

Update from the Inter EURL working group on NGS

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INTER EURLs WORKING GROUP ON NGS



AIM

to promote the use of WGS 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 WGS mandate sent by the Commission

from
November 2017

Meeting twice a year
(10 meetings done)



Inter EURLs WG webpages

- EURL *E. coli*:
<https://www.iss.it/en/web/guest/-/inter-european-union-reference-laboratories-working-group-on-next-generation-sequencing-1>
- EURL *Salmonella*
- EURL *Campylobacter*
- EURL AMR
- EURL *Listeria*
- EURL Coagulase Positive *Staphylococci*
- EURL Parasites



Direct link!

[Overview of conducted and planned PTs](#) – curated by EURL Antimicrobial Resistance

[Reference Whole Genome Sequencing collection](#) – curated by EURL *Salmonella*

[Guidance document for WGS-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

Documents prepared/1

Drafting of a position paper:

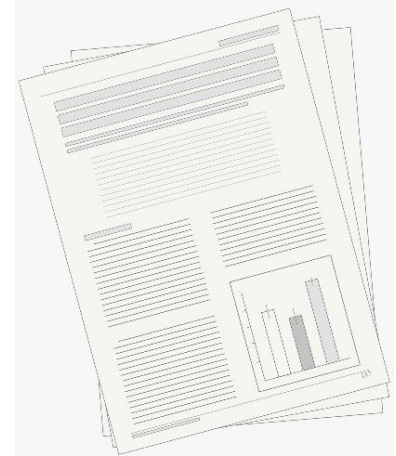
Adoption of NGS across NRLs

Harmonization of procedures

Proficiency tests

Benchmarking guidelines

Training and capacity building



Updated reference **WGS collection** curated by EURL *Salmonella*

Including *E. coli*, *Salmonella* and *Campylobacter*

To be updated soon with *Listeria monocytogenes* and more *Salmonella*

E. coli: Multiple sequences of the same six **O26 STEC strains** obtained during the 1st PT on WGS (anonymized)

Sequences available upon direct request to: crl.vtec@iss.it

Documents prepared/2

NGS laboratory procedures curated by EURL Parasites

DNA extraction from bacteria

DNA extraction from parasites

RNA extraction from viruses

Library preparation

Standard Operating Procedures linked or appended in Appendix, including protocols developed at the EURLs:

DNA extraction for Lm

DNA extraction for CPS

Library preparation (Illumina) for Lm

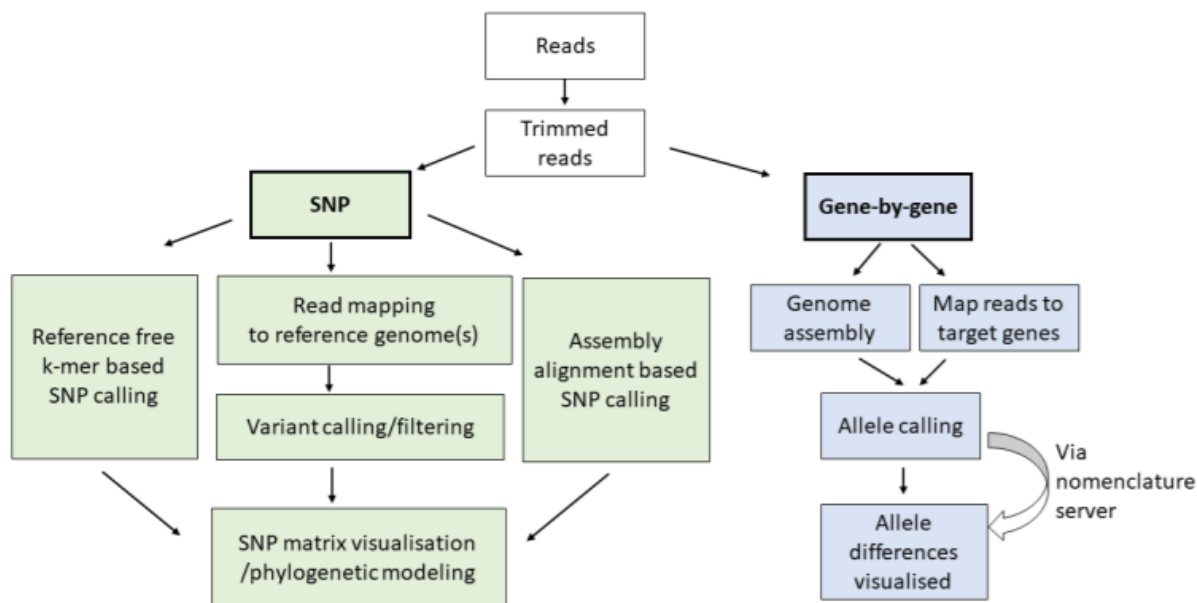
Library preparation (for Ion Torrent) for *E. coli*



