Oxford Nanopore Technologies

Microbial sequencing with Oxford Nanopore

Introduction

Microorganisms are the most abundant life forms on Earth, with over 1 trillion species estimated[1]. They are fundamental to the maintenance of all ecosystems and play crucial roles in health, disease, and many environmental, industrial, and pharmaceutical processes.

Full characterisation of microbial genomes is advancing numerous fields of research. Sequencing of the human microbiome is shedding light on its role in health and disease, whilst sequencing of the pathogens behind outbreaks is improving our understanding of their origins, transmission, antimicrobial resistance (AMR) profiles, and virulence factors. In agriculture, timely detection of animal and plant pathogens is of great importance to animal welfare and food production.

The rapid and significant decrease in sequencing costs means that next-generation sequencing has emerged as a critical tool for microbiologists. Although advances have been enabled by short-read sequencing, approximately 93% of bacterial genomes in GenBank remain incomplete[2]. Complex genomic regions, such as repetitive sequences and structural variants (SVs), are inaccessible to short-read sequencing technologies, leading to incomplete assembly of microbial genomes.

High-accuracy Oxford Nanopore sequencing overcomes the limitations of legacy technologies to characterise complete, contiguous, high-quality microbial genomes (Table 1). Our technology generates reads of any length, from short to ultra long (20 bp to >4 Mb). Long Oxford Nanopore reads enhance *de novo* genome assembly (Figure 1), enabling more accurate analysis of known and novel microbes, precise differentiation of closely related microbes, and complete plasmid reconstruction.

Table 1. Advantages of Oxford Nanopore technology for microbial sequencing

Complete genome assembly
Generate high-quality microbial genome assemblies and resolve plasmids and mobile elements with long, accurate nanopore reads

Thorough AMR characterisation
Identify AMR genes and their locations within mobile genetic elements or chromosomes, allowing monitoring and understanding of transmission

Structural variant and repeat resolution
Span entire SVs and repeat segments — characteristic of AMR genes — in single long reads

Accurate microbe characterisation
Differentiate between closely related organisms and assemble genomes from complex metagenomic samples

Native DNA sequencing
Detect modifications, avoid GC bias, and access genomic regions which cannot be amplified

End-to-end workflows
Access simple workflows, from extraction to data analysis, with bioinformatics solutions for all levels of expertise

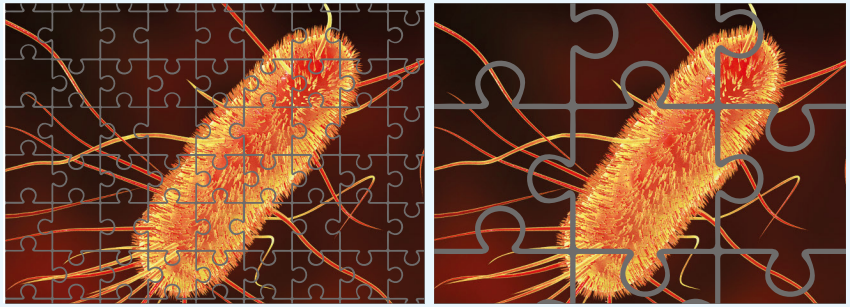
Cost effective and scalable
Scale to suit your project size and experimental goals with nanopore sequencing solutions that offer flexible throughput and sample batching

Rapid turnaround times
Rapidly characterise microbes for outbreak surveillance using fast workflows and real-time data analysis

Sequencing at the sample source
Characterise microbes in the lab or in the field with portable MinION™ devices

Figure 1. Long Oxford Nanopore reads simplify genome assembly

Like a jigsaw with large pieces, long reads are much easier to assemble than short reads. The *Escherichia coli* genome comprises 4.6 million bases, which would equate to 92,000 fragments of 50 bp or just nine fragments of 500,000 bp in length.



