

**European Union Reference Laboratory (EURL)
for *Escherichia coli*, including Shiga toxin-
producing *E. coli* (STEC)
(EURL VTEC)**

<https://www.iss.it/web/iss-en/vtec>

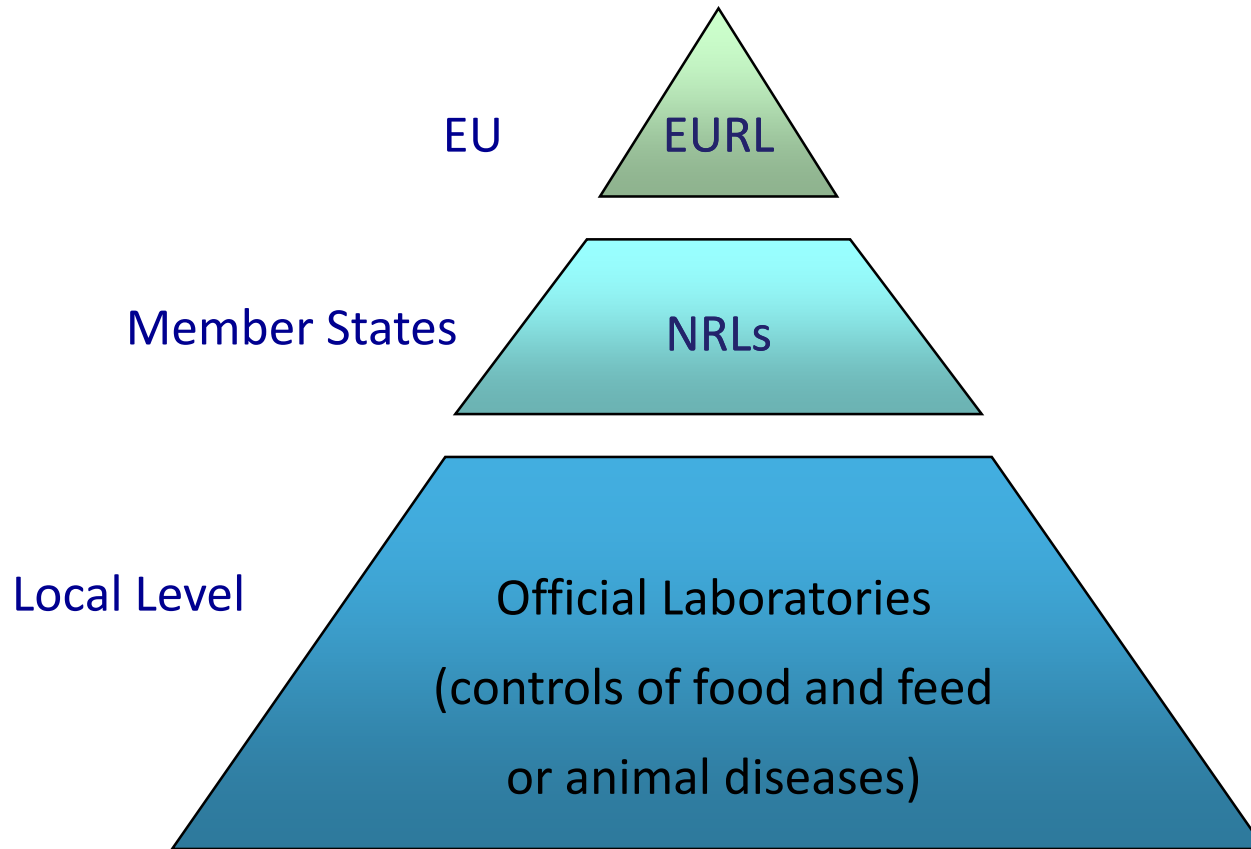
crl.vtec@iss.it



**Istituto Superiore di Sanità, Food Safety, Nutrition and Veterinary Public Health Dep.
European Reference Laboratory for *Escherichia coli***



The Istituto Superiore di Sanità was designated as Reference Laboratory for STEC by the European Commission on July the 1st, 2006 (Commission Regulation 776/2006)



Responsibilities and tasks of EURLs (625/2017, Art. 94)

- ✓ To provide NRLs with analytical reference methods
- ✓ To produce and distribute reference materials (reference strains for pathogenic *E. coli*; PCR targets; reference genomes)
- ✓ To organise comparative testing (Proficiency Tests)
- ✓ To conduct training and courses for NRLs
- ✓ To organise annual workshop
- ✓ To provide scientific and technical assistance to the Commission