Documents prepared/3

Updated version of Guidance document for **WGS - cluster analysis**

curated by EURL Campylobacter



List of tools and pipelines for the different approaches for cluster analysis

Updated with sections dedicated to visualization tools and interpretation guidelines

Documents produced/3

Supporting document for **DNA preparation and quality check** curated by EURL VTEC

Following a request made by EFSA in the framework of the Joint EFSA-ECDC Steering Committee on Molecular Typing Data collection

AIM: supporting the laboratories in the first steps of the NGS protocol, starting from the experiences on *E. coli*, *Listeria* and *Salmonella*, representing the first three pathogens for which WGS data collection has started

In preparation

Inventory of **training supports** curated by EURL CPS

Updated with links to new resources



First Inter EURLs joint training – June 14th-15th 2022

Introduction to Bioinformatics for genomic data mining

- EURL *E. coli*
- EURL *Listeria monocytogenes*
- EURL *Salmonella*

First edition in 2019

- EURL Coagulase Positive *Staphylococci*
- EURL Parasites
- EURL Foodborne viruses
- EURL AMR
- EURL *Campylobacter* **NEW!**

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, webserver) (20') (Joakim Skarin, EURL Viruses)

10.40 **Coffee break**

11.10 Amplicon-based sequencing of viral genomes (20') (Luca De Sabato, ISS)

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 webservice (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:

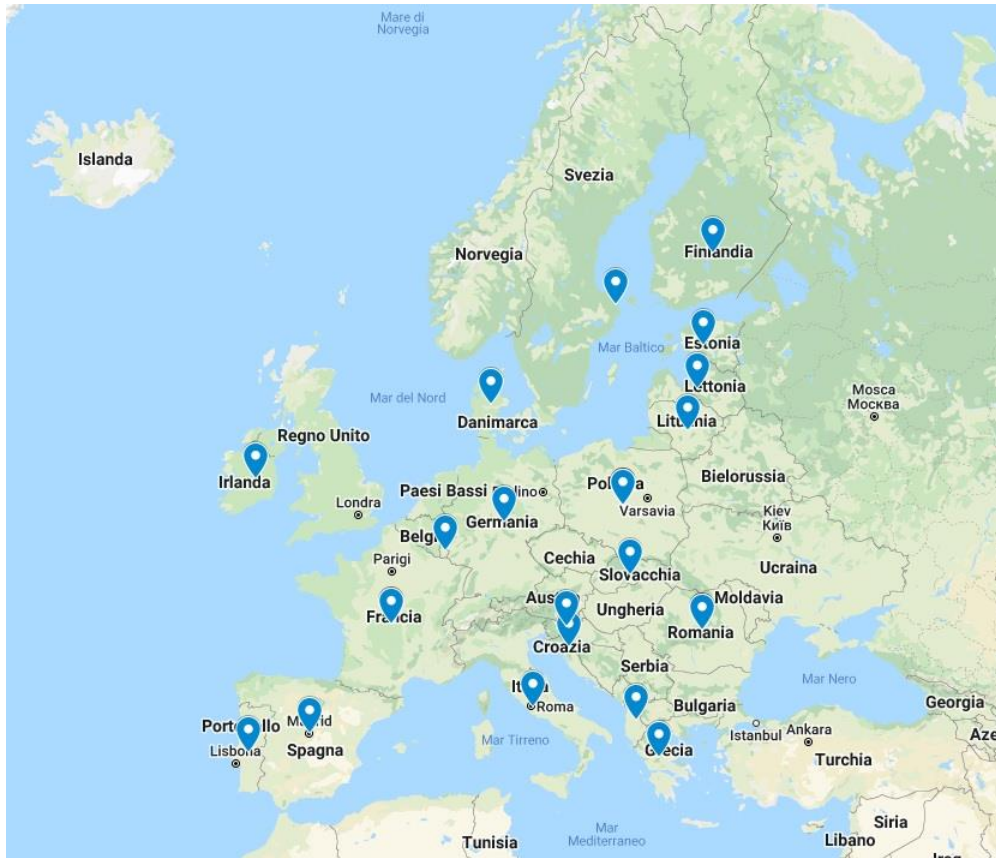
- [Basic analytical tools \(Document on bioinformatics tools for basic analysis\)](#) (Valeria Michelacci, EURL VTEC)
- [Reference Whole Genome Sequencing collection](#) (Angela van Hoek, EURL *Salmonella*)
- [Guidance document for NGS-Benchmarking](#) (Maroua Sayeb, EURL *Listeria*)
- [Inventory of training supports](#) (Marina Cavaiuolo, EURL CPS)

