



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 DNA¹ and >20 kb RNA²).

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.

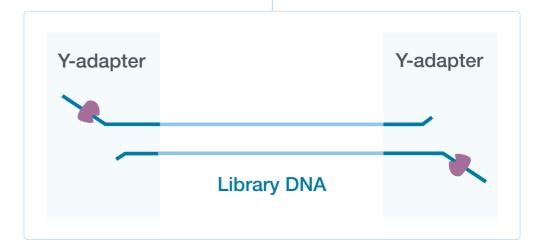
Nanopore reader

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 resistant membrane means all current must pass through the nanopore, ensuring a clean signal.

Library prep

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



Translocation

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









Template...

...Template...

(Exit)

Next molecule...

^{1.} Payne, A. et al. Bioinformatics. bty841 (2018). 2. Viehweger, A. et al. Genome Res. 29:9 (2019).

Discover the benefits of nanopore technology



Unrestricted read length short to ultra-long (longest 2.3 Mb¹)

- Ultimate flexibility optimise for your application
- Easier genome assembly
- Resolve structural variants, repeats, and phasing
- Characterise and quantify full-length transcripts





- 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™



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

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



Direct sequencing

- Sequence native DNA or RNA, not a copy
- Eliminate amplification bias
- Identify base modifications



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



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
- 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

