Our goal is to enable the analysis of any living thing, by anyone, anywhere.

Nanopore DNA and direct RNA sequencing has been performed on board the International Space Station. Image credit: NASA's Johnson Space Center.
Nanopore sequencing — how it works

Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of DNA or RNA fragments of any length. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore. The resulting signal is decoded to provide the specific DNA or RNA sequence.

The nanopore processes the length of DNA or RNA presented to it. The user can control fragment length through the library preparation protocol utilised, allowing the generation of any desired read length — from short to ultra-long (e.g. >2 Mb DNA\(^1\) and >20 kb RNA\(^2\)).

An enzyme motor controls the translocation of the DNA or RNA strand through the nanopore. Once the DNA or RNA has passed through, the motor protein detaches and the nanopore is ready to accept the next fragment.

A library preparation results in the addition of a sequencing adapter and motor protein at each end of the fragment.

Both the template and complement strands carry the motor protein which means both strands are able to translocate the nanopore.

DNA or RNA fragments pass through a nano-scale hole. The fluctuations in current during translocation are used to determine the DNA or RNA sequence (see page 30).

An electrically resistive membrane ensures all current must pass through the nanopore, ensuring a clean signal.

Unrestricted read length — short to ultra-long (longest 2.3 Mb) • Ultimate flexibility — optimise for your application • Easier genome assembly • Resolve structural variants, repeats, and phasing • Characterise and quantify full-length transcripts

Real-time analysis • Immediate access to actionable results • Rapid species identification • Early sample insights and QC • Enough data? Stop, wash, store, or run another sample

Direct sequencing • Sequence native DNA or RNA, not a copy • Eliminate amplification bias • Identify base modifications

Scalable — portable to ultra-high throughput • One technology across all devices — scale to your needs • Sequence at sample source with Flongle™ and MinION™ • Compact, high-throughput benchtop sequencing with GridION™ and PromethION™

Streamlined library prep • Rapid 10-minute (DNA) library prep • Automated, portable prep — VolTRAX™ • High DNA and RNA yields from low input amounts • Maximise throughput with barcoding

On-demand sequencing • Sequence what you need when you need it • No sample batching required • Flexible throughput with modular GridION and PromethION

Discover more at nanoporetech.com

Discover the benefits of nanopore technology

Using the MinION in Antarctica. Image courtesy of Dr. Sarah Stewart Johnson, Georgetown University.

Generate new biological insights

Whole genome sequencing
- De novo assembly
- Scaffolding and finishing
- Variant analysis: structural variation, SNVs, phasing, base modifications
- Resequencing

Targeted sequencing
- Amplicon and PCR-free enrichment
- 16S rRNA analysis
- Variant analysis: structural variation, SNVs, phasing, base modifications

RNA sequencing
- Direct RNA, direct cDNA, and cDNA
- Characterise and quantify full-length transcripts
- Identify splice variants and gene fusions
- Sequence complete viral genomes
- Detect base modifications

Metagenomics
- Real-time, unbiased analysis of mixed samples
- Enhanced species identification using long reads

Epigenetics
- Methylation
- Histone modification
- Non-coding RNA activity

Clinical research
Cancer research
Transcriptome analysis
Environmental research
Animal research
Plant research
Basic genome research
Human genetics
Microbiology
Microbiome
A complete and streamlined workflow – real-time answers to biological questions

### PREPARE
- Library preparation kits
- Flongie
- MinION
- MinION Max
- GridION
- PromethION
- VoLTRAX

### SEQUENCE
- Compatible with Flongie

### ANALYSE
- Bioinformatic capability needed
  - **EPI2ME™**
    - Use the cloud-based or local EPI2ME platform for real-time analysis workflows.
    - nanoporetech.com/analyse
  - **EPI2ME Labs**
    - Explore your data and develop your bioinformatics skills with interactive, best practice workflows and tutorials.
    - nanoporetech.com/analyse
  - **Community-developed tools**
    - Run open-source tools written and developed by the Nanopore Community.
    - community.nanoporetech.com
  - **Custom analysis pipelines**
    - All the data, raw or basecalled, can be used in custom analysis pipelines written by the user for specific applications.
DNA library preparation

For maximum throughput

Ligation Sequencing Kit

- DNA ends are repaired and dA-tailed
- Sequencing adapters are ligated onto the prepared ends
- Fragment lengths can be controlled by fragmentation or size selection

- For maximum throughput
  - Ligation Sequencing Kit

- For minimal preparation time
  - Rapid Sequencing Kit with transposase

- For minimal preparation time
  - Rapid Sequencing Kit with transposase

Which DNA kit?

