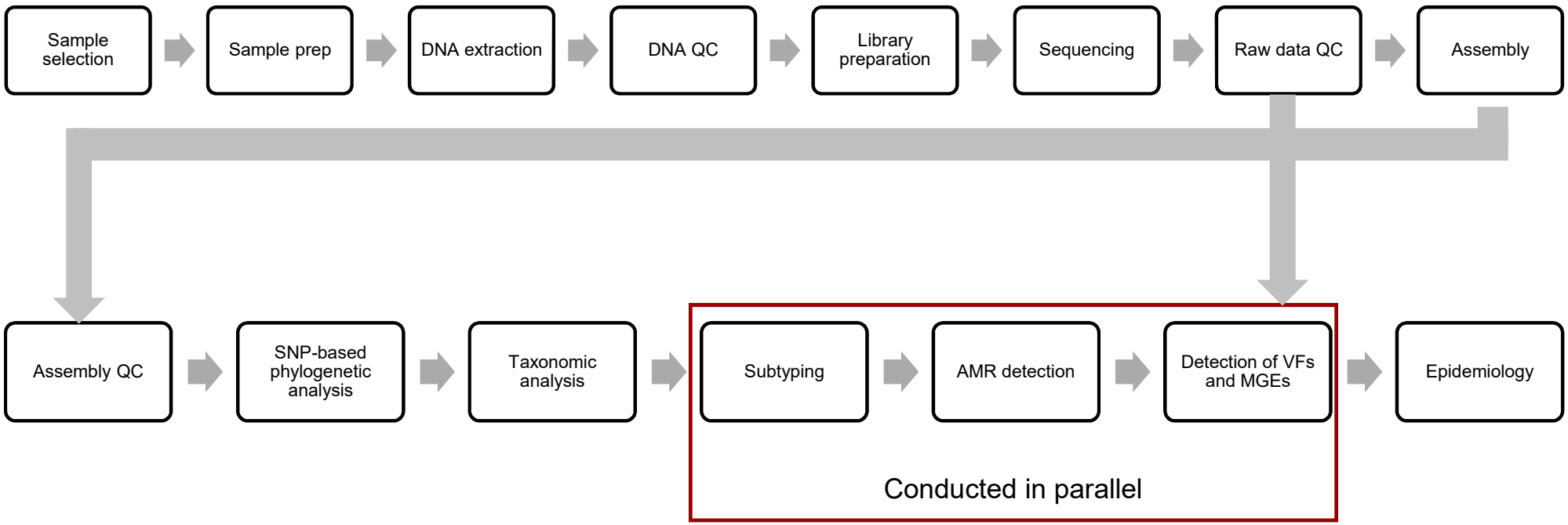
Day 7. Bridging the gaps in bioinformatics

# Annotation and AMR

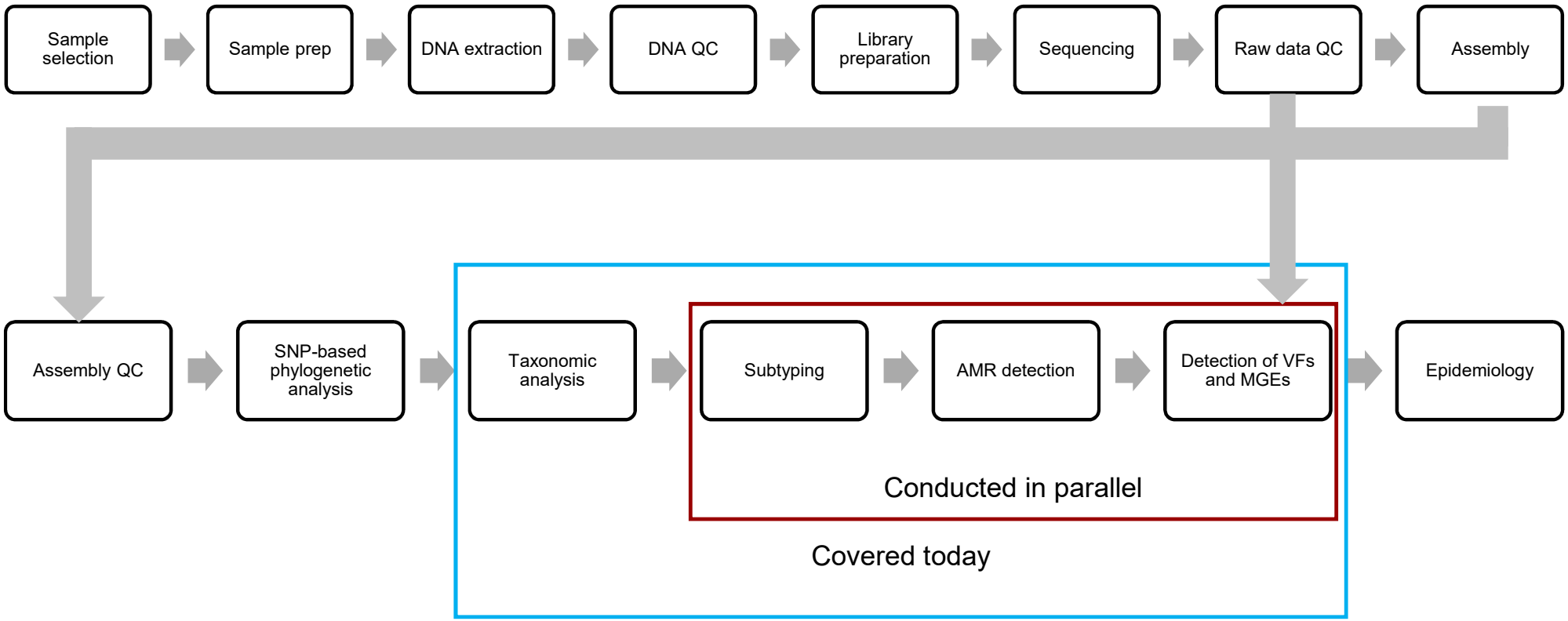
# Subjects this morning

- Annotation
  - Full genome annotation
  - Functional annotation
  - How do we predict?
    - Kmer
    - Seed and extend
  - Identification of genes of interest
    - Database considerations
    - AMR
    - How to make your own database
    - Replicons

# Example of workflow



# Example of workflow



# Annotation

- “The annotation process infers the structure and function of the assembled sequences. Protein-coding genes are often annotated first, but other features, such as non-coding RNAs or presence of regulatory or repetitive sequences, can also be annotated.” (Dominguez Del Angel et al, 2018)

- Once we have the assembled the genome annotation is straight forward:
  - Conformation of species
  - Prediction of genes
  - Prediction of function
    - Conserved modules
  - Typing
  - Other features

- If only certain features are of interest, we can use raw sequence data directly

# Full genome annotation

- With Whole genome sequencing (WGS) we capture (almost) everything in the cell
  - Prokka: rapid prokaryotic genome annotation (GitHub - tseemann/prokka: Rapid prokaryotic genome annotation)
  - ANNOVAR: Higher organisms (ANNOVAR Documentation (openbioinformatics.org))
  - NCBI-PGAP: Prokaryotic annotation (NCBI Prokaryotic Genome Annotation Pipeline (nih.gov))
  - Predictive annotation: eggNOG-mapper (eggNOG-mapper (embl.de))

- These pipelines usually generate multiple output files, which can be used for further data handling or visualization

- There are multiple visualization tools, e.g. IGV, which can be installed locally or used online.


E. Coli strain K-12 from NCBI visualized with gff in IGV (IGV)

# Data is everything

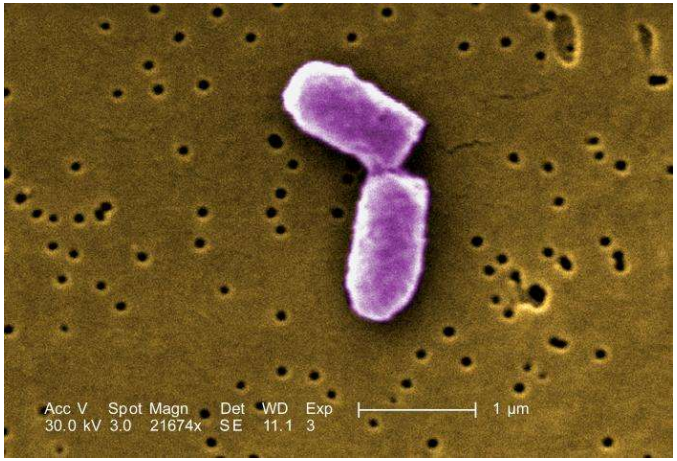
- We can produce a lot of data, but is it useful?
  - Bioinformatics relies heavily on findings produced in the wet lab
    - We cannot prove biological functions purely *in silico*
    - We can make prediction based on previous findings
    - We can track changes down to the nucleotide level
  - We are limited by our databases
    - Some genes have no annotation information
      - Gene with unknown function, but recognized modules or motives
    - Pseudo genes
  - We are only interested in a small subset of information
    - If we are interested in a given subject, we can reduce the amount of data
    - In E. coli K-12 there are 4639 predicted genes of which 3 are related to AMR
    - Specialized databases
    - Simplifies analysis and clarifies message

# Data is everything

- Translating classic biochemical methods to bioinformatics
  - Serotyping of *E. coli* is based on testing the O- and H-antigens with antisera
  - 186 O-antigens and 53 H-flagellar antigens determines the serotype
  - Time consuming and sometimes inconclusive

