EURL CPS
European Union Reference Laboratory for
Coagulase Positive Staphylococci
http://eurl-staphylococci.anses.fr

European Union Reference Laboratory
Foodborne Viruses

EURL Salmonella

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

EURL Campylobacter

EU-RL VTEC

Foreword

The WG has been established by the European Commission with the aim to promote the use of NGS across the EURLs networks, build WGS capacity within the EU and ensure liaison with the work of the EURLs and the work of EFSA and ECDC on the NGS mandate sent by the Commission. The WG includes all the EURLs operating in the field of the microbiological contamination of food and feed and this document represent a deliverable of the WG and is meant to be diffused to all the respective networks of NRLs.

Inventory of training supports

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Introduction

From the survey sent in 2018 by the EURLs to the NRLs network, an inventory of training supports used by different organisations was performed and presented to the working group in November 2018. As all NRLs expressed their need for training in each step of NGS analyses (wet-lab and dry-lab), it was decided by the working group to draft a guidance document on training possibilities, with the links to the available training supports for the EURLs.

1   Training programs available within EURLs

EURL *Listeria monocytogenes* (*Lm*) organised a training session in **2019**:
- First day: Cell preparation and extraction
- Second day: Quality control of DNA
- Third day: bioinformatics analysis

EURL AMR organised seven training courses:
- In **2024** (5, 11, 12, and 18 December, online and on-site event): Four days of training on Oxford Nanopore Sequencing – the first day included a virtual session on Oxford Nanopore Technologies, the second and third day included a wet-lab session at DTU on how to extract HMW gDNA and sequencing using MinION and the last day was dedicated to bioinformatic analysis of long-read sequencing data
- In **2022** (21 and 23 November, online event): Whole genome sequencing prediction of AMR (MRSA)
- In **2021** (26-29 April, online event): Whole genome sequencing prediction of AMR
- In **2020** (19-23 October, on-site event): Whole genome sequencing prediction of AMR
- In **2019** (24-27 September, on-site event): Enhancing WGS capacity of AMR surveillance in the EURL-AR network
- In **2018** (25-28 September, on-site event): Four days of training with wet-lab and dry-lab about NGS for surveillance of AMR
- In **2017** (27-29 September, on-site event): Three days of training with wet-lab and dry-lab on The Use of Whole Genome Sequencing (WGS) in Monitoring of Antimicrobial Resistance

EURL VTEC organised seven training sessions, based on bioinformatics analysis:
- In 2022 and 2024, two days for [Bioinformatics for tools for aiding STEC outbreak investigation](online event)
- In 2021 and 2023, two days for [Bioinformatics for NGS data mining for typing pathogenic *E. coli*](online event)
- in 2020, two days for Bioinformatics (online event);
- in 2019, two days for Bioinformatics;
- in 2018, two days for Bioinformatics analysis;
- in 2016, two days for Bioinformatics analysis;
- in 2015, two days for Bioinformatics analysis.

EURL *Campylobacter* organized a two-days online training course in **2020** describing the process from generating whole genome sequences to quality control and sequence analysis. The training was accompanied with a tutorial for home practice, which can be accessed upon contact with the EURL *Campylobacter*.

EURL for Coagulase positive staphylococci (CPS) organized a two-days training course on NGS:
- The first session was held on October 5th-6th 2022 at the National Food Chain Safety Office (Budapest, Hungary).
- The second edition was held on October 15th-16th 2024 at the EVH Institute of Food safety, Feed and Environment (Ljubljana, Slovenia).
The training provided description of sequencing technologies, tools for analysis, concepts in NGS and data formats and analysis. The course included practical sessions carried out with the trainee with hands-on exercises on reads quality control, assembly, typing and BLAST search of genetic features specific to *Staphylococcus aureus*. The supports of the training can be accessed upon contact with the EURL for CPS.

2 Training courses organized by the Inter EURL working group on NGS

The Inter EURL working group on NGS organises annual joined training sessions on NGS data analysis to their respective NRL networks. Each year the number of participants to be invited is around 30 and up to three candidates are funded by each EURL.