Read any length of DNA — from short to ultra-long. Simplify genome assembly, variant detection, phasing, and metagenomic species identification with ultra-long reads. Use direct, PCR-free approaches to analyse native DNA and detect modified bases.

<table>
<thead>
<tr>
<th>Use for...</th>
<th>Ligation (SQK-LSK110)</th>
<th>Rapid (SQK-RAD004-4)</th>
<th>PCR (SQK-PSK004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prep time</td>
<td>60 mins</td>
<td>10 mins</td>
<td>PCR + 60 mins</td>
</tr>
<tr>
<td>Input amount</td>
<td>1,000 ng dsDNA</td>
<td>400 ng HMW gDNA (&gt;30 kb)</td>
<td>100 ng dsDNA</td>
</tr>
<tr>
<td>Fragmentation</td>
<td>Optional</td>
<td>Transposase based</td>
<td>N/A</td>
</tr>
<tr>
<td>Read length</td>
<td>Equal to fragment length</td>
<td>Random distribution, dependent on input fragment length</td>
<td>Equal to fragment length post-PCR</td>
</tr>
<tr>
<td>PCR required</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Multiplexing options</td>
<td>Native Barcoding (PCR free)*; PCR Barcoding Expansion pack</td>
<td>Use Rapid Barcoding Kit</td>
<td>Use PCR Barcoding Kit</td>
</tr>
</tbody>
</table>

Also available:

- Cas9 Sequencing Kit — streamlined, PCR-free enrichment of long targeted regions with maintenance of base modifications
- Field Sequencing Kit — get all the benefits of rapid sequencing with the added convenience of ambient shipping and storage
- Application-specific library preparation kits (e.g. 16S sequencing)
- Ligation Sequencing Kit XL — plate-based ligation sequencing kit for high-throughput workflows
- Automatable workflows

* Currently available for SQK-LSK109; coming soon for SQK-LSK110.

PREPARE

12 13
Which RNA kit?

Characterise and quantify full-length RNA transcripts, splice variants, and fusions using long nanopore sequencing reads. Sequence native RNA directly, without amplification or reverse transcription, and identify base modifications.

**Direct RNA Sequencing Kit**

- **Use for:** Sequence RNA molecules directly and preserve base modifications
- **Prep time:** 105 mins
- **Input recommendation:** 500 ng RNA (poly-A+)
- **Read length:** Equal to RNA length
- **PCR required:** No
- **Reverse transcription:** Optional
- **Multiplexing options:** In development
- **Sequencing adapters:** Attached to prepared ends
- **Read length:** Reflects length of molecules in sample

**PCR-cDNA Sequencing Kit**

- **Use for:** Full-length transcripts with high throughput
- **Prep time:** 165 mins
- **Input recommendation:** 1 ng RNA (poly-A+)
- **Read length:** Enriched for full-length cDNA
- **PCR required:** Yes
- **Reverse transcription:** Yes
- **Multiplexing options:** PCR-cDNA Barcoding Kit
- **Sequencing adapters:** Attached to amplified cDNA

**Direct cDNA Sequencing Kit**

- **Use for:** Full-length transcripts without PCR bias
- **Prep time:** 275 mins
- **Input recommendation:** 100 ng RNA (poly-A+)
- **Read length:** Enriched for full-length cDNA
- **PCR required:** No
- **Reverse transcription:** Yes
- **Multiplexing options:** Native Barcoding Expansion pack
- **Sequencing adapters:** Attached to amplified cDNA
Cost-effective analysis of multiple samples

Barcoding

Barcoding kits allow users to multiplex samples to generate maximum data from a single flow cell, to separate the reads from sequential library loadings, and to lower the cost per sample.

- Native Barcoding Kit for a PCR-free approach (up to 96 samples)
- PCR Barcoding Kits (up to 96 samples)
- Native and PCR barcoding can be combined to increase multiplexing capabilities to thousands of samples
- Barcode libraries of gDNA, amplicon, or cDNA either with a dedicated barcoding kit or a barcoding expansion pack

Washing

The wash kit allows re-use of flow cells after short sequencing runs, meaning multiple libraries can be run sequentially.

PromethION

High-throughput, high-sample number benchtop systems

Maximising flow cell usage

Barcode multiple samples → Pool and sequence → Separate and analyse

More information nanoporetech.com
VolTRAX

Automated library preparation solution for nanopore sequencing

VolTRAX is a small USB-powered device that automates laboratory processes upstream of nanopore sequencing — from sample extraction to library preparation. Predefined or custom protocols can be utilised, enabling complete optimisation of sample preparation and the development of novel methods.