- O- and H- genes have been extracted and their associated phenotype determined
  - Predicting the Serotype based on genetic profile showed >98% concordance with phenotype
  - Analysis can be conducted in minutes rather than hours
  - It is still only an estimate

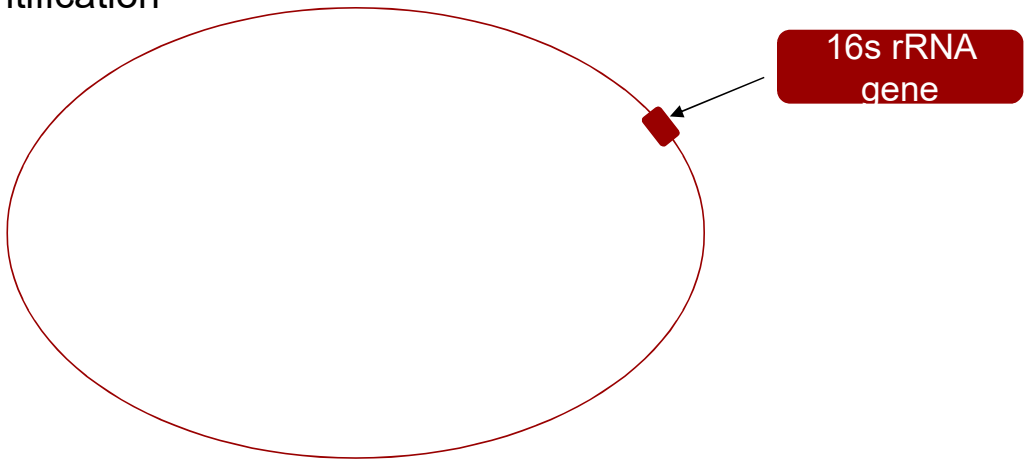
(Joensen et al., 2015, Fratamico et al., 2016)




*E. coli in scanning electron microscopic image, CDC/ Evangeline Sowers, Janice Haney Carr, 2005, Public domain image, https://phil.cdc.gov/Details.aspx?pid=10042*

# Prediction of species

- 16s rRNA gene formed the basis as the first method for sequenced based taxonomy
- Other approaches:
  - gyrB gene, rMLST, species-specific functional domain profiles
  - Shortcomings:
    - Only represents a small fraction of the entire genome
    - Today we have WGS data, better to ultilize the whole genome instead of just one gene for identification



# Prediction of species - Kmerfinder

- With WGS we can use all the genetic information to predict the species
- Kmerfinder works by breaking a genome into little pieces (k-mers) and identifying the species from these pieces (k-mers)

# k-mers

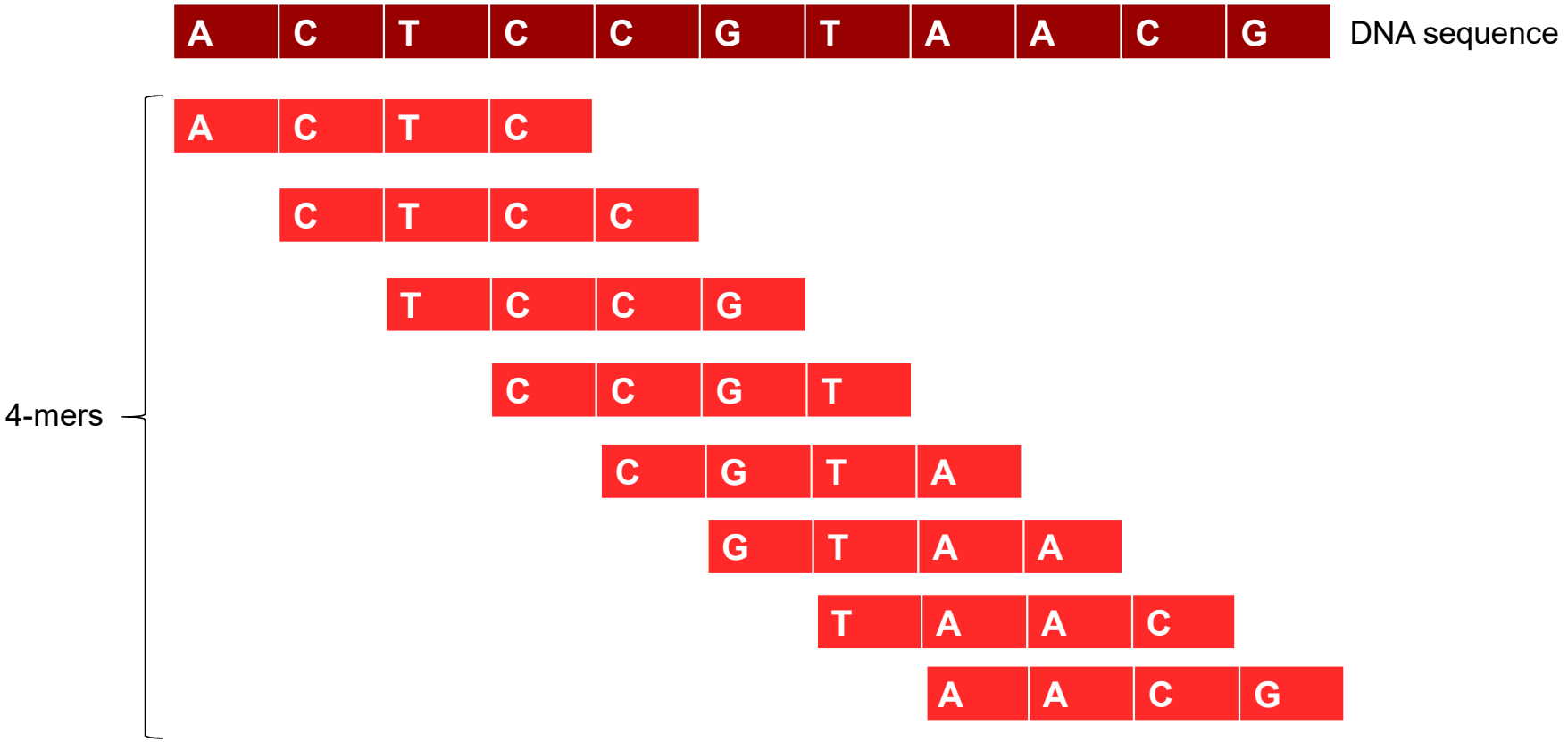
- A k-mer is a continuous sequence of k bases
  - e.g a certain length of DNA, RNA or protein
- k is any positive integer
- Sequences with high similarity must share k-mers

- Consider the nucleotide sequence 'ACTCCGTAACG'.

| A | C | T | C | C | G | T | A | A | C | G |
|---|---|---|---|---|---|---|---|---|---|---|

- We have this long sequence that we can cut into smaller pieces or k-mers
- We can extract all the 4-mers (substrings of length 4) in this DNA sequence

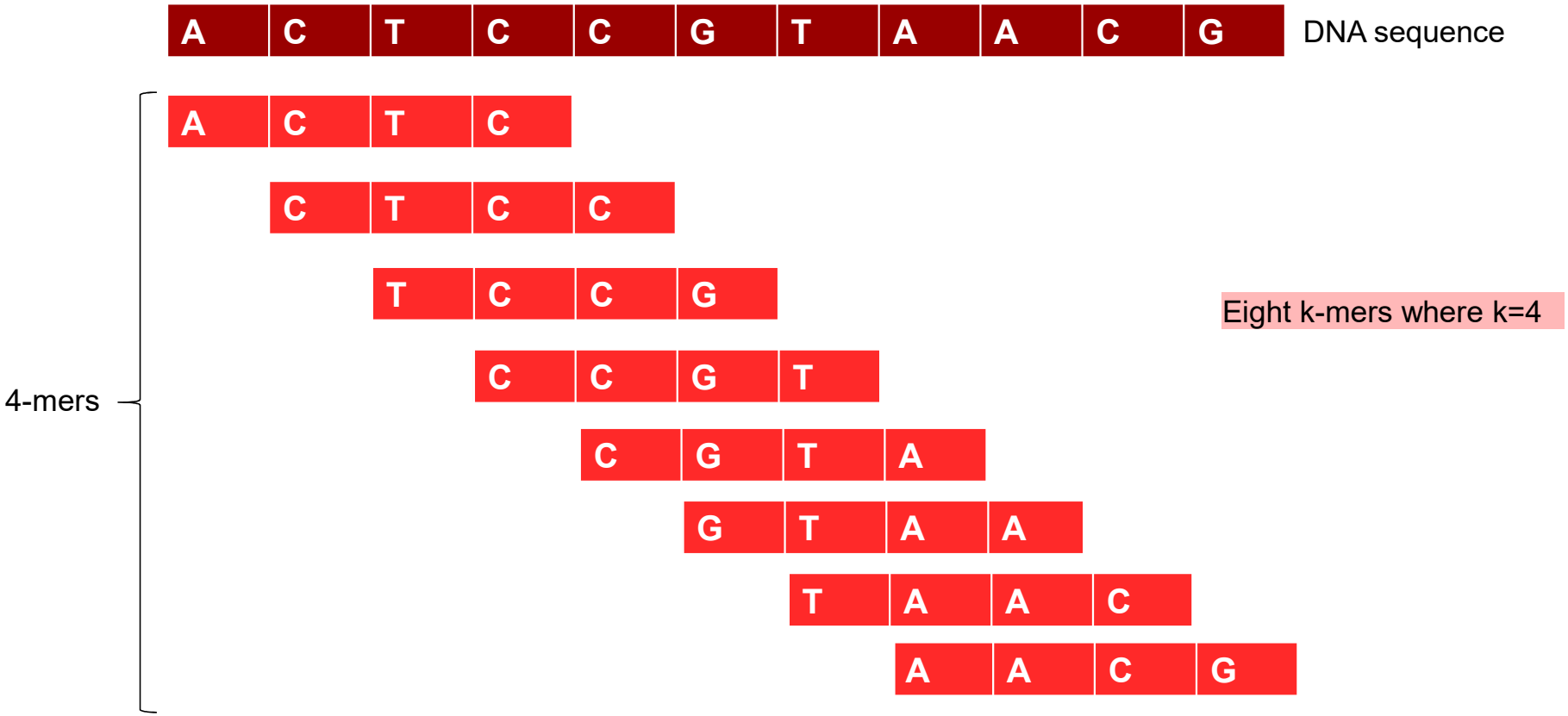
# k-mers


A C T C C G T A A C G
DNA sequence
4-mers
A C T C
C T C C
T C C G
C C G T
C G T A
G T A A
T A A C
A A C G

