



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport



The inter EURL working group on NGS

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Inter European Union Reference Laboratories (EURL) Working Group (WG) on Next Generation Sequencing (NGS)

- From November 2017
- Meets twice a year
- EURLs operating in the field of the microbiological contamination of food and feed;
 - EURL-Antimicrobial Resistance (EURL-AR)
 - EURL-*Campylobacter*
 - EURL-Coagulase Positive Staphylococci (EURL-CPS)
 - EURL-*Escherichia coli*, including Shiga toxin-producing *E. coli* (EURL-VTEC)
 - EURL-Foodborne Viruses
 - EURL-*Listeria monocytogenes* (EURL-Lm)
 - EURL-Parasites
 - EURL-*Salmonella*



Aims

- Promote the use of NGS/WGS across the network of the EURLs
- Build NGS/WGS capacity within the EU
- Ensure liaison with the work of the EURLs and the work of EFSA & ECDC on the WGS mandate sent by the Commission



Drafted guidance documents

- Overview of conducted and planned proficiency tests (PTs)
- Reference WGS collection
- NGS laboratory procedures
- Preparing high quality DNA for WGS
- Bioinformatics tools for basic analysis of NGS data
- Cluster analysis of WGS data
- NGS-Benchmarking
- Inventory of training supports
- Survey on the use of NGS across the NRLs networks



Overview of conducted and planned PTs – curated by EURL-AR

- In the fall of 2019, a survey among the members of the inter EURL WG was conducted
- Five of the EURLs had performed NGS-based PTs
- Guidance document version 01 (November 25th, 2020) summarizes:
 - Previously conducted NGS-based PTs.
 - Planned NGS-based PTs for the EURL work programs 2020-2022.
 - Lessons learned in relation to NGS-based PTs
- The guidance document is currently being updated

- Dedicated online webinar on September 29th 2023 'Proficiency Tests on Next Generation Sequencing: approaches in use at the European Union Reference Laboratories'
 - For the benefit of the NRLs



Reference WGS collection – curated by EURL-Salmonella

- Collection was built from the NGS raw data files of PT participants for the validation and benchmarking of bioinformatic tools
- Sequences are available upon request for the NRLs
- Guidance document version 02 (March 6th, 2023) contains:
 - ***Escherichia coli*** reference collection: six O26:H11 STEC isolates from 24 PT participants, Illumina and Ion Torrent data
 - ***Salmonella enterica*** reference collection: six *S.* (monophasic) Typhimurium isolates from 14 PT participants and six *S.* Enteritidis isolates from 15 PT participants, Illumina data
 - ***Campylobacter*** reference collection: one *C. jejuni* and one *C. coli* (DNA and culture) from 6 PT participants and six *C. jejuni* DNA samples from 17 PT participants, Illumina data
 - ***Listeria monocytogenes*** reference collection: ten *L. monocytogenes* isolates (different serogroups/CCs) from 8 PT participants, Illumina data
 - **AMR *Campylobacter*** reference collection: four *Campylobacter* STs (DNA and culture) from >30 PT participants, Illumina data
 - **AMR *Escherichia coli*** reference collection: four *E. coli* serotypes (DNA and culture) from >30 PT participants, Illumina data
 - **AMR *Salmonella enterica*** reference collection: four *S. enterica* serovars (DNA and culture) from >30 PT participants



NGS laboratory procedures – curated by EURL-Parasites

- Guidance document version 01 (March 22nd, 2021) contains:
 - Identification of SOPs and LOPs available
 - Methodologies for DNA extraction for
 - Bacteria
 - Parasites
 - Viruses
 - Not pathogen-specific, i.e. specific matrices
 - Library preparation and workflows
 - Appendix 1. List of the SOPs and LOPs
 - Appendix 2. List of the web links for direct downloading of the protocols



Preparing high quality DNA for WGS – curated by EURL-VTEC

- Supporting document version 01 (December 23rd, 2022) highlights:
 - Important topics to ensure pure and high quality of extracted DNA
 - DNA extraction
 - Protocols based on magnetic-beads extraction and spin-columns kits are preferred
 - Selection of an isolated bacterial colony for DNA extraction
 - Control of the main genetic features characterizing the isolate
 - Plasmids
 - DNA manipulation
 - Elution buffers
 - DNA quality check to ensure its pureness and inspect fragmentation
 - DNA concentration to fulfil minimum criteria of the library preparation kits used for WGS



Bioinformatics tools for basic analysis of NGS data – curated by EURL-VTEC

- Guidance document version 01 (December 23rd, 2022) describes:
 - Analytical steps for basic NGS analysis
 - Quality check of the raw NGS DNA sequences
 - Trimming
 - Assemblers
 - 7-MLST, Serotype identification, Virulotyping, and inference of AMR
 - Tools
 - Open-source command line
 - Commercial software
 - EFSA One Health WGS System and analytical pipeline
 - Webservers
 - Other webservers dedicated to selected tools



