Foreword

The Working Group (WG) has been established by the European Commission 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 and this document represents a deliverable of the WG and is meant to be diffused to all the respective networks of NRLs.

Supporting document for preparing high quality DNA for Whole Genome Sequencing

Version 02 - 21/01/2026

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Co-funded by the European Union

Co-funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA). Neither the European Union nor HaDEA can be held responsible for them.

1. Introduction

In the framework of the activities of the Inter EURLs Working Group on Next Generation Sequencing, the need for guidelines for checking the quality of extracted DNA to be used for sequencing was highlighted. This document is meant to provide guidance in this crucial preliminary step of the laboratory work to produce NGS data with short-read sequencing technologies.

The present document considers the main food-borne pathogens object of the Commission Implementing Regulation (EU) 2025/179 (1), i.e. *Listeria monocytogenes*, *Salmonella enterica, Escherichia coli* including Shiga toxin-producing *E. coli* (STEC), *Campylobacter jejuni*, and *Campylobacter coli*, and on which EFSA and ECDC One Health WGS system is currently focused.

General requirements and guidance for generating whole genome sequencing (WGS) data of bacteria obtained from the food chain are also detailed in ISO 23418:2022 (2). As a general principle, all the material which will be subjected to sequencing (bacterial strains or extracted genomic DNA) should be handled and stored in such a way that the risk of degradation, wrong identification and cross-contamination of the samples is reduced (2).

2. DNA extraction

A document dedicated to wet-lab procedures performed as preliminary steps for WGS, including DNA extraction protocols, was prepared and published in the framework of the activities of this working group (3). Numerous DNA extraction protocols based on different principles have been shown to extract suitable DNA in terms of purity and concentration to be used for downstream protocols needed for sequencing. On the other hand, some common practices such as preparing boiling lysates to be used as DNA preparation or DNA extraction protocols based on salt and ethanol precipitation are generally not recommended to be used for WGS (4). Protocols based on magnetic-beads extraction and spin-column kits, either used through automatic workstations or by single-sample treatment, have proved to produce DNA of adequate quality for WGS. Nevertheless, performing a quality check of the extracted DNA before using it for WGS application is advisable to avoid submitting low quality samples to WGS, which could provide issues in the library preparation and in the sequencing protocol. Despite requiring some additional time, this is meant to avoid possible repetition of shipment of the DNA samples to the sequencing centre and double treatment of samples for sequencing, finally resulting in saving time, which is crucial especially in situations of emergency such as, for example, during the investigation of a potential outbreak.

For this reason, here we summarize the main steps considered crucial for preparing pure and high-quality DNA for WGS of pathogenic bacteria.

Selection of an isolated bacterial colony for DNA extraction

When testing bacteria, selecting a single isolated colony for DNA extraction is the first preliminary step. A minimal contamination of the starting material, either with other bacterial species or with other isolates of the same species potentially present in the sample, would impair the genomic analyses. For this reason, it is important to start from a plate containing a pure culture of the strain intended for genomic sequencing, from where a single colony is selected for the chosen DNA extraction protocol. It is advisable to minimize the number of passages on solid media to reduce the risk of genetic modification (such as loss of plasmids or

accumulation of polymorphisms during growth) (2). The strains should be stored frozen at -70 °C or lower to ensure stability.

Control of the main genetic features characterizing the isolate

In order to avoid unintended sequencing of non-target strains present in the initial culture or sequencing of strains which may have lost virulence genes (e.g. loss of Shiga toxin-encoding genes from STECs) checking the presence of the main (pathogenic) characteristics of the strains by PCR in the single colony selected for sequencing, or directly in the DNA extracted for sequencing, is advisable. This is especially important for pathogens for which loss of virulence genes is occasionally reported (e.g. STEC).

