Download FASTA files

In the folder named "**fastas**", download all 7 files, or use the files you have previously assembled.

Web-based tools we would use during the exercise

ResFinder

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

VirulenceFinder

https://cge.cbs.dtu.dk/services/VirulenceFinder/

PlasmidFinder

https://cge.cbs.dtu.dk/services/PlasmidFinder/

Instructions for running AMR prediction of WGS using ResFinder

1. Click the link under ResFinder above

2. Click the checkboxes if you want to search for **Chromosomal point mutations** and/or **Acquired antimicrobial resistance genes.**

2.1. To adjust the **threshold %ID** (minimum percentage of identity between the best matching resistance gene in the database and your query sequences) and **minimum length** (coverage that a sequence must overlap a resistance gene to count as a hit for that gene), use the dropdown menu.

NOTE: If you are not sure about this, better to use the default parameters

3. Select Species. If the species of your query sequence is not included on the list in the dropdown menu, select **Other**.

4. Select type of your read. If you are using the raw reads, you may select the sequencing technology used to generate them or you may conveniently choose to use the **Assembled Genome/Contigs**.

5. Isolate file. Click this tab to locate the downloaded FASTA file on your computer.

6. Click **Upload.** This uploads your file

7. You have the option of staying on the page and waiting for your job to complete or input your email address to receive a notification when the job is done.

8. When the job is done, you are provided several formats in which you may choose to save the output of your genome analysis.

Instructions for running Virulence prediction of WGS using VirulenceFinder

1. Click the VirulenceFInder link above

2. Select species. At the moment only *Escherichia coli*, *Enterococcus, Listeria* and *Staphylococcus aureus* are available.

> **2.1** Use the ***Select threshhold for %ID*** drop-down menu to select the minimum percentage of nucleotides that are identical between the best matching virulence gene in the databse and the corresponding sequence in the genome.

NOTE: If you are not sure about this, better to use the default parameters.

3. Select the type of your reads. **You have the option of using** Assembled or Draft Genome/Contigs or Raw Sequencing Reads (fastq)

4. Choose File. Click this to go to the location of the downloaded FASTA files on your computer. Select the file, then click **upload**

5. You have the option of staying on the page and waiting for your job to complete or input your email address to receive a notification when the job is done.

6. You are provided several formats in which you may choose to save the output of your genome analysis.

Instructions for running Plasmid replicon type prediction of WGS using PlasmidFinder

1. Depending on the identity of your genomes, **select the appropriate database.**

NOTE: If need be, you can select more than one database by using Ctrl-Click (or Cmd-Click on mac).

2. Using the dropdown menu, you may select the **threshold for minimum % identity** and **minimum % coverage**.

NOTE: If you are not sure about this, better to use the default parameters. By default, the threshold for minimum % identity and **minimum % coverage is 95% and 60% respectively**.

3. Select the type of your reads. You have the option of using **Assembled or Draft Genome/Contigs** or **Raw Sequencing Reads (fastq)**.

4. Choose File. Click this to go to the location of the downloaded FASTA files on your computer. Select the file and then, click **upload**

5. You have the option of staying on the page and waiting for your job to complete or input your email address to receive a notification when the job is done.

6. You are provided several formats in which you may choose to save the output of your genome analysis.

Exercises

1. As described above, run all your genome sequences on ResFinder. Set the following parameters for chromosomal point mutations and acquired antimicrobial resistance genes;

   Threshold % ID: 90%

   Minimum length: 80%
2. Run your genomes on VirulenceFinder and PlasmidFinder, using default %identity and %coverage parameters.
3. Enter results into an excel sheet. Indicate a "yes" or "no" for the presence or absence of genes respectively.

For example;

GENOME NAMES	blaNDM-1	qnrA	tetC	floR	parC	gyrA	ompK35	fimH	HlyA	IncHIB	IncX	IncY
Isolate_xyz	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no
Isolate_quv	yes	yes	no	yes	no	yes	no	yes	no	yes	yes	yes

4. Which antimicrobial resistance genes are present in all your genomes and what are their predicted AMR phenotypes?
5. What are the commonly occurring chromosomal mutations genes in the genomes?
   a. What are the predicted AMR phenotypes for these genes?
   b. Why should their predicted AMR phenotypes be used with caution?
6. How many genomes harbour an IncX3 plasmid replicon?
7. In your analysis, what would you say is a limitation to the use of the CGE tool in terms of virulence gene detection?
8. Submit your excel sheets and answers (in word format) to instructor "Odion Ikhimiukor" as a direct message on the slack channel