50 bp reads
92,000 'pieces'

500,000 bp reads
9 'pieces'

1. Lennon, J.T. and Locey, K.J. More support for Earth's massive microbiome. *Biol. Direct* 15(1):5 (2020). DOI: https://doi.org/10.1186/s13062-020-00261-8

2. Amoutzias, G.D. et al. The notable achievements and the prospects of bacterial pathogen genomics. *Microorganisms* 10(5):1040 (2022). DOI: https://doi.org/10.3390/microorganisms10051040

Long Oxford Nanopore reads can span large genomic regions such as insertion sequences and transposable elements, enhancing AMR gene and virulence factor analysis. Similarly, long reads resolve repeat sequences, revealing their location and structure; repeats are characteristic of plasmids, for example, which can be sequenced in single nanopore reads.

Our technology offers solutions across a range of microbial sequencing techniques. The end-to-end nanopore-only microbial isolate sequencing solution (NO-MISS) delivers high-quality, complete, whole-genome assemblies from microbial isolates, spanning bacteria and fungi. Comprehensive, best-practice data analysis is delivered through a dedicated EPI2ME™ pipeline, supporting *de novo* or reference-based genome assembly, annotation of regions of interest, species and plasmid identification, sequence typing, and AMR profiling. The same EPI2ME pipeline powers the end-to-end *Salmonella* serotyping workflow.

Long, highly accurate nanopore reads also greatly enhance metagenomic analysis of mixed microbial samples. Oxford Nanopore offers a dedicated metagenomic workflow for rapid identification of bacterial, fungal, and viral pathogens from respiratory samples. Furthermore, long nanopore reads enable the generation of high-quality metagenome-assembled genomes (MAGs) and distinction between very similar microbial genomes.

We also provide efficient, sample-to-answer workflows for targeted approaches. Full-length 16S ribosomal RNA (rRNA) gene sequencing provides greater taxonomic resolution than short-read approaches that cannot span the full gene, enabling genus or species-level bacterial identification. The same approach as used for 16S characterisation also identifies fungi through full-length internal transcribed spacer (ITS) region sequencing. Workflows are available for the rapid sequencing of viruses, including influenza A/B and SARS-CoV-2, providing timely data for pathogen surveillance and to inform vaccine and public health programmes. Meanwhile, our end-to-end plasmid validation workflow equips you to conduct rapid analysis of full plasmid sequences securely in your own lab.

With a range of nanopore sequencing devices providing flexibility and scalability for your experimental goals, our technology delivers high-resolution data to enhance your microbial research. From sequencing at the sample source — even in extreme, remote environments — using the portable MinION, to flexible sequencing on the benchtop GridION™, to high- and ultra-high-output sequencing using the PromethION™ range, all your microbial sequencing needs can be met (**page 8**).

With streamlined Oxford Nanopore workflows and real-time analysis, microbial sequencing data can be rapidly obtained in-house and analysed as soon as sequencing starts.

View Oxford Nanopore sequencing devices: nanoporetech.com/sequence

No microbe left behind

Complete bacterial genome assemblies

Oxford Nanopore sequencing generates unrestricted read lengths, which is one of the most impactful benefits of our technology, enabling complete microbial genomes and plasmids to be resolved. Conversely, short-read legacy technologies generate fragmented and incomplete bacterial genome assemblies as the short reads often cannot span repetitive regions and SVs — common features in bacterial genomes and plasmids (**Figure 2**)[3].

Long Oxford Nanopore reads resolve repetitive regions and SVs, allowing high-quality bacterial genome assembly — including of metagenome-assembled genomes (MAGs) from mixed microbial samples (**Figure 3**)[4]. From this, AMR genes, virulence factors, phages, and mobile genetic elements such as plasmids can be thoroughly characterised. Determining the presence of AMR and virulence genes together with their locations within chromosomes or mobile genetic elements is important in investigating outbreaks and understanding drivers of resistance.

Profiling microbial communities

Oxford Nanopore sequencing provides high taxonomic resolution of microbial communities, supporting accurate species identification. A single long read can span the entire 16S rRNA gene. In contrast, legacy technologies limit identification to genus-level resolution as the short reads cannot span the entire gene — instead, sequencing partial fragments of the variable regions.

