

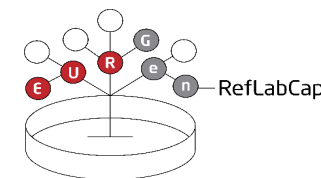
DTU



Simulated exercise on CPO outbreak – *Pseudomonas aeruginosa*

EURGen-RefLabCap
Virtual multidisciplinary training workshop
September 2023

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Simulated exercises - background

- Series of multidisciplinary training workshops
 - Sept/Oct 2022 – introduction to SNP analysis and cgMLST for cluster analysis (WS1)
 - May 2023 – Simulated exercise on outbreak analysis (*Klebsiella pneumoniae*; WS1)
 - Sept 2023– Simulated exercise on outbreak analysis (*Pseudomonas aeruginosa*; WS2)
 - 2024: Simulated exercises on outbreak analysis (WS1 and WS2)

WS1: CCRE/ *E. coli* and *Klebsiella* spp.

WS2: CPO/ *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Purpose of the workshop

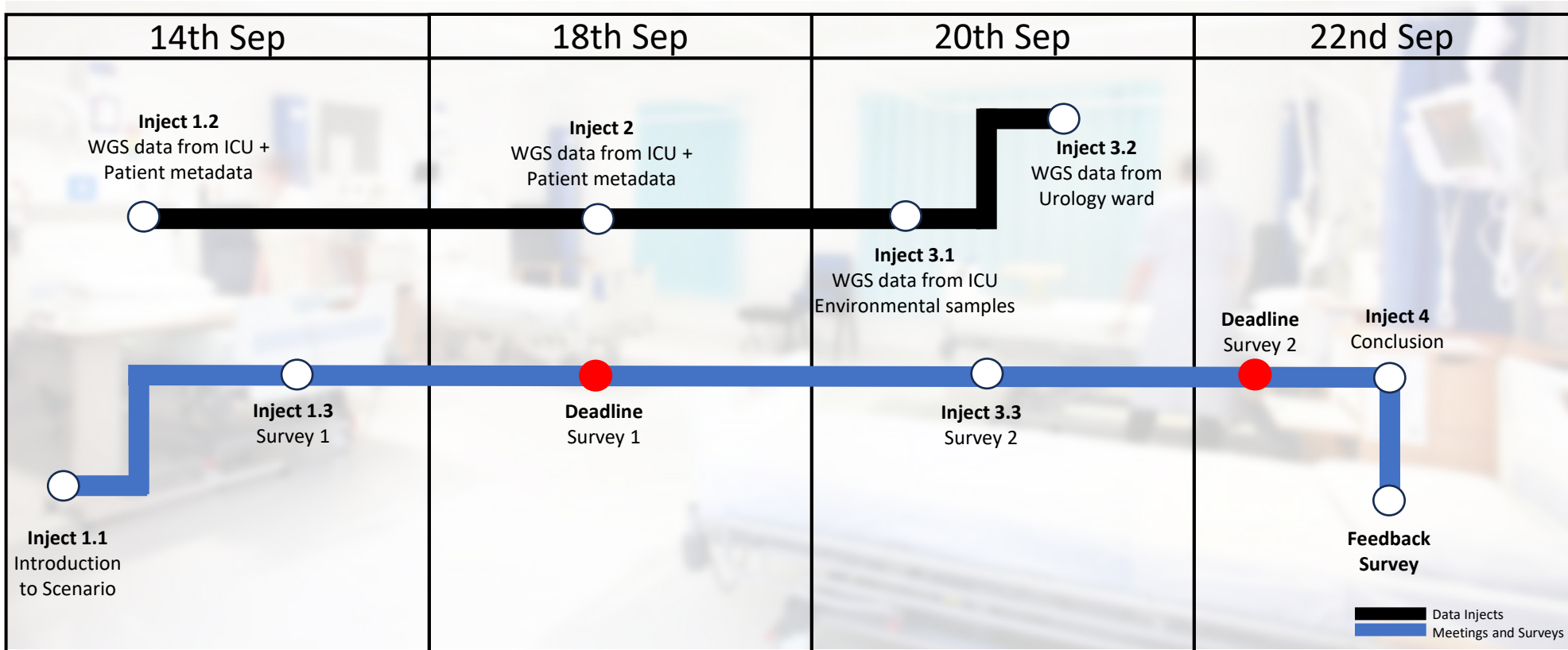
- To build capacity to work with outbreak investigations
 - background information about bacterial subtyping and cluster analysis
 - Web-based bioinformatics tools to get started on bacterial phylogenetics and outbreak detection
- To work with larger sets of sequencing data and metadata and analyse outputs from SNP analyses

