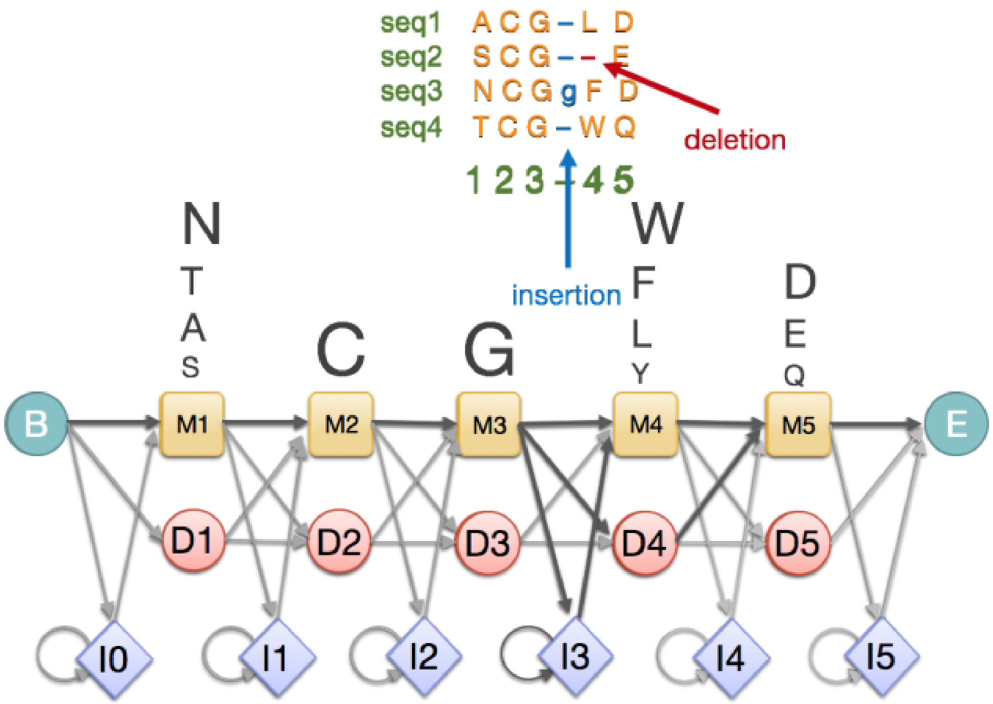
Insertions / deletions can be modelled

⇩

Occupancy and amino acid frequency at each position in the alignment are encoded

⇩

Profile created



The probabilistic **model** considers **observed frequencies** of the amino acids in each position as well as **their transitions** from the observed occupancy of each position in a multiple sequence alignment.

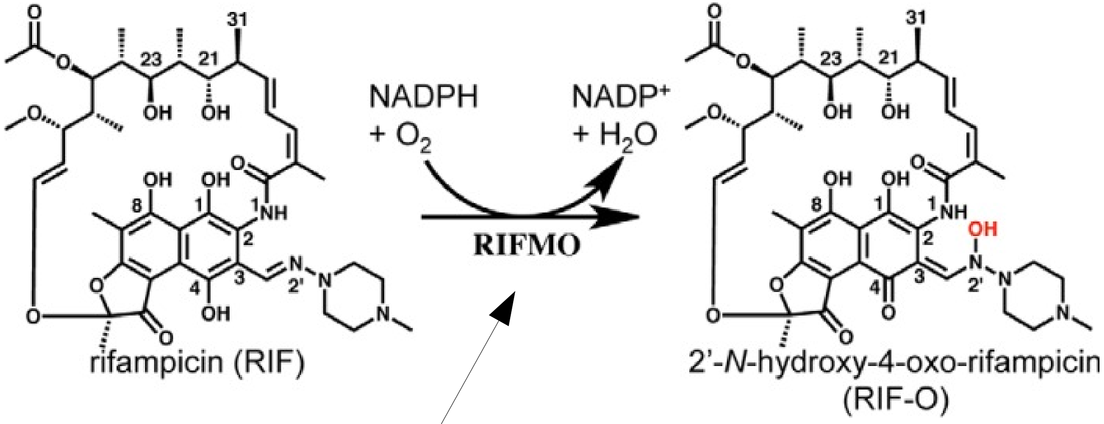
www.ebi.ac.uk

Deactivation by modification

Rifampicin is a potent frontline antibiotic against tuberculosis and other mycobacterial infections. It is produced in *Amycolaptosis rifamycinica* and then modified in vitro. It acst by **blocking RNA Polymerase**

Two mechanisms are known to deactivate it:

- point mutations in RNA polymerase in mycobacteria
- **enzyme-mediated inactivation** in soil actinomycetes

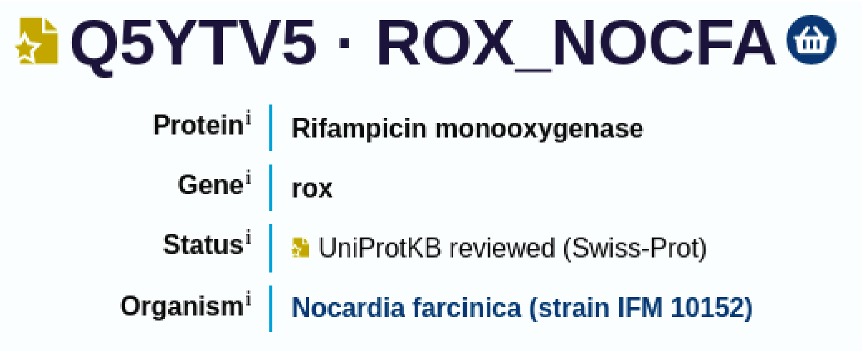


rifampicin (RIF)