From extraction and library preparation to data analysis with EPI2ME, the Oxford Nanopore microbial amplicon barcoding workflow offers an end-to-end approach for full-length 16S and ITS sequencing. EPI2ME generates interactive graphics for visualisation of taxonomy, diversity, and relative abundance for microbiome characterisation, food safety surveillance, pathogen detection, and environmental monitoring. Researchers have shown 16S nanopore sequencing to deliver superior resolution of unidentifiable bacterial pathogens in a shorter turnaround time when compared with Sanger sequencing[5], and identification up to the species level[6], outperforming that of short-read sequencing[7].

Figure 2. Oxford Nanopore sequencing generates complete genome assemblies, whereas short-read technologies typically generate fragmented bacterial genome assemblies[3]

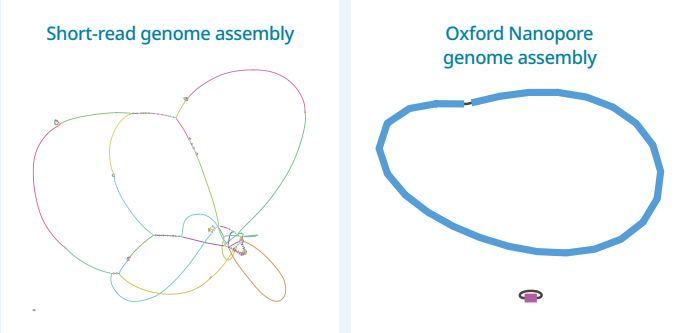
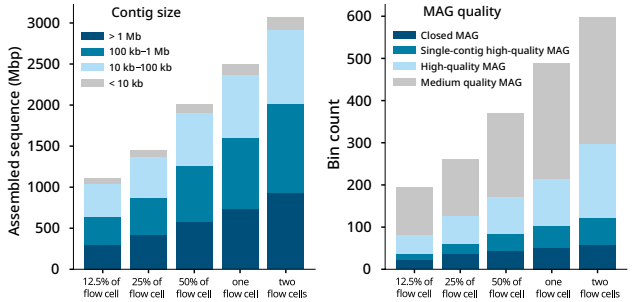


Figure 3. Metagenomic sequencing of a mixed microbial sample on PromethION delivers long contigs (left) and MAGs (right)[4]



3. Oxford Nanopore Technologies. Oxford Nanopore whole-genome sequencing of foodborne pathogens. Available at: https://nanoporetech.com/resource-centre/nanopore-whole-genome-sequencing-of-foodborne-pathogens [Accessed 03 February 2026]

4. Oxford Nanopore Technologies. Oxford Nanopore sequencing provides superior metagenome-assembled genome recovery and strain-level resolution from a complex microbiome. Available at: https://nanoporetech.com/resource-centre/oxford-nanopore-sequencing-provides-superior-metagenome-assembled-genome-recovery-and-strain-level-resolution-from-a-complex-microbiome [Accessed 04 February 2026]

5. Campodónico, V.L. et al. Evaluation of long-read 16S rRNA next-generation sequencing for identification of bacterial isolates in a clinical diagnostic laboratory. *J. Clin. Microbiol.* 63(5):e0167024 (2025). DOI: https://doi.org/10.1128/jcm.01670-24

6. Zhang, T. et al. The newest Oxford Nanopore R10.4.1. full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling. *Appl. Environ. Microbiol.* 89(10):e00605-23 (2023). DOI: https://doi.org/10.1128/aem.00605-23

7. Gupta, A., Cooper, V.S., and Zemke, A.C. Evaluation of V3-V4 and FL-16S rRNA amplicon sequencing approach for microbiota community analysis of tracheostomy aspirates. *mSphere* 10(8):e0038825 (2025). DOI: https://doi.org/10.1128/msphere.00388-25

Which approach do I choose for known microbial samples?