# k-mers

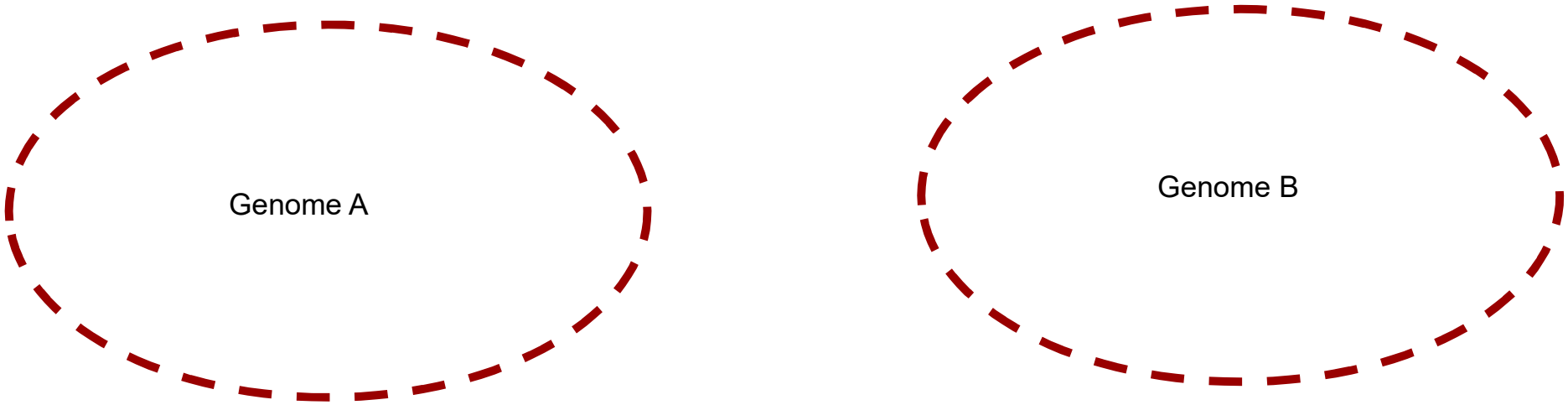
- We can extract all the 4-mers (substrings of length 4) in this DNA sequence.
- We created a window of length 4 and slide it from left to right, shifting one character at a time.
- If the length of a given DNA sequence is N, we end up with N - k+1 k-mers
- Total number of k-mers = N – k + 1
- In the previous slide, the DNA sequence is 11 characters long (N = 11) and k = 4, so we get eight k-mers (11- 4 + 1)

# k-mers

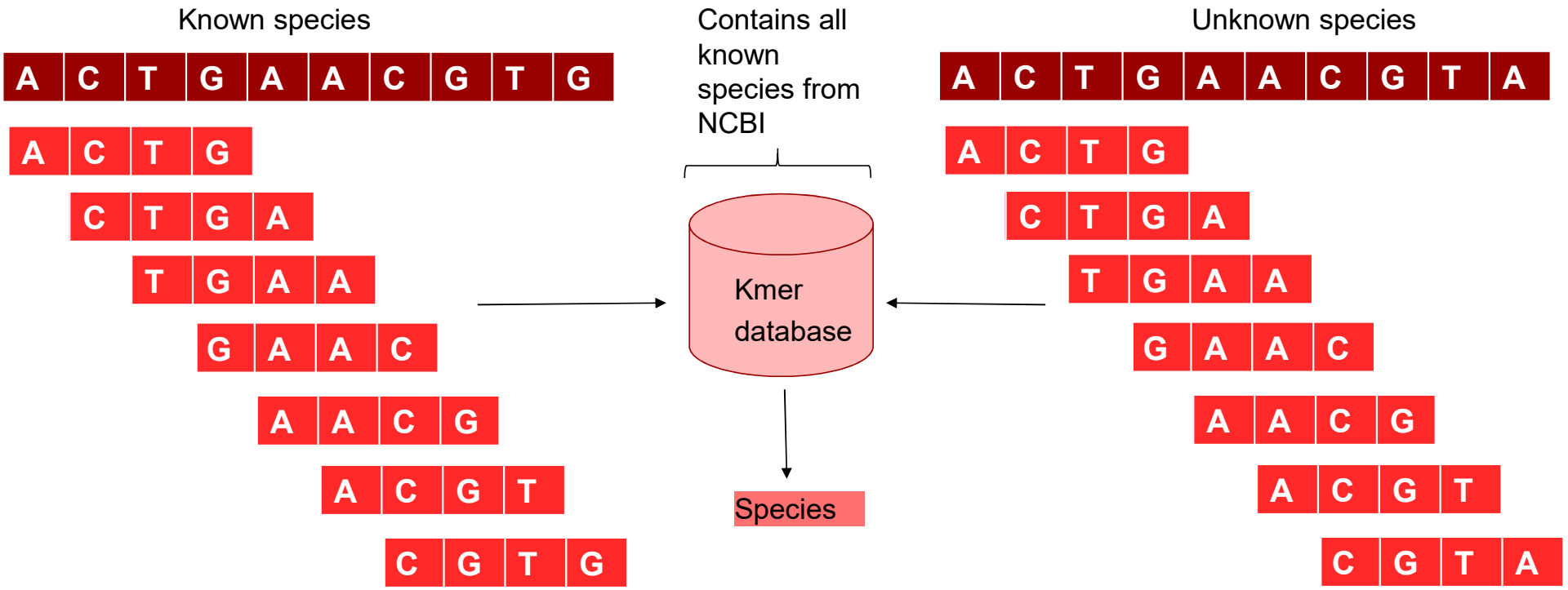

A C T C C G T A A C G
DNA sequence
4-mers
A C T C
C T C C
T C C G
C C G T
C G T A
G T A A
T A A C
A A C G
Eight k-mers where k=4

# Species prediction with k-mers

- Sequences with high simlarity must share k-mers
- We can break genomes up into k-mers and compare them



