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### NANOPORE Technologies

Biomolecule Sequencer

P/N BS-BS S/N 02

### Our goal is to enable Ø 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. >4 Mb DNA<sup>1</sup> and >20 kb RNA<sup>2</sup>).

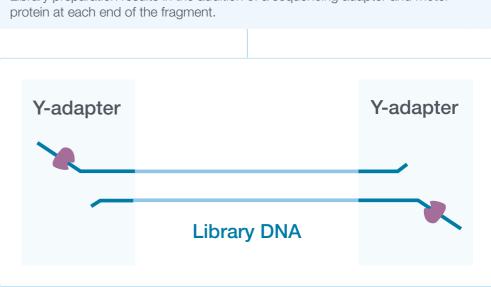
An **enzyme motor** controls the translocation of the DNA or RNA strand through the nanopore. Once the DNA or RNA has passed through, the motor protein detaches and the nanopore is ready to accept the next fragment.

#### 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.

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

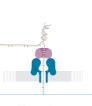


#### Translocation

Library prep

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...

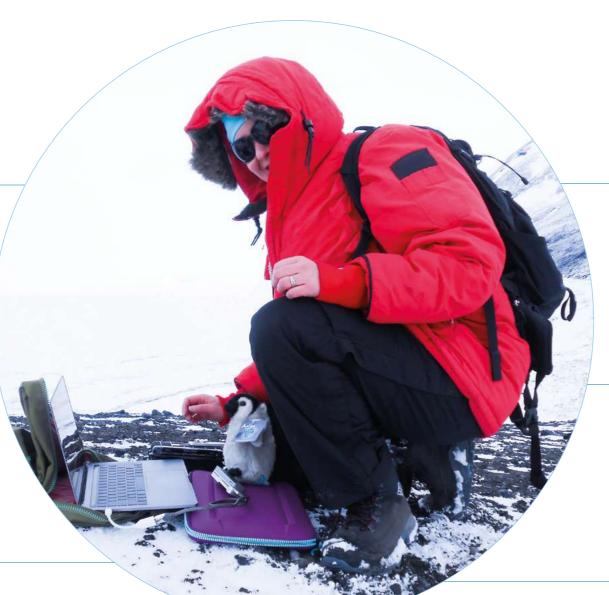
### Discover the benefits of nanopore technology

#### Unrestricted read length short to ultra-long (longest >4 Mb<sup>1</sup>)

- 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



- Maximise throughput with barcoding

### **Direct sequencing**

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

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

### Scalable – portable to ultra-high throughput

• One technology across all devices - scale to your needs Sequence at sample source with Flongle<sup>™</sup> and MinION<sup>™</sup> • Compact, high-throughput benchtop sequencing with

GridION<sup>™</sup> and PromethION<sup>™</sup>



### **Streamlined library prep**

- Rapid 10-minute (DNA) library prep
- Automated, portable prep VolTRAX™
- High DNA and RNA yields from low input amounts



### **On-demand sequencing**

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



# 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

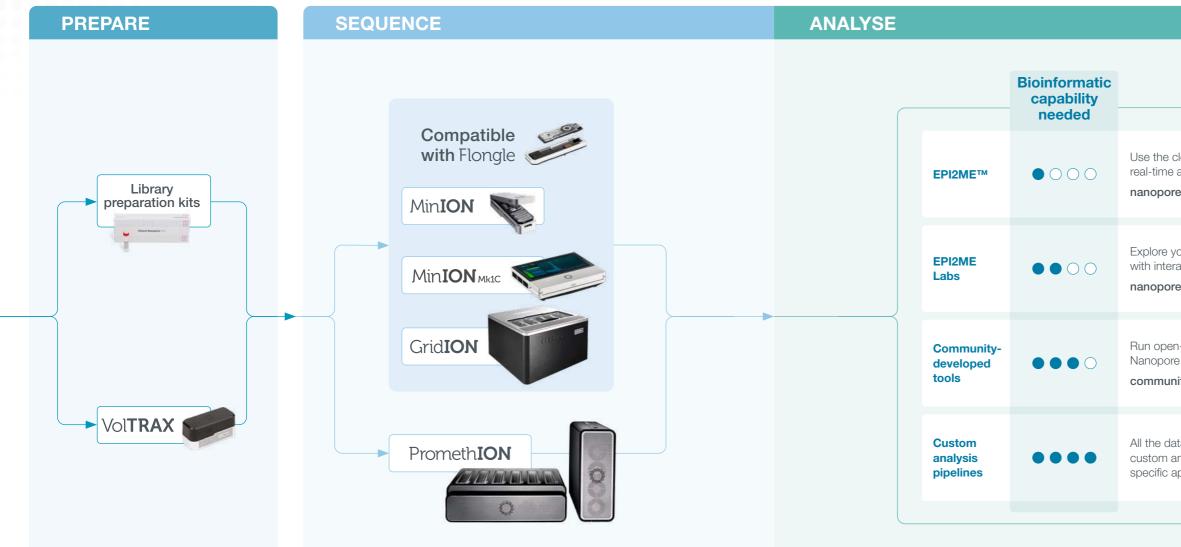


### Epigenetics

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



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



Use the cloud-based or local EPI2ME platform for real-time analysis workflows.