START HERE

What do you want to investigate?

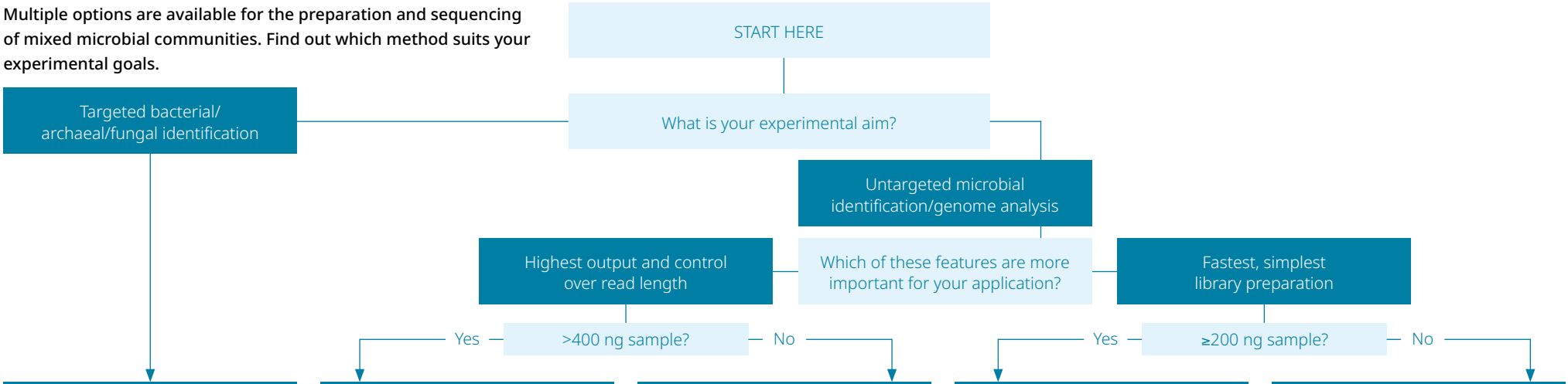
	Influenza genome	*Salmonella* serotyping	Microbial isolate genome	SARS-CoV-2 genome	Amplicon target
End-to-end protocol	Influenza virus sequencing	Direct-from-colony rapid microbial sequencing for *Salmonella* serotyping	Nanopore-only isolate sequencing solution (NO-MISS)	SARS-CoV-2 virus PCR tiling and sequencing	Amplicon sequencing
Library prep kit	Native Barcoding Kit	Rapid PCR Barcoding Kit	Rapid Barcoding Kit	Rapid Barcoding Kit	Rapid Barcoding Kit
Input required per sample	1–2 µl RNA	Single colony of *Salmonella* 2–3 mm in diameter	200 ng extracted gDNA	8 µl RNA	50 ng extracted gDNA
Total number of samples	Up to 24 or 96	Up to 24	Up to 24 or 96*	Up to 24 or 96	Up to 24 or 96
Library prep time	~2 hours + PCR	1 hour + PCR	~25–60 minutes	~5 hours	~60 minutes
EPI2ME analysis workflow	wf-flu	wf-bacterial-genomes	wf-bacterial-genomes		wf-165 or wf-amplicon

View Oxford Nanopore end-to-end sequencing workflows:
nanoporetech.com/application-workflows

* The sample number can be increased or decreased depending on flow cell type chosen and/or the desired application, such as serotyping, AMR gene analysis, and genus or species-level identification.

Which library prep kit do I choose for mixed microbial communities?

Multiple options are available for the preparation and sequencing of mixed microbial communities. Find out which method suits your experimental goals.