Training stages and Courses

- 5-days training on the detection of STEC in food matrices according to the ISO TS 13136:2012 and the characterization of the isolated STEC strains
- 4-days training on the identification and characterization of the different groups of pathogenic *E. coli* by Real Time PCR amplification of their virulence genes
- 5-days training on the design and preparation of proficiency tests (PTs) on the detection of STEC in food matrices

Participants

NRLs from EU MS;

Non-EU NRLs;

Italians OLS



Training stages and Courses, NGS

- 2-days training course on WGS data use: bioinformatics for Next Generation Sequencing data mining for typing pathogenic *E. coli*
- 2-days training course on WGS data use: bioinformatics tools for aiding STEC outbreak investigation NGS nostro outbreak investigation
- 2-days Joint Training Course of the inter EURLs Working Group on NGS: Introduction to Bioinformatics for genomic data mining

Participants

NRLs from EU MS;

Non-EU NRLs;

Italians OLs



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European Reference Laboratory for *Escherichia coli*



Proficiency Tests

- PTs on the detection of pathogenic *E. coli* in food and related matrices
- PTs on the identification and typing of pathogenic *E. coli* strains
- PTs on the enumeration of *E. coli* in Live Bivalve Molluscs

Participants

NRLs from EU MS;

Non-EU NRLs;

Italians OLs



Laboratory methods

<https://www.iss.it/web/iss-en/vtec>

- International Standard ISO/TS 13136:2012 for the detection of STEC in food
- Conventional PCR for the identification of characterization of: STEC strains; subtypes of *stx* genes; STEC serogroups mainly associated with human infections
- Real Time PCR for the detection and characterization of: *E. coli* pathogroups (STEC, EAEC; ETEC; EIEC; EPEC); STEC serogroups mainly associated with human infections; STEC O104:H4 in food



E. coli genomics - methods

Galaxy ARIES

Analyze Data Workflow Visualize Shared Data Admin Help User

Using 99%

Tools

search tools

---PHYLOGENY TOOLS---

MLST 7 Loci

Phylogenetics

kSNP3

---HREVP TOOLS---

HReVAP

---E COLI TOOLS---

E coli typing

---LISTERIA TOOLS---

Listeria typing

---SALMONELLA TOOLS---

Salmonella typing

---OTHER BACTERIA TOOLS---

Klebsiella typing

---VIRUS TOOLS---

Virus typing

---NGS TOOLS---

Istituto Superiore di Sanita'

ARIES - Advanced Research Infrastructure for Experimentation in Genomics - Galaxy Instance at ISS

ARIES

ISTITUTO SUPERIORE DI SANITA'

EU-RL VTEC

Please read our disclaimer before using ARIES.

⚠️ "Science meets Policy" conference:

Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU

[Download the slides here](#)

[Read about it in FoodSafetyNews](#)

History

search datasets

Metagenomica

91 shown, 385 deleted, 15 hidden

222.6 GB

- 486: Filter on data 484
- 485: Filter on data 484
- 484: Filter on data 483
- 483: megablast BM_ED1 110_Ion-phantastic_contigs.fasta vs 'PLSDB_2210623'
- 482: BM_ED1110_Ion-phantastic_contigs.fasta
- 480: Diamond on ISS_18
- 478: ISS_18_scaffolds
- 473: Diamond on ISS_6
- 471: ISS_6_scaffold
- 466: Diamond on ISS_5_2_scaffolds

<https://aries.iss.it>

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European Reference Laboratory for *Escherichia coli*



Galaxy ARIES

Tools

search tools

---SALMONELLA TOOLS---

Salmonella typing

---OTHER BACTERIA TOOLS---

Klebsiella typing

---VIRUS TOOLS---

Virus typing

---NGS TOOLS---

NGS: QC and manipulation

NGS: Assembly

NGS: Alignment

NGS: Mapping

NGS: SAM Tools

NGS: BED Tools

---METAGENOMICS TOOLS---

MetaGenomics

---VARIOUS TOOLS---

Commet

javascript:void(0)

Tools

blastn

Show Sections

blast_to_scaffold Generate DNA scaffold from blastn or tblastx alignment of Contigs

NCBI BLAST+ blastn Search nucleotide database with nucleotide query sequence(s)

EURL VTEC WGS PT performs various typing tools

qiime quality-control exclude-seqs - Exclude sequences by alignment

qiime feature-classifier classify-consensus-blast - BLAST+ consensus taxonomy classifier

sistr_cmd Salmonella In Silico Typing Resource commandline tool for serovar prediction

JBrowse genome browser

WORKFLOWS

All workflows

NCBI BLAST+ blastn Search nucleotide database with nucleotide query sequence(s) (Galaxy Version 2.10.1+galaxy0) Favorite

Nucleotide query sequence(s)

482: BM_ED1110_Ion-phantastic_contigs.fasta

(-query)

Subject database/sequences

Locally installed BLAST database

Nucleotide BLAST database

Select/Unselect all

Multilocus sequence typing

NCBI NT 20220719

Plasmids 20151117

PlasmidFinder 20230317

PLSDB v.2021_06_23_v2

ResistanceGenes ResFinder 20170427

Salmo...