The main objectives of these joined training courses are the following:

- Providing an overview of the different possible approaches for NGS data analysis;

- Understand the vocabulary connected to NGS data analysis

- Illustrating the basic steps for data analysis (quality check, trimming, assembly, search of genetic features);

- Providing guidelines for cluster analysis and understand the concepts behind different cluster analysis approaches;

- Evaluate and interpret the results obtained;

- Illustrating guidance documents on NGS analysis published by the Inter EURLs Working;

- Exchanging experiences among participants and reinforcing networking.

Four editions of joined training session were organized.

The first session was organized by **EURL *Salmonella*, EURL *Lm*** and **EURL VTEC** for the respective NRLs networks. The session was held on the 17th - 18th October 2019 at the ANSES (Maisons-Alfort, FR).

The second session was organized by **EURL VTEC, EURL *Lm*, EURL *Salmonella*, EURL CPS, EURL Parasites, EURL Foodborne viruses, EURL AMR, EURL *Campylobacter***. The session was held on the 14th - 15th June 2022 at the Istituto Superiore di Sanità (ISS) in Rome (IT).

The third joined training session was organized by **EURL VTEC, EURL *Lm*, EURL *Salmonella*, EURL CPS, EURL Parasites, EURL Foodborne viruses, EURL AMR, EURL *Campylobacter***. The session was held on the 20th – 21st June 2023 at the National Institute for Public Health and the Environment (RIVM) in Bilthoven (NL).

The fourth joined training session was organized by **EURL VTEC, EURL *Lm*, EURL *Salmonella*, EURL CPS, EURL Parasites, EURL Foodborne viruses, EURL AMR, EURL *Campylobacter***. The session was held on the 25th and 26th June 2024 at the Swedish Veterinary Agency (SVA) and the Swedish Food Agency (SFA) (Uppsala, Sweden).

3   Supports for training courses

Several training courses supports are available for both wet-lab and dry-lab procedures related to NGS. These supports were created by EURLs or by companies providing sequencing technologies and programs for bioinformatic analyses. Hyperlinked supports are listed in the following subsections.

3.1   Wet-lab

Supports from **YouSeq**
-   Beginners guide to NGS and video for support of NGS (from wet-lab to dry-lab)

3.1.1   DNA extraction

EURL AMR (available upon request):
-   DNA extraction – kits and procedures
-   From Isolate to Whole Genome Sequencing (Part 1)

Promega:
-   DNA Purification guide

Roche Diagnostics:
-   Video of MagnaPure 24 and 96 systems
-   Webinar: Automated DNA extraction for NGS using the MagNA Pure System family

3.1.2   DNA quality control

Support from **Promega**:
-   How do I determine the concentration, yield and purity of a DNA sample?

Support from **Illumina**:
-   DNA/RNA Isolation Considerations for illumina library preparation

3.1.3   Library preparation: short reads and long reads

EURL AMR for Illumina library preparation with the Nextera kit (available upon request):

-   Whole genome sequencing (WGS) - there's a new tool in town
-   From isolate to whole genome sequence (Part 2)

Illumina:
-   Illumina Training Opportunities
-   Library Preparation Tutorials for NGS

- Best Practices for Nextera Library Prep Expert Video Tip
- Illumina DNA Prep (formerly Nextera DNA Flex): Introduction

Ion Torrent

- Courses proposed by Thermofisher
- Video of IonTorrent sequencing: The Workflow | Ion S5 Next Generation Sequencing System

PacBio

- Training on library preparation proposed by **PacBio**

Oxford Nanopore

- Nanopore training: Essentials to advanced

3.1.4 Sequencing technologies

EURL AMR (available upon request):

- Sequencing Platforms

EURL VTEC:

- Next Generation Sequencers: from the bacterial culture to raw data

Illumina

- Video on sequencing by synthesis technology

PacBio

- Video on Single Molecule Real Time Sequencing technology

Ion Torrent

- Ion Torrent Next-Generation Sequencing Technology

Oxford Nanopore

- How nanopore sequencing works

3.2 Dry-lab

Inter-EURL Joined-training sessions:

- The presentations from the 2022 edition of the joint course in Rome (IT) are available at this link;

- The presentations from the 2023 edition of the joint course in Bilthoven (NL) are available at this link.

