



# 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. >4 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.

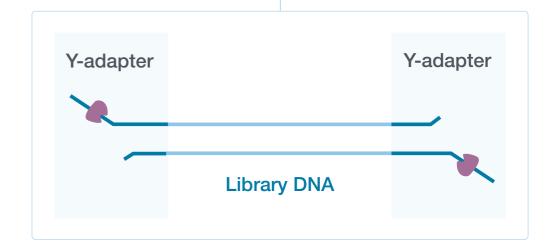
#### Nanopore reader

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.



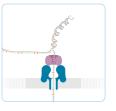
#### **Translocation**

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. Internal data generated using the Ultra-Long DNA Sequencing Kit. 2. Viehweger, A. et al. Genome Res. 29:9 (2019)

# Discover the benefits of nanopore technology



#### Unrestricted read length short to ultra-long (longest >4 Mb¹)

- 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
- Enrich regions of interest without additional sample prep using adaptive sampling
- Early sample insights and QC
- Enough data? Stop, wash, store, or run another sample





- 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



### **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.

1. Internal data generated using the Ultra-Long DNA Sequencing Kit (September 2020).

Discover more nanoporetech.com

# Generate new biological insights



#### Whole genome sequencing

- De novo assembly and resequencing
- Scaffolding and finishing
- Variant analysis: structural variation, SNVs, phasing, base modifications
- Chromatin conformation



#### **Targeted sequencing**

- Amplicon and PCR-free enrichment
- Real-time targeting with adaptive sampling (see page 15)
- 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
- Sequence complete viral genomes
- Variant analysis: splice variants, gene fusions, SNVs, base modifications



#### Metagenomics

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

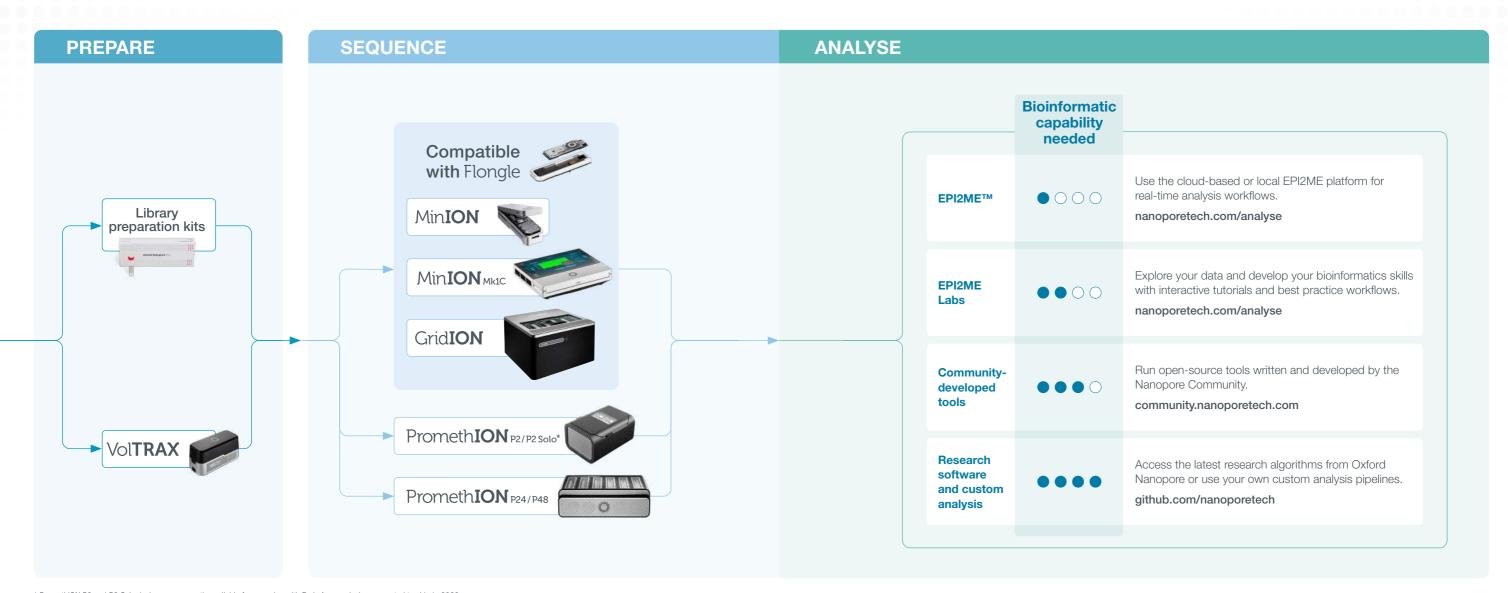


#### **Epigenetics**

- Base modifications (e.g. methylation)
- Histone modification
- Non-coding RNA activity (e.g. IncRNA)