The workshops build on the previous workshops
– video recordings of previous workshops are available

Learning objectives

- To be able to perform a **cluster analysis of bacteria** to look into possible genetic relatedness in a dataset
- To learn about other relevant **tools for sequence analysis** and work with a **real outbreak data set** to characterise and analyse
- Hands-on work on using the results from the cluster analysis, bacterial typing, and simulated patient metadata to elucidate a possible nosocomial outbreak

Exercise overview



Outbreak scenario background

Location: Hospital X, Europe

- Increased detection of carbapenem resistant *P. aeruginosa* (CRPa) in the ICU was observed.
- The infection prevention and control team (IPCT) has been alerted and initiated the screening of ICU for the multidrug resistant organisms (MDRO) upon admission, discharge, and twice weekly for patients in the ICU.
- There was an endemic problem with *Stenotrophomonas maltophilia* in the ICU detected in 2021 with a water reservoir as suspected source.
- Since *P. aeruginosa* and *S. maltophilia* are closely related environmental species and commonly associated with nosocomial infections, screening of environmental surfaces is being carried out in the ICU.

Fictitious

Scenario-Roles

Outbreak Management Team (OMT)

- Inter-department communication
- Patient health records
- Environmental assessment (e.g. identification of contaminated food or food handling equipment, infection control breaches, cleaning, environmental sampling)
- epidemiological data (e.g. movements and contacts of cases)
- Laboratory data (e.g. whole genome sequencing)

Participant's Role

The role of exercise participants is to support OMT in the analyses of epidemiology and laboratory data (including WGS data) to generate a hypothesis of the most likely exposure that has caused the outbreak.

Injects 1.1 to 1.3

Sep 14,
2023

- Introduction to outbreak scenario
- Tools for plasmid replicon typing

Inject 1.1 (Introduction)

10 carbapenem resistant *P. aeruginosa* isolates from different patients at ICU were identified in Hospital X
MLST, cluster analysis, AMR, Plasmid replicon analysis

Inject 1.2

Survey 1
Submit answers by Sep 18 12:00 CET

Inject 1.3

Inject 2

Sep 18,
2023

Further **08** *P. aeruginosa* identified at ICU, Hospital X
cluster analysis, MLST, Plasmid replicon analysis

Inject 2

Deadline for Survey 1
12:00 CET

Injects 3.1 to 3.3

Sep 20,
2023

09 *P. aeruginosa* identified from sinks, pipes, etc, **ICU**,
Hospital X

cluster analysis, AMR genes, plasmid analysis

Inject 3.1

02 Cases from **urology ward** in were identified.
cluster analysis, AMR genes, plasmid analysis

Inject 3.2

Survey 2

Submit answers by Sep 22 09:00 CET

Inject 3.3

Inject 4 – Results/discussion

Sep 22,
2023

- Results and conclusion of the exercise
 - Additional analysis for plasmid typing – MOB-Recon
- Questions

Inject 4

Deadline for Survey 2
09:00 CET

A total of 29 *P. aeruginosa* isolates

Tasks - analyses

Analyses

- Characterisation:
 - Carbapenem resistance mechanism?
 - Plasmid replicons
- Subtyping:
 - MLST
- Cluster analysis

Bioinformatics Tools

- ResFinder/CARD/AmrFinderPlus
- MOB-Suite (Galaxy server)
- MyDbFinder with custom replicon database
- MLST at CGE (or Pasteur)
- CSIPhylogeny

No need characterize all isolates! Analyse isolates that are relevant to answer the survey questions.