# Species prediction with KmerFinder

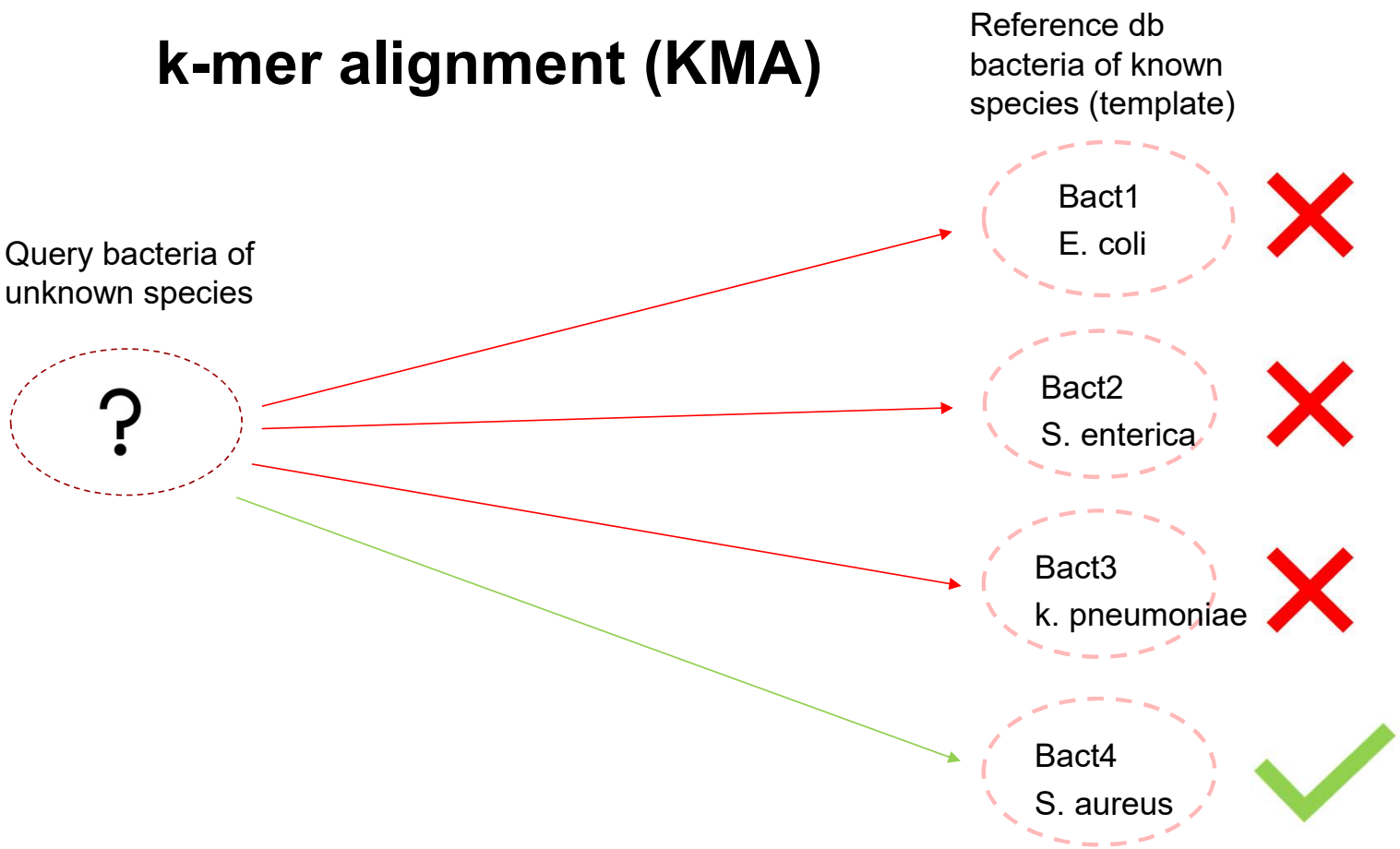


# Species prediction with KmerFinder

- Genomes are spilt into 16-mers
- But all these k-mers won't be in the database
- Tool will take too long to compute
- So redundant data is reduced
- Only 16-mers with specific prefixes are kept e.g ATGAG
- Reduced redundancy speeds up the tool

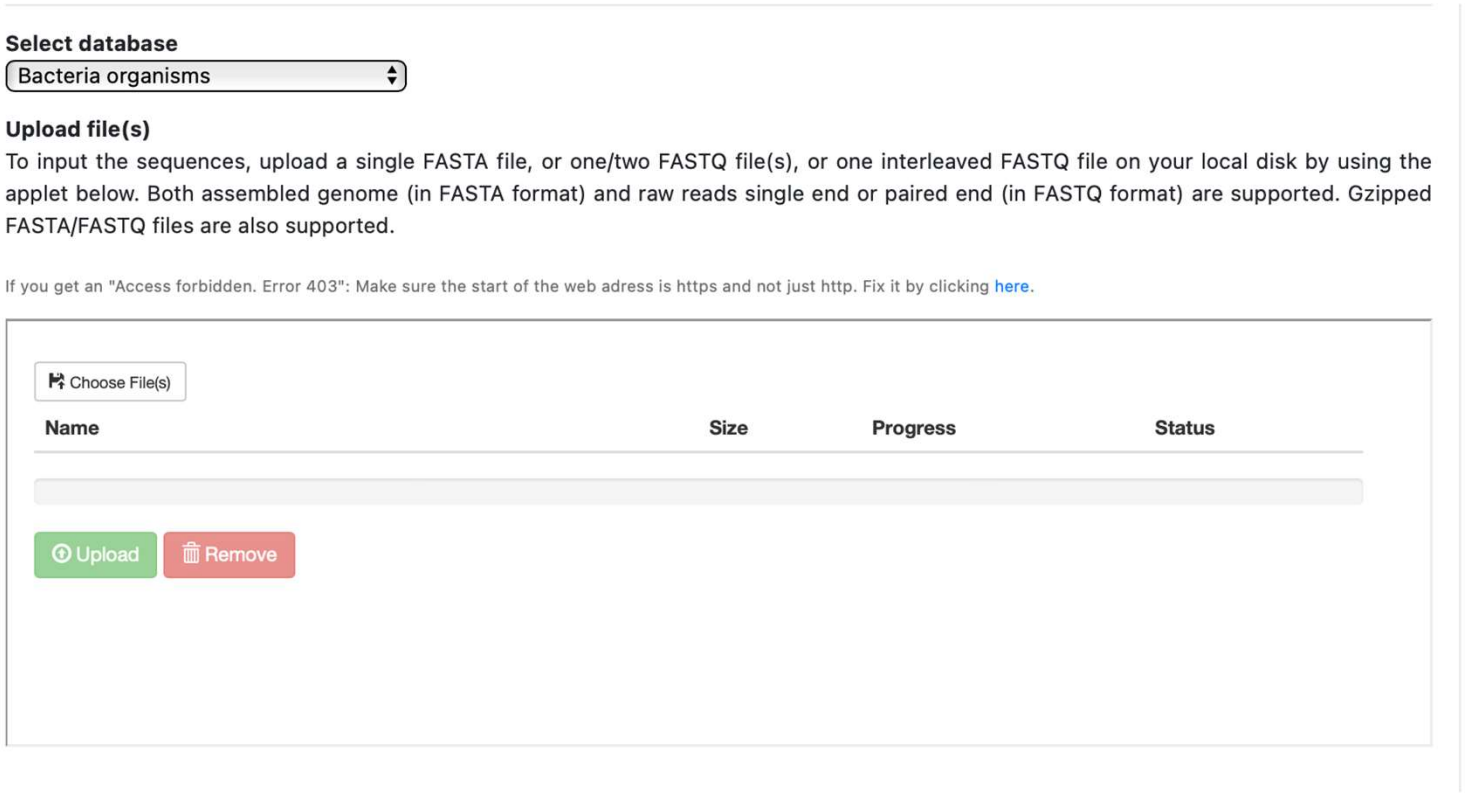
- But how does the tool the compare k-mers?

# k-mer alignment (KMA)




*Adapted from: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

# KmerFinder webtool




*https://cge.food.dtu.dk/services/KmerFinder/*

# Kmer webtool output

| Hit | Score | z-score | Query Coverage [%] | Template Coverage [%] | Depth | Total Query Coverage [%] | Total Template Coverage [%] | Total Depth |
|---|---|---|---|---|---|---|---|---|
| Escherichia coli, Escherichia coli O104:H4, Escherichia coli O104:H4 str. 2011C-3493 get sequence | 10879 | 527.6 | 97.08 | 99.79 | 1.00 | 97.08 | 99.79 | 1.00 |
| Escherichia coli, Escherichia coli NA114 get sequence | 16 | 9.3 | 0.14 | 0.15 | 0.00 | 54.87 | 58.92 | 0.60 |
| Salmonella enterica, Salmonella enterica subsp. enterica, Salmonella enterica subsp. enterica serovar Typhimurium, Salmonella enterica subsp. enterica serovar Typhimurium str. T000240 get sequence | 12 | 6.9 | 0.11 | 0.11 | 0.00 | 6.59 | 6.87 | 0.08 |

*Source: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

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**Score**
number of k-mers

*Source: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

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z-score
statistical significant

*Source: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

# Kmer webtool output

| Hit | Score | z-score | Query Coverage [%] | [illegible] | | | | [illegible]pth |
|---|---|---|---|---|---|---|---|---|
| Escherichia coli, Escherichia coli O104:H4, Escherichia coli O104:H4 str. 2011C-3493 get sequence | 10879 | 527.6 | 97.08 | 99.79 | 1.00 | 97.08 | 99.79 | 1.00 |
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**Query Coverage**
percentage of k-mers from unknown sample that map to a template

*Source: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

# Kmer webtool output

| Hit | Score | z-score | Query Coverage [%] | Template Coverage [%] | | | | |
|---|---|---|---|---|---|---|---|---|
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**Template Coverage**
percentage of k-mers from template genome that covered by k-mers from an unknown sample

*Source: Introduction to CGE tools, presentation by Pimpalas Leekitcharoenphon*

# Functional annotation AMR

- Attaching biological, chemical or otherwise functional information to a DNA sequence

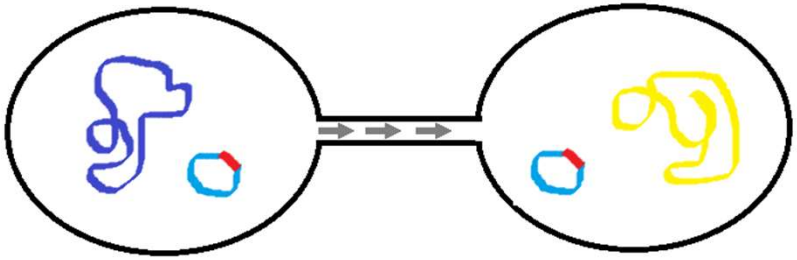
- Often you are only interested in a limited set of genes, we will look further into AMR

- AMR is a large threat to public health
  - Carried on mobile genetic elements (MGE) -> horizontal gene transfer
  - Estimated 1.27 million people died due to AMR in 2019 and estimated up to 10 million deaths by 2050 (Murray et al., 2019)
  - Development of new drugs is slow (Norrby et al., 2005)

# Genetic basis of AMR

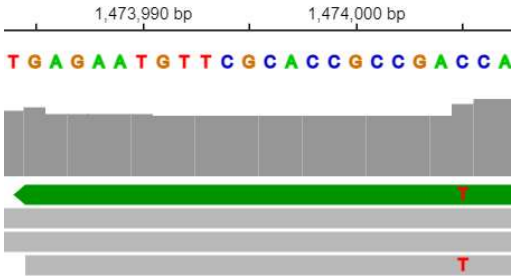
- AMR is conferred by different mechanisms:
  - Acquired resistance genes
  - Mutation
  - (Copy numbers)

