DTU Food
National Food Institute

PROTOCOL FOR WHOLE GENOME SEQUENCING AND BIOINFORMATIC ANALYSIS OF BACTERIAL ISOLATES RELATED TO THE EU MONITORING OF ANTIMICROBIAL RESISTANCE

AUTHORED BY THE EURL-AR

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https://www.eurl-ar.eu/wgs.aspx

Background for protocol

- The Commission Implementing **Decision 2020/1729** on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria
- Authorising the **use of WGS as an alternative method** for prediction of resistance in relation to the specific monitoring of ESBL- or AmpC- or carbapenemase-producing *E. coli* and *Salmonella*
- The EURL-AR has produced the present protocol for guidance in these matters

- The whole genome sequencing (WGS) processes divides into three overall processes:

  – Bacterial isolation, DNA preparation and DNA quality and quantity assessment
  – Library preparation, library quality and quantity assessment and sequencing
  – Sequence QC and bioinformatics analyses

Purpose of protocol

- **Ensure that WGS data reported to EFSA is obtained in a harmonised and comparable way**

  – Less important
    - How the bacteria, DNA and sequences are obtained

  – Very imporant
    - Assure the sequence quality control
      – Using the same QC criteria
    - Harmonised AMR gene analysis
      – using the same methods and settings for analysis
    - Reporting adequate data

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Links Generic protocol – not one method that fits all

Table 2: Collection of links referred to in the protocol, including last date of accession

Link#	Method or content	Last accessed
Link 1	Illumina website https://www.illumina.com/	December 2020
Link 2	Oxford Nanopore website https://nanoporetech.com/products	December 2020
Link 3	Thermofisher website https://www.thermofisher.com/dk/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-products-services.html	December 2020
Link 4	EURL-AR website – Inter-EURLs WG on NGS https://www.eurl-ar.eu/inter-eurls-working-group-on-ngs.aspx	December 2020
Link 5	Document on bioinformatics tools for basic analysis of Next Generation Sequencing data https://www.iss.it/documents/20126/0/Bioinformatics_tools_for_basic_analysis_of_Next_Generation_Sequencing_data_Del4.pdf/02c8f77b-db2c-6b8d-e2ba-416144f89f7e?t=1602603602556	December 2020
Link 6	Methods for isolation of ESBL, ampC and carbapenemase-producing E. coli from meat and caecal samples https://www.eurl-ar.eu/protocols.aspx	December 2020
Link 7	Method for detection of *Salmonella* in food and animal feed https://www.eurlsalmonella.eu/publications/eurl-manual	December 2020
Link 8	Method for detection of Campylobacter https://www.sva.se/en/about-us/eurl-campylobacter/laboratory-procedures/	December 2020
Link 9	DNA extraction protocol EasyDNA https://assets.thermofisher.com/TFS-Assets/LSG/manuals/easydna_man.pdf	December 2020
Link 10	Automated DNA extraction Magna Pure https://lifescience.roche.com/en_dk/products/magna-pure-96-instrument-382411-1.html	December 2020
Link 11	Overview of applications of Qubit www.invitrogen.com/qubit	December 2020
Link 12	Protocol for Qubit 4 DNA quantification https://assets.thermofisher.com/TFS-Assets/BID/manuals/MAN0017210_Qubit_4_Assays_QR.pdf	December 2020

Links to protocols

- Continuously updated
- Dependent on lab
  - Equipment
  - Throughput
  - Prerequisites

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Bacterial isolation, DNA preparation and DNA quality and quantity assessment

- Methods for isolation of ESBL, ampC and carbapenemase-producing E. coli from meat and caecal samples (EURL-AR)
- Method for detection of Salmonella in food and animal feed (EURL-Salmonella)

  – Ensure purity and correct species

- Examples of DNA extraction kits
  – Laboratory routine methods

- Examples of DNA quality/quantity assessment

Library preparation, library quality and quantity assessment and sequencing

- Dependent on the laboratory equipment
  - Majority using Illumina sequencing equipment

At present the EURL-AR recommends Illumina sequencing
- QC of sequences
- Tools for analysis

- Suggestion for library preparation
- Quantification and QC of library prep
- Illumina instrument-specific sequencing reagents, flow cells, cluster generation reagents
  - MiSeq and NextSeq

Sequence QC and bioinformatics analyses

- Trimming of raw reads
  - Can be performed, but is not crucial for Illumina sequences

- File format
  - it is recommended to perform the assembly of fastq files into fasta files
    - part of the quality control

- Check for contamination
  - E.g. using KmerFinder for species determination and look into QC parameters

- Assembly
  - Using SPAdes 3.14 or newer
  - Accessible as CGE tool with output of important QC parameters
    - https://cge.cbs.dtu.dk/services/SPAdes-3.14/

QC parameters

- The process of raw reads assembly into contigs outputs a range of QC paramters

- **number of reads**
- **depth of coverage**
- **average read length** (as specified by the sequencing equipment)
- **size of assembled genome** (+/- 0.5 million bases deviation from expected size)
- **total number of contigs** (<500 contigs)
- **N50** (>30.000 bp)

Assembly with SPAdes v 3.14

- The SPAdes 3.14 tool will output the contigs file (.fasta) and additionally a .txt file with some basic statistics and QC parameters.