(Disclaimer: In the real outbreak, you need to characterize all the suspected case isolates)

Survey questions

- AMR genes (carbapenemase gene and gene variant?)
- MLST's?
- How many clusters?
- Part of a cluster?
 - How many SNPs difference within core-cluster?
- Plasmid carrying carbapenemase gene?
- Possible source of the outbreak?
 - Environmental vs clinical isolates

PDFs of the survey questions (Inject 1.3 + 3.3) available



New ResFinder server & entry

ResFinder 4.1

Service

Instructions

Output

Article abstract

Citations

Overview of genes

Database history

New ResFinder Server:

Click here for the new ResFinder server: [ResFinder \(new\)](#)

The new server employs identical applications and databases as its predecessor, ensuring consistent server outputs.

Nonetheless, significant modifications have been introduced to ResFinder, including its runtime environment, queuing system, and interface.

Select species

Campylobacter spp.*
Campylobacter spp.*
Campylobacter jejuni*
Campylobacter coli*
Escherichia coli*
Salmonella spp.*
Plasmodium falciparum*
Neisseria gonorrhoeae*
Mycobacterium tuberculosis*
Enterococcus faecalis*
Enterococcus faecium*
Klebsiella*
Helicobacter pylori*
Staphylococcus aureus*
Other

Old:

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

New:

<http://genepi.food.dtu.dk/resfinder>

Both are working and run-time should be faster!

Be aware – ResFinder does not have a point mutation scheme for *Pseudomonas*
– you can only search for acquired genes

Carbapenem resistance in *P. aeruginosa*

- Several mechanisms of resistance in action
 - Acquired carbapenemase genes
 - Point mutations
 - Porin modification/deletion
 - Efflux pumps
- Acquired genes are often on plasmids
 - Plasmid content is continuously evolving
 - High volume of small mobile genetic elements (MGE) on plasmids
 - Other MGEs can also carry resistance genes

Detection of plasmids from WGS data

- Several bioinformatics tools and databases are available for plasmid identification
- Usually, only the plasmid replicon is detected
- Numerous variants of some replicons
- Limited analysis possible using Illumina short reads!
 - Plasmid DNA can be assembled into several contigs
 - Hybrid plasmids are hard to detect
- ONT MinION or other long read sequence data typically used for confirmation and full assembly/characterisation of plasmids

Co-localisation of plasmid replicons and resistance genes on same contig

- For *E. coli*, *Klebsiella* and other *Enterobacterales* there are several suitable tools
 - We have available CGE tools
 - **Limited** to Gram Positive & *Enterobacterales* plasmids
- It is more challenging to analyse other pathogens like:
 - *Pseudomonas aeruginosa*
 - *Acinetobacter baumannii*

<https://cge.food.dtu.dk/services/MobileElementFinder/>

<https://cge.food.dtu.dk/services/PlasmidFinder/>



MobileElementFinder: *E. coli* vs. *P. aeruginosa*

MobileElementFinder Results

Sample name: ***E. coli***

Date:

MGEfinder version: 1.0.3

MGEdb version: 1.0.2

Displaying: 21 of 166 mobile elements

Contig	Plasmid	#MGEs	Resistance
NODE 57 length 12689 cov 1.779...		1	sul2
NODE 83 length 3462 cov 1.5576...		0	blaA17, aadA5
NODE 45 length 31502 cov 5.278...	IncX4	0	mcr-1.26
NODE 79 length 3955 cov 2.1724...		0	tet(A)
NODE 41 length 41695 cov 5.106...	IncX1	1	blaTEM-32, blaTEM-48
NODE 35 length 44591 cov 1.855...		1	blaCTX-M-1
NODE 6 length 182344 cov 3.375...		2	
NODE 22 length 84699 cov 3.299...		2	
NODE 70 length 7174 cov 2.9338...		0	
NODE 31 length 49762 cov 2.263...	IncFII(pSE11)	0	
NODE 60 length 10308 cov 3.487...		0	
NODE 16 length 115775 cov 3.17...		0	
NODE 61 length 10163 cov 2.768...		0	
NODE 12 length 137967 cov 3.19...		0	

