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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EURL for Coagulase Positive Staphylococci

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 express 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 two training sessions:
-        In **2018**, four days of training with wet-lab and dry-lab;
-        In **2017**, 3 days of training with wet-lab and dry-lab.

EURL VTEC organised six training sessions, based on bioinformatics analysis:
-        In 2022, two days for bioinformatics for tools for aiding STEC outbreak investigation (online event) (agenda to be published);
-        in 2021, two days for bioinformatics for NGS data mining for typing pathogenic E. coli (online event) (agenda to be published);
-        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 Coagulase positive staphylococci (CPS) organized a two-days training course for the National Food Chain Safety Office (Nébih) (Budapest, Hungary) on October 5th-6th **2022**. The training provided description of sequencing technologies, tools for analysis, concepts in NGS and data formats, data analysis. The course included practical sessions carried out with the trainee with hands-on exercises on reads quality control, assembly and BLAST search of genetic features.

2 Guidance for training organization

The working group proposed to organise joined training sessions with several EURLs to their respective NRL networks, in particular concerning the data analysis.

Two types of training courses could be offered: wet-lab and dry-lab.

The wet-lab training course could include:

- DNA extraction;
- Quality control of DNA;
- Library preparation;
- Sequencing;
- Quality control of raw data and assembly.

The dry-lab training course could include:

- Annotation;
- Cluster analysis;
- Gene detection.

The first joined training 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 program included:
- Introduction to WGS:
  - ✓ Sequencing platform & output data
  - ✓ Quality check and basic analytical tools
  - ✓ WGS data analysis (cg/wgMLST, wgSNPs, k-mer approach)
- Hands on exercises for NGS analyses using Bionumerics, Seqsphere, and ARIES-Galaxy tools.

A second 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 14th - 15th June 2022 at the Istituto Superiore di Sanità (ISS) in Rome (IT).

The course objectives were:
- overview of different approaches for NGS data analysis;
- basic steps for data analysis (quality check, trimming, assembly, search of genetic features);
- guidelines for cluster analysis;
- guidance documents on NGS analysis published by the Inter EURLs Working Group.

A 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 course objectives were:
- overview of different platform for NGS;
- basic steps for NGS data analysis (quality check, trimming, assembly, search of genetic features);
- guidelines for cluster analysis;
- presentation of pipeline of analysis;
- guidance documents on NGS analysis published by the Inter EURLs Working Group.

3   Supports to prepare training courses

The supports to organise training courses 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. Hyperlink 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   Support for DNA extraction

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

Support from **Promega**:
- DNA Purification guide

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

3.1.2   Control for DNA extraction

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 for NGS

Illumina technology

EURL AMR performed training for 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)

Training courses available from **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 presentation about sequencing technologies (available upon request):

- Sequencing Platforms

EURL VTEC presentation about sequencing technologies:

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

Illumina technology

- 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 Joint-training
- 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.

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:

- Quality control (QC) metrics for WGS data

Bionumerics (Please note that the Bionumerics software is currently phasing out. Consequently, Bionumerics will no longer be supported after December 31, 2024)

- Video on Quality assessment of next generation sequencing runs

Galaxy training

- [Tutorials on quality control of NGS raw data](#)

- [Quality Control](#)

3.2.2 Assembly, annotation and gene detection

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](#)

BioNumerics (Please note that the Bionumerics software is currently phasing out. Consequently, Bionumerics will no longer be supported after December 31, 2024):

- [Calculate a de novo assembly locally](#)

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

- MLST typing

Bionumerics (Please note that the Bionumerics software is currently phasing out. Consequently, Bionumerics will no longer be supported after December 31, 2024)**:**

- Multilocus sequence typing (MLST) analysis

- Whole genome multi locus sequence typing (wgMLST)
  - wgMLST analysis
  - Quality assessment window
  - Cluster analysis of wgMLST data
  - Whole genome MLST typing in only a few clicks
- Whole genome SNP analysis
  - An overview of the wgSNP workflow in BioNumerics

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