- The output file contains data on:
- Input files :
  - Total number of reads
  - Total number of bases
- Contigs file :
  - Number of contigs
  - Number of bases (assembled genome size)
  - N50

- Using this output, it is also possible to calculate the average read length= Number of bases/Number of reads (input files)

AMR gene and point mutation prediction

- The EURL-AR recommends using ResFinder v4.1 or newer
- For harmonisation of the AMR data reported by different laboratories, it is important to use the defined settings.
- The EURL-AR recommends running the ResFinder analysis on the contigs **assembly files (.fasta**) using specific **settings**

- ResFinder can be run as a web-tool (CGE) or as local installation (available on BitBucket)
  – Web-tool limited to analysing one sequence at a time

ResFinder settings

For chromosomal point mutations:

- Select threshold for % ID: **90 %**
- Select minimum length: **60 %**

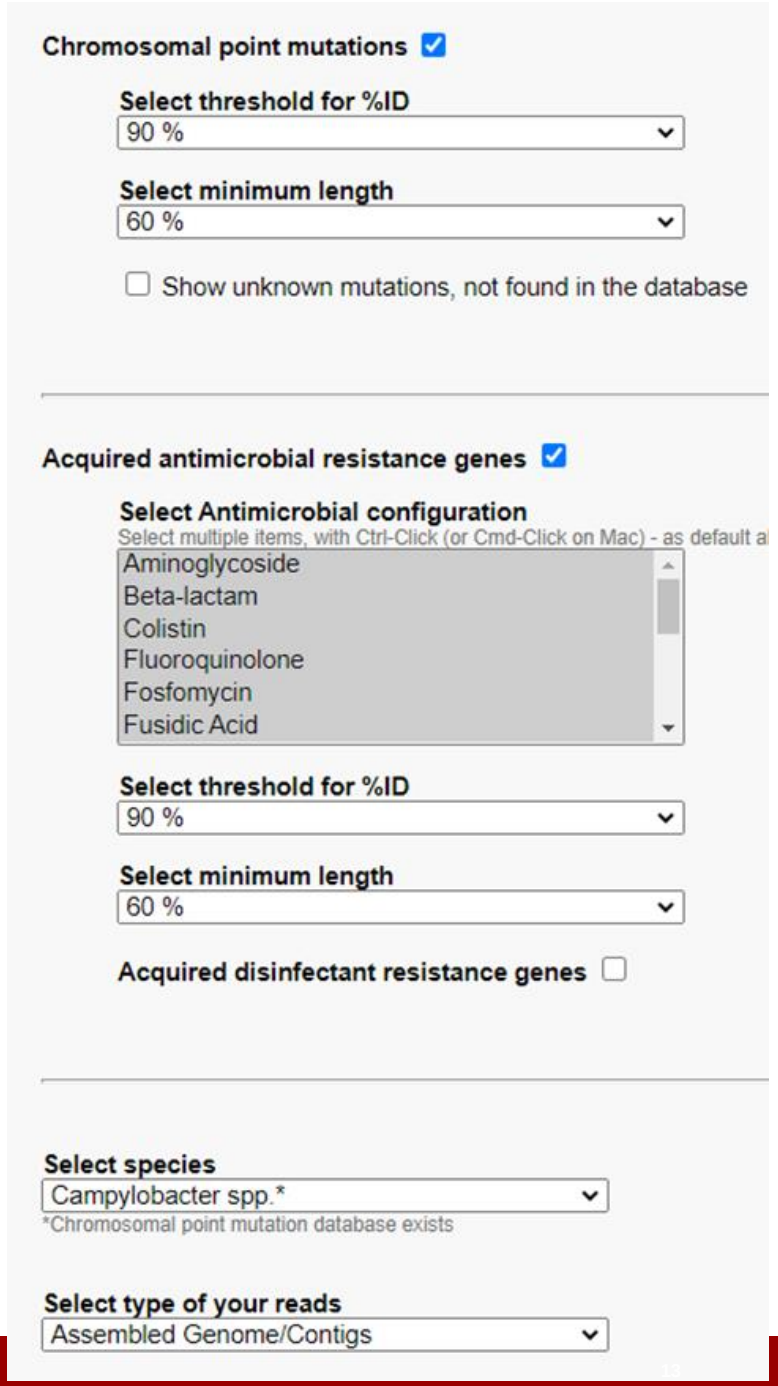
For acquired antimicrobial resistance genes:

Select all antimicrobial databases (default setting)

- Select threshold for % ID: **90 %**
- Select minimum length: **60 %**

Select species: as appropriate

Select type of your reads: Assembled genome/Contigs



Data to report to EFSA

- Beyond the sampling and isolate data, the results reported in relation to Decision 2020/1729 should include:

- Date of sequencing
- Sequencing technology used
- Library preparation used
- Version of the predictive tool (ResFinder)
- AMR-conferring genes data:
  - Gene name
  - Output information on % identity
  - Output information on % coverage (length)
- Date of ResFinder analysis

- The protocol will be added a template sheet for collection of metadata, including examples of how to report data.

Questions and discussion?