MobileElementFinder Results

Sample name: ***P. aeruginosa***

Date:

MGEfinder version: 1.0.3

MGEdb version: 1.0.2

Displaying: 4 of 38 mobile elements

Contig	Plasmid	#MGEs	Resistance
contig00005 len=433304 cov=38....		0	fosA
contig00029 len=5266 cov=40.7 ...		0	tet(G)
contig00001 len=743927 cov=38....		0	blaPAO, aph(3')-IIb
contig00028 len=5992 cov=37.8 ...		0	rmtB
contig00022 len=84655 cov=38.1...		2	qacE, qnrVC1, ...
contig00010 len=272534 cov=39....		1	blaOXA-395
contig00051 len=930 cov=78.0 c...		0	sul1
contig00023 len=83036 cov=39.1...		0	catB7
contig00039 len=1569 cov=39.4 ...		0	blaNDM-1
contig00003 len=517527 cov=38....		0	crpP
contig00004 len=472663 cov=38....		1	

Plasmid replicon typing: alternative tools

- MOB-Typer is a tool from MOB-suite
 - Command-line tool and has no official web-based application
- Two ways we can still use MOB-suite without command-line interface
 - CGE tools: MyDBFinder (<https://cge.food.dtu.dk/services/MyDbFinder/>)
 - Simple tool for BLASTing your query sequence (.fa) against your specified database
 - Use the MOB Suite database to search for plasmid replicons
 - one isolate at a time!
 - European Galaxy server (<https://usegalaxy.eu/>)
 - MOB-Suite incorporated
 - Can handle batch-upload

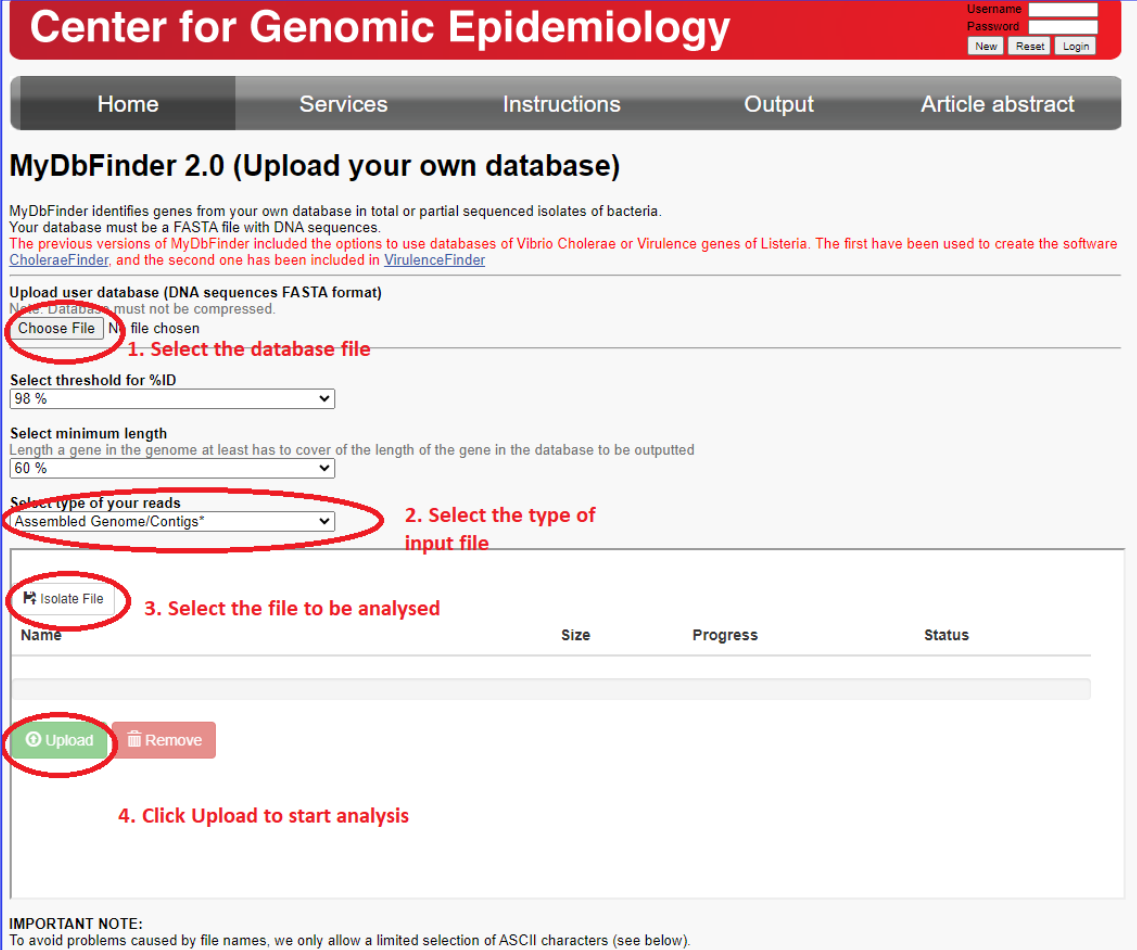
MOB-suite: Software tools for clustering, reconstruction and typing of plasmids from draft assemblies