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



<sup>\*</sup> PromethION P2 and P2 Solo devices are currently available for preorder, with Early Access devices expected to ship in 2022.

# Library preparation kits

Select the library preparation kit that matches your specific experimental needs — your choice of read length (short to ultra-long), turnaround time, input amount, sample multiplexing, modification detection, and output requirements. Find out more and view our complete library prep portfolio at store.nanoporetech.com.

	Native DNA		Amplified DNA		RNA		Targeted			
	Ligation	Rapid/Field	Ultra-Long	PCR	Rapid PCR	PCR-cDNA	Direct RNA	16S	Cas9	Adaptive sampling
Prep time	60 mins	10 mins	90 min + 1 x O/N incubation	60 mins + PCR	15 mins + PCR	160 mins + PCR	105 mins	10 mins + PCR	110 mins	-
△ △ Input	1,000 ng dsDNA	From 50 ng HMW gDNA	6M cells / 1 ml blood	100 ng dsDNA	1-5 ng gDNA	4 ng poly-A+ RNA, or 200 ng total RNA	500 ng RNA	10 ng gDNA	1-10 μg dsDNA	-
Multiplexing options	Yes	Yes	-	Yes	Yes	Yes	In development	Yes	Coming soon	-
Read length	Equal to fragment length	Random distribution, dependent on input fragment length	N50 >50 kb	Equal to fragment length post-PCR	~2 kb	Enriched for full-length cDNA	Equal to RNA length	Full-length 16S gene (~1.5kb)	Equal to fragment length	Equal to fragment length
PCR required	No	No	No	Yes	Yes	Yes	No	Yes	No	-
Product range highlights	Detect modified bases for free. Automatable workflows and XL kits enable production-scale sequencing			Ideal for low input amounts		Detect modified bases for free with direct RNA kits		Read more about adaptive sampling on page 15. Generate your own or view pre-defined panels on the Nanopore Community		

### 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.

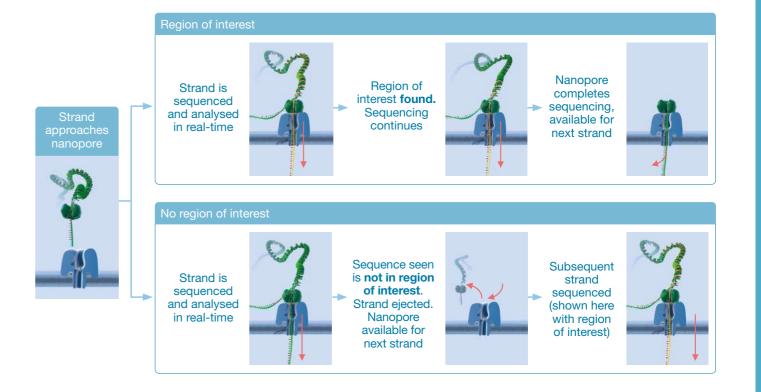
### Maximising flow cell usage **Barcode multiple samples** Pool and sequence Separate and analyse

### A novel approach to targeted sequencing

#### Adaptive sampling

Adaptive sampling is a unique, on-device approach to targeted sequencing, which requires no upfront library enrichment steps. Using real-time basecalling, DNA fragments can be accepted or rejected for further sequencing based on their initial sequence composition. Furthermore, adaptive sequencing can be implemented in advance of, or even during, a run to increase coverage of specific targets.

- Target multiple regions of interest without lengthy lab-based enrichment steps
- No limit on read length expand targeted assays to include SNVs, SVs, and phasing
- Enrich long, native DNA molecules retain base modifications
- Adjust enrichment in real time enhance coverage of key regions or low-abundance species





### 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

> > Fluorescence detector, magnetic array, heater, and Peltier for flexible sample preparation

**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

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

Specification

Weight

301 g including cartridge

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.