- MGEs can transfer resistance genes between isolates closely or distantly related
- Resistance genes tend to aggregate, meaning MGEs often confer resistance to multiple classes
- May integrate into host chromosome



Transfer of plasmid with resistance gene

- Point mutations can confer resistance by various mechanisms:
  - Change the target of a drug, making the strain resistant
  - Upregulate the expression of a gene
  - Downregulate the expression of a gene
  - Change target specificity of protein
  - Usually species specific




Possible point mutation

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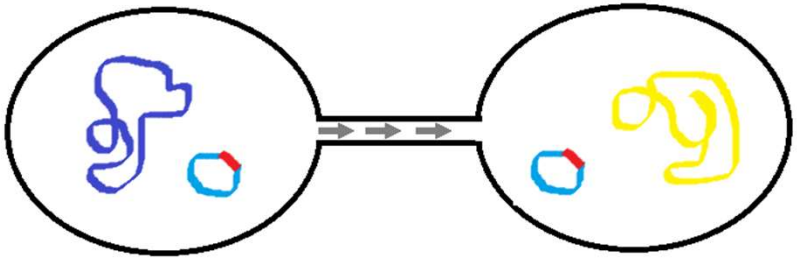
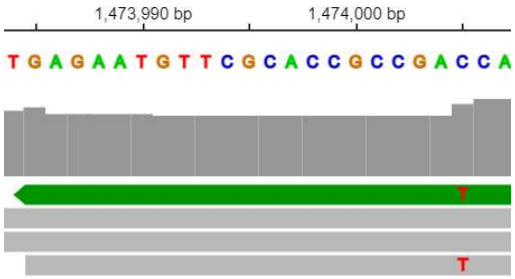


Transfer of plasmid with resistance gene

Note!

We also have intrinsic resistance in certain species, e.g. *Mycobacterium tuberculosis* inherently possess erm(37) protecting against macrolides, lincosamide and streptogramin

- Point mutations can confer resistance by various mechanisms:
  - Change the target of a drug, making the strain resistant
  - Upregulate the expression of a gene
  - Downregulate the expression of a gene
  - Change target specificity of protein
  - Usually species specific




Possible point mutation

# AMR tools and databases

- There are multiple tools which all utilize their own and/or each others databases for predicting antimicrobial resistance
  - Resfinder (ResFinder 4.1 (dtu.dk)), AMRfinderplus (Releases · ncbi/amr (github.com)) , CARD (https://card.mcmaster.ca/home), KmerResistance, ARIBA
  - Differences exists due to
    - How the database is created and curated
    - How the database is maintained
    - How the tool conducts its search
  - The correct tool/database will likely depend on the type of analysis or workflow you are using
  - Approach results from tools with a critical mindset!

**EXAMPLE**

CARD output:

Data was complete genome of E. Coli strain

44 hits in total!

Let us take a closer look

| RGI Criteria | ARO Term | SNP | Detection Criteria | AMR Gene Family | Drug Class | Resistance Mechanism | % Identity of Matching Region | % Length of Reference Sequence |
|---|---|---|---|---|---|---|---|---|
| Perfect | acrB | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | fluoroquinolone antibiotic, cephalosporin, glycylcycline, penam, tetracycline antibiotic, rifamycin antibiotic, phenicol antibiotic, disinfecting agents and antiseptics | antibiotic efflux | 100.0 | 100.00 |
| Perfect | Escherichia coli acrA | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | fluoroquinolone antibiotic, cephalosporin, glycylcycline, penam, tetracycline antibiotic, rifamycin antibiotic, phenicol antibiotic, disinfecting agents and antiseptics | antibiotic efflux | 100.0 | 100.00 |
| Perfect | Escherichia coli emrE | | protein homolog model | small multidrug resistance (SMR) antibiotic efflux pump | macrolide antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | kdpE | | protein homolog model | kdpDE | aminoglycoside antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | msbA | | protein homolog model | ATP-binding cassette (ABC) antibiotic efflux pump | nitroimidazole antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | mdtG | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | phosphonic acid antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | mdtH | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | fluoroquinolone antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | H-NS | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump | macrolide antibiotic, fluoroquinolone antibiotic, cephalosporin, cephamycin, penam, tetracycline antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | marA | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump, General Bacterial Porin with reduced permeability to beta-lactams | fluoroquinolone antibiotic, monobactam, carbapenem, cephalosporin, glycylcycline, cephamycin, penam, tetracycline antibiotic, rifamycin antibiotic, phenicol antibiotic, penem, disinfecting agents and antiseptics | antibiotic efflux, reduced permeability to antibiotic | 100.0 | 100.00 |
| Perfect | ugd | | protein homolog model | pmr phosphoethanolamine transferase | peptide antibiotic | antibiotic target alteration | 100.0 | 100.00 |
| Perfect | mdtA | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminocoumarin antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | mdtB | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminocoumarin antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | mdtC | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminocoumarin antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | baeS | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminoglycoside antibiotic, aminocoumarin antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | baeR | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminoglycoside antibiotic, aminocoumarin antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | YojI | | protein homolog model | ATP-binding cassette (ABC) antibiotic efflux pump | peptide antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | PmrF | | protein homolog model | pmr phosphoethanolamine transferase | peptide antibiotic | antibiotic target alteration | 100.0 | 100.00 |
| Perfect | emrY | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | tetracycline antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | emrK | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | tetracycline antibiotic | antibiotic efflux | 100.0 | 110.26 |
| Perfect | evgA | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump | macrolide antibiotic, fluoroquinolone antibiotic, penam, tetracycline antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | evgS | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump | macrolide antibiotic, fluoroquinolone antibiotic, penam, tetracycline antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | acrD | | protein homolog model | resistance-nodulation-cell division (RND) antibiotic efflux pump | aminoglycoside antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | emrR | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | fluoroquinolone antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | emrA | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | fluoroquinolone antibiotic | antibiotic efflux | 100.0 | 100.00 |
| Perfect | emrB | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump | fluoroquinolone antibiotic | antibiotic efflux | 100.0 | 100.00 |

| Filename |
| --- |
| GCF_000005845.2_ASM584v2_genomic |

**EXAMPLE** CARD output:

- EmrY, emrK and emrB
- Perfect hits!
  - Expect for emrK, ID and COV are 100%
- Should we expect resistance to tetracycline and fluoroquinolones in this isolate?

| RGI Criteria | ARO Term | SNP | Detection Criteria | AMR Gene Family |
| --- | --- | --- | --- | --- |
| Perfect | emrY | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump |
| Perfect | emrK | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump |
| Perfect | emrB | | protein homolog model | major facilitator superfamily (MFS) antibiotic efflux pump |

| Drug Class | Resistance Mechanism | % Identity of Matching Region | % Length of Reference Sequence |
| --- | --- | --- | --- |
| tetracycline antibiotic | antibiotic efflux | 100.0 | 100.00 |
| tetracycline antibiotic | antibiotic efflux | 100.0 | 110.26 |
| fluoroquinolone antibiotic | antibiotic efflux | 100.0 | 100.00 |

Lets try a different tool for the strain: ResFinder

- No resistance at all?

## ResFinder-4.1 Server - Results

**Input Files:** *GCF_000005845.2_ASM584v2_genomic.fna*

**Warning:**
One or more resistance genes does not exist in the phenotype database. The Summary table does not take this into account.

escherichia coli | complete

| Antimicrobial | Class | WGS-predicted phenotype | Genetic background |
|---|---|---|---|
| amikacin | aminoglycoside | No resistance | |
| tigecycline | tetracycline | No resistance | |
| tobramycin | aminoglycoside | No resistance | |
| cefepime | beta-lactam | No resistance | |
| chloramphenicol | amphenicol | No resistance | |
| piperacillin+tazobactam | beta-lactam | No resistance | |
| cefoxitin | beta-lactam | No resistance | |
| ampicillin | beta-lactam | No resistance | |
| ampicillin+clavulanic acid | beta-lactam | No resistance | |
| cefotaxime | beta-lactam | No resistance | |
| ciprofloxacin | quinolone | No resistance | |
| colistin | polymyxin | No resistance | |
| sulfamethoxazole | folate pathway antagonist | No resistance | |
| imipenem | beta-lactam | No resistance | |
| trimethoprim | folate pathway antagonist | No resistance | |
| nalidixic acid | quinolone | No resistance | |
| ertapenem | beta-lactam | No resistance | |
| tetracycline | tetracycline | No resistance | |
| fosfomycin | fosfomycin | No resistance | |
| ceftazidime | beta-lactam | No resistance | |
| temocillin | beta-lactam | No resistance | |
| gentamicin | aminoglycoside | No resistance | |
| meropenem | beta-lactam | No resistance | |
| azithromycin | macrolide | No resistance | |

Lets try a different tool for the strain: ResFinder

- No resistance at all?
- No resistance to tetracycline or quinolones?

## ResFinder-4.1 Server - Results

**Input Files:** *GCF_000005845.2_ASM584v2_genomic.fna*

**Warning:**
One or more resistance genes does not exist in the phenotype database. The Summary table does not take this into account.