<https://github.com/phac-nml/mob-suite>

Finding plasmids using MyDBFinder

- Choose the MOB Suite database (text file) as user data base (located in Inject 1.1 folder on ScienceData)
- Adjust threshold for ID if you like
- Analyse one strain per upload

Database file: *rep.dna.fas*



Center for Genomic Epidemiology

Username: Password:
New Reset Login

Home Services Instructions Output Article abstract

MyDbFinder 2.0 (Upload your own database)

MyDbFinder identifies genes from your own database in total or partial sequenced isolates of bacteria. Your database must be a FASTA file with DNA sequences. The previous versions of MyDbFinder included the options to use databases of *Vibrio Cholerae* or Virulence genes of *Listeria*. The first have been used to create the software *CholeraeFinder*, and the second one has been included in *VirulenceFinder*.

Upload user database (DNA sequences FASTA format)
Note: Database must not be compressed.

1. Select the database file
Choose File No file chosen

Select threshold for %ID
98 %

Select minimum length
Length a gene in the genome at least has to cover of the length of the gene in the database to be outputted
60 %

2. Select the type of input file
Select type of your reads
Assembled Genome/Contigs*

3. Select the file to be analysed
Isolate File

Name	Size	Progress	Status
------	------	----------	--------

4. Click Upload to start analysis
Upload Remove

IMPORTANT NOTE:
To avoid problems caused by file names, we only allow a limited selection of ASCII characters (see below).

Latest database can also be downloaded from MOB-Suite github page. See Annex. 3 in Inject 1.1

Output from MyDBFinder

- MOB Suite DB fasta header
 - Accession number: CP15000
 - Rep_cluster_#: 2025
 - Identity %
 - Length of replicon
 - Which contig (NODE_44)
 - Length of contig (51,041 bp)
 - Position in contig
- Extended output
 - Variations in bases

Input Files: *SRR12301191_1.fa*

Antibiotic(s): *Database*

Database				
Fasta header	Identity	Query / Template length	Contig	Position in contig
001460__CP015000 rep_cluster_2025	100	243 / 243	NODE_44_length_51041_cov_54.074262	5676..5918