2'-*N*-hydroxy-4-oxo-rifampicin (RIF-O)

Rifampicin monooxigenase

The uniprot reference is in Nocardia farcinica

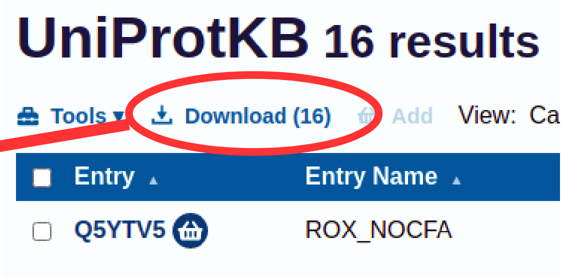


Q5YTV5 · ROX_NOCFA

Protein i	Rifampicin monooxygenase
Gene i	rox
Status i	UniProtKB reviewed (Swiss-Prot)
Organism i	Nocardia farcinica (strain IFM 10152)

The sequence similarity search at 90% indicate only 3 more proteins...not sufficient for a good modeling.

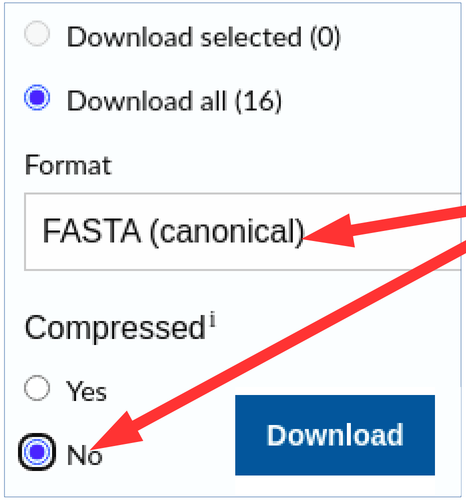
Let's search for **"rifampicin monooxygenase"**



UniProtKB 16 results

Tools ▾ | Download (16) | Add | View: Ca

☐	Entry ▲	Entry Name ▲
☐	Q5YTV5	ROX_NOCFA



○ Download selected (0)

● Download all (16)

Format

FASTA (canonical)

Compressed i

○ Yes

● No

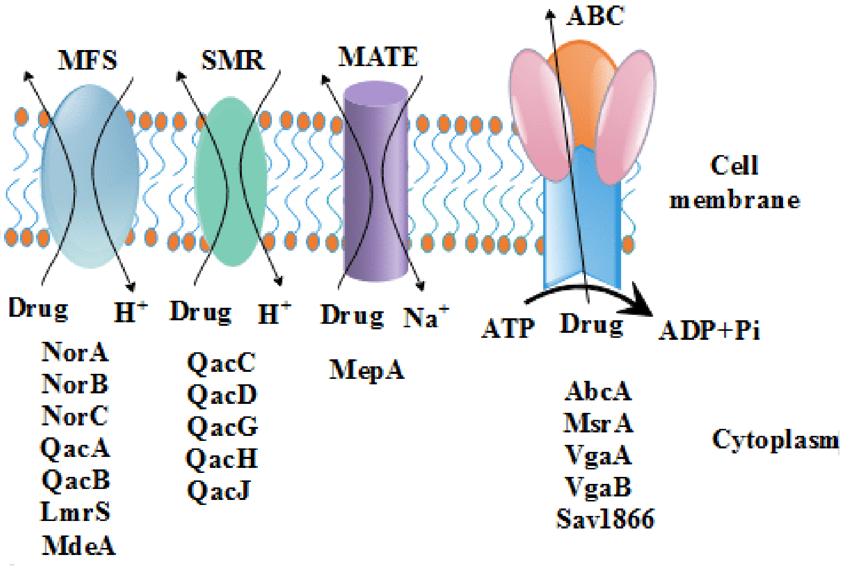
Download

Generate URL for API

just download, align and model

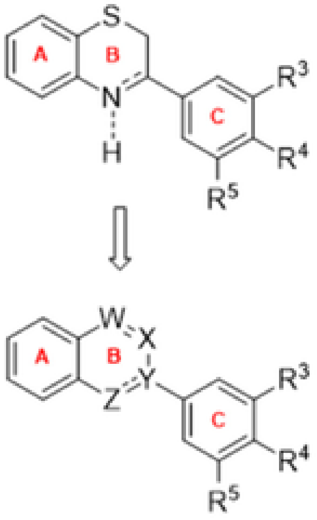
wget -O rifampicin.fasta "https://rest.uniprot.org/uniprotkb/stream?format=fasta&query=%28%22rifampicin+monooxygenase%22%29"

Deactivation by extrusion with efflux pumps

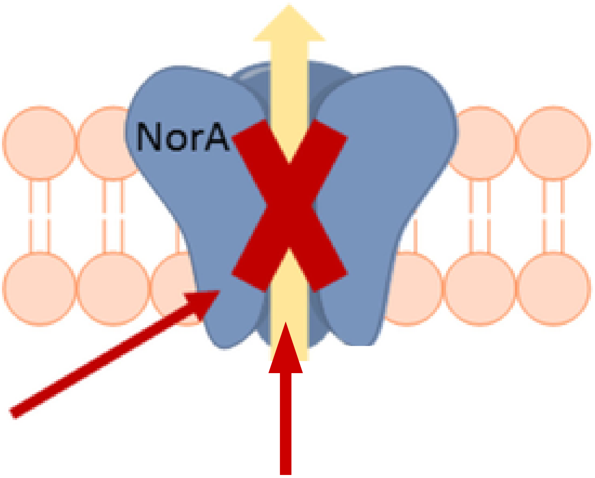


Efflux pumps overexpression is a major facilitator of multidrug resistance.

