Rapid identification of respiratory pathogens with Oxford Nanopore metagenomics

Metagenomic sequencing is a key technique for the identification of potential pathogens without the need for prior knowledge of microbial sample composition, providing important insights for outbreak surveillance to inform public health measures. However, the use of legacy short-read technologies can limit the confident identification of microbes, especially when distinguishing between closely related strains. Furthermore, legacy techniques require sample batching, which can result in lengthy turnaround times.

Oxford Nanopore technology can sequence any fragment length, enabling thorough characterisation of mixed microbial samples. Long nanopore reads can more reliably identify antimicrobial gene context (plasmid-borne or chromosomal) than short-read sequencing, providing information that can inform control and intervention measures. The scalable technology — with devices ranging from portable to benchtop, and no need to batch samples — ensures rapid access to results where and when needed.

The rapid metagenomic nanopore sequencing workflow provides fast identification of bacterial, fungal, and viral pathogens from a single sample. With streamlined, multiplexed library preparation, real-time sequencing, and intuitive data analysis requiring no prior experience, this sample-to-answer workflow delivers rapid access to data that has the potential to be critical for outbreak control.

This workflow overview introduces how to rapidly identify bacterial, fungal, and viral pathogens from respiratory samples using metagenomic sequencing on a MinION™ or GridION™.

Sample and library prep:
preparing mixed microbial samples for nanopore sequencing

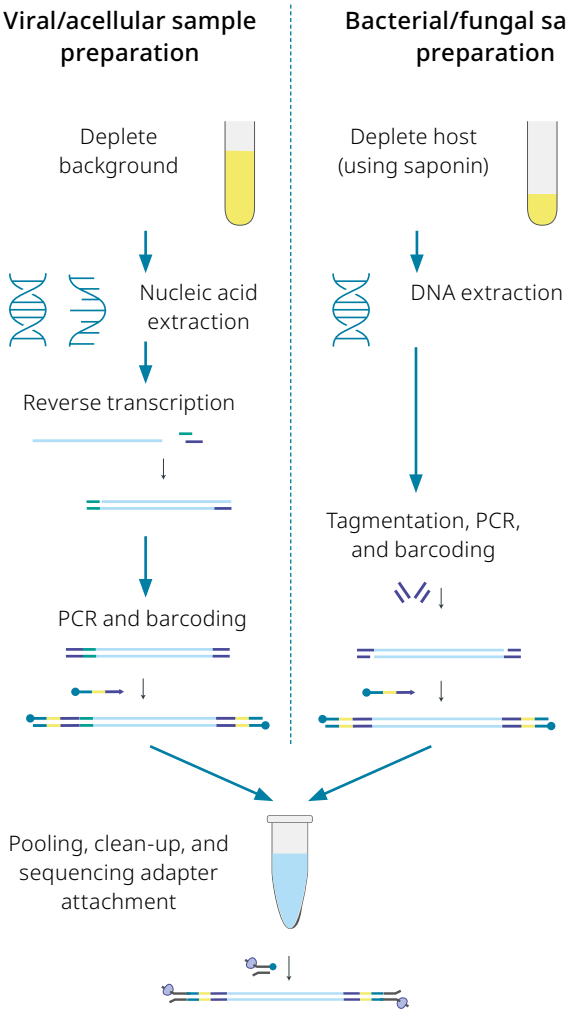
View the protocol, featuring detailed sample information:
nanoporetech.com/rapid-metagenomic-sequencing

Sample preparation begins with depletion of host material from the respiratory samples. Early in the process, the samples are centrifuged and split: some supernatant is taken forward for viral/acellular sample preparation, while the remaining supernatant and pellet can be processed for bacterial and fungal sample preparation, provided that sufficient material is available. Next, the depletion steps are completed side by side for the split samples.

For the viral/acellular sample arm of the workflow, both DNA and RNA are extracted. The RNA is then reverse transcribed using a method derived from that established by Claro *et al.*[1] For the bacterial and fungal sample arm, DNA is extracted, then 'tagmented' — fragmented and tagged with PCR adapters in a single ten-minute step — using the Oxford Nanopore **Rapid PCR Barcoding Kit 24**.

At this point, all samples are separately amplified with barcoded primers, using a different barcode for every sample. Following PCR, all samples are pooled and the single pooled sample is prepared for nanopore sequencing via the rapid attachment of sequencing adapters.

Excluding the two recommended negative controls, this method enables up to 22 individual-arm or 11 dual-arm samples to be sequenced in multiplex. The process takes around six hours from extraction to sequencing-ready metagenomic library.



Sequencing:
scalable sequencing in the lab or field

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

Multiplexed sequencing on a single MinION Flow Cell generates sufficient data for up to 22 barcoded samples (and two controls) in one sequencing run.

MinION Flow Cells are compatible with the compact **MinION** sequencer, for portable sequencing anywhere — in the lab, or at the point of sampling. For higher throughput needs, the flexible, benchtop **GridION** delivers sequencing on up to five independent MinION Flow Cells, allowing rapid processing of larger sample batches or usage by multiple users for different projects.

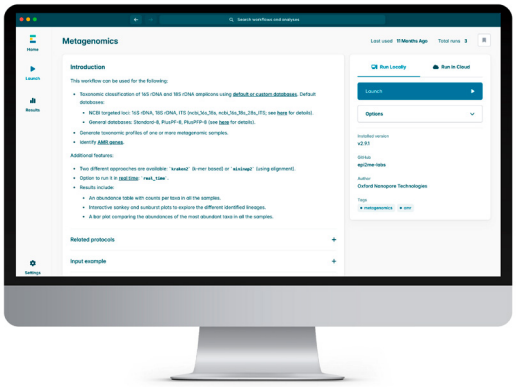
We recommend performing real-time basecalling using the high accuracy (HAC) basecalling mode to optimise for rapid turnaround time and simplest data analysis.

Analysis:
intuitive data analysis with EPI2ME

Learn more about EPI2ME:
nanoporetech.com/epi2me

The metagenomic nanopore sequencing data can be easily analysed using the intuitive **EPI2ME™** platform, providing data analysis workflows for all levels of experience. From a FASTQ or BAM file input and a CSV sample sheet, the EPI2ME workflow **wf-metagenomics** outputs an interactive HTML report providing identification and abundance information for the taxa present in each microbial sample. The all-in-one workflow delivers accurate identification of viral, bacterial, fungal, archaeal, protozoal, and human sequences.

The workflow can be accessed locally or in the cloud; those with prior bioinformatics experience can also choose to run it via the command-line.



Learn more about infectious disease research with Oxford Nanopore:
nanoporetech.com/infectious-disease

View the end-to-end protocol: nanoporetech.com/rapid-metagenomic-sequencing

Oxford Nanopore Technologies

oxford-nanopore-technologies
@nanopore
@nanoporetech.com
www.nanoporetech.com

References:

1. Claro, I.M. et al. Rapid viral metagenomics using SMART-9N amplification and nanopore sequencing. *Wellcome Open Res.* 6:241 (2023). DOI: https://doi.org/10.12688/wellcomeopenres.17170.2

WF_1288(EN)_V1_14Mar2025