extended output

001460__CP015000|rep_cluster_2025

```

template query ATGGCAGTGGACCTATCCGCGATGCGCAAGCTTTTCGAAGTTGGGGCCTTGAAAGAGGCC
template query ATGGCAGTGGACCTATCCGCGATGCGCAAGCTTTTCGAAGTTGGGGCCTTGAAAGAGGCC

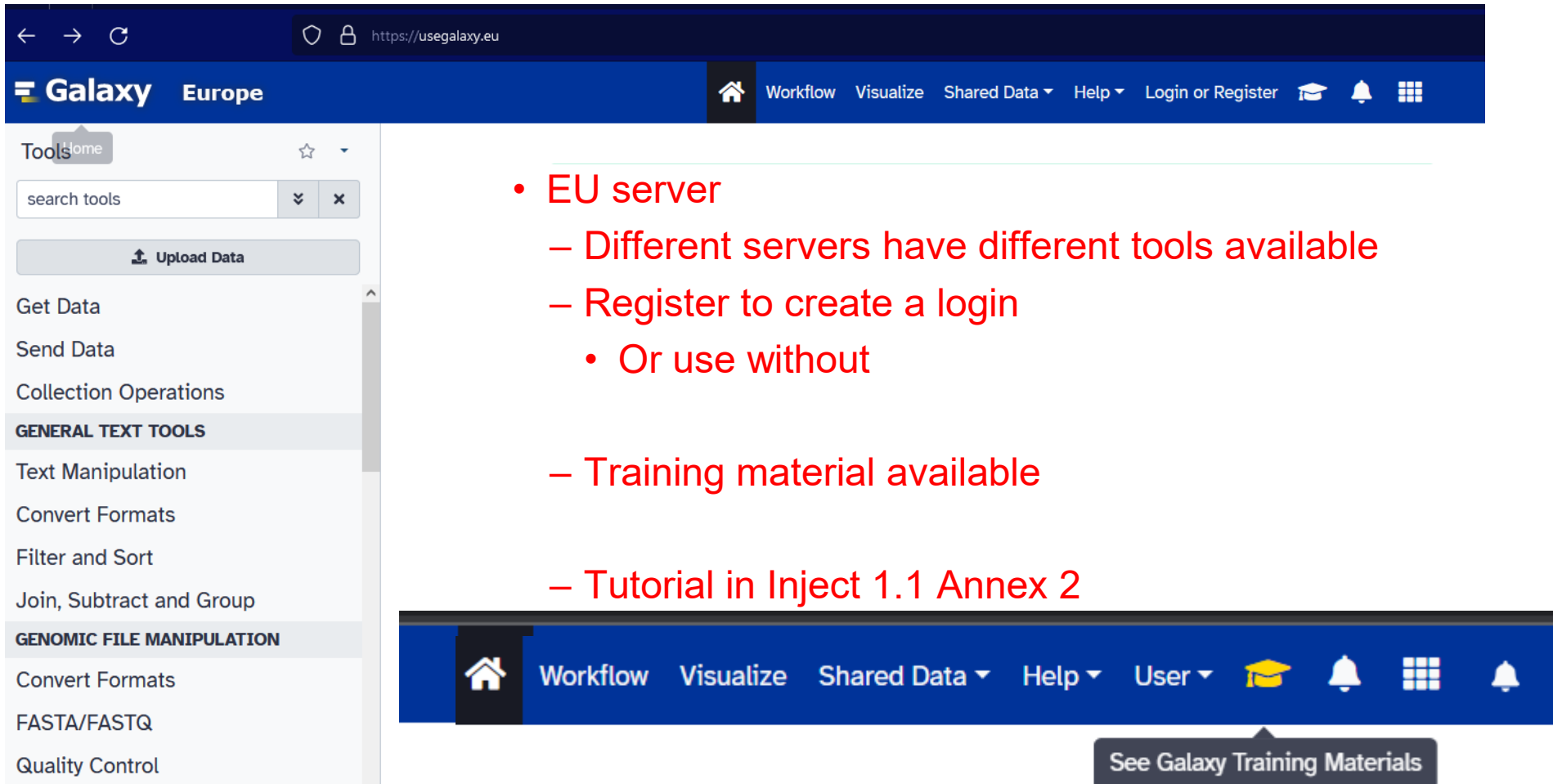
template query ATAGTCGCACCGGCTCCCATGAAAAAGGGGGGCTGGCTGCTGCTGGTCAACCGTTCGGAC
template query ATAGTCGCACCGGCTCCCATGAAAAAGGGGGGCTGGCTGCTGCTGGTCAACCGTTCGGAC

template query GGTGCTCAAGAGCACCTGACGTTGGCGCGCTCTACGCGGCCGAAGATCTACAAGAGCCTG
template query GGTGCTCAAGAGCACCTGACGTTGGCGCGCTCTACGCGGCCGAAGATCTACAAGAGCCTG

template query GAACATGTCCGGGCAGACGCTGAACGTGTGGGGTTTCGTGAAGTGAGGCTACAGGTAGCG
template query GAACATGTCCGGGCAGACGCTGAACGTGTGGGGTTTCGTGAAGTGAGGCTACAGGTAGCG

template query TAG
template query TAG
  