#### nanoporetech.com/analyse

Explore your data and develop your bioinformatics skills with interactive, best practice workflows and tutorials.

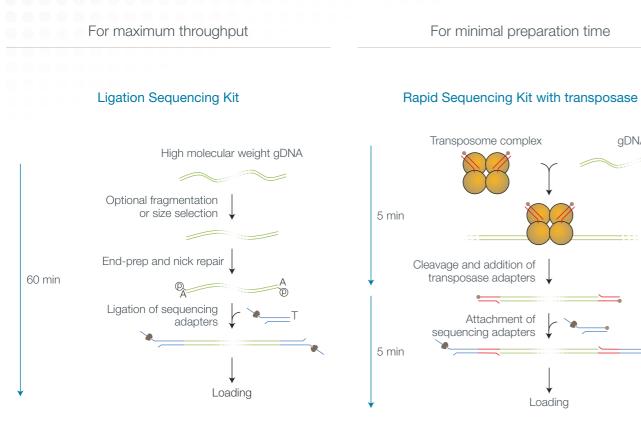
#### nanoporetech.com/analyse

Run open-source tools written and developed by the Nanopore Community.

#### community.nanoporetech.com

All the data, raw or basecalled, can be used in custom analysis pipelines written by the user for specific applications.

## DNA library preparation



- 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

Loading

**gDNA** 

- 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.

(SQK-LSK110)	Rapid (SQK-RAD004)	PCR (SQK-PSK004)
Highest throughput	Rapid and simple prep	Control over read length or amplicon sequencing
60 mins	10 mins	PCR + 60 mins
1,000 ng dsDNA	400 ng HMW gDNA (>30 kb)	100 ng dsDNA
Optional	Transposase based	N/A
Equal to fragment length	Random distribution, dependent on input fragment length	Equal to fragment length post-PCR
No	No	Yes
Native Barcoding (PCR free)*; PCR Barcoding Expansion pack	Use Rapid Barcoding Kit	Use PCR Barcoding Kit
	60 mins 1,000 ng dsDNA Optional Equal to fragment length No Native Barcoding (PCR free)*; PCR Barcoding	60 mins10 mins1,000 ng dsDNA400 ng HMW gDNA (>30 kb)OptionalTransposase basedEqual to fragment lengthRandom distribution, dependent on input fragment lengthNoNoNative Barcoding (PCR free)*; PCR BarcodingUse Rapid Barcoding kit

#### Also available:

- New: Ultra-Long DNA Sequencing Sequencing Kit optimised for ultra-long DNA fragments to routinely generate read N50s of 50-100 kb plus.
- Cas9 Sequencing Kit streamlined, PCR-free enrichment of long targeted regions with maintenance of base modifications
- Application-specific library preparation kits (e.g. 16S sequencing)

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

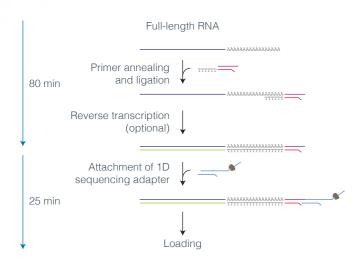
- Field Sequencing Kit get all the benefits of rapid sequencing with the added convenience of ambient shipping and storage
- Ligation Sequencing Kit XL plate-based ligation sequencing kit for high-throughput workflows
- Automatable workflows

## RNA library preparation

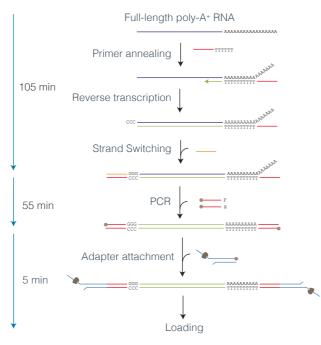
For sequencing the RNA molecule directly