Active research is ongoing to block such aspecific pumps

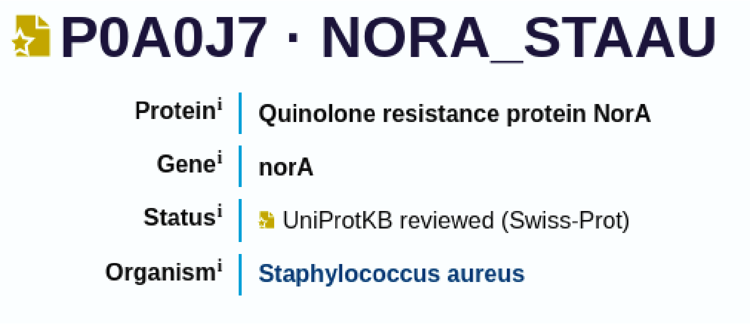


β-lactam,
erythromycin,
clindamycin,
ciprofloxacin (CPX)
tetracycline



β-lactam,
erythromycin,
clindamycin,
ciprofloxacin (CPX)
tetracycline

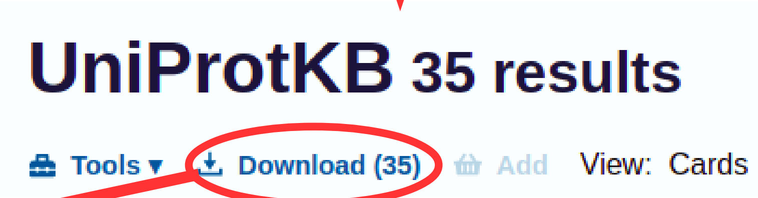
The uniprot reference is in Staphylococcus aureus



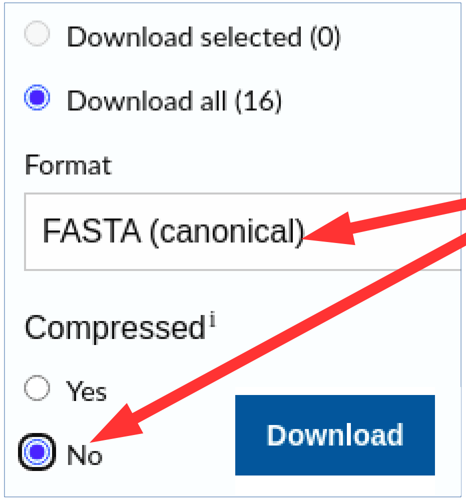
P0A0J7 · NORA_STAAU

Protein | **Quinolone resistance protein NorA**
Gene | **norA**
Status | UniProtKB reviewed (Swiss-Prot)
Organism | **Staphylococcus aureus**

The sequence similarity search at 90% indicate 35 proteins, sufficient for a good modeling.



UniProtKB 35 results



○ Download selected (0)

● Download all (16)

Format

FASTA (canonical)

Compressed

○ Yes

● No

Generate URL for API

just download, align and model

```
wget -O quinolone.fasta "https://rest.uniprot.org/uniprotkb/stream?format=fasta&query=%28uniref_cluster_90%3AUniRef90_P0A0J4%29
```

Antibiotic resistance databases

CARD

Comprehensive Antibiotic Resistance Database

Species	New Isolates	Total Isolates
Salmonella enterica	606	677,770
E.coli and Shigella	27	428,411
Campylobacter jejuni	24	129,117
Listeria monocytogenes	22	69,389

See more organisms...

https://doi.org/10.1128/aac.00419-13

https://www.ncbi.nlm.nih.gov/pathogens/

Remote computer: access and folder structure

To access the remote server download MobaXterm.exe,

- click on new session/ssh
  - ip address: 150.217.159.17
  - username: bioinfo11
- click connect
  - password: #14ott24#   --- you won't see anything during password typing

