Streamlined identification of bacteria, archaea, and fungi with Oxford Nanopore microbial amplicon barcoding

The identification of prokaryotes through targeted analysis of the 16S ribosomal RNA gene is a technique utilised across microbiology, from infectious disease research through to environmental studies. Though the method provides a faster, more cost-efficient workflow than microbial whole-genome sequencing, legacy short-read technology cannot capture the entire ~1.5 kb 16S gene. As a result, short reads typically restrict taxonomic resolution to the genus level, limiting the depth of insight that can be drawn from microbial samples.

Oxford Nanopore reads of unrestricted length span the complete 16S gene with ease, often achieving greater taxonomic resolution of mixed microbial samples than possible with short-read 16S sequencing. The Microbial Amplicon Barcoding Kit provides a fast, fragmentation-free method of preparing 16S amplicons for sequencing. Furthermore, the kit also enables amplification of the full-length internal transcribed spacer (ITS) region for the identification of fungi. From efficient library preparation to scalable sequencing and intuitive data analysis with EPI2ME™ — no prior experience needed — the simple Oxford Nanopore workflow delivers the clarity and resolution you need to unravel your microbial samples.

Here we present a streamlined workflow for identification of bacteria, archaea, and fungi from microbial samples, using 16S and ITS amplicon sequencing on a MinION™ or GridION™.

Sample preparation:
amplifying and preparing full-length 16S and ITS sequences

View extraction protocols for a range of sample types: **nanoporetech.com/extraction-methods**

First, extract DNA from your microbial samples. Oxford Nanopore offers protocols for the extraction of high-quality DNA from a range of sample types, as well as information on how to check the quality of your samples for optimal results.

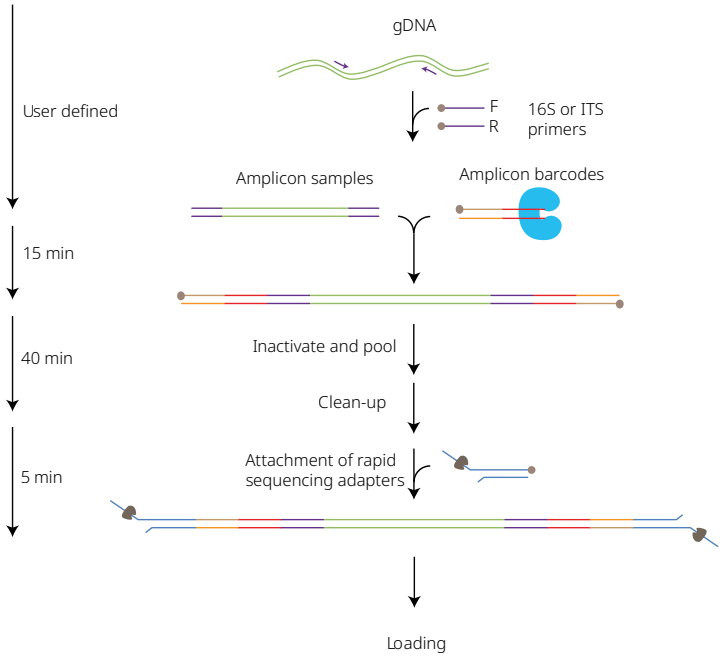
To prepare your microbial DNA samples for targeted nanopore sequencing, use the **Microbial Amplicon Barcoding Kit**. The sample prep process begins with amplification of the full-length 16S gene from prokaryotic samples or the ITS region from fungal samples using the primers provided.

After this, select a unique barcode for each sample that will be sequenced in multiplex, for up to 24 samples in total. These barcodes are attached to your full-length amplicons in a simple incubation step, with no need for fragmentation, ligation, or further PCR.

Then, pool together all barcoded samples that will be sequenced on the same flow cell. For best results, we recommend sequencing 16S and ITS amplicons in separate runs; if you wish to run them together, we advise adjusting molarity to ensure balanced representation of each sample in sequencing.

To prepare your barcoded amplicons for Oxford Nanopore sequencing, attach the provided rapid sequencing adapters to the pooled sample. Using rapid attachment chemistry, this process requires only five minutes.

From extracted microbial DNA to sequencing-ready amplicon library, the library prep process takes one hour plus PCR time.



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

Sequencing:
generating high depth of coverage at the scale you need

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

To achieve a high depth of coverage of up to 24 microbial amplicon samples, we recommend sequencing your library on one MinION Flow Cell using a **MinION** or **GridION** device. The compact MinION can be plugged into a laptop for sequencing in any location, while the flexible GridION is ideal for higher throughput requirements, featuring sequencing on up to five independent flow cells and integrated compute.

Set up your sequencing run using the software **MinKNOW™**, which controls Oxford Nanopore devices. We recommend sequencing for 12 hours, which will generate 100,000 reads or more per barcoded sample, and performing live basecalling using the high accuracy (HAC) model.

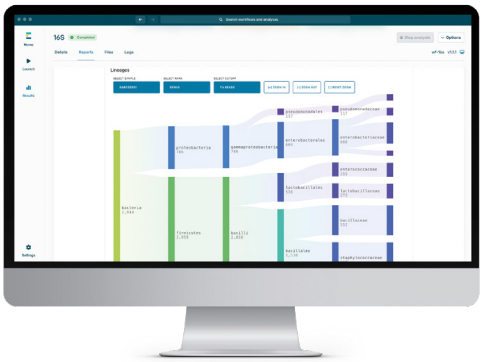
After your sequencing run has finished, you can use the **Flow Cell Wash Kit** to prepare your flow cell for reuse or for storage until it is needed again.

Analysis:
exploring microbial taxa using EPI2ME

Find out more about EPI2ME:
nanoporetech.com/epi2me

To analyse your data, we recommend the easy-to-use **EPI2ME** platform from Oxford Nanopore. EPI2ME provides bioinformatics pipelines for all levels of experience, which can be accessed via an intuitive user interface or the command line, locally or in the cloud. The EPI2ME 16S workflow, **wf-16s**[1], enables taxonomic classification from your 16S and ITS amplicons to the genus or species level.

The 16S workflow takes an input FASTQ or BAM file, produced by MinKNOW, then performs either k-mer or alignment-based classification of each sample. The pipeline outputs abundance information for the bacterial, archaeal, and fungal taxa in each sample, plus interactive plots through which you can explore the identified lineages.



Discover data analysis with wf-16s: nanoporetech.com/wf-16s

View the end-to-end protocol: nanoporetech.com/MAB-protocol

References:

1. GitHub. wf-16s. Available at: https://github.com/epi2me-labs/wf-16s [Accessed 10 September 2025]

Oxford Nanopore Technologies

www.nanoporetech.com

phone +44 (0)845 034 7900
email support@nanoporetech.com
oxford-nanopore-technologies
@nanopore
@nanoporetech.com

WF_1321(EN)_V1_24Sep2025