Plasmids

It has been shown that some DNA extraction protocols are impaired in terms of plasmid extraction (4, 5). Virulence genes of pathogenic bacteria and antimicrobial resistance genes are often carried on (large) plasmids. Impaired plasmid extraction would still allow strain-by-strain comparison through cgMLST, as the genes representing the target of such analysis are harboured on the chromosome, but would hinder a proper characterization of the isolate. For this reason, it is advisable to avoid salting-out extraction methods based on alcoholic precipitation, in order to obtain a complete picture of bacterial genome through WGS.

DNA manipulation

It is important to avoid fragmentation of DNA, for example minimizing the steps of freeze/thawing. Fragmentation would impact the result of sequencing (2).

Elution buffers

Library preparation kits used for DNA sequencing generally make use of enzymes whose activity could be impaired by the presence of EDTA in the DNA solution. On the other hand, many DNA extraction kits involve a final elution/rehydration step in EDTA-containing buffers. It was demonstrated that 10mM Tris-HCl (pH 8.5) or nuclease-free water can be used as valid alternatives preventing problems in downstream WGS protocols (5). Nevertheless, the presence of EDTA in the buffer is beneficial for DNA preservation. As it has been shown that data quality, isolate characterization, and relatedness are not affected by low concentration of EDTA in the buffer (< 1.0 mM) (5), Low TE buffer (10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA) may also be used, when long-term DNA preservation is required.

3. DNA quality check

Despite no specific quality criteria are generally required for input DNA by library preparation kits, assessing the quality of the DNA sample is advisable after extraction, to ensure its pureness and inspect fragmentation and to avoid problems in downstream protocols needed for WGS.

As a quality check, agarose gel or capillary electrophoresis can be performed, to inspect if fragmentation appears with smeared bands at low molecular weight and to visualize absence of evident rRNA bands.

Spectrophotometric analysis of the DNA can also be performed to assess the ratio between the absorbance at 260 nm and 280 nm. The value of the 260/280 absorbance ratio should within the range 1.75-2.05 which implies pure DNA (2).

4. DNA concentration

The concentration of the DNA samples must be measured to fulfil minimum criteria of the library preparation kits used for WGS. The DNA extraction kits available on the market are generally able to extract adequate quantity of DNA of the target organisms for the majority of the library preparation kits. The amount of input DNA can significantly vary between different library preparation kits and sequencing services. In detail, some library preparation protocols require only 1 ng of DNA, but others may require at least 100 ng (3). Additionally, when sequencing is outsourced to an external service provider, the shipment of up to 600 ng of DNA may be required.

The concentration should be measured for each DNA sample using a fluorometric system (e.g. the Qubit fluorometer) and the corresponding reagents kit, avoiding spectrophotometric methods measuring absorbance, which provide useful information in the quality check step but are less accurate for DNA quantification.

If the above-mentioned quality and quantity parameters are not fulfilled, the DNA isolation step should be repeated and troubleshooted.

5. Bibliography

1. Commission Implementing Regulation (EU) 2025/179 of 31 January 2025 on the collection and transmission of molecular analytical data within the frame of epidemiological investigations of food-borne outbreaks in accordance with Directive 2003/99/EC of the European Parliament and of the Council, OJ L, 2025/179, 3.2.2025.
2. ISO 23418:2022 “Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance”. Technical Committee: ISO/TC 34/SC 9 Microbiology; Publication date: June 2022
3. Inter EURLs Working Group on NGS “Guidance document for WGS-laboratory procedures” https://doi.org/10.5281/zenodo.10406850
4. European Union Reference Laboratory for Antimicrobial Resistance “Protocol for Whole Genome Sequencing and bioinformatics analysis of bacterial isolates related to the EU monitoring of antimicrobial resistance” https://www.food.dtu.dk/english/-/media/institutter/foedevareinstituttet/temaer/antibiotikaresistens/eurl-ar/wgs/628_protocol-for-wgs-v2-2.pdf
5. Nouws S. et al. “Impact of DNA extraction on whole genome sequencing analysis for characterization and relatedness of Shiga toxin-producing *Escherichia coli* isolates” Scientific Reports (2020) 10:14649.