Salmo...

AMR typing by using blastn and ResFinder db

Tools

search tools

coli

pathogenic typing In silico pathogenic typing directly from raw Illumina reads

ABRicate performs mass screening of contigs for antimicrobial and virulence genes

---MLST---

agMLST by Assembly

ABRicate performs mass screening of contigs for antimicrobial and virulence genes (Galaxy Version 1.0)

Dataset

482: BM_ED1110_Ion-phantastic_contigs.fasta

Fasta files to be included in the analysis

Email notification

Yes No

Send an email notification when the job completes.

Execute

AMR typing by using ABRicate tool



Antimicrobial resistance in biosolids and water from WWTPs

Hazards characterization of:

- Biosolids of different origin
- Irrigation waters



municipal sludge-derived compost;
manure and compost from intensive animal farming systems;
compost from green and household wastes



reclaimed water
surface water

Shotgun metagenomics approach as a strategy to screen for the presence of AMR

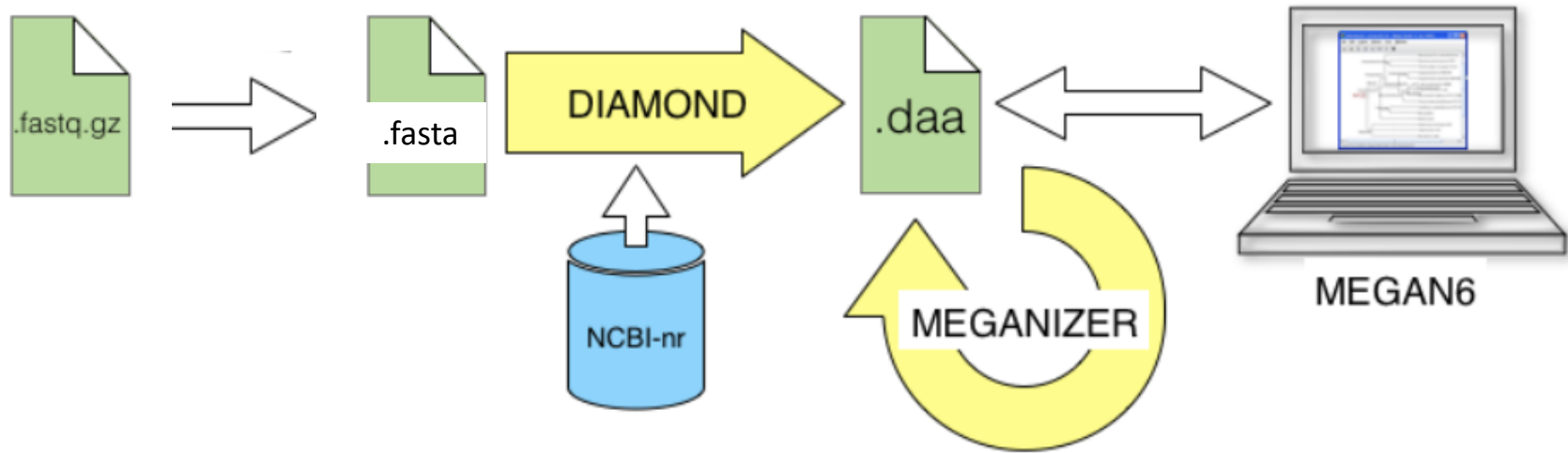
determinants



MEGAN6 - Metagenome Analyzer



DIAMOND + MEGAN pipeline

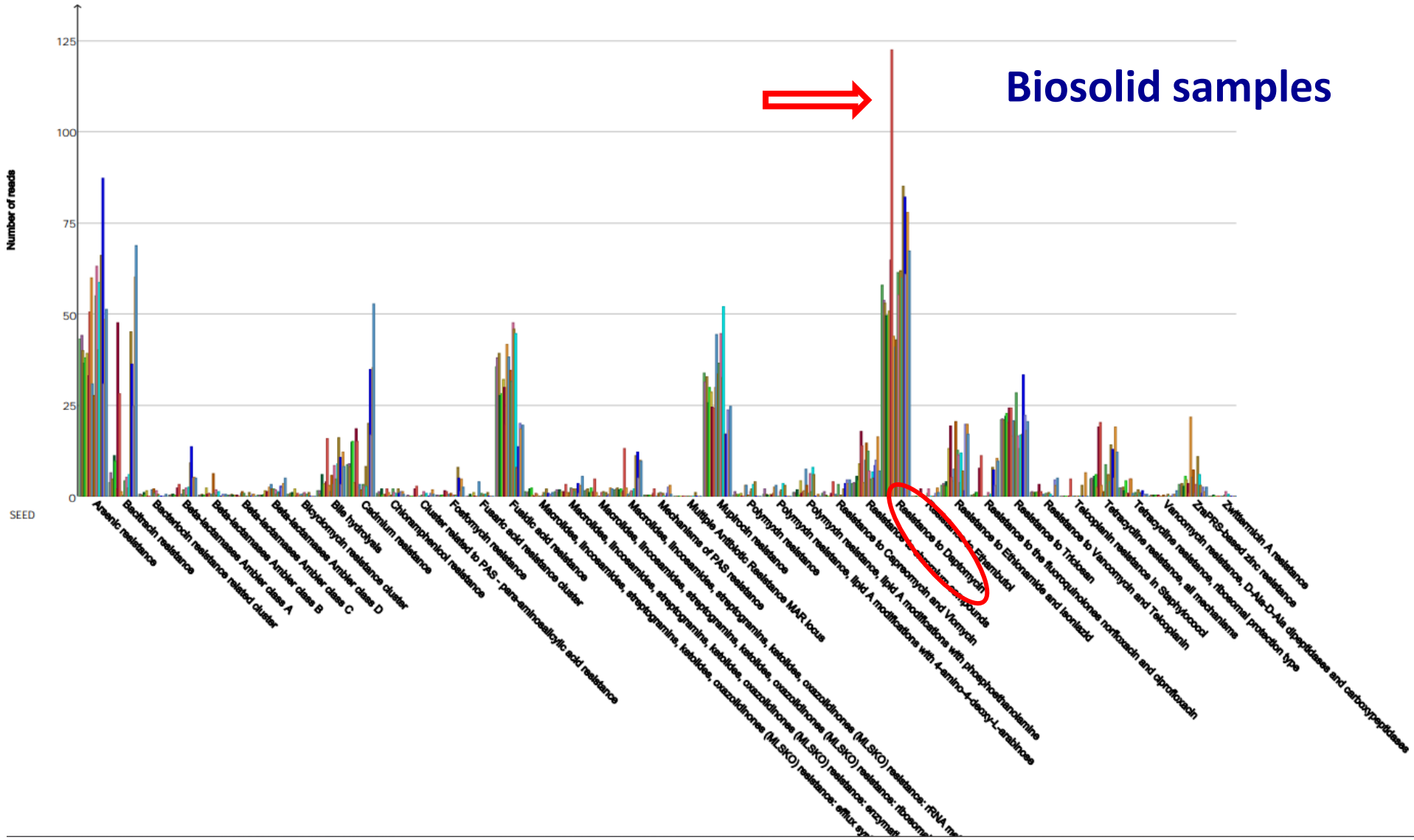


On ARIES:

- Assembly by SPAdes tool
- Alignment of nt sequences vs protein db by DIAMOND tool

On MEGAN6:

- Analysis of SEED content using the MEGAN internal Reference sequence map