Microbial Amplicon Barcoding Kit Targeted sequencing	
Preparation time	~25 minutes + PCR
Input required per sample	10 ng gDNA
Read length	Full-length 16S gene (~1.5 kb) or full-length ITS region (variable)
Total number of samples	Up to 24
End-to-end workflows	Microbial amplicon barcoding for 16S and ITS

Native Barcoding Kit Output and native data optimised	
Preparation time	~140 minutes
Input required per sample	400–1,000 ng gDNA
Read length	Equal to fragment length
Total number of samples	Up to 24 or 96

Ligation Sequencing Kit + PCR Barcoding Expansion Low input optimised	
Preparation time	~140 minutes + PCR
Input required per sample	35–100 fmol or up to 300 ng depending on input type
Read length	Equal to fragment length post-PCR
Total number of samples	Up to 12 or 96

Rapid Barcoding Kit Speed and native data optimised	
Preparation time	~60 minutes
Input required per sample	200 ng gDNA
Read length	Random distribution
Total number of samples	Up to 24 or 96

Rapid PCR Barcoding Kit Low input and speed optimised	
Preparation time	~15 minutes + PCR
Input required per sample	1–5 ng gDNA
Read length	~2 kb
Total number of samples	Up to 24
End-to-end workflows	Rapid metagenomic sequencing for surveillance of bacterial, fungal, and viral pathogens

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

From sample to answer

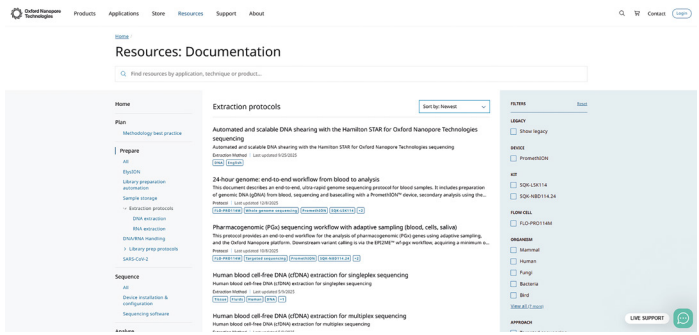
Extraction

How do I extract high-quality DNA from my samples?

The Documentation section of the Oxford Nanopore website features recommended DNA extraction protocols for a wide range of sample types, including cell culture, fluids, tissue, and environmental samples. In this section, you will also find information sheets covering effective sample storage, DNA and RNA handling, size selection, and more.

View extraction protocols:
nanoporetech.com/extraction-methods

Extraction protocols



Library prep

How can I sequence multiple samples at once?

Sample multiplexing improves the cost effectiveness of sequencing, but will also reduce depth of coverage per genome, so the rarity of microbes, sample complexity, and presence of host DNA should be considered prior to choosing this approach.

Barcoding options are available for ligation-based and rapid library preparations, for both PCR-based and PCR-free protocols.

Ligation

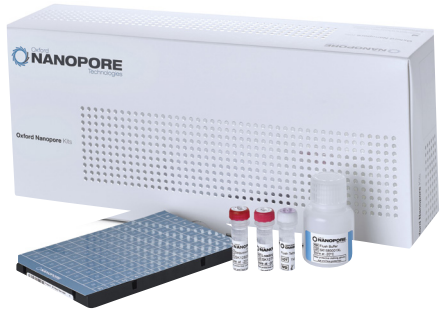
PCR-free:	Native Barcoding Kit (up to 24 or 96 samples)
PCR-based:	Ligation Sequencing Kit + PCR Barcoding Expansion (up to 12 or 96 samples)

Rapid

PCR-free:	Rapid Barcoding Kit (up to 24 or 96 samples)
PCR-based:	Rapid PCR Barcoding Kit (up to 24 samples)

For 16S or ITS sequencing, up to 96 samples can be prepared.

Library preparation kit



Find out more about nanopore library prep kits:
nanoporetech.com/prepare

From sample to answer

Library prep

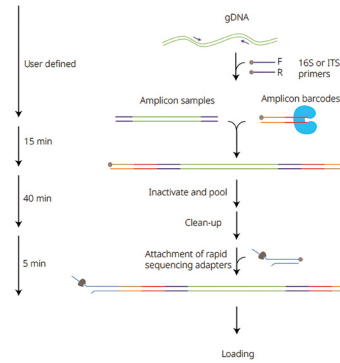
How do I sequence the 16S rRNA gene or ITS region?