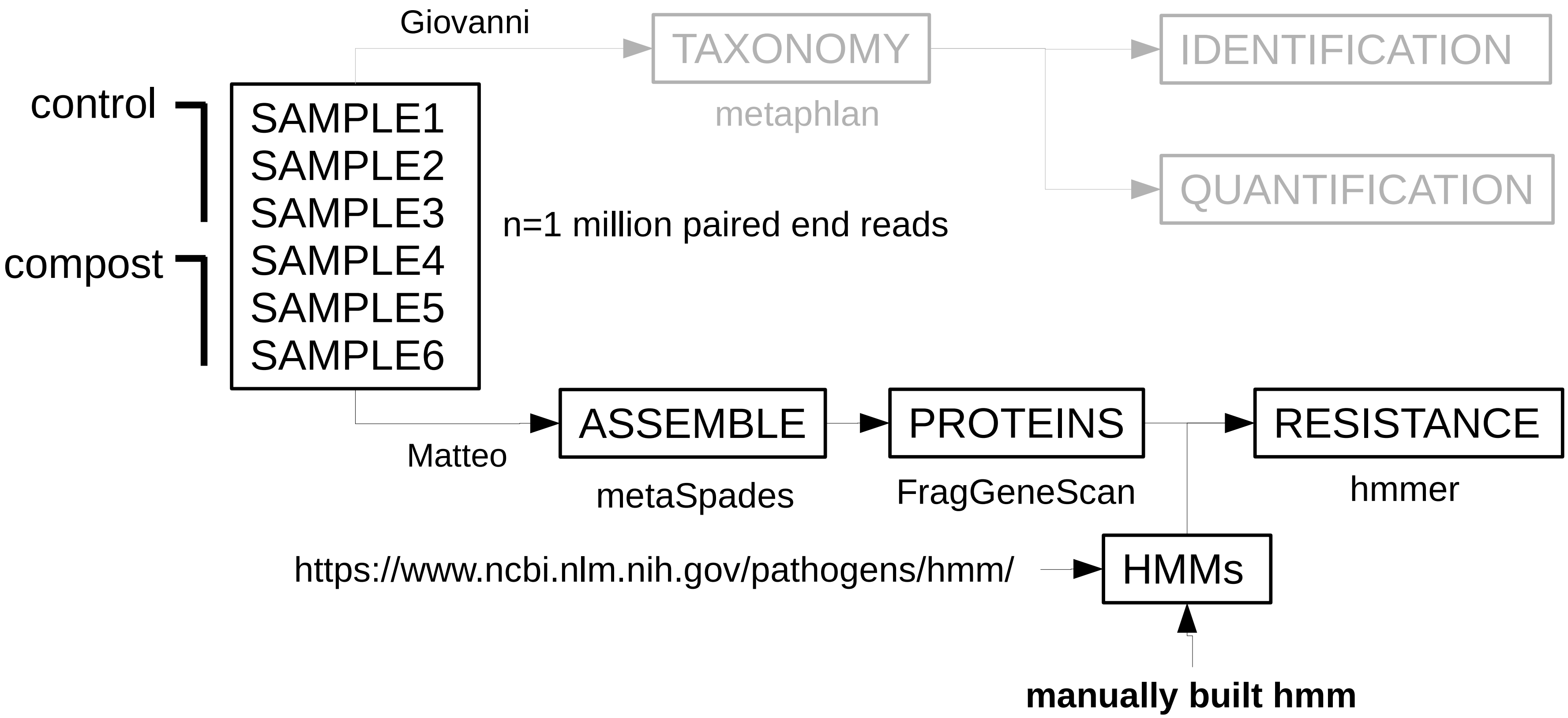
Once in type **ls** [enter]

You'll find the following folders:

reads : paired-end reads of 3+3 samples
data : processed reads – assembled and transformd to potential proteins
tax: processed reads – taxonimized and quantified
stud01 - 20 : once in the computer we will us assign you a number N
- cd studN – this will move you into the folder N
- **stay there = never type "cd .."**

Lab workflow

data from SRR7414903 - PMC9541739



Giovanni

TAXONOMY

metaphlan

IDENTIFICATION

QUANTIFICATION

control

compost

SAMPLE1
SAMPLE2
SAMPLE3
SAMPLE4
SAMPLE5
SAMPLE6

n=1 million paired end reads

Matteo

ASSEMBLE

metaSpades

PROTEINS

FragGeneScan

RESISTANCE

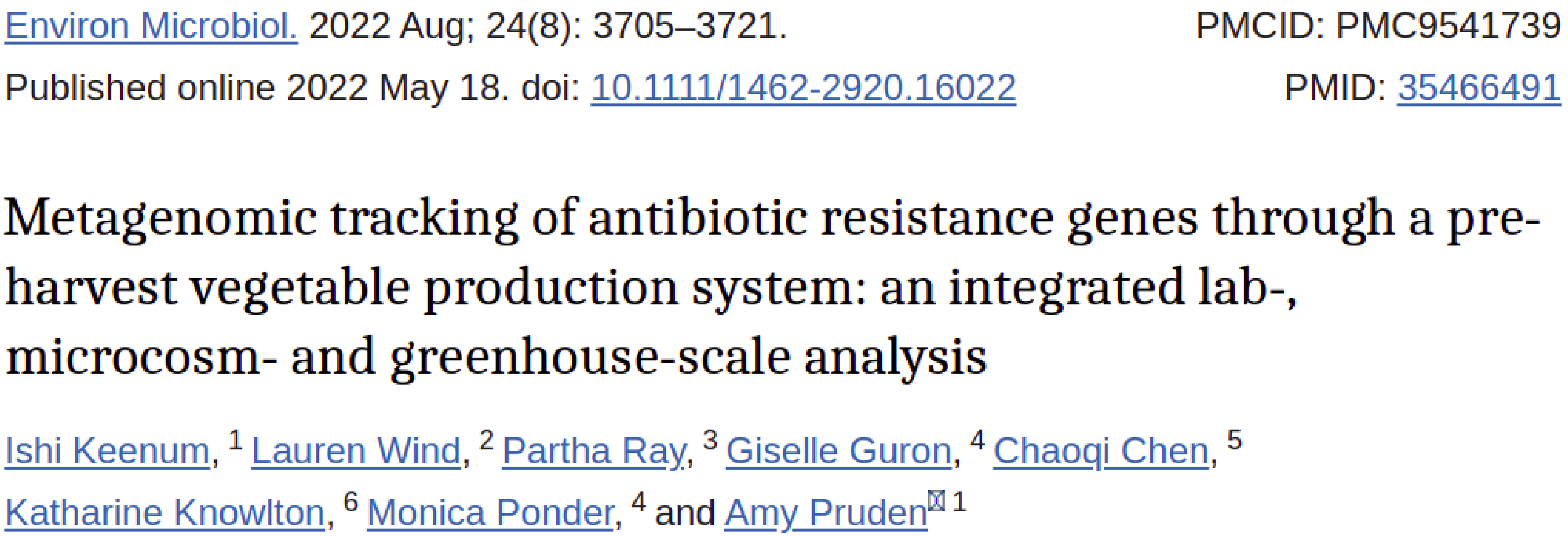
hmmer

https://www.ncbi.nlm.nih.gov/pathogens/hmm/

HMMs

manually built hmm

Input dataset – a matter of antibiotic resistance



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Metagenomic tracking of antibiotic resistance genes through a pre-harvest vegetable production system: an integrated lab-, microcosm- and greenhouse-scale analysis