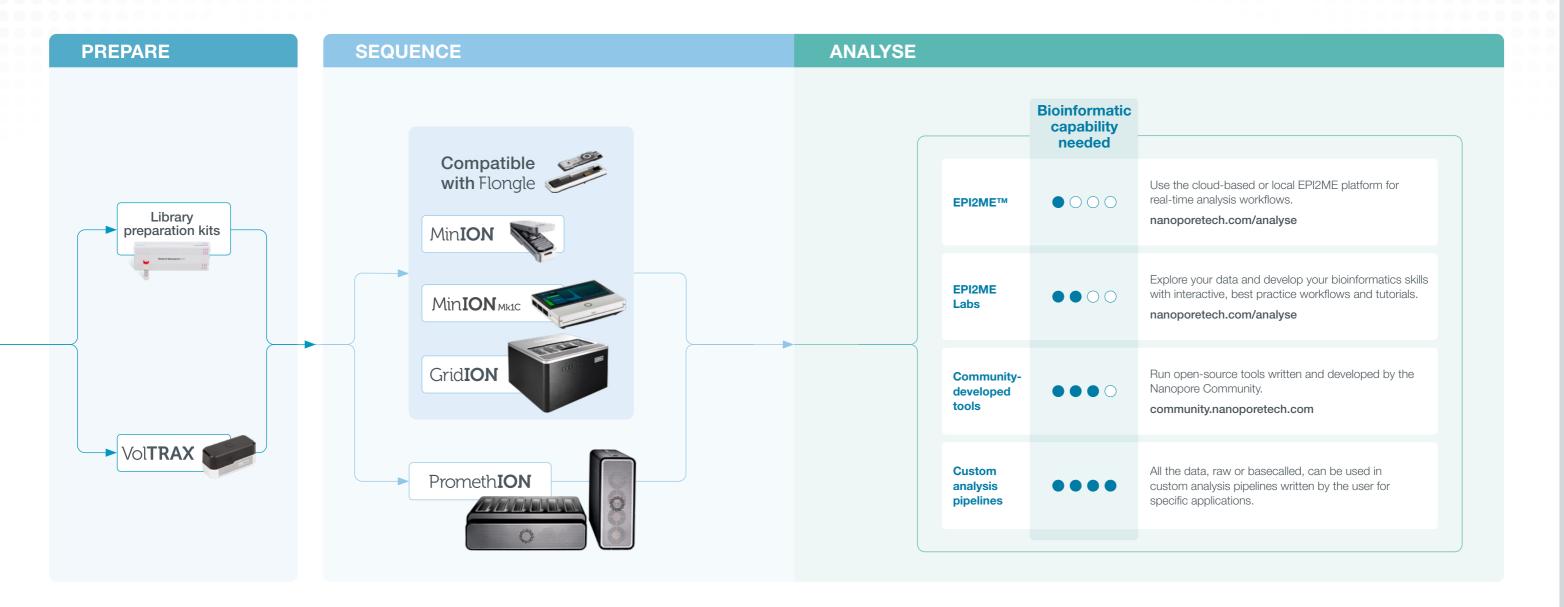


Epigenetics

- Methylation
- Histone modification
- Non-coding RNA activity



A complete and streamlined workflow – real-time answers to biological questions

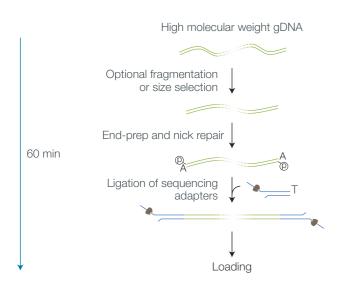


DNA library preparation

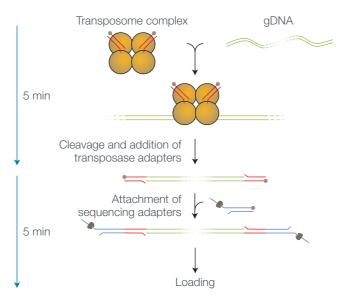
For maximum throughput

For minimal preparation time

Ligation Sequencing Kit



Rapid Sequencing Kit with transposase



- 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
- The transposase simultaneously cleaves template molecules and attaches tags to the cleaved ends
- Rapid sequencing adapters are added to the tagged ends
- Fragment lengths are a result of the random cleavage

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.

	Ligation (SQK-LSK110)	Rapid (SQK-RAD004)	PCR (SQK-PSK004)
Use for	Highest throughput	Rapid and simple prep	Control over read length or amplicon sequencing
Prep time	60 mins	10 mins	PCR + 60 mins
Input amount	1,000 ng dsDNA	400 ng HMW gDNA (>30 kb)	100 ng dsDNA
Fragmentation	Optional	Transposase based	N/A
Read length	Equal to fragment length	Random distribution, dependent on input fragment length	Equal to fragment length post-PCR
PCR required	No	No	Yes
Multiplexing options	Native Barcoding (PCR free)*; PCR Barcoding Expansion pack	Use Rapid Barcoding Kit	Use PCR Barcoding Kit

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

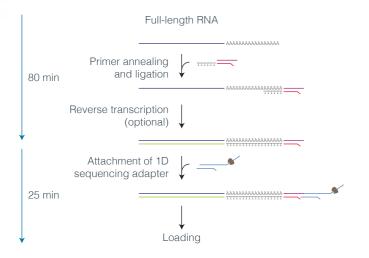
^{*} Currently available for SQK-LSK109; coming soon for SQK-LSK110.

RNA library preparation

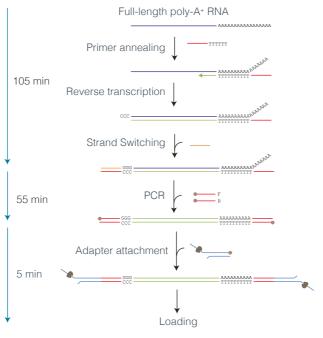
For sequencing the RNA molecule directly

For full-length transcript analysis with high throughput

Direct RNA Sequencing Kit



PCR-cDNA Sequencing Kit



- Optional reverse transcription step improves throughput cDNA strand is not sequenced
- Sequencing adapters attached to prepared ends
- Read length reflects length of molecules in sample

- cDNA is synthesised using reverse transcription and strandswitching method, and then is amplified with PCR
- Strand-switching before PCR enriches for full-length transcripts
- Sequencing adapters are attached to the amplified cDNA

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 (SQK-RNA002)	PCR-cDNA (SQK-PCS109)	Direct cDNA (SQK-DCS109)
Use for	Sequence RNA molecules directly and preserve base modifications	Full-length transcripts with high throughput	Full-length transcripts without PCR bias
Prep time	105 mins	165 mins	275 mins
Input recommendation	500 ng RNA (poly-A+)	1 ng RNA (poly-A+)	100 ng RNA (poly-A+)
Read length	Equal to RNA length	Enriched for full-length cDNA	Enriched for full-length cDNA
PCR required	No	Yes	No
Reverse transcription	Optional	Yes	Yes
Multiplexing options	In development	PCR-cDNA Barcoding Kit	Native Barcoding Expansion pack

Cost-effective analysis of multiple samples

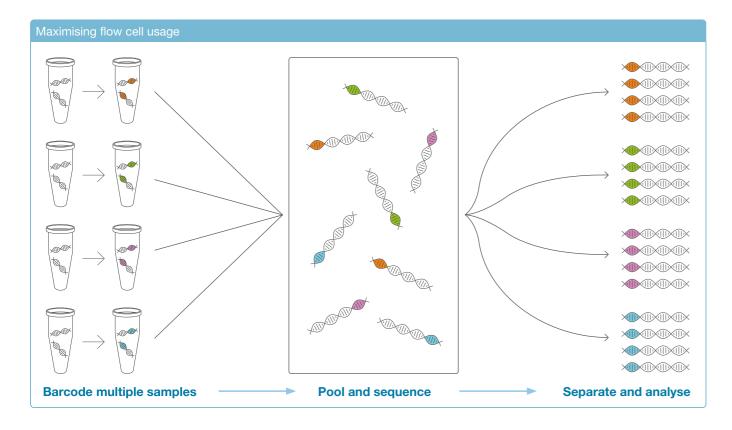
Barcoding

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.