Nanopore sequencing characterises full-length 16S and ITS amplicons. Using the Microbial Amplicon Barcoding Kit, up to 24 samples can be prepared for multiplexed sequencing on a single flow cell. As only the 16S or ITS region is amplified per sample, this targeted workflow provides a rapid and cost-effective method of identifying bacteria, archaea, and fungi.

The end-to-end workflow spans from extraction recommendations to downstream analysis with the EPI2ME wf-16S workflow, which provides taxonomic classification from your 16S and ITS amplicons to the genus or species level (**page 10**).

Find out more about the microbial amplicon barcoding workflow:
nanoporetech.com/mab-workflow

Microbial Amplicon Barcoding Kit workflow



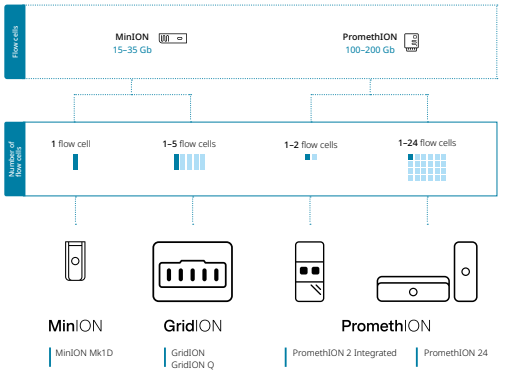
Sequencing

Which device should I choose?

The chemistry underpinning nanopore sequencing can be scaled up or down; our devices make full use of this. The portable MinION is ideal for sequencing at sample source, even in the most extreme environments, where it can be operated from a contemporary laptop. To scale up, the flexible GridION provides benchtop sequencing on up to five MinION Flow Cells. Meanwhile, the ISO9001-qualified GridION Q provides a fixed configuration and standardised format, enabling the implementation of routine, long-term workflows for in-house validated assays. For high- to ultra-high-output requirements, such as genome assembly of metagenomic samples, the PromethION range can accommodate up to two (PromethION 2 Integrated) or 24 (PromethION 24) PromethION Flow Cells.

To maximise sequencing output, the Flow Cell Wash Kit can be used to remove >99.9% of a sequenced library, leaving the flow cell ready for either sequencing of a fresh library or for storage. This is useful in situations when the sufficient depth of coverage has been met early in a sequencing run: the run can be stopped when target coverage is met and the flow cell washed and reloaded with a new sequencing library.

One technology — any scale



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

From sample to answer

Sequencing

How much data do I need?

The recommended sequencing data yield depends on your experimental aims and the sample being analysed (e.g. rarity of microbe, complexity of sample, presence of host DNA).

For metagenomic sequencing, we suggest the following as rough guidelines for sequencing depth of coverage per microbe:

- Confirm presence of organism of interest: 10x
- Species-level identification: 20x
- AMR gene analysis: 20x
- Assembly: 40x
- Variant calling: 50x

For single microbial genomes, we suggest the following as guidelines for sequencing depth of coverage per microbe:

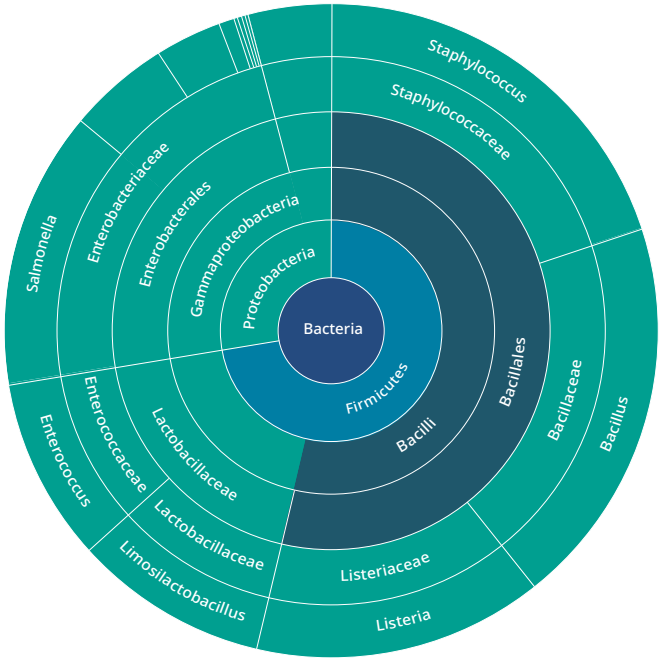
- Isolate genome assembly and annotation: 50x
- *Salmonella* serotyping: 10x
- Influenza A and B strain typing: 30x
- *De novo* plasmid assembly: 30x
- Methylation detection in bacterial genomes: 10x

Microbial identification with 16S versus whole-genome sequencing

For 20x depth of coverage of the ~1.5 kb 16S locus, 30 kb of sequencing data is required per microbe. Therefore, bacteria/archaea with an abundance of ≥0.003% can be detected with 1 Gb of data.

For 20x depth of coverage of a 3 Mb genome, 60 Mb of sequencing data is needed. From 1 Gb of data, microbes with ≥6% abundance can therefore be identified. Sensitivity can be increased or decreased by altering the amount of data obtained accordingly. Sample multiplexing will decrease sensitivity but increase cost effectiveness.

Example 16S data generated using the EPI2ME platform showing taxonomic composition of a sample



From sample to answer

Data analysis

How can I analyse my data?

We provide a range of solutions for analysing sequencing data for metagenomic and single microbial genomes, covering all levels of bioinformatics expertise. Scientists without bioinformatics experience can easily access the EPI2ME desktop application for cloud-based or local analysis, which offers intuitive point-and-click analysis and reports, as well as real-time analysis for selected workflows for quick turnaround times. Best-practice workflows and detailed documentation are also available to support the analysis of nanopore sequencing data.

EPI2ME data analysis pipelines are also available as open-access, open-source Nextflow workflows via GitHub and can be run from the command line. In addition, custom-developed Nextflow workflows can be integrated into the EPI2ME desktop application, enabling sharing and collaboration.

Pre-packaged with a wide range of open-source workflows in EPI2ME and paired with our extraction and library preparation protocols, we provide an end-to-end solution for sequencing microbial genomes, from sample to data analysis.

EPI2ME is compatible with Windows, macOS, and Linux. The application can be run from your computer, cluster or cloud service, PromethION, or GridION to best suit your lab set-up.

Find the full range of available EPI2ME workflows at:
nanoporetech.com/epi2me

Multiple EPI2ME tools are available for microbial analysis

Workflow	Description
wf-bacterial-genomes	Performs assembly, calls variants, and annotates bacterial genomes.
wf-clone-validation	Performs *de novo* assembly of plasmid sequences.
wf-metagenomics	Performs taxonomic classification of single reads from amplicon-targeted and shotgun metagenomics.
wf-16S	Classifies 16S/18S/ITS amplicons.
wf-flu	Performs influenza A and B typing.

Find out more about nanopore sequencing data analysis:
nanoporetech.com/analyse

Case studies

Case study 1: identifying urinary tract infection pathogens and AMR in hours, not days

Urinary tract infections (UTIs) are the second most common form of infection globally[8]. Rapid identification of the pathogens underlying these infections and any AMR is essential in ensuring effective treatment and preventing serious complications such as sepsis. However, the culture-based methods used to routinely diagnose UTIs take ~2–4 days to return answers. In this time, clinicians may prescribe ineffective antibiotics, which fail to treat the infection and further add to the growing threat of resistance. Also, difficult-to-culture pathogens can be missed entirely.