Ishi Keenum,[1] Lauren Wind,[2] Partha Ray,[3] Giselle Guron,[4] Chaoqi Chen,[5] Katharine Knowlton,[6] Monica Ponder,[4] and Amy Pruden✉[1]

Prior research demonstrated the potential for **agricultural production systems** to **contribute to the environmental spread of antibiotic resistance genes** (ARGs)

1. control compost x 3

2. compost with antibiotics x 3

3. control manure

4. manure with antibiotics

Step 1 : from short reads to scaffolds and contigs

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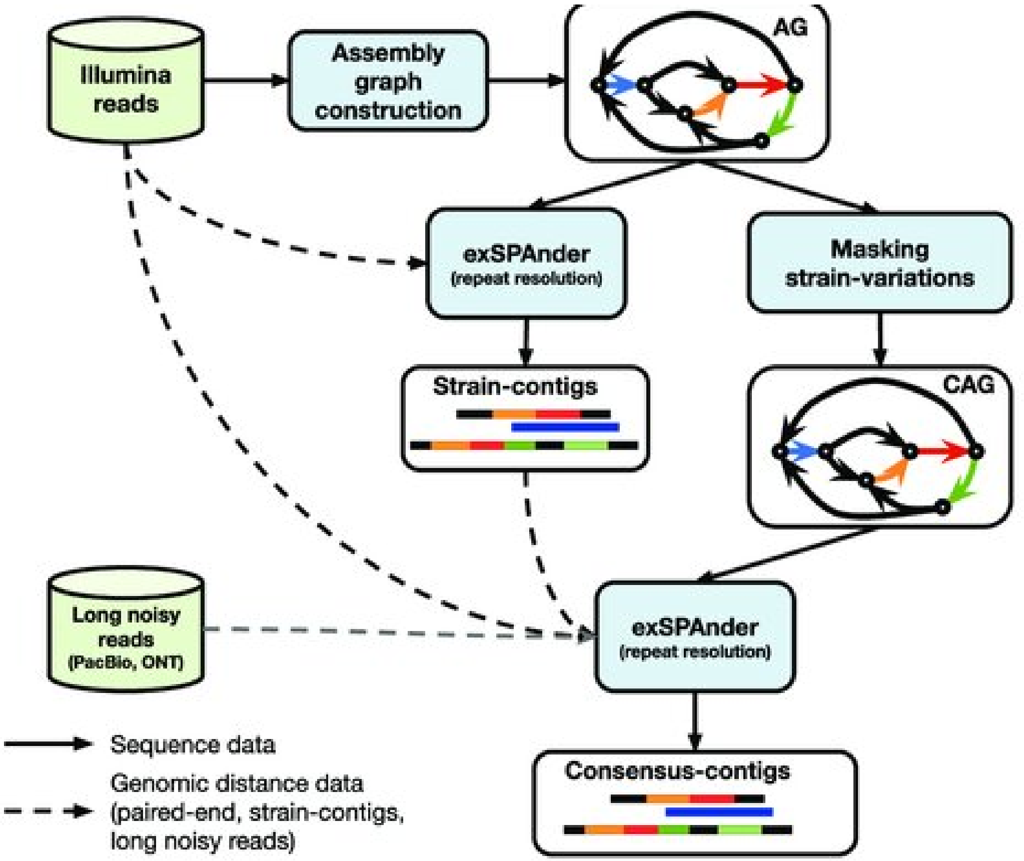
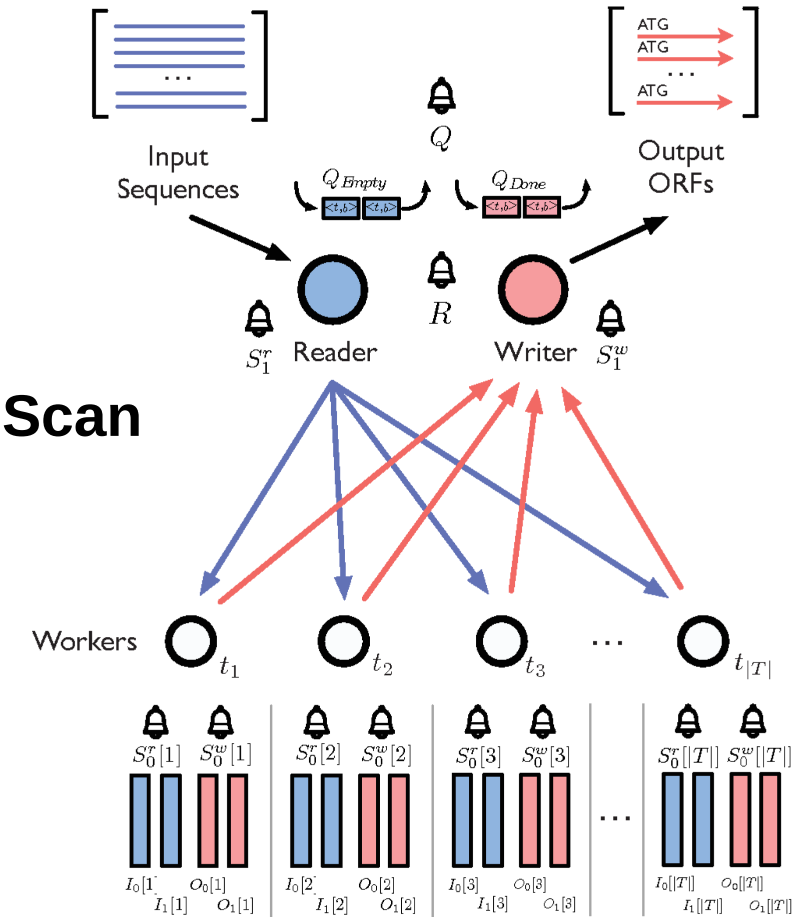
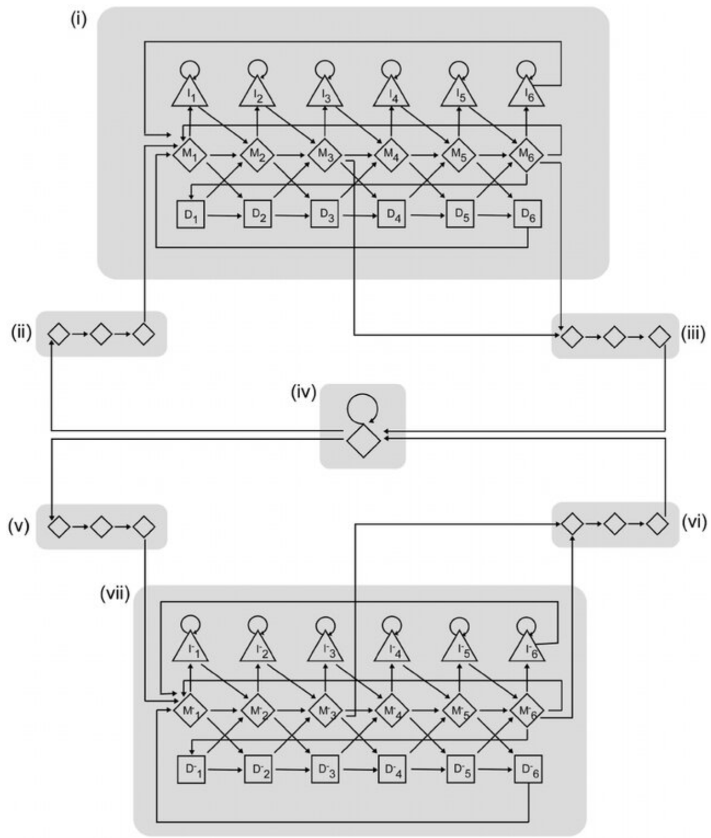