```

Use Galaxy – <https://usegalaxy.eu/>



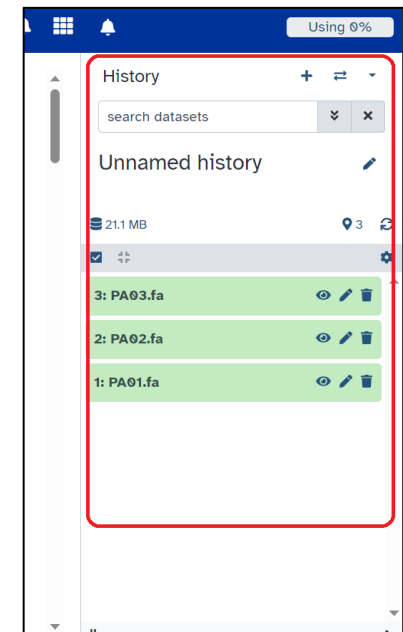
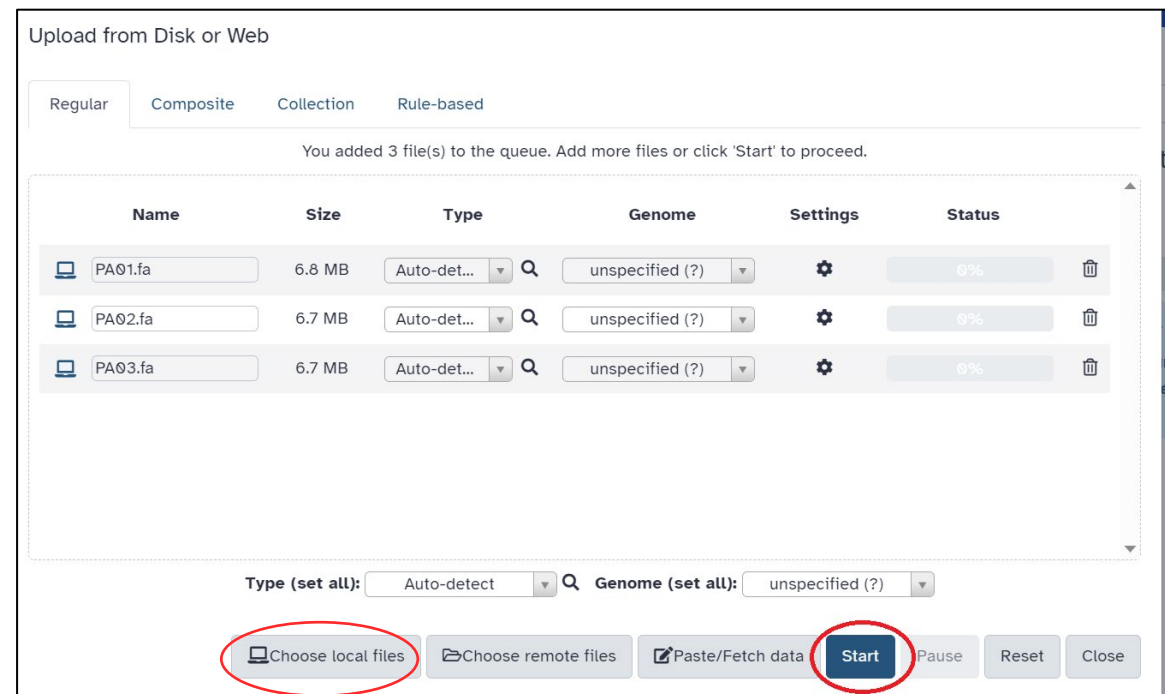
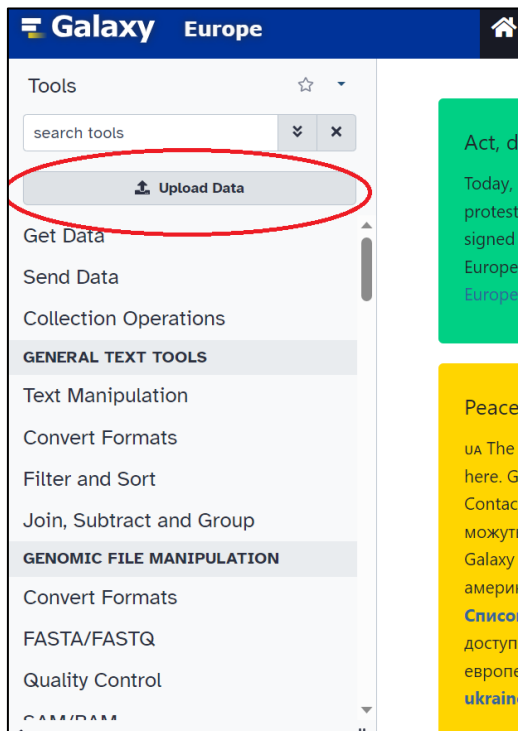
The screenshot shows the Galaxy Europe web interface. The top navigation bar includes links for Workflow, Visualize, Shared Data, Help, Login or Register, and a user profile icon. The left sidebar contains a search bar, an Upload Data button, and a list of tool categories including GENERAL TEXT TOOLS and GENOMIC FILE MANIPULATION. The main content area displays a list of tools, with the first tool being 'EU server'.