Now, scientists are investigating the potential of metagenomic Oxford Nanopore sequencing to deliver this data without the wait. Bellankimath and Branders *et al*. used this culture-free approach to analyse 78 urine clinical research samples from UTI cases where the individuals had an increased risk of complications[9]. The team selected an optimised, in-house sample preparation technique to extract DNA and deplete host material, then prepared libraries using the Oxford Nanopore Rapid Barcoding Kit. They sequenced the metagenomic samples on MinION Flow Cells and analysed the incoming data in real time.

With a limit of detection of 103 CFU/ml and a bacteria-to-host-cell ratio of 0.5, the researchers identified the pathogens underlying both mono- and polymicrobial infections with 99% accuracy when compared to routine microbiology results. Plus, they found 13 pathogens that were missed by culture-based testing. They also obtained both antibiotic susceptibility predictions, with 90% concordance with existing tests, and virulence gene information. The authors noted that the method is also up to 30% more cost effective than published studies and commercial kits.

Crucially, from receiving the sample to analysing the data, all of this was possible in just four hours. While current diagnostic methods leave patients waiting for days, the authors highlighted that this rapid, target-agnostic approach 'could prevent the unnecessary use of 1.62 billion daily doses … of empirical broad-spectrum antibiotics each year before initiating personalised, targeted therapy against an identified pathogen'.

Read the publication (December 2025):
nanoporetech.com/metagenomic-sequencing-UTIs

Case study 2: assembling complete genomes from bacterial isolates with a single platform

Access to complete bacterial genome and plasmid sequences is key in both outbreak surveillance and clinical settings, unlocking insights into AMR and its spread via transmission vectors. While short-read sequencing can struggle to assemble these sequences, especially repetitive regions commonly found in mobile genetic elements, long reads can span them to produce highly complete assemblies. In the past, researchers have used hybrid assembly approaches that combine long and short reads, but this requires two platforms and adds costs and complexity. With our technology, scientists are now demonstrating why this is no longer necessary.

Nagy *et al*. compared Oxford Nanopore-only bacterial assembly with hybrid approaches for clinically relevant *Enterobacterales* isolates from a national surveillance program in England[10]. The group extracted DNA from the samples and prepared libraries using the Rapid Barcoding Kit, before sequencing them in multiplex on a PromethION Flow Cell. They also sequenced the samples using Illumina short-read technology. They then used a range of tools to generate both nanopore-only assemblies and hybrid assemblies combining Oxford Nanopore and short-read Illumina data, as well as polishing with reads from either platform.

The scientists found that Autocycler[11]-generated assemblies using only Oxford Nanopore reads circularised 95% of chromosomes — the highest of the methods they tested. Polishing their assemblies with the tool Medaka[12] and un-subsampled nanopore reads achieved accuracies comparable to those of the hybrid approaches tested, without the need for a second platform. Plasmid sequence reconstruction was consistent for all but one tool, while multilocus sequence typing (MLST) and AMR, virulence, and stress gene annotation was equivalent across the techniques.

The researchers concluded that their high-throughput, Oxford Nanopore-only workflow produced bacterial assemblies with similar accuracy and possibly higher completeness than the hybrid assemblies. As well as this, they emphasised that Oxford Nanopore technology offered 'cost effectiveness, real-time data generation and decentralised implementation' to enhance outbreak detection.

Read the publication (September 2025):
nanoporetech.com/enterobacterales-assembly

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10. Nagy, D. et al. Nanopore long-read only genome assembly of clinical Enterobacterales isolates is complete and accurate. *bioRxiv* 676237 (2025). DOI: https://doi.org/10.1101/2025.09.15.676237

11. Wick, R.R., Howden, B.P., and Stinear, T.P. Autocycler: long-read consensus assembly for bacterial genomes. *Bioinformatics* 41(9):btaf474 (2025). DOI: https://doi.org/10.1093/bioinformatics/btaf474

12. Github. Medaka. Available at: https://github.com/nanoporetech/medaka [Accessed 05 February 2026]

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Find out more at
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