Step 2 : locating possible ORFs



FragGeneScan



Viomycin phosphotrasferase HMM preparation protocol

```
### go to www.uniprot.org ###
```

observe

```
# viomycin resistance has been studied in Streptomyces vinaceus
# go to https://www.uniprot.org/uniprotkb/P18623/entry
# last section is "similar_proteins"
```

compile

```
# click on 90%identity
# we see the preformed uniref_cluster_90:UniRef90_P18623 with 15 proteins
# click on View all
# thick the selector to select all proteins
# click on tools -> align
# we notice a warning, indicating to remove some proteins because they are identical
```

refine

```
# go back and remove protein 8(A0A1V0UK02),11(A0A1G6NDG2),12(A0A6G3PQN2),13(D3K8V0)
# click on tools -> align. No more warning, we can go on with 11 proteins
```

download

```
# scroll and evaluate the alignment to study variations in similar organisms. Seems good.
# click on download, choose to save in "raw submitted sequence".
# Open the downloaded text file (or copy if text is shown to screen).
# type cat > viomycin.fasta [enter]
# paste the copied text in the terminal
# type ctrl-c
```

align

```
# now you should have a file names viomycin.fasta in your space
# let's realign sequences using a local cluslalo
# clustalo -i viomycin.fasta -o viomycin.aln
# inspect the alignment by typing
# ../bin/alan viomycin.aln   (press q to exit)
```

build

```
# build the hidden markov model
# hmmbuild -n viomycin viomycin.hmm viomycin.aln
# stamp the model in binary format (makes calculatio faster)
# hmmpress viomycin.hmm
```

scan

```
for i in $(ls ../assembled/sample*/scaffolds.fasta.prot.faa)
do
hmmsearch rifampicin.hmm $i | grep -c ">>"
done
```

Rifampicin monooxigenase HMM preparation protocol

go to www.uniprot.org

observe

```
# rifampicin resistance has been studied in Nocardia farcinica
# go to https://www.uniprot.org/uniprotkb/Q5YTV5/entry
# using the strategy seen before (90% similarity) just returns 4 proteins form different strain of the same genus
# we can then switch to the query "rifampicin monooxygenase": we obtain 16 valid proteins, with no redundancy
```

download

```
# select all entries and click Download: this time click on "Generate URL for API"
# this returns a link that can be used to directly download proteins to our terminal
wget -O rifampicin.fasta  "https://rest.uniprot.org/uniprotkb/stream?format=fasta&query=%28%22rifampicin+monooxygenase%22%29"
# we now have all 16 proteins in our space, ready to be aligned and checked
```

align

```
clustalo -i rifampicin.fasta -o rifampicin.aln
../bin/alan viomycin.aln
# it seems consistent, let's then build the hidden Markov model
```

build

```
hmmbuild -n rifampicin rifampicin.hmm rifampicin.aln
hmmpress rifampicin.hmm
```

scan

```
for i in $(ls ../assembled/sample*/scaffolds.fasta.prot.faa)
do
hmmsearch rifampicin.hmm $i | grep -c ">>"
done
```