- EU server
 - Different servers have different tools available
 - Register to create a login
 - Or use without
 - Training material available
 - Tutorial in Inject 1.1 Annex 2

At the bottom of the interface, there is a button labeled 'See Galaxy Training Materials'.

Galaxy: MOB typer

- Upload the genomes (fasta files) that you want to analyse with MOB-typer.



Galaxy: MOB typer

- Run **MOB-typer** from Galaxy Toolshed

1. Type MOB-Typer in search

MOB-Typer Get the plasmid type and mobility given its sequence

2. Select MOB-Typer

Search MOB-Typer

1. Select the file from the dropdown list

2. Click "Run Tool"

Tip: Multiple files can also be selected and analysed simultaneously

Run the analysis

1. View results in Galaxy

2. Download results

IncU (accession : KF23109)

Interpret the results

Co-localisation of plasmid replicons and resistance genes on same contig?

- Use the Contig outputs from fasta file analysis
 - ResFinder or other AMR detection tool
 - MyDbFinder on plasmid database
- Keep in mind that lack of consensus does not necessarily mean that the gene is not on a plasmid

Beta-lactam					
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig
blaNDM-1	100.0	813/813	1..813	contig00039 len=1569 cov=39.4 corr=0 origname=NODE_39_length_1569_cov_39.422869 sw=shovill-spades/1.1.0 date=20230316	208..1020

Database				
Fasta header	Identity	Query / Template length	Contig	Position in contig
001460__CP015000 rep_cluster_2025	100	243 / 243	NODE_44_length_51041_cov_54.074262	5676..5918

Resources

- Slack
 - Injects (except sequence data)
 - Video recordings
 - Link to the surveys
- ScienceData folder: <https://sciencedata.dk/shared/dd60004f4cccc3452d49f24cfb938af3>
 - Sequence data
 - MOB-suite database (text file)
 - Inject text
 - Link to the surveys and PDF file of survey questions
- Data Injects: both fasta and fastq files for each isolate (Illumina)
 - No suitable MinION data available

Next Session (Friday, 22nd September 13:00-14:30 CET)

- Walkthrough of results and survey questions
- Discussion about cluster analysis
 - Input from participants
- Discussion about replicon typing tools
 - Input from participants
- MOB-Recon tool
- Feedback about the exercise

Questions/Comments?