# Nanopore-only microbial isolate sequencing solution (NO-MISS): a flexible and rapid approach for whole-genome sequencing of bacterial isolates

Whole-genome sequencing of microbial isolates provides valuable information for public health, clinical microbiology research, food safety, and microbial ecology. It is especially important during outbreak investigations, including detection and characterisation of antimicrobial resistance genes, virulence genes, plasmids and other mobile genetic elements, and additional genomic features that may affect the phenotypic properties of the strain. Reconstruction of complete, contiguous, high-quality microbial genomes by *de novo* assembly is limited with short-read sequencing. Nanopore sequencing can generate reads of unrestricted length and overcome these limitations to deliver comprehensive genome assemblies.

High-accuracy nanopore sequencing shows no bias in GC-rich regions[1] and can span repeat-rich sequences and structural variants that are inaccessible to legacy sequencing technologies. Furthermore, nanopore sequencing is a scalable solution that offers flexible sample batching and provides rapid turnaround time from sample to answer. By obtaining complete, reference-quality microbial genome sequences in one experiment and in-house, the need to outsource or use multiple techniques for validation is no longer required.

In addition, ElysION™ — an all-in-one, sample-to-answer device — offers complete end-to-end, fully automated nanopore sequencing for many applications such as microbial isolate whole-genome sequencing. It enables automated extraction, library preparation, sequencing, basecalling, and data analysis for multiple samples, ensuring a streamlined, hands-free, and simplified choice for researchers. Find out more about ElysION at: **nanoporetech.com/elysion**.

**Here we present a rapid end-to-end workflow for whole-genome sequencing of bacterial isolates, using MinION™ Flow Cells on MinION or GridION™ sequencing devices and the EPI2ME™ analysis platform.**

## **EXTRACTION:** cell lysis and obtaining high-quality DNA

View extraction protocol recommendations for your sample type, plus guidance on DNA storage and contaminants: **community.nanoporetech.com/docs/prepare**

To ensure high outputs of long reads in nanopore sequencing, it is important to select an extraction method that yields high-quality DNA. Depending on your research requirements and microbial isolate, we recommend different cell lysis and extraction methods. This workflow is suitable for multiple microbial isolate types — see the **full NO-MISS protocol on the Nanopore Community** for more information.

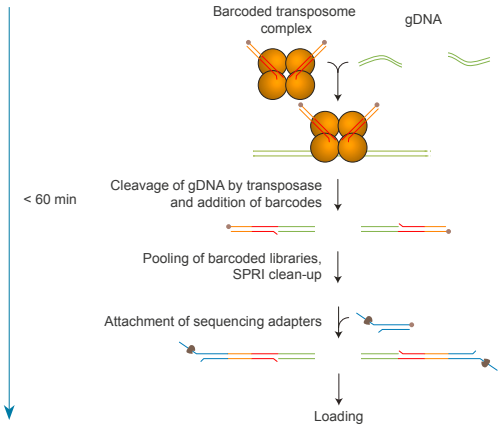
To manually extract DNA from a general bacterial isolate, we recommend the **NEB Monarch Genomic DNA Purification Kit**. Alternatively, you can use a universal automated extraction method that uses bead-beating and the **Promega Maxwell RSC PureFood Pathogen Kit**. Prior to proceeding to library preparation, we recommend using a **Qubit fluorometer** for accurate DNA quantification.

## **LIBRARY PREPARATION:** sample multiplexing

Find out more about library preparation: **nanoporetech.com/prepare**

To prepare your library for sequencing and downstream analysis, you can choose from either the 24- or 96-plex **Rapid Barcoding Kits**. These PCR-free kits use a transposase to fragment and attach barcodes to your bacterial isolate DNA before the addition of a sequencing adapter. We recommend multiplexing 4–24 bacterial isolate genomes per MinION Flow Cell.

Through multiplexing a number of bacterial isolate libraries on a single MinION Flow Cell, the cost per bacterial isolate can be considerably reduced; when using 24 barcodes, data can be generated for as little as ~$25 per bacterial isolate. Flow cells that are not run at full sample capacity can be washed and reused, facilitating efficient sample batching while maintaining low cost per bacterial isolate. The **Flow Cell Wash Kit** provides a cost-effective method to wash and re-run a flow cell multiple times.



## **SEQUENCING:** achieving ultimate flexibility with MinION Flow Cells

Find out more about nanopore sequencing devices:
**nanoporetech.com/sequence**

We recommend sequencing your bacterial isolate libraries on MinION Flow Cells, which can be run on the portable **MinION** device for easily accessible, routine sequencing. Alternatively, the benchtop **GridION** device enables on-demand, scalable sequencing of up to five individually addressable flow cells at one time, allowing you to increase sample throughout, as required.

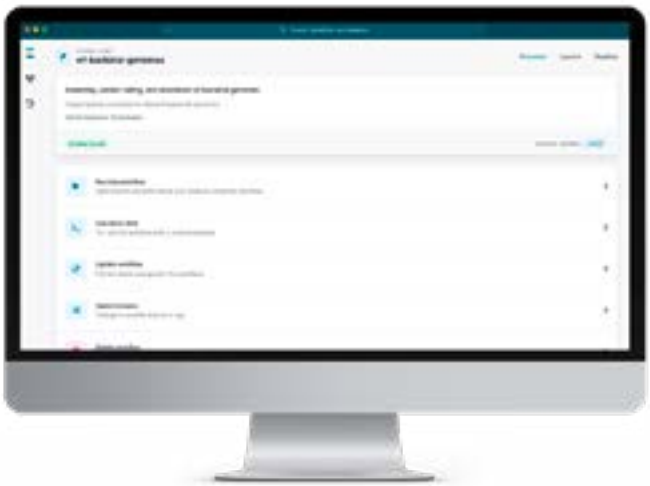
For complete, high-quality genome assembly, we recommend sequencing to 50x depth of coverage and basecalling in high accuracy (HAC) mode using the MinKNOW™ software. For a 24-plex run, this can be achieved by sequencing on one MinION Flow Cell for up to 72 hours.

## **ANALYSIS:** using the EPI2ME bacterial genomes workflow

View the dedicated bacterial genomes workflow:
**labs.epi2me.io/workflows/wf-bacterial-genomes**

**EPI2ME** workflows enable nanopore data analysis for all levels of expertise. The pre-configured analysis packages are free to access from a desktop application with an easy-to-use graphical interface or the command line and can be run on local compute or in the cloud.

The **wf-bacterial-genomes** workflow enables *de novo* assembly of your bacterial isolate genomes, annotation of regions of interest within the assemblies, species identification and sequence typing, and identification of genes and single nucleotide variants associated with antimicrobial resistance[2].



Learn more about data analysis solutions: **nanoporetech.com/analyse**

## Find out more at: **nanoporetech.com/microbiology**

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**References:**

1. Browne, P. D. et al. *GigaScience* 9(2):giaa008 (2020). DOI: https://doi.org/10.1093/gigascience/giaa008
2. GitHub. wf-bacterial-genomes. Available at: https://github.com/epi2me-labs/wf-bacterial-genomes [Accessed 20 Mar 2024]



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