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| Antimicrobial | Class | WGS-predicted phenotype | Genetic background |
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| tigecycline | tetracycline | No resistance | |
| tobramycin | aminoglycoside | No resistance | |
| cefepime | beta-lactam | No resistance | |
| chloramphenicol | amphenicol | No resistance | |
| piperacillin+tazobactam | beta-lactam | No resistance | |
| cefoxitin | beta-lactam | No resistance | |
| ampicillin | beta-lactam | No resistance | |
| ampicillin+clavulanic acid | beta-lactam | No resistance | |
| cefotaxime | beta-lactam | No resistance | |
| ciprofloxacin | quinolone | No resistance | |
| colistin | polymyxin | No resistance | |
| sulfamethoxazole | folate pathway antagonist | No resistance | |
| imipenem | beta-lactam | No resistance | |
| trimethoprim | folate pathway antagonist | No resistance | |
| nalidixic acid | quinolone | No resistance | |
| ertapenem | beta-lactam | No resistance | |
| tetracycline | tetracycline | No resistance | |
| fosfomycin | fosfomycin | No resistance | |
| ceftazidime | beta-lactam | No resistance | |
| temocillin | beta-lactam | No resistance | |
| gentamicin | aminoglycoside | No resistance | |
| meropenem | beta-lactam | No resistance | |
| azithromycin | macrolide | No resistance | |

Lets try a different tool for the strain: ResFinder

- No resistance at all?
- No resistance to tetracycline or quinolones?
- One tool gives 44 hits, another gives 0 what is the truth?

## ResFinder-4.1 Server - Results

**Input Files:** *GCF_000005845.2_ASM584v2_genomic.fna*

**Warning:**
One or more resistance genes does not exist in the phenotype database. The Summary table does not take this into account.

escherichia coli | complete

| Antimicrobial | Class | WGS-predicted phenotype | Genetic background |
|---|---|---|---|
| amikacin | aminoglycoside | No resistance | |
| tigecycline | tetracycline | No resistance | |
| tobramycin | aminoglycoside | No resistance | |
| cefepime | beta-lactam | No resistance | |
| chloramphenicol | amphenicol | No resistance | |
| piperacillin+tazobactam | beta-lactam | No resistance | |
| cefoxitin | beta-lactam | No resistance | |
| ampicillin | beta-lactam | No resistance | |
| ampicillin+clavulanic acid | beta-lactam | No resistance | |
| cefotaxime | beta-lactam | No resistance | |
| ciprofloxacin | quinolone | No resistance | |
| colistin | polymyxin | No resistance | |
| sulfamethoxazole | folate pathway antagonist | No resistance | |
| imipenem | beta-lactam | No resistance | |
| trimethoprim | folate pathway antagonist | No resistance | |
| nalidixic acid | quinolone | No resistance | |
| ertapenem | beta-lactam | No resistance | |
| tetracycline | tetracycline | No resistance | |
| fosfomycin | fosfomycin | No resistance | |
| ceftazidime | beta-lactam | No resistance | |
| temocillin | beta-lactam | No resistance | |
| gentamicin | aminoglycoside | No resistance | |
| meropenem | beta-lactam | No resistance | |
| azithromycin | macrolide | No resistance | |

# Differences in output example

- The strain run in this example is a standard laboratory strain E. coli K-12 substrain MG1655
- It is not expected to have any phenotypic resistance to tetracycline (Zhang et al., 2022)
  - Not actually expected to have any particular phenotypic resistance different from wild-type e. coli
- If run on AMRfinderplus, no resistance genes are found either.
- Approach databases with care and select based on your scope
  - How does results translate to the laboratory, genotypic =/= phenotypic
  - How much expertise is demanded to utilize findings
  - What is the aim of your analysis

# Resfinder

- Refinder is a tool and database for detection of genes and point mutations conferring AMR.

- The tool has (3) databases:
- Poinfinder_db
- Resfinder_db
- (DesinFinder_db)

https://cge.food.dtu.dk/services/ResFinder/

Databases with antimicrobial resistance genes and chromosomal point mutations

**ResFinder database** **PointFinder database**

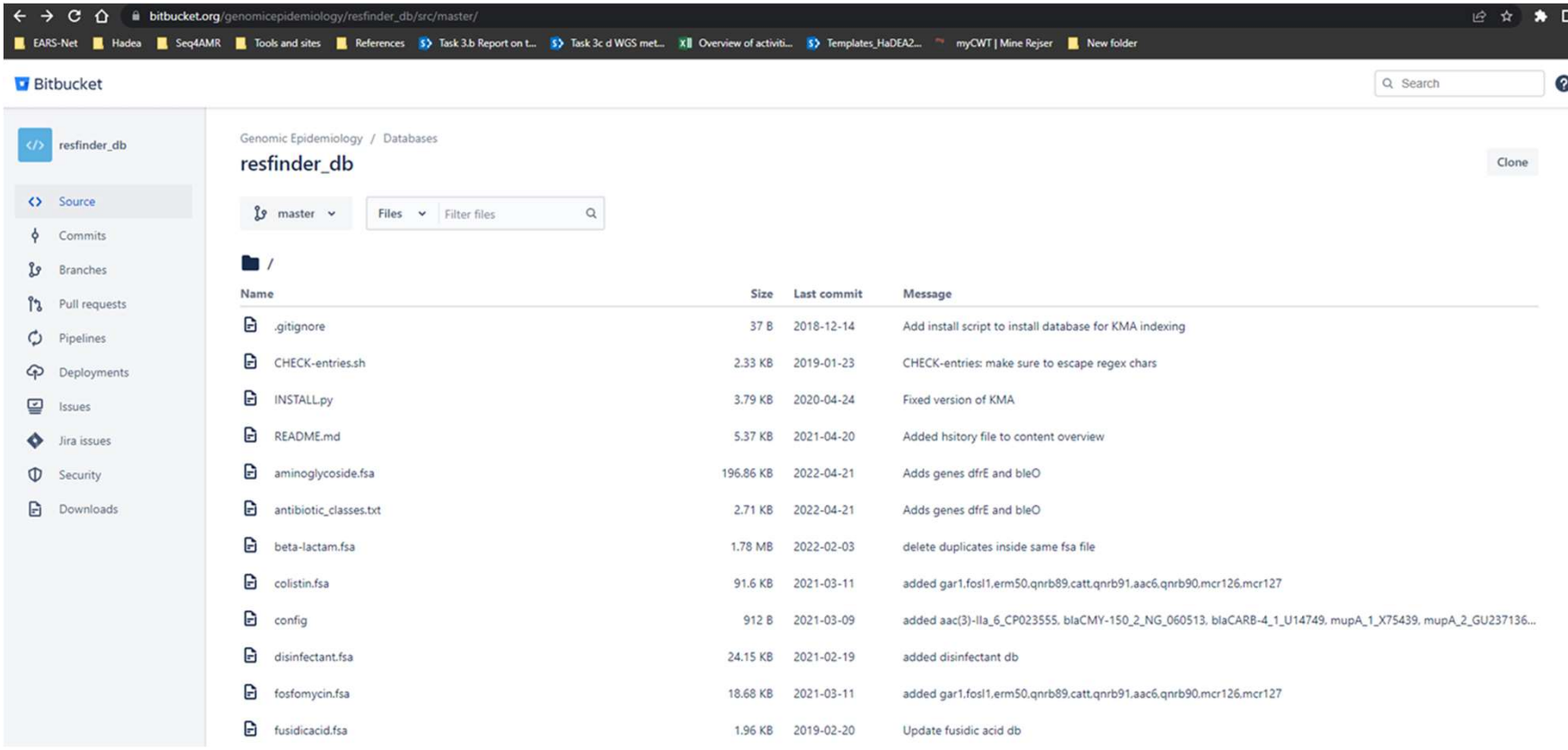
https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/

https://bitbucket.org/genomicepidemiology/pointfinder_db/src/master/

*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# Resfinder

- The tool and databases are freely available on github
- The main page contains instructions for installation of the tool and how to download and install the associated databases
- We will be using this tool today