GridION, MinION, and MinION Mk1C Sample added to flow cell here Specification

**COMPATIBLE WITH** 

Consumable flow cell with 126 channels Reusable adapter that allows docking of smaller flow cell Same MinION device Weight W 105 mm | H 23 mm | D 8 mm 20 g

Order now store.nanoporetech.com

### MinION

### Your personal, portable DNA and RNA sequencer

Get complete control and creativity over when, where, and how often you sequence. MinION provides the power of nanopore sequencing in an accessible, fully portable device. Weighing only 100 g and running off a laptop, MinION generates tens of gigabases of real-time data in the field or lab.

> 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

Sensor array with multiple nanopores for scaled-up sequencing

Sensor chip works with custom ASIC for control and data acquisition



Weight

87 g (103 g with flow cell) W 105 mm | H 23 mm | D 33 mm



440

Order now store.nanoporetech.com

### MinION Mk1C

### Your all-in-one, portable DNA and RNA sequencer

MinION Mk1C provides the power of nanopore sequencing in a fully portable device with integrated real-time basecalling and data analysis, touchscreen operation, and wireless connectivity. Sequence and analyse your samples in the lab or field, and easily standardise assays across multiple sites or collaborators.

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

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

Connected: Ethernet 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

Data files are written to an onboard, 1 TB SSD; data can then be transferred to your own system

Specification

Weight 420 g

W 140 mm | H 30 mm | D 114 mm



Order now store.nanoporetech.com

### GridION Mk1

### Self-contained, easily deployable DNA/RNA benchtop nanopore sequencer

A flexible, self-contained, benchtop nanopore sequencer, running up to five MinION or Flongle Flow Cells (or combinations of each) that can respond to the needs of multiple users on demand, across varied applications. Integrated, high-performance data processing alleviates the need for complex IT infrastructure.

#### Consumable flow cell

where the biology and electronics come together for nanopore sequencing

> Onboard data analysis offering real-time basecalling and adaptive sampling

Sample added to flow cell here

### Five individual MinION or Flongle Flow Cells

can be operated individually or together, suitable for research labs and service providers

Specification

Weight 11 kg

W 370 mm | H 220 mm | D 365 mm





Service provider certification is available for the GridION

### PromethION 2 Solo and PromethION 2

### Low-cost access to high-yield PromethION sequencing

Offering the flexibility of two independent, high-output PromethION Flow Cells, the compact PromethION 2 (P2) devices deliver the benefits of high-coverage nanopore sequencing to users with lower sample processing requirements. Get fully integrated sequencing and analysis with P2 or expand your GridION/existing compute infrastructure with P2 Solo.\*



PromethION 2 Solo

Two high-output flow cells can be operated individually or together for flexible, on-demand sequencing

> Connect to GridION or existing compute infrastructure

Service provider certification is available for the PromethION



<sup>\*</sup> PromethION P2 and P2 Solo devices are currently available for preorder, with Early Access devices expected to ship in 2022.

Pre-order now

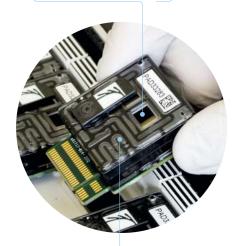
store.nanoporetech.com

### PromethION 24 and PromethION 48

### Flexible DNA/RNA high-throughput nanopore sequencers

Offering the flexibility of 24 independently controllable, high-output flow cells and leveraging state-of-the-art algorithms and GPU technology, PromethION 24 provides single or multiple users with on-demand access to terabases of sequencing data. PromethION 48, our most powerful sequencing device, delivers twice the capacity and output of PromethION 24 — ideal for large- and production-scale sequencing projects.

Sample added to flow cell here



**Each flow cell** comprises up to 2,675 active channels

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

Sequencing Unit

PromethION 24 can deliver up to 7 Tb\* of data in a single run Specification

Weight

**Sequencing module:** 28 kg **Compute module:** 25 kg

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

Theoretical max output (TMO). Assumes system is run for 72 hours at 420 bases / second.

Actual output varies according to library type, run conditions, etc. TMO noted may not be available for all applications or all chemistries.