Consumable cartridge preparing any biological sample ready for nanopore sequencing

USB powered and portable, liquids are moved around the cartridge in a path programmed by software, performing individual reactions in sequence

Fluorescence detector for DNA and RNA QC

Automation of library preparation methods integrating capabilities such as PCR

Only minutes of hands-on time, even for novel/complex experiments

**Specification**

<table>
<thead>
<tr>
<th>Weight</th>
<th>301 g including cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>W 58 mm</td>
</tr>
</tbody>
</table>

Order now [store.nanoporetech.com/devices](store.nanoporetech.com/devices)
Adapting MiniON and GridION for smaller rapid tests and analyses

Flongle is an adapter for MiniON or GridION that enables direct, real-time DNA or RNA sequencing on smaller, single-use flow cells. Providing immediate access to sequence data, Flongle is designed to be the most rapid, accessible, and cost-efficient sequencing system for smaller or more frequently performed tests and experiments.

Specification

<table>
<thead>
<tr>
<th>Weight</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 g</td>
<td>W 105 mm</td>
</tr>
</tbody>
</table>
MinION

Portable DNA/RNA sequencing for anyone

MinION is a powerful, portable sequencing device that delivers cost-effective, real-time access to gigabases of data. Small enough to fit in a pocket and capable of reading any length of DNA or RNA fragment, the USB-powered MinION allows researchers in any environment to rapidly generate actionable biological insights across a wide range of application areas.

Sensor chip works with custom ASIC for control and data acquisition

Custom sensor array with multiple nanopores for scaled-up sequencing

Flow cell with 512 active channels

Sample added to flow cell here

Consumable flow cell where the biology and electronics come together for nanopore sequencing

USB powered device; link to laptop or desktop computer to operate

Specification

<table>
<thead>
<tr>
<th>Weight</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>87 g (103 g with flow cell)</td>
<td>W 105 mm</td>
</tr>
</tbody>
</table>

Portable DNA/RNA sequencing for anyone

MinION is a powerful, portable sequencing device that delivers cost-effective, real-time access to gigabases of data. Small enough to fit in a pocket and capable of reading any length of DNA or RNA fragment, the USB-powered MinION allows researchers in any environment to rapidly generate actionable biological insights across a wide range of application areas.
MinION Mk1C

A complete, portable, connected device for sequencing and analysis

MinION Mk1C combines the real-time, rapid, portable sequencing of MinION and Flongle with powerful integrated compute and a high-resolution touchscreen — offering a complete, go-anywhere solution for DNA and RNA sequencing.

**High-resolution** touchscreen display allowing complete device control and easy visualisation of results

**Integrated, powerful, real-time compute** with pre-installed basecalling and analysis software

**Data files are written to an onboard, high-capacity SSD; data can then be transferred to your own system**

**Connected:** Bluetooth and Wi-Fi enabled — upload and share your data, wherever you are

**Use Flongle** for smaller tests and analyses, or **MinION Flow Cells** for tens of gigabases of data

---

**Specification**

<table>
<thead>
<tr>
<th>Weight</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>420 g</td>
<td>W 140 mm</td>
</tr>
</tbody>
</table>
GridION Mk1

High-throughput, benchtop system with integrated compute module

With the capacity to run five flow cells either concurrently or individually, GridION provides busy labs and service providers with cost-efficient, on-demand access to the advantages of real-time nanopore sequencing. Integrated, high-performance data processing alleviates the need for complex IT infrastructure.

Up to 2,560 active channels can be sequencing at one time on the GridION

Sample added to flow cell here

5 individual flow cells can be operated individually or together, suitable for fee-for-service operations

Consumable flow cell where the biology and electronics come together for nanopore sequencing

Onboard data analysis offering real-time local analysis

Service provider certification is available for the GridION

<table>
<thead>
<tr>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>11 kg</td>
</tr>
<tr>
<td>Size</td>
</tr>
<tr>
<td>W 370 mm</td>
</tr>
</tbody>
</table>

High-throughput, benchtop system with integrated compute module

With the capacity to run five flow cells either concurrently or individually, GridION provides busy labs and service providers with cost-efficient, on-demand access to the advantages of real-time nanopore sequencing. Integrated, high-performance data processing alleviates the need for complex IT infrastructure.

Up to 2,560 active channels can be sequencing at one time on the GridION

Sample added to flow cell here

5 individual flow cells can be operated individually or together, suitable for fee-for-service operations

Consumable flow cell where the biology and electronics come together for nanopore sequencing

Onboard data analysis offering real-time local analysis

Service provider certification is available for the GridION

<table>
<thead>
<tr>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>11 kg</td>
</tr>
<tr>
<td>Size</td>
</tr>
<tr>
<td>W 370 mm</td>
</tr>
</tbody>
</table>
PromethION 24 and PromethION 48

High-throughput, high-sample number benchtop systems

PromethION devices deliver flexible, high-yield, benchtop sequencing ideal for large-scale projects and high-throughput laboratories. Up to 24 (PromethION 24) or 48 (PromethION 48) high-capacity flow cells can be run either simultaneously or individually, delivering on-demand access to terabases of sequencing data at your desired read length — from short to ultra long (e.g. >2 Mb). Integrated, high-performance compute allows real-time base calling and onward analysis for rapid access to results.