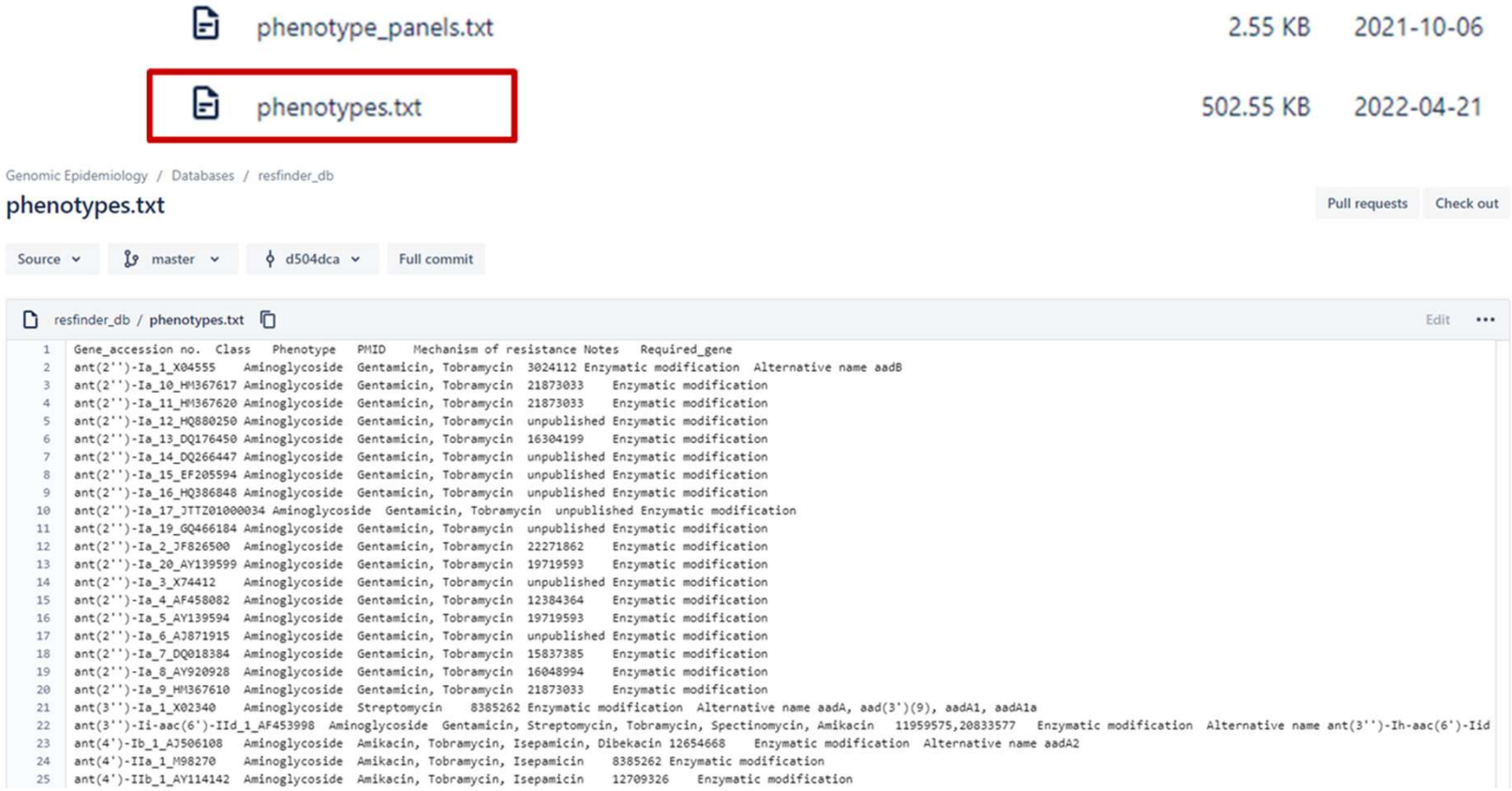



*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# Resfinder

- The databases contain:
  - Nucleotide references for AMR elements
  - Curation for each genes
  - Phenotypes associated with specific species

- We can get:
  - Class of AMR gene
  - Specific phenotypes
  - Supporting sources
  - Mechanism
  - Other notes

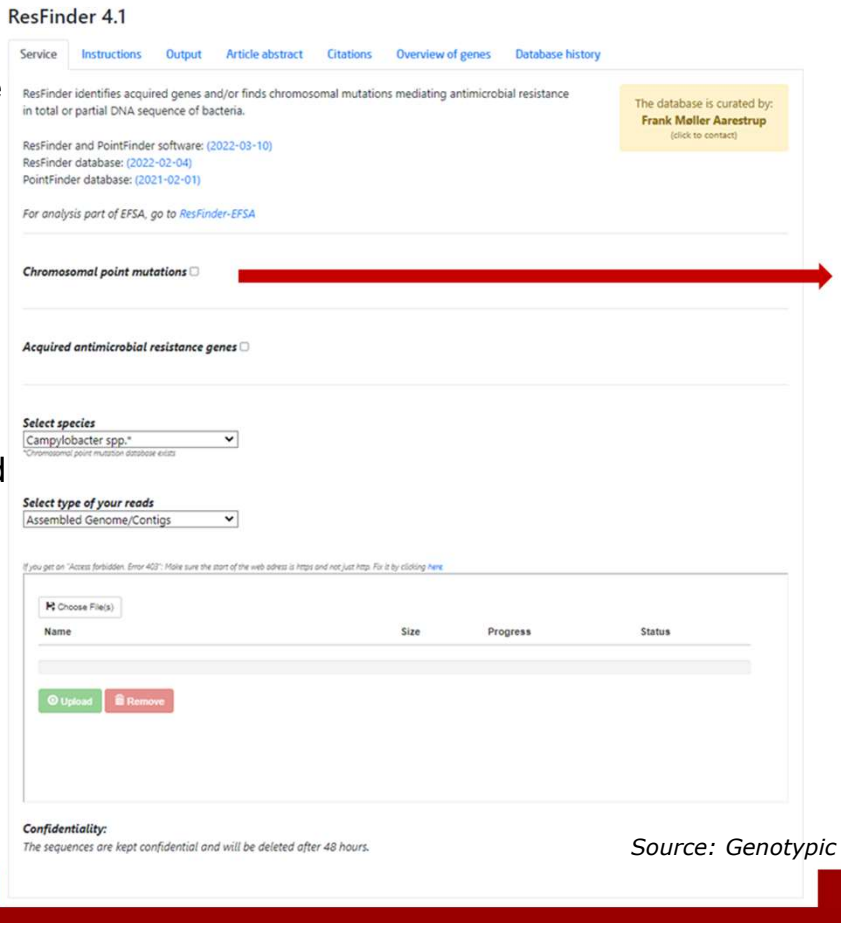


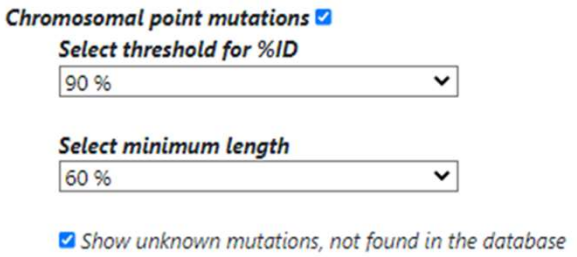

| Gene_accession no. | Class | Phenotype | PMID | Mechanism of resistance | Notes | Required_gene |
|---|---|---|---|---|---|---|
| ant(2'')-Ia_1_X04555 | Aminoglycoside | Gentamicin, Tobramycin | 3024112 | Enzymatic modification | Alternative name aadB | |
| ant(2'')-Ia_10_HM367617 | Aminoglycoside | Gentamicin, Tobramycin | 21873033 | Enzymatic modification | | |
| ant(2'')-Ia_11_HM367620 | Aminoglycoside | Gentamicin, Tobramycin | 21873033 | Enzymatic modification | | |
| ant(2'')-Ia_12_HQ880250 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_13_DQ176450 | Aminoglycoside | Gentamicin, Tobramycin | 16304199 | Enzymatic modification | | |
| ant(2'')-Ia_14_DQ266447 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_15_EF205594 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_16_HQ386848 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_17_JTTZ01000034 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_19_GQ466184 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_2_JF826500 | Aminoglycoside | Gentamicin, Tobramycin | 22271862 | Enzymatic modification | | |
| ant(2'')-Ia_20_AY139599 | Aminoglycoside | Gentamicin, Tobramycin | 19719593 | Enzymatic modification | | |
| ant(2'')-Ia_3_X74412 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_4_AF458082 | Aminoglycoside | Gentamicin, Tobramycin | 12384364 | Enzymatic modification | | |
| ant(2'')-Ia_5_AY139594 | Aminoglycoside | Gentamicin, Tobramycin | 19719593 | Enzymatic modification | | |
| ant(2'')-Ia_6_AJ871915 | Aminoglycoside | Gentamicin, Tobramycin | unpublished | Enzymatic modification | | |
| ant(2'')-Ia_7_DQ018384 | Aminoglycoside | Gentamicin, Tobramycin | 15837385 | Enzymatic modification | | |
| ant(2'')-Ia_8_AY920928 | Aminoglycoside | Gentamicin, Tobramycin | 16048994 | Enzymatic modification | | |
| ant(2'')-Ia_9_HM367610 | Aminoglycoside | Gentamicin, Tobramycin | 21873033 | Enzymatic modification | | |
| ant(3'')-Ia_1_X02340 | Aminoglycoside | Streptomycin | 8385262 | Enzymatic modification | Alternative name aadA, aad(3')(9), aadA1, aadA1a | |
| ant(3'')-Ii-aac(6')-IId_1_AF453998 | Aminoglycoside | Gentamicin, Streptomycin, Tobramycin, Spectinomycin, Amikacin | 11959575,20833577 | Enzymatic modification | Alternative name ant(3'')-Ih-aac(6')-Iid | |
| ant(4')-Ib_1_AJ506108 | Aminoglycoside | Amikacin, Tobramycin, Isepamicin, Dibekacin | 12654668 | Enzymatic modification | Alternative name aadA2 | |
| ant(4')-IIa_1_M98270 | Aminoglycoside | Amikacin, Tobramycin, Isepamicin | 8385262 | Enzymatic modification | | |
| ant(4')-IIb_1_AY114142 | Aminoglycoside | Amikacin, Tobramycin, Isepamicin | 12709326 | Enzymatic modification | | |

*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# Resfinder

- The tool is also available online, where isolates can be uploaded
- The stand-alone tool utilize the same inputs, as we will find out in the exercises
- Select the databases to use
- Choose thresholds for ID and COV
- Provide species
- Specify input format
- Give input files

