Nanopore-only microbial isolate sequencing solution (NO-MISS): flexible, rapid bacterial and fungal isolate whole-genome sequencing

Across public health, clinical microbiology research, food safety, and the biopharma sector, whole-genome microbial isolate sequencing is a valuable tool. It is especially important during outbreak investigations, where it delivers crucial information on antimicrobial resistance genes, plasmids and other mobile genetic elements, and further features that may affect strain phenotypes. For the biopharma sector, whole-genome sequencing of production strains ensures that they are well characterised, stable, and free from mutations and contamination.

Complete, contiguous, high-quality microbial genome assembly, however, is limited with legacy short-read sequencing technology. Oxford Nanopore sequencing overcomes these limitations, generating reads of unrestricted length to deliver comprehensive genome assemblies. Plus, high-accuracy nanopore sequencing shows no bias in GC-rich regions[1] and spans repeat-rich sequences and structural variants that are inaccessible to short-read methods. Oxford Nanopore sequencing offers scalability, flexible sample batching, and rapid turnaround time from sample to answer. You can now generate complete, reference-quality microbial genome sequences in-house, in one go, without needing to outsource or use multiple validation techniques.

Here we present a rapid end-to-end workflow for whole-genome sequencing of microbial isolates, compatible with any of our sequencing devices and including analysis with the EPI2ME™ platform.

Extraction:
lysing cells and obtaining high-quality DNA

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

To ensure high outputs of long reads in Oxford Nanopore sequencing, it is important to select an extraction method that yields high-quality DNA. Depending on your research requirements and sample, we recommend different cell lysis and extraction methods. Our workflow is suitable for multiple microbial isolate types.

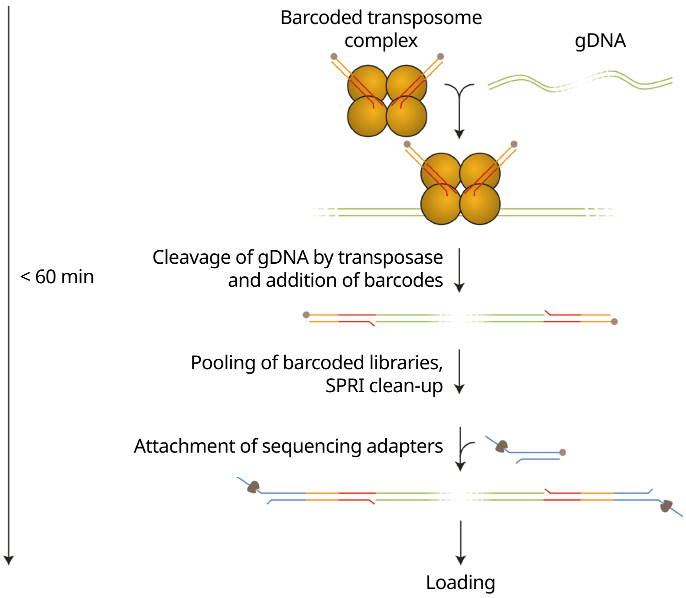
We provide four options for extracting DNA from your isolates, comprising a universal bead-beating method for high-throughput requirements and manual column-based techniques for bacteria, hard-to-lyse bacteria, and fungi. Our protocols also include guidance on how to perform quality checks.

Library preparation:
multiplexing samples

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 microbial isolate DNA before adding a sequencing adapter. We recommend multiplexing 4–24 microbial isolate genomes per MinION™ Flow Cell or 4–96 genomes per PromethION™ Flow Cell.

Through multiplexed sequencing on a single flow cell, the cost per isolate can be considerably reduced. You can wash and reuse flow cells that are not run at full sample capacity to ensure efficient sample batching while maintaining a low cost per sample. The **Flow Cell Wash Kit** provides a cost-effective method to wash and re-run your flow cell multiple times.



Sequencing: scaling for your throughput needs

Find out more about Oxford Nanopore sequencing devices: nanoporetech.com/sequence

The NO-MISS workflow is compatible with our full range of nanopore sequencing devices, so you can scale to suit your experimental goals.

You can sequence up to 24 isolates on one MinION Flow Cell on a **MinION** device for easily accessible, routine sequencing, or on a benchtop **GridION**™ for flexible sequencing on up to five independent flow cells.

To scale up for higher throughputs, you can sequence up to 96 isolates on one PromethION Flow Cell. These are compatible with the **PromethION 2 Integrated** or **PromethION 24**, for powerful, on-demand sequencing on up to 2 or 24 independent flow cells, respectively.

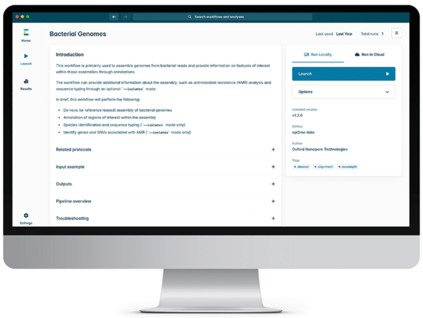
For complete, high-quality genome assembly, we recommend sequencing to 50x depth of coverage and basecalling in high accuracy (HAC) mode using the **MinKNOW™** software. For a 24-plex run on a MinION Flow Cell or 96-plex run on a PromethION Flow Cell, this can be achieved by sequencing for up to 72 hours.

Analysis: using the EPI2ME wf-bacterial-genomes workflow

View the dedicated EPI2ME workflow: epi2me.nanoporetech.com/workflows/wf-bacterial-genomes

EPI2ME workflows enable Oxford Nanopore data analysis for all levels of expertise. The pre-configured workflows are free to access through an easy-to-use graphical interface or the command line and can be run on local compute or in the cloud.

Our **wf-bacterial-genomes**[2] workflow assembles your bacterial or fungal isolate genomes, annotates regions of interest, performs species identification and typing, detects plasmids, and identifies antimicrobial resistance genes.



Bacterial Genomes

Introduction

This workflow is primarily used to assemble genomes from bacterial reads and provide information on features of interest within those assemblies through annotations.

The workflow can provide additional information about the assembly, such as antimicrobial resistance (AMR) analysis and sequence typing through an optional `--isolates` mode.

In brief, this workflow will perform the following:

- De novo (or reference-based) assembly of bacterial genomes
- Annotation of regions of interest within the assembly
- Species identification and sequence typing (`--isolates` mode only)
- Identify genes and SNVs associated with AMR (`--isolates` mode only)

Installed version
v1.2.0

GitHub
epi2me-labs

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Tags
denovo alignment mosdepth

View the end-to-end protocol: nanoporetech.com/no-miss-protocol

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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 26 Mar 2026]

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