Channels Panel **PromethION** High-throughput, high-sample number benchtop systems

Automated library preparation solution for nanopore sequencing

VoITRAX 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



Automation of library preparation methods integrating capabilities such as PCR

Fluorescence detector for DNA and RNA QC

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

Specification

Weight

301 g including cartridge

Size

W 58 mm | H 64 mm | D 134 mm

Flongle

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.

COMPATIBLE WITH GridION, MinION, and MinION Mk1C

Sample added to flow cell here



Consumable flow cell with 126 channels

Reusable adapter that allows docking of smaller flow cell

Same MinION device

444

Weight

W 105 mm | H 23 mm | D 8 mm 20 g

Specification

store.nanoporetech.com/devices

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.

> Sample added to flow cell here

Flow cell with 512 active channels

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

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

Custom sensor array with multiple nanopores for scaled-up sequencing

Sensor chip works with custom ASIC for control and data acquisition

Specification

Weight

87 g (103 g with flow cell)

W 105 mm | H 23 mm | D 33 mm



444

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.

own system

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

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

Weight 420 g

W 140 mm | H 30 mm | D 114 mm



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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

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

sequencing

Onboard data analysis offering real-time local analysis

Sample added to flow cell here

1111..0

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

Specification

Weight 11 kg

W 370 mm | H 220 mm | D 365 mm





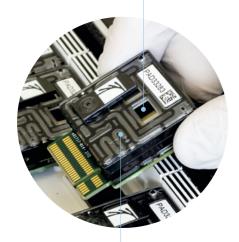
Service provider certification is available for the GridION

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, highperformance compute allows real-time base calling and onward analysis for rapid access to results.

> Sample added to flow cell here



Each flow cell comprises up to 3,000 active channels 24 (P24) or 48 (P48) individual flow cells can be operated individually or together for flexible, on-demand sequencing

Sequencing module

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

Specification

Weight

Sequencing module: Compute module:

Compute module

28 kg 25 kg W 590 mm | H 190 mm | D 430 mm W 178 mm | H 440 mm | D 470 mm



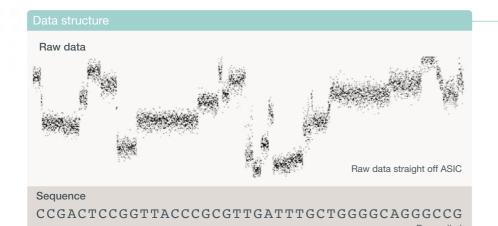
Service provider certification is available for the PromethION



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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.



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

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

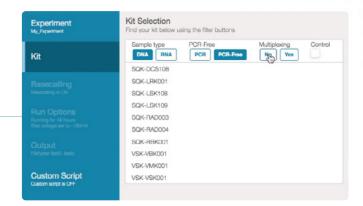


Nanopore data is provided in standard FASTQ and FAST5 formats suitable for analysis using a range of downstream real-time EPI2ME platform.

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.

MinKNOW enables complete control of sequencing parameters: start runs, set run parameters, and group experiments



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

Live output of basecalled reads in .fastq or .fast5 formats for immediate analysis. Basecalling can also be performed after the sample run using a range of algorithms



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:

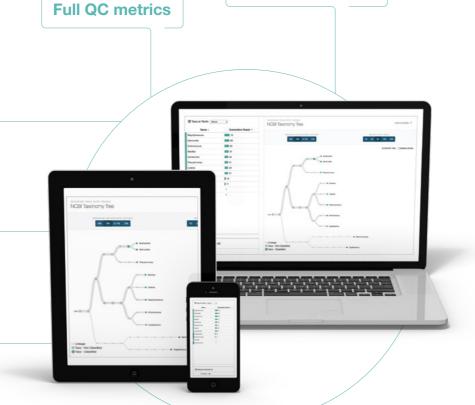
- Metagenomic species identification
- Antimicrobial resistance profiling
- 16S-based bacteria and archaea identification
- Human structural variation analysis
- Reference alignment

Intuitive graphical interface

Cloud-based or local analysis

No bioinformatics experience required

Immediate access to actionable results



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

	EPI2ME	EPI2ME Labs
Location	Cloud-based or local	Local
Aim	Simple, one-click analysis solutions	Bioinformatics best practices and training
Configurability	Pre-configured	Configurable
Shareability	Limited	Extensive
Focus	Simple, rapid, real-time analysis	Customisable, exploratory, post-run analysis

In development



Plongle™

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

Biology for anyone, anywhere



Real-time nanopore sequencing and analysis on a smartphone



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