Performing accurate species-level bacterial identification with nanopore sequencing

16S sequencing is the predominant method for microbial identification and has a wide range of applications, including food safety, environmental and conservation monitoring, pathogen detection, and clinical microbiology. The 16S ribosomal RNA (rRNA) gene is ~1.5 kb and comprised of nine variable regions divided by highly preserved sequences[1]. Using legacy sequencing technology, species-level bacterial identification is challenging because the short reads cannot span the full gene, limiting resolution. Instead, only partial fragments of the gene are sequenced, for example the V3–V4 or V4–V5 regions[1].

However, nanopore technology can overcome these limitations by generating long reads spanning the V1–V9 regions of the 16S rRNA gene in a single read. By sequencing the entire gene rather than subsets of exons, greater taxonomic resolution is achieved for accurate species identification from polymicrobial samples.

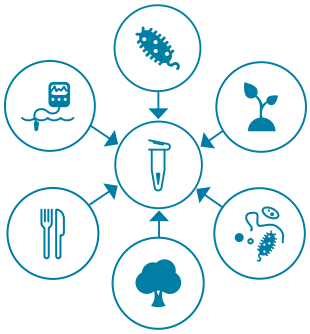
In this targeted workflow, the 16S rRNA gene is first amplified by PCR with 16S primers and then sequenced with long nanopore reads, providing a rapid and cost-effective method of species-level microbial identification.

Here we present a rapid workflow for full-length 16S rRNA sequencing of polymicrobial samples, using MinION™ Flow Cells on a MinION or GridION™ sequencing device with an EPI2ME™ analysis solution.

EXTRACTION: obtaining high-quality DNA

View extraction protocol recommendations for your sample type: **community.nanoporetech.com/docs/prepare**

Selecting a suitable extraction method to obtain high-quality DNA depends on the sample type. For analysing polymicrobial samples, we have a range of sample-specific extraction protocols available alongside the following kit recommendations: for environmental water samples, we recommend the **ZymoBIOMICS DNA Miniprep Kit**; for soil samples, the **QIAGEN DNeasy PowerMax Soil Kit** is recommended; and for stool samples, we recommend either the **QIAmp PowerFecal DNA Kit** to extract microbiome DNA or the **QIAGEN Genomic-tip 20/G** for an even mix of host and microbiome DNA.

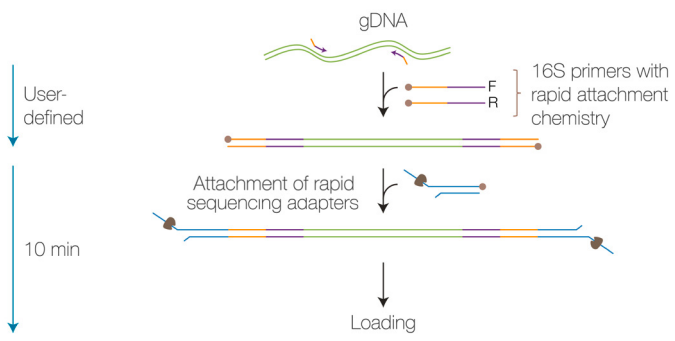


LIBRARY PREPARATION: preparing DNA for targeted PCR and barcoding

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

To prepare your library for sequencing and downstream analysis, use the **16S Barcoding Kit 24** to multiplex up to 24 DNA samples in a single prep. This kit uses PCR to amplify the entire ~1.5 kb 16S rRNA gene from extracted gDNA using barcoded 16S primers, before the addition of a sequencing adapter.

Only the amplified region of interest is sequenced for fast bacterial identification and economical sequencing. In addition, by multiplexing your libraries on a single flow cell, the cost per sample is further reduced. Flow cells that are not run at full sample capacity can be washed and reused multiple times using the **Flow Cell Wash Kit**, facilitating efficient sample batching whilst maintaining low cost per sample.



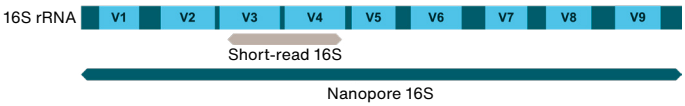
Find out more about the Flow Cell Wash Kit: **store.nanoporetech.com/flow-cell-wash**

SEQUENCING:
generating full-length 16S rRNA reads

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

To achieve high taxonomic resolution, we recommend sequencing your amplified library to 20x coverage per microbe. To achieve this for a 24-plex library, we recommend sequencing on a MinION Flow Cell using the high accuracy (HAC) basecaller on the MinKNOW™ software for ~24–72 hours, depending on microbial sample complexity. For lower-plex libraries, we recommend sequencing until enough data is generated to reach optimal coverage.

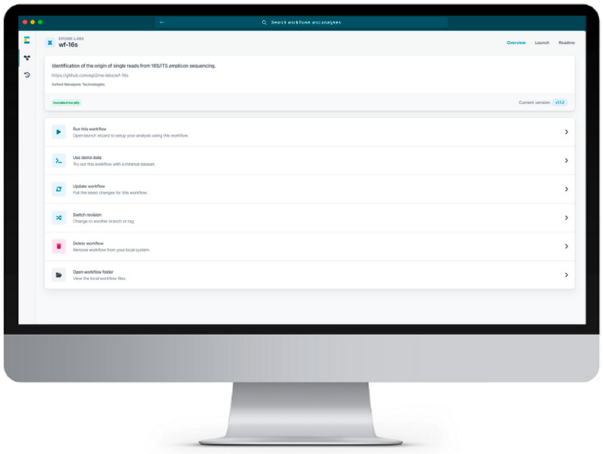
We recommend sequencing your library on MinION Flow Cells, which can be run on the portable MinION device for at-source sequencing. To scale up, up to five MinION Flow Cells can be run on the benchtop GridION device, enabling sample throughput to be increased as required.



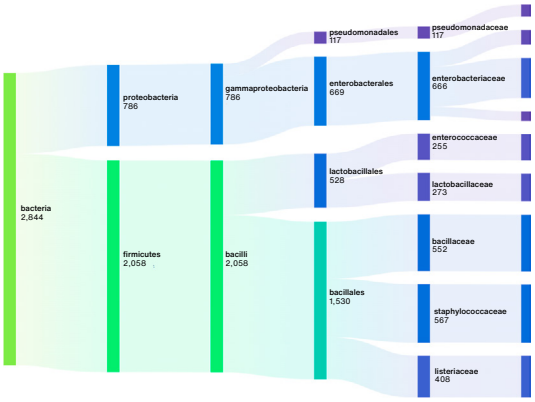
ANALYSIS:
identifying species rapidly

View the dedicated 16S analysis pipeline:
labs.epi2me.io/workflows/wf-16s

EPI2ME offers multiple workflows to analyse nanopore sequencing data, accessed through an easy-to-use graphical interface or the command line. Both options can be run on either local compute or in the cloud.



The **wf-16s** pipeline is designed to analyse 16S sequencing data for species-level identification. There are two options available with this workflow: a real-time option for rapid results and a post-run analysis option for high-accuracy results. As part of the workflow, an abundance table with counts per taxa in all samples is generated, along with a bar plot comparing abundances, and interactive Sankey and sunburst plots to explore lineages[2].



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

Find out more at:
nanoporetech.com/applications/techniques/targeted-sequencing

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References:

1. Zhang, T. et al. *Appl. Environ. Microbiol.* 89(10):e00605-23 (2023). DOI: https://doi.org/10.1128/aem.00605-23
2. GitHub. wf-16s. Available at: https://github.com/epi2me-labs/wf-16s [Accessed 29 May 2024]

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