Each flow cell comprises up to 3,000 active channels

Up to 72,000 (P24) or 144,000 (P48) active channels can be sequencing at one time on the PromethION

PromethION 48 can deliver over 7 Tb of data in a single run

Service provider certification is available for the PromethION

<table>
<thead>
<tr>
<th>Specification</th>
<th>Weight</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing module:</td>
<td>28 kg</td>
<td>W 590 mm</td>
</tr>
<tr>
<td>Compute module:</td>
<td>25 kg</td>
<td>W 178 mm</td>
</tr>
</tbody>
</table>

Sample added to flow cell here

24 (P24) or 48 (P48) individual flow cells can be operated individually or together for flexible, on-demand sequencing

Compute module
Data analysis and basecalling

Nanopore sequencing provides real-time data streaming, enabling basecalling and subsequent data analysis to be performed in parallel for immediate access to results.

Data structure

- **Raw data**
  - Raw data straight off ASIC

- **Sequence**
  - `CCGACTCCGGTATTACCGCCGTGATTTGCTGGGGCAGGGCCG`
  - Basecalled

The facility of nanopore technology to sequence native DNA and RNA without the requirement for amplification or reverse transcription, allows the retention and detection of base modifications alongside nucleotide sequence.

As a DNA or RNA strand passes through the nanopore, the current is measured several thousand times per second. These current samples are known as raw data, which is subsequently processed using machine learning techniques into basecalled data — the sequence of DNA or RNA bases.

Nanopore data is provided in standard FASTQ and FAST5 formats suitable for analysis using a range of downstream analysis tools (see page 11), including Oxford Nanopore’s real-time EPI2ME platform.

Basecalling and device control

MinKNOW™, the device control and primary analysis software for all nanopore devices, provides easy experimental setup and real-time visualisation of sequencing performance.

- **Basecalling and device control**
  - **MinKNOW**
    - Enables complete control of sequencing parameters: start runs, set run parameters, and group experiments
  - **Live output**
    - Basecalled reads in .fastq or .fast5 formats for immediate analysis. Basecalling can also be performed after the sample run using a range of algorithms
  - **Visualise**
    - Sequencing progress and performance in real time. Quality check your run, and if there’s a problem with the library, stop sequencing, wash the flow cell, and start again
Real-time insights with EPI2ME

The cloud-based or local data analysis platform EPI2ME provides easy access to a growing number of real-time data analysis workflows.

Workflows include:
- Metagenomic species identification
- Antimicrobial resistance profiling
- 16S-based bacteria and archaea identification
- Human structural variation analysis
- Reference alignment

Analyse your nanopore sequencing data and develop your bioinformatics skills using fully customisable, best practice EPI2ME Labs workflows and tutorials.

EPI2ME Labs delivers:
- Web browser-based platform with minimal installation requirements
- Interactive tutorials and workflows with extensive data visualisation tools
- Full customisation — include your own code, or copy between workflows
- Community enabled — submit and share your workflows

Simplified analysis with EPI2ME Labs

Analyse your nanopore sequencing data and develop your bioinformatics skills using fully customisable, best practice EPI2ME Labs workflows and tutorials.

EPI2ME Labs delivers:
- Web browser-based platform with minimal installation requirements
- Interactive tutorials and workflows with extensive data visualisation tools
- Full customisation — include your own code, or copy between workflows
- Community enabled — submit and share your workflows

<table>
<thead>
<tr>
<th>EPI2ME</th>
<th>EPI2ME Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Cloud-based or local</td>
</tr>
<tr>
<td>Aim</td>
<td>Simple, one-click analysis solutions</td>
</tr>
<tr>
<td>Configurability</td>
<td>Pre-configured</td>
</tr>
<tr>
<td>Shareability</td>
<td>Limited</td>
</tr>
<tr>
<td>Focus</td>
<td>Simple, rapid, real-time analysis</td>
</tr>
</tbody>
</table>
In development

**Ubik™**
Rapid and portable, single-tube sample preparation

**SmidgION™**
Real-time nanopore sequencing and analysis on a smartphone

**Plongle™**
High-throughput analysis of smaller, frequently preformed tests and assays in a 96-well plate format

Biology for anyone, anywhere