Legend (Samples):

- 21-125880
- 21-125883
- 21-125893
- 21-132910
- 21-47142
- 21-47146
- CRPA_2746
- CRPA_2747
- CRPA_2749
- CRPA_2751
- CRPA_2752

- CRPA_2753
- CRPA_2754
- CRPA_2755
- ISS_18
- ISS_2
- ISS_3
- ISS_5.2
- ISS_5
- ISS_6

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
(11 meetings done)

- EURL *E. coli*
- EURL *Listeria monocytogenes*
- EURL *Salmonella*
- EURL Coagulase Positive *Staphylococci*
- EURL Parasites
- EURL Foodborne viruses
- EURL AMR
- EURL *Campylobacter*



Documents prepared/1

Guidance documents released through the webpages of each EURL



- Reference **WGS collection** curated by EURL *Salmonella*

Including STEC, *Salmonella enterica*, *Campylobacter*, *L. monocytogenes*

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

- **NGS laboratory procedures** curated by EURL Parasites

DNA extraction from bacteria

DNA extraction from parasites

RNA extraction from viruses

Library preparation



Documents prepared/2

- Inventory of **training supports**
- Supporting document for **DNA preparation and quality check**
- **Bioinformatics tools for basic analysis of NGS data**
Quality check, Trimming, Assembly, MLST, Virulotyping, Serotyping, AMR
Open source, commercial software, webservers
- **Guidance document for NGS-Benchmarking**
Quality and quantity metrics