Quinolone resistance HMM preparation protocol

```
### go to www.uniprot.org ###
```

observe

```
# quinolone resistance has been studied in Staphilococcus aureus
# go to https://www.uniprot.org/uniprotkb/P0A0J7/entry
# using the strategy seen before (90% similarity) returns 36 proteins form different strain of the same genus
```

download

```
# select all entries and click Download: click on "Generate URL for API"
# this returns a link that can be used to directly download proteins to our terminal
wget -O qinolone.fasta  "https://rest.uniprot.org/uniprotkb/stream?format=fasta&query=%28uniref_cluster_90%3AUniRef90_P0A0J4%29
# we now have all 16 proteins in our space, ready to be aligned and checked
```

align

```
clustalo -i quinolone.fasta -o quinolone.aln
../bin/alan quinolone.aln
# it seems consistent, let's then build the hidden Markov model
```

build

```
hmmbuild -n quinolne quinolone.hmm quinolone.aln
hmmpress qinolone.hmm
```

scan

```
for i in $(ls ../assembled/sample*/scaffolds.fasta.prot.faa)
do
hmmsearch qinolone.hmm $i | grep -c ">>"
done
```

Scanning NCBI pre-formed HMMs

```
#from user's home
for j in $(ls ../hmm/db/hmm_NCBI/HMM/)
do
cp ../hmm/db/hmm_NCBI/HMM/$j tmp
hmmpress –f tmp
cat tmp | grep –B1 DESC
for i in $(ls ../assembled/sample*/scaffolds.fasta.prot.faa)
do
echo $i | perl –ne '/.+?(sample\d+_\w+)\//;print $1,": "'
hmmsearch tmp $i | grep –c ">>"
done
rm tmp*
read
done
```

This bash script streams all hmms from the NCBI models and reports the model name (so the resistance type) and the counts of scaffolds potentially containing such resistance.

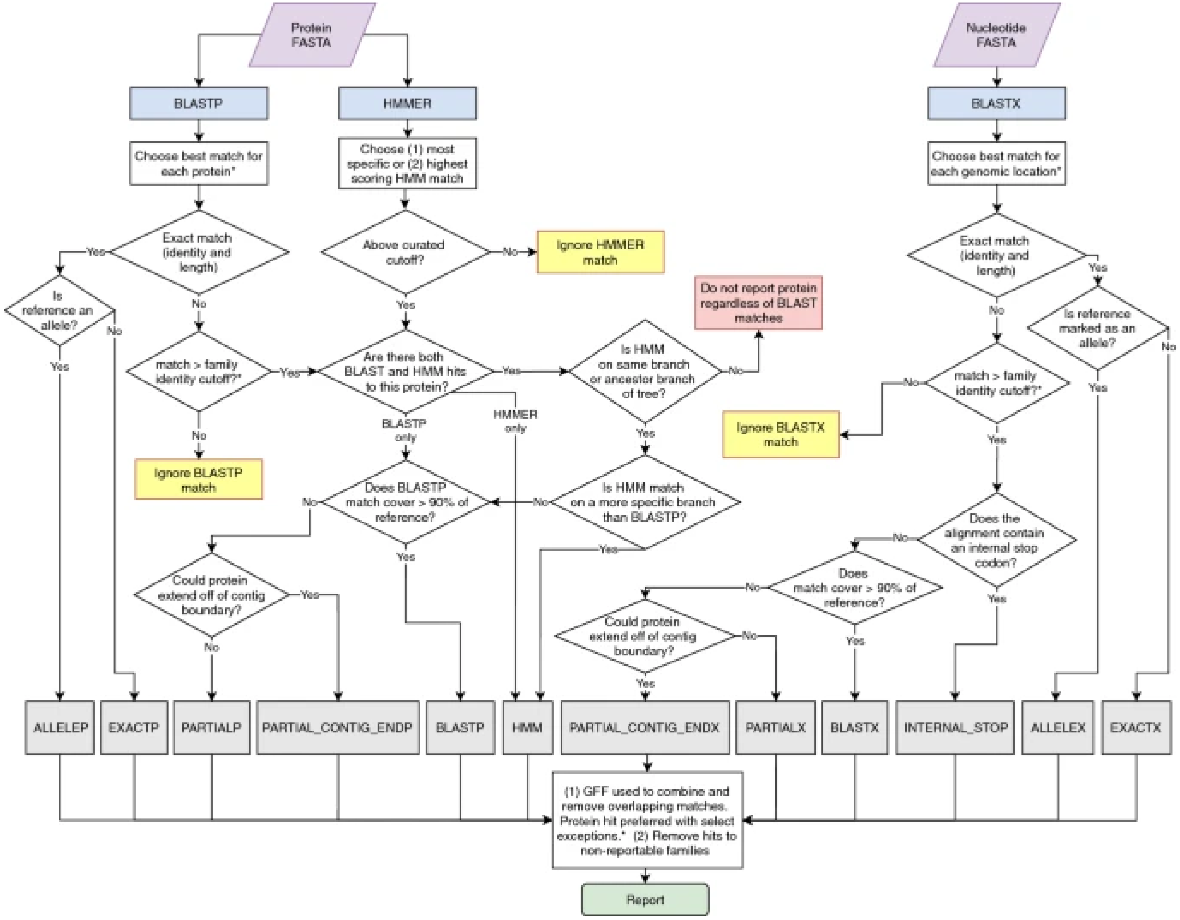
You'll see that samples 1,2,3 (untreated controls) are usually less affected by resistance than 4,5,6 (treated with antibiotics)