14:00 Wrap up

14.30 **Closure**



Participants – geographic distribution



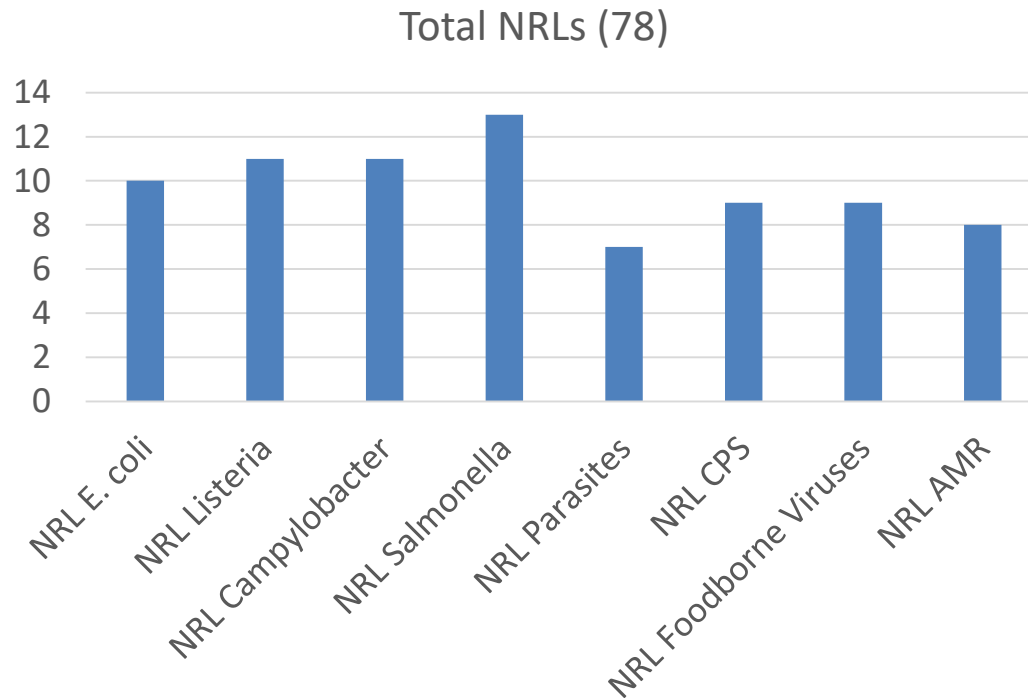
24 participants from different NRLs
(3 supported by EURL VTEC)

20 different countries
(19 Member States + 1 Candidate Country)



Participants – NRLs representation

Many participants come from labs appointed as NRLs for multiple pathogens:





Satisfaction survey

4. ORGANIZATION - Event location

[Altri dettagli](#)

● Poor	0
● Fair	0
● Good	2
● Very good	10
● Excellent	12



8. TOPICS - Relevance

[Altri dettagli](#)

● Poor	0
● Fair	1
● Good	3
● Very good	5
● Excellent	15



11. TOPICS - transfer of knowledge

[Altri dettagli](#)

● Poor	0
● Fair	0
● Good	6
● Very good	6
● Excellent	12