Cluster analysis of WGS data – curated by EURL-Campylobacter

- Guidance document version 02 (April 28th, 2022) contains:
 - Approaches
 - SNP analysis
 - Gene-by gene analysis (cgMLST, wgMLST))
 - *k*-mer
 - SNP-based versus gene-by gene approach
 - Data differences and resolution
 - Comparability of results and nomenclature
 - Numerous tables regarding online and software options
 - Visualisation solutions of clustering data
 - Interpretation of clustering data



NGS-Benchmarking – curated by EURL-Lm

- Guidance document version 01 (April 8th, 2021) contains a checklist for bacterial genome analysis using WGS technology
 - Wet-Bench
 - Quality and quantity input DNA
 - DNA library quality and quantity
 - Quality of the sequencing reads
 - Comparison between sequencing platforms
 - Validation stage
 - Repeatability and reproducibility
 - Sensitivity and specificity
 - Validation of WGS workflow
 - Dry-bench
 - Accuracy of WGS-derived MLST assay
 - Accuracy of genotyping assay
 - Comparison of phylogenetic tree
 - Detection of specific genes



Inventory of training supports – curated by EURL-CPS

- Guidance document version 03 (December 15th, 2022) describes
 - Summary of training programs available within the different EURLs
 - Suggestions for training organisation; wet-lab as well as dry-lab
 - Training courses, wet-lab as well as dry-lab, by
 - EURLs
 - Companies like Illumina, Ion Torrent, Oxford Nanopore, PacBio
 - Companies like BioNumerics, Galaxy, Geneious, Ridom SeqSphere



Joint Trainings on NGS

- ANSES (Paris), EURL-Lm, October 2019 (**EURL-Lm, EURL-Salmonella, EURL-VTEC**)
'Analysis of molecular typing data, Bioinformatics tools for WGS data analysis'
12 participants from different NRLs from 10 countries
- ISS (Rome), EURL-VTEC, June 2022 (**All EURLs from the Inter EURL WG**)
'Introduction to Bioinformatics for genomic data mining'
24 participants from different NRLs from 20 countries
- RIVM (Bilthoven), EURL-Salmonella, June 2023 (**All EURLs from the Inter EURL WG**)
'Introduction to Bioinformatics for genomic data mining'
25 participants from different NRLs from 17 countries





Survey on the use of NGS across the NRLs networks – curated by EURL-VTEC

- Document version 01 (April 15th, 2020) describes the first action by the Inter EURL WG
 - In 2018 a survey was conducted across the NRLs to acquire knowledge on the level of adoption of NGS in the EU
 - Twenty questions about NGS at the NRLs, wet-lab protocols, dry-lab procedures, participation in PTs on NGS, trainings, experience in benchmarking of NGS protocols and one option to add free text comments
 - 178 NRLs replied, 88 reported not to have access to or use the NGS technology
 - The answer to the questions were summarized
- In 2019 a follow-up survey was performed to determine the cause



Drafted a position paper

- Illustrating the results of the first 5 years of activity of the inter EURL WG
 - NGS adoption across NRLs
 - Harmonization of procedures
 - NGS laboratory procedures
 - Bioinformatic tools
 - Quality control support
 - Proficiency tests
 - Reference whole genome sequences
 - Wet and dry benchmarking guidelines
 - Training and capacity building

MICROBIAL GENOMICS

SHORT COMMUNICATION

Michelacci *et al.*, *Microbial Genomics* 2023;9:001074

DOI 10.1099/mgen.0.001074



European Union Reference Laboratories support the National food, feed and veterinary Reference Laboratories with rolling out whole genome sequencing in Europe

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Abstract

The Inter European Union Reference Laboratories (EURLs) Working Group on Next Generation Sequencing (NGS) involves eight EURLs for microbiological food and feed hazards and has been working since 2017 to promote the adoption of NGS by the National Reference Laboratories (NRLs) in the European Union. This work illustrates the results of the first 5 years of activity. By working together, the EURLs involved have released guidance documents for assisting NRLs in all the steps of NGS, helping the transition from classical molecular methods towards whole genome sequencing while ensuring harmonization, with the final aim of improving preparedness in the use of NGS to characterize microbial hazards and trace the sources of infection.

<https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.001074>



“Science Meets Policy” conferences

- Online conference September 25th 2020, hosted by ISS, Rome (Italy)
“Modern technologies to enable response to crises: Next Generation Sequencing to tackle food-borne diseases in the EU”
Over 500 participants from 49 countries worldwide
- Hybrid conference September 5th – 6th 2023, hosted by EFSA, Parma (Italy)
“EU initiatives towards the large-scale use of Next Generation Sequencing (NGS) to tackle foodborne threats”



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