The Working Group (WG) on Next Generation Sequencing (NGS) has been established by the European Commission in 2017, with the aim to promote the use of NGS across the EURLs' networks, build NGS 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.

The WG is led by the EURL *E. coli* (Dr. Stefano Morabito, ISS, Rome) and comprises the EURL for *Listeria*, *Salmonella*, *Staphylococcus*, *Campylobacter*, AMR, Foodborne viruses and Parasites.

The WG met regularly twice a year in Brussels

Development of guidance documents or SOPs for the following topics :

1) Proficiency Testing: (**EURL AR lead**)

2) WGS laboratory procedures: (**EURLP lead)**

3) Bioinformatics tools: (**EURL VTEC lead**)

4) WGS Cluster Analysis: (**EURL Campy lead**)

5) Bench marking: (**EURL Listeria lead**)

6) Trainings on WGS: (**EURL CPS lead**)

7) Reference and confirmatory testing using WGS. (**EURL Salmonella Lead)**

This job has been completed and the related documents have been published on the website of each participating EURL

The documents linked in this section are meant to provide guidance to the laboratories in the area of application of NGS and have been produced by the working group with the aim of being diffused to all the networks of NRLs.

- Overview of conducted and planned PTs – curated by EURL Antimicrobial Resistance
- Reference Whole Genome Sequencing collection – curated by EURL Salmonella
- NGS laboratory procedures – curated by EURL Parasites
- Bioinformatics tools for basic analysis of Next Generation Sequencing data – curated by EURL VTEC
- Guidance document for Whole Genome Sequencing - cluster analysis – curated by EURL Campylobacter
- Guidance document for NGS-Benchmarking – curated by EURL Listeria
- Inventory of training supports – curated by EURL Coagulase Positive Staphylococci
- Survey on the use of NGS across the NRLs networks – curated by EURL VTEC

Inter-EURLs Working Group on Next Generation Sequencing (NGS) - ISS (EN) - ISS

Inter-EURLs Working Group on NGS (NEXT GENERATION SEQUENCING)

Joint Training Course of the inter EURLs Working Group on NGS:

Introduction to Bioinformatics for genomic data mining

Istituto Superiore di Sanità

AULA ROSSI

Via Giano della Bella, 34

00161 Rome, Italy

14-15 June

Organised by:

EURL VTEC

EURL *Listeria monocytogenes*

EURL *Salmonella*

EURL Coagulase Positive *Staphylococci*

EURL Parasites

EURL Foodborne viruses

EURL AMR

EURL *Campylobacter*

It was estimated that each EURL could invite up to 3 colleagues from their networks. A call was launched and candidates selected based on background knowledge in molecular biology and computer analyses.
About 40 people, trainers and trainees, came to ISS in Rome.
From the network of the NRLs for Parasites, three colleagues were selected (from NRL Finland, Denmark and Poland). Additionally, a colleague from NRL Romania was invited by another EURL.

Course objectives:

- Providing an overview of different possible approaches for NGS data analysis
- Illustrating the basic steps for data analysis (quality check, trimming, assembly, search of genetic features of interest)
- Providing guidelines for cluster analysis
- Illustrating guidance documents on NGS analysis published by the Inter EURLs Working Group
- Exchanging experiences among participants and reinforcing networking

After the course, the participants will be able to:

- Choose a solution for NGS data analysis
- Understand the vocabulary connected to NGS data analysis
- Perform basic analysis on NGS data (quality check, trimming, assembly, search of genetic features of interest)
- Evaluate and interpret the results obtained
- Understand the concepts behind different cluster analysis approaches

PROGRAM

June 14th 2022

9.30   **Registration**

9.45   Welcome and general overview on the joint training activities
            (Stefano Morabito, EURL VTEC)

10.00   Introduction to WGS
            1. Sequencing platforms & output data (20') (Maroua Sayeb,
               EURL *Listeria*)
            2. Bioinformatics analysis of NGS data: approaches and
               opportunities (command-line tools, commercial software,
               webservers) (20') (Joakim Skarin, EURL Viruses)

10.40   **Coffee break**

11.30   Introduction on quality check and trimming (Valeria Michelacci,
            EURL VTEC)

11.50   **Hands on exercises – Quality check and trimming**
            Quality check and trimming using different tools and platforms.
            Results interpretation.
            *Hands-on: FastQC and Positional and Quality Trimming on ARIES
            (Valeria Michelacci, EURL VTEC)*
            *Demo: Quality check and trimming with SeqSphere (Ásgeir
            Ástvaldsson, EURL Campylobacter)*

12.50   **LUNCH BREAK**
            (a list of suggested snack bars and restaurants will be provided)

14.30   Introduction on assembly and assembly statistics (Lauge Holm
            Sørensen, EURL AMR)

14.45   **Hands on exercises – Assembly statistics**
            Assembly statistics using different tools and platforms. Results
            interpretation.
            *Hands-on: SPAdes and Quast from ARIES (Federica Gigliucci, EURL
            VTEC)*
            *Demo: SeqSphere Assembly Quality Check (Angela van Hoek, EURL
            Salmonella)*

15.30   From BAM to BCF and beyond, making sense of aligned data (Paolo
            Vatta, EURL Parasites)

16.00   **Hands on exercises – Mapping approach**
            Demonstration of mapping through different platforms.
            *Hands-on: E. coli virulotyping using a mapping approach (Federica
            Gigliucci, EURL VTEC)*
            Demo: Mapping with SeqSphere (Maroua Sayeb, EURL Listeria)

16.45   **End of the first day**

June 15th 2022

09.30 **Hands on exercises – Search of genetic features on contigs**
Identification of virulence and AMR genes using different tools and platforms. Results interpretation.

Demonstration of genes identification on contigs through different platforms
Hands-on ResFinder on CGE webserver (Lauge Holm Sørensen, EURL AMR)
Demo: Salmonella virulotyping with SeqSphere (Angela van Hoek, EURL Salmonella)

10.10 Introduction to genome comparisons: gene-by-gene VS SNPs (Guidance document on cluster analysis) (*Ásgeir Ástvaldsson, EURL Campylobacter*)

10.40 Demonstration of gene-by-gene approach through different platforms:
ARIES (Federica Gigliucci, EURL VTEC)
Startflow (Maroua Sayeb, EURL *Listeria* and Marina Cavaiuolo, EURL CPS)
SeqSphere (Angela van Hoek, EURL *Salmonella*)

11.15 Parasites WGS: opportunities and challenges (Simone M. Cacciò, EURL Parasites)

12.00 Update on the collection and management of NGS data for molecular typing (Mirko Rossi, EFSA)

12:30 **LUNCH**
(a list of suggested snack bars and restaurants will be provided)

13:30 Strategies and opportunities for NGS analysis applied to food-borne threats. Overview of documents released by the Inter EURLs WG:
- Basic analytical tools (Document on bioinformatics tools for basic analysis) (Valeria Michelacci, EURL VTEC)
- Reference Whole Genome Sequencing collection (Angela van Hoek, EURL *Salmonella*)
- Guidance document for NGS-Benchmarking (Maroua Sayeb, EURL *Listeria*)
- Inventory of training supports (Marina Cavaiuolo, EURL CPS)

14:00 Wrap up

14.30 **Closure**

Friendly atmosphere, and a lot of exchanges!