- The presentations from the 2024 edition of the joint course in Uppsala (SE) are available at the Inter Biorisks-EURLs WG on NGS page on Zenodo:
    1. Introduction of the inter EURLs Working Group on NGS and the guidance documents released (EURL-VTEC)
    2. Sequencing platforms & output data (EURL-Listeria monocytogenes)
    3. Verification of the integrity of the raw data files (like md5sum) (EURL-Salmonella)
    4. Bioinformatics analysis of NGS data: approaches and opportunities (command-line tools, commercial software, webservers) (EURL-Viruses)
    5. Introduction on quality check and trimming (EURL-VTEC)
    6. Intro ARIES and FastQC and Positional and Quality Trimming on ARIES (EURL-VTEC)
    7. Demo: Quality check and trimming with alternative tools (EURL-Campylobacter)
    8. Introduction on assembly and assembly statistics (EURL-AMR)
    9. Hands-on SPAdes and Quast from ARIES (EURL-VTEC)
    10. Data alignment (EURL-Parasites)
    11. Hands-on E. coli virulotyping using a mapping approach (EURL-VTEC)
    12. Demo: Mapping with Seqsphere (EURL-Listeria monocytogenes)
    13. Amplicon-based sequencing of viral genomes (ISS and EURL-Foodborne Viruses)
    14. Introduction to gene detection using BLAST approach (EURL-CPS)
    15. Hands-on ResFinder on CGE webserver (EURL-AMR)
    16. Introduction to genome comparisons: gene-by-gene vs SNP approach (EURL-Campylobacter)
    17. Demonstration of gene-by-gene approach through different platforms:
        - ARIES (EURL-VTEC)
        - Starflow (EURL-Listeria monocytogenes and EURL-CPS)
        - Seqsphere (EURL-Salmonella)
    18. Parasites WGS: opportunities and challenges (EURL-Parasites)
    19. Demonstration via Grapetree (EURL-Listeria monocytogenes)
    20. EFSA One Health WGS database and demo (EFSA)

EURL VTEC general aspects for data analysis:

- Basic Course on bioinformatics tools for next generation sequencing data mining
- VTEC strains typing: from traditional methods to NGS
- Introduction to NGS data formats, basic tools and servers for analysis
- Systems and servers for NGS data analysis

EURL AMR general aspects for data analysis (available upon request):

- WGS data sharing for improved public health

EURL VTEC quality control of raw data:

- Introduction to NGS data formats, quality check and analytical tools

EURL AMR quality control of raw data (available upon request):

- Quality control (QC) metrics for WGS data

Galaxy training

- Tutorials on quality control of NGS raw data
- Quality Control
- Quality control: contamination

3.2.2 Assembly, annotation and typing

EURL VTEC presentation of assembly and annotation methods:

- From Reads To Contigs
- Assembly, assembly stats, virulotyping, serotyping
- Decode NGS data: search for genetic features
- Basic characterization: Serotyping, 7-genes Multi Locus Sequence Typing (MLST) and Virulotyping

EURL AMR presentation of assembly and annotation methods (available upon request):

- Tools - ENGAGE benchmarking for AMR gene detection
- AMR prediction based on WGS data
- Finding the appropriate method, with a special focus on: Mapping and alignment

Galaxy training:

- Assembly
- Genome annotation with Prokka
- Detection AMR genes

EURL CPS presentation of research of enterotoxin coding genes (available upon request)

Geneious:

- De novo assembly

Ridom SeqSphere:

- Tutorial for SeqSphere+ Assembly and cgMLST Analysis Pipeline

3.2.2 Cluster analysis

EURL VTEC presentations:

- Introduction to core genome MLST (cgMLST)

- Whole genome SNPs comparison

EURL AMR presentations (available upon request):

- WGS clustering (SNP, cgMLST)

Galaxy Training:

- Microbial Variant Calling

Ridom SeqSphere:

- cgMLST Typing with Illumina Data

- tutorial for cgMLST scheme

- MLST typing

3.2.3 Tools for bioinformatics analysis

EURL-VTEC:

Concept of galaxy servers

- NGS solutions in Microbiology

- The Galaxy architecture and ARIES cluster

- Introduction to the ARIES webserver user-interface

EURL-AMR (available upon request):

- EURL-AR hub