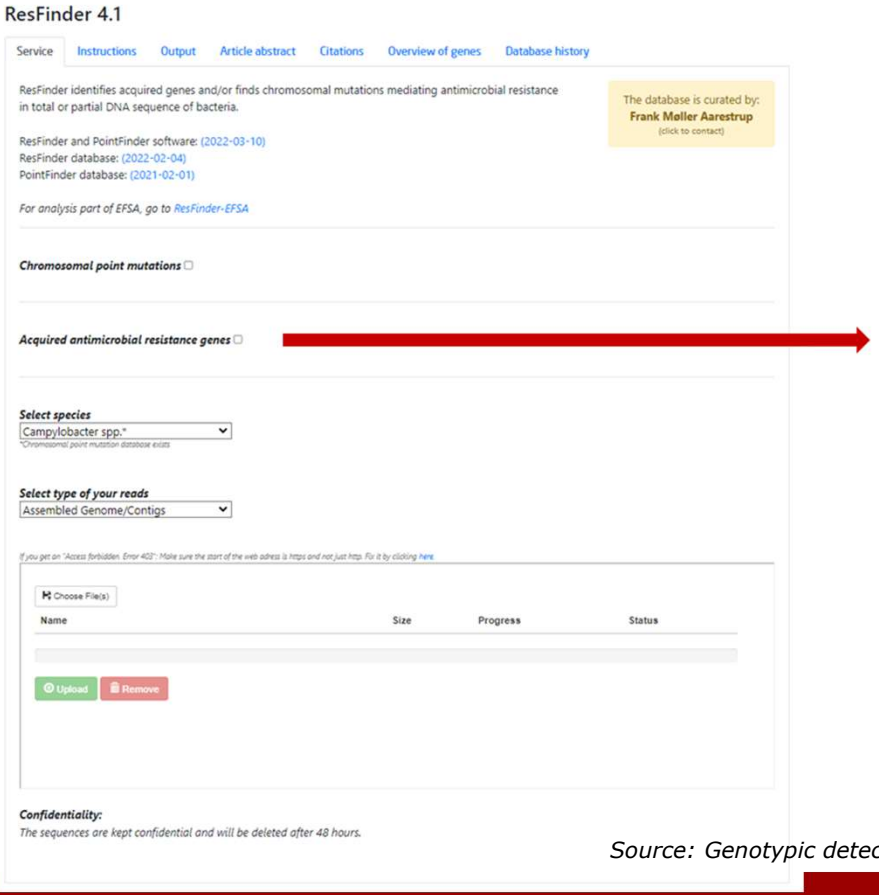


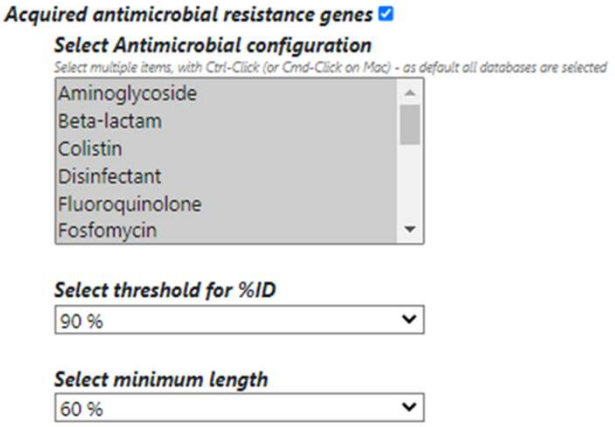




*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# Resfinder

- The tool is also available online, where isolates can be uploaded
- The stand-alone tool utilize the same inputs, as we will find out in the exercises
- Select the databases to use
- Choose thresholds for ID and COV
- Provide species
- Specify input format
- Give input files
- For AMR genes, specific class can be chosen

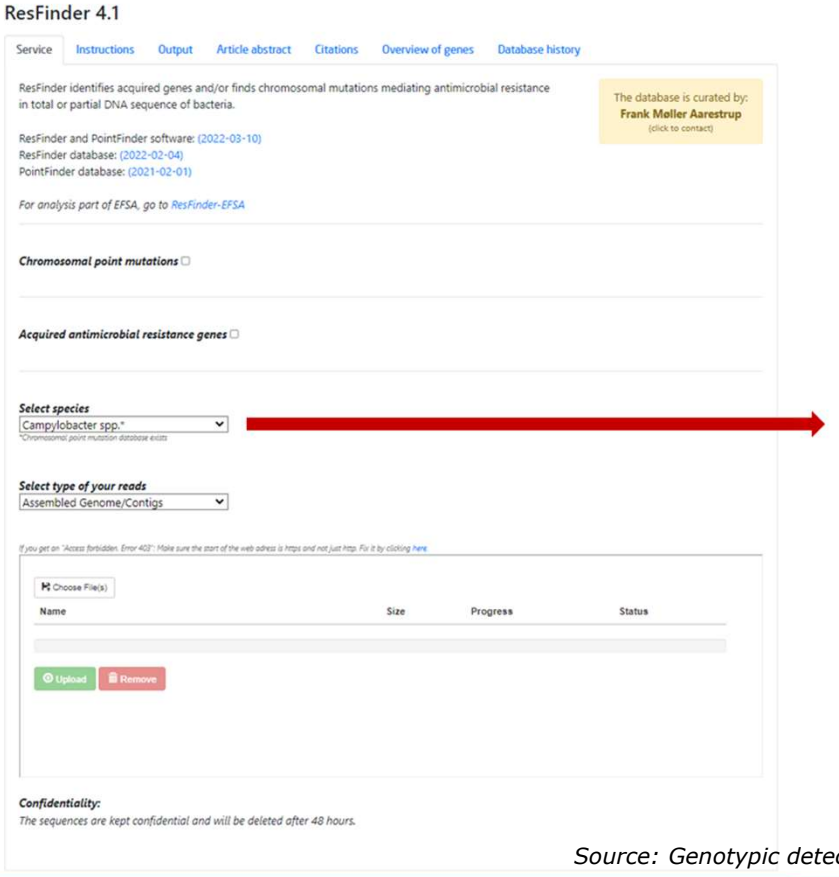


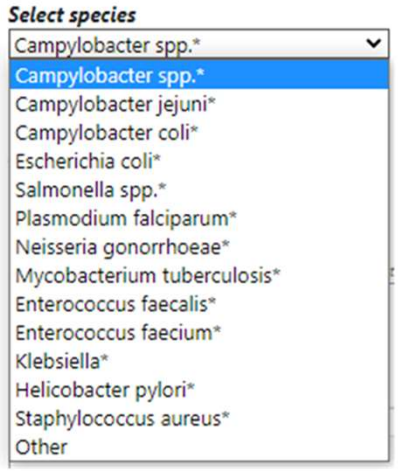




*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# Resfinder

- Species are neccesary for point mutations
- Not all species are present
- "Other" allows us to search for AMR genes




*Chromosomal point mutation database exists*

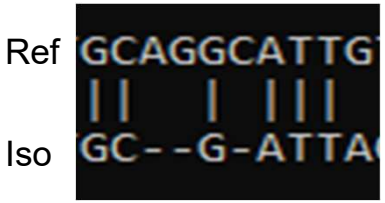
*Source: Genotypic detection of AMR in Salmonella, presentation by Ana Rita Rebelo*

# ID and COV

- What is identity (ID)?
  - Proportion of matching nucleotides:


9/10 bp align = 90% ID

- What is coverage/length (COV)?
  - Proportion of nucleotides covered:




7/10 bp covered = 70% COV, 6/10 bp align = 60% ID

# Note on databases

- Make your own database!
  - MyDBFinder (https://cge.food.dtu.dk/services/MyKMAfinder/)
  - MyKMAFinder (https://cge.food.dtu.dk/services/MyDbFinder/)
  - NCBI-Blast (BLAST+ executables — BLASTHelp documentation (nih.gov))
- Organize genes of interest into a fasta file
  - Upload to online solution
  - Make blast database with makeblastdb (part of standalone blast tools)
- Large range of customization
- Better options likely exist for larger datasets

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# References


- Dominguez Del Angel V, Hjerde E, Sterck L *et al.* Ten steps to get started in Genome Assembly and Annotation [version 1; peer review: 2 approved]. *F1000Research* 2018, **7**(ELIXIR):148 (https://doi.org/10.12688/f1000research.13598.1)
- Murray, Christopher J. L., et al. “Global Burden of Bacterial Antimicrobial Resistance in 2019: a Systematic Analysis.” Lancet, vol. 399, no. 10325, Elsevier B.V., 2022, pp. 629–55, doi:10.1016/S0140-6736(21)02724-0.
- Norrby, S. Ragnar, et al. “Lack of Development of New Antimicrobial Drugs: A Potential Serious Threat to Public Health.” Lancet Infectious Diseases, vol. 5, no. 2, Lancet Publishing Group, 2005, pp. 115–19, doi:10.1016/S1473-3099(05)70086-4.
- Zhang, P., Mao, D., Gao, H. et al. Colonization of gut microbiota by plasmid-carrying bacteria is facilitated by evolutionary adaptation to antibiotic treatment. ISME J 16, 1284–1293 (2022). https://doi.org/10.1038/s41396-021-01171-x
- Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM, Feng P. Advances in Molecular Serotyping and Subtyping of Escherichia coli. Front Microbiol. 2016 May 3;7:644. doi: 10.3389/fmicb.2016.00644. PMID: 27199968; PMCID: PMC4853403.
- Joensen, Katrine Grimstrup, et al. “Rapid and Easy In Silico Serotyping of Escherichia Coli Isolates by Use of Whole-Genome Sequencing Data.” Journal of Clinical Microbiology, edited by K. C. Carroll, vol. 53, no. 8, American Society for Microbiology, 2015, pp. 2410–26, doi:10.1128/JCM.00008-15.