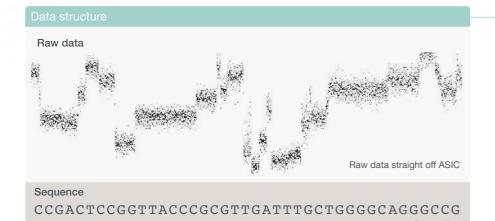
**Data Acquisition Unit** 



Service provider certification is available for the PromethION



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 (e.g. methylation) alongside nucleotide sequence



Nanopore data is provided in standard FASTQ and FAST5 formats suitable for analysis using a range of downstream best practice EPI2ME pipelines.

### 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.

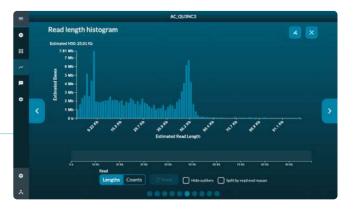
MinKNOW enables complete control of sequencing parameters: start runs, set run parameters, and group experiments





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



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

#### Workflows include:

- SARS-CoV-2 analysis generate consensus sequences and identify genetic variants
- Metagenomic species identification
- Antimicrobial resistance profiling
- 16S-based bacteria and archaea identification
- Human structural variation analysis
- Reference alignment
- Plasmid sequencing

Cloud-based or local analysis

No bioinformatics experience required

**Immediate access** to actionable results



# Simplified analysis with EPI2ME Labs

EPI2ME Labs offers two streamlined approaches to analysing your nanopore sequencing data.

- Tutorials: develop your bioinformatics skills with interactive tutorials and customisable, best practice analysis pipelines, using your own data or model datasets
- Workflows: utilise a growing number of simplified, standardised pipelines, from plasmid assembly to small variant calling — ideal for high-throughput, automated analyses

	EPI2ME	EPI2ME Labs Tutorials	EPI2ME Labs Workflows		
Location	Cloud-based or local	Local	Local & distributed (cluster and/or cloud)		
Aim	Simple, one-click analysis solutions	Bioinformatics best practices and training	Formalised workflows		
Configurability	Pre-configured	Configurable	Configurable		
Shareability	Limited	Extensive	Extensive		
Focus	Simple, rapid, real-time analysis	Customisable, exploratory, post-run analysis	Standardised, high-throughput analysis		

## In development

#### MinION Mk1D

An accessory keyboard with integrated sequencer for tablet devices





#### Ubik™

Rapid and portable, single-tube sample preparation

### Plongle™

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

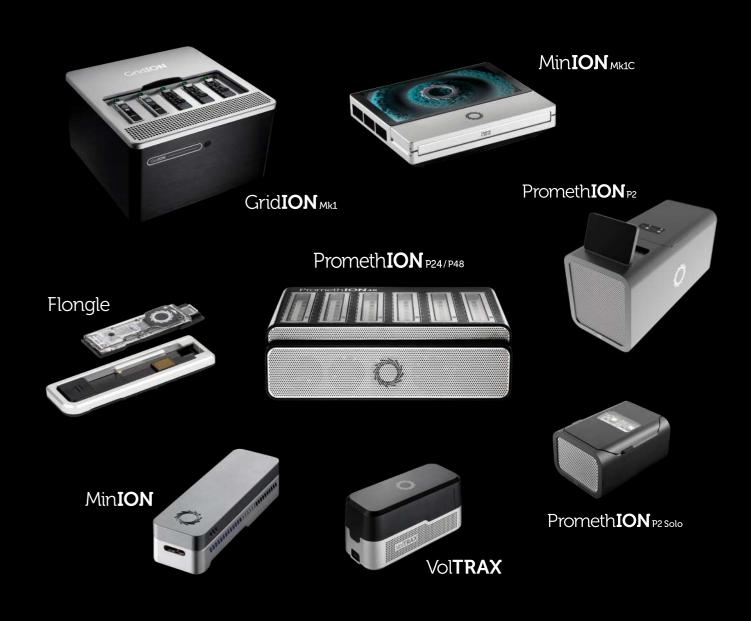




### **SmidgION**™

Real-time nanopore sequencing and analysis on a smartphone

# Biology for anyone, anywhere



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