The latest tool: NCBI AMRFinderPlus

It can now use

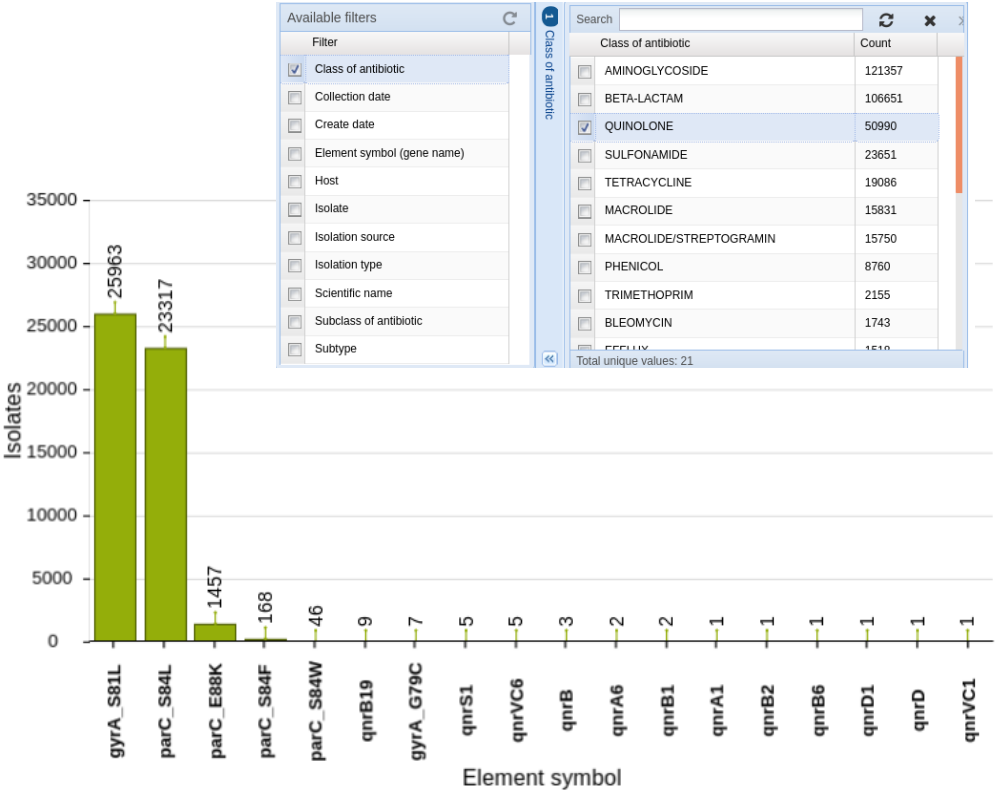
- nucleotide
- protein
- **both**

Using both, poymorphisms can be taken into account



Mapping isolates of concern @ NCBI

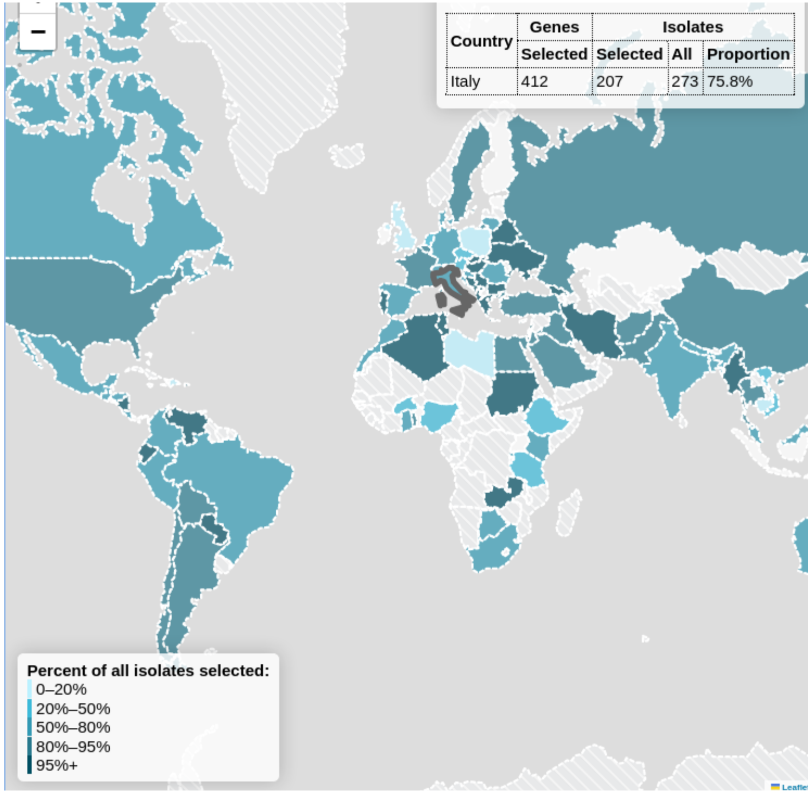
https://www.ncbi.nlm.nih.gov/pathogens/microbigge_map/



	Filter
☑	Class of antibiotic
☐	Collection date
☐	Create date
☐	Element symbol (gene name)
☐	Host
☐	Isolate
☐	Isolation source
☐	Isolation type
☐	Scientific name
☐	Subclass of antibiotic
☐	Subtype

	Class of antibiotic	Count
☐	AMINOGLYCOSIDE	121357
☐	BETA-LACTAM	106651
☑	QUINOLONE	50990
☐	SULFONAMIDE	23651
☐	TETRACYCLINE	19086
☐	MACROLIDE	15831
☐	MACROLIDE/STREPTOGRAMIN	15750
☐	PHENICOL	8760
☐	TRIMETHOPRIM	2155
☐	BLEOMYCIN	1743

Total unique values: 21



Country	Genes	Isolates		
	Selected	Selected	All	Proportion
Italy	412	207	273	75.8%

Percent of all isolates selected:
- 0–20%
- 20%–50%
- 50%–80%
- 80%–95%
- 95%+