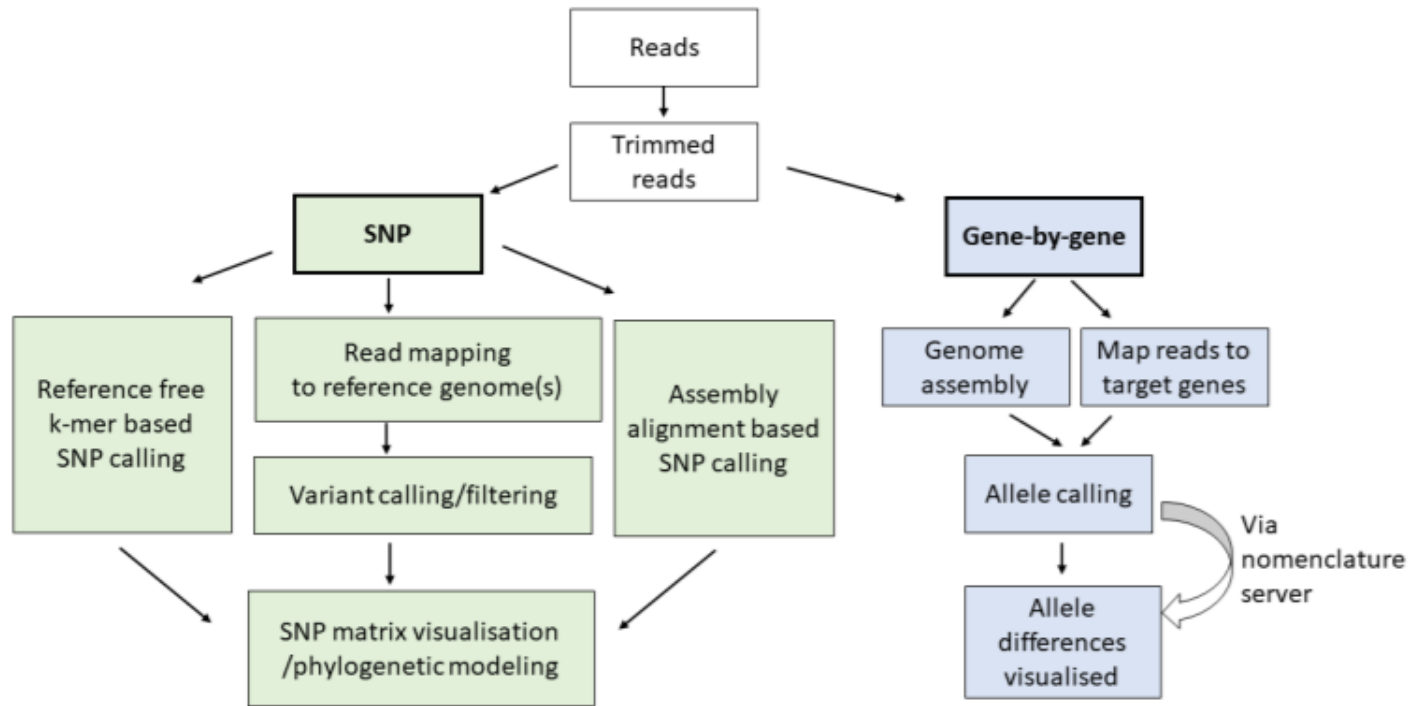
Wet and dry lab analyses

Accuracy, repeatability, reproducibility



Guidance document for WGS - cluster analysis

curated by EURL *Campylobacter*



List of tools and pipelines for the different approaches for cluster analysis, including visualization tools and interpretation guidelines

Inter EURLs initiatives

Joint trainings: “Introduction to Bioinformatics for genomic data mining”

- **ISS (EURL *E. coli*), Rome, June 2022**

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

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



- **NEW EDITION: at RIVM (EURL *Salmonella*), June 2023**

“Science Meets Policy”

- First edition: virtual conference, September 2020: over 500 participants from 49 countries worldwide
- **“EU initiatives towards the large-scale use of NGS to tackle foodborne threats”** organized by EFSA and the Inter EURLs WG on NGS
EFSA, Parma, Italy and online, September 5th-6th 2023 **Free of charge!**
<https://www.efsa.europa.eu/en/events/science-meets-policy-conference-using-next-generation-sequencing-tackle-foodborne-threats>



crl.vtec@iss.it

federica.gigliucci@iss.it

valeria.michelacci@iss.it