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)
Sequence RNA molecules directly and preserve base modifications	Full-length transcripts with high throughput	Full-length transcripts without PCR bias
105 mins	165 mins	275 mins
500 ng RNA (poly-A+)	1 ng RNA (poly-A+)	100 ng RNA (poly-A+)
Equal to RNA length	Enriched for full-length cDNA	Enriched for full-length cDNA
No	Yes	No
Optional	Yes	Yes
In development	PCR-cDNA Barcoding Kit	Native Barcoding Expansion pack
	(SQK-RNA002) Sequence RNA molecules directly and preserve base modifications 105 mins 500 ng RNA (poly-A <sup>+</sup> ) Equal to RNA length No Optional	(SQK-RNA002)(SQK-PCS109)Sequence RNA molecules directly and preserve base modificationsFull-length transcripts with high throughput105 mins165 mins500 ng RNA (poly-A+)1 ng RNA (poly-A+)Equal to RNA lengthEnriched for full-length cDNANoYesOptionalYes

## Cost-effective analysis of multiple samples

### Barcoding

Washing

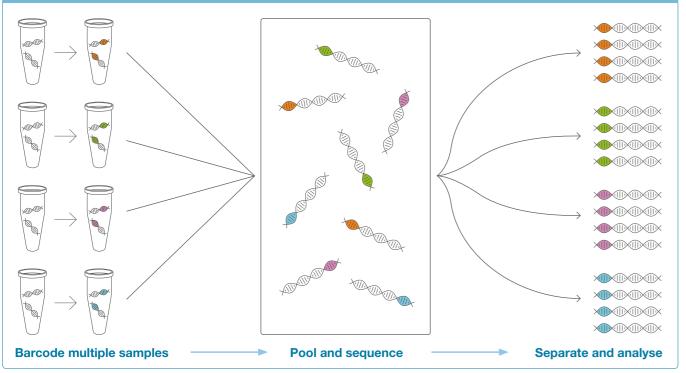
The wash kit allows re-use of flow cells after short sequencing

runs, meaning multiple libraries can be run sequentially.

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

#### Maximising flow cell usage





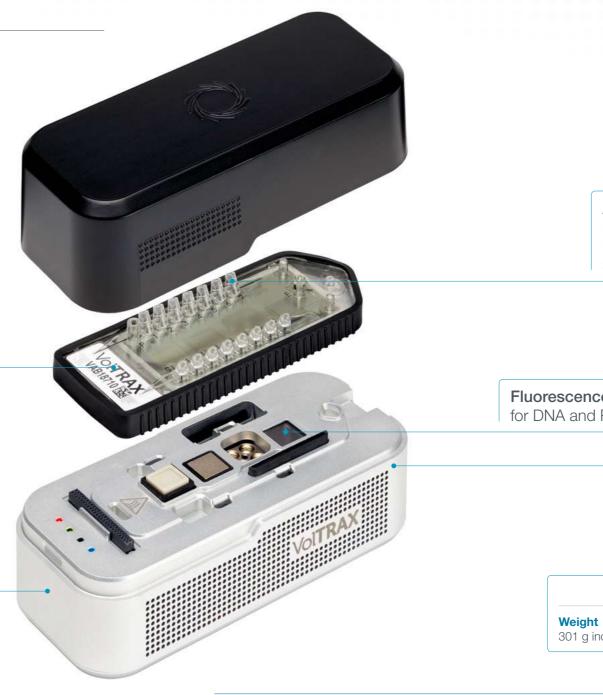
## VoITRAX

### 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

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.



20

**COMPATIBLE WITH** GridION, MinION, and MinION Mk1C



20 g

W 105 mm | H 23 mm | D 8 mm

# 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.



Custom

sensor array with multiple

nanopores

for scaled-up

sequencing

22

87 g (103 g with flow cell)

Sensor chip works with custom ASIC

W 105 mm | H 23 mm | D 33 mm



# 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.



**Connected:** LAN 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

**Size** W 140 mm | H 30 mm | D 114 mm



# 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





Service provider certification is available for the GridION

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

### Specification

**Size** W 370 mm | H 220 mm | D 365 mm



Order now store.nanoporetech.com/devices

### 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. >4 Mb). Integrated, highperformance compute allows real-time base calling and onward analysis for rapid access to results.

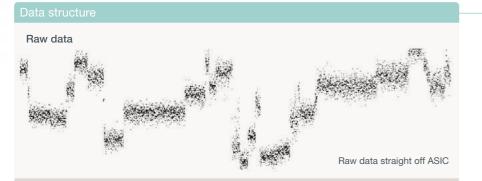
Up to 72,000 (P24) or 144,000 (P48) active channels can be sequencing at one time on the PromethION



W 590 mm | H 190 mm | D 430 mm W 178 mm | H 440 mm | D 470 mm

### 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

#### Sequence CCGACTCCGGTTACCCGCGTTGATTTGCTGGGGGCAGGGCCG 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 (e.g. methylation) alongside nucleotide sequence



## Basecalling and device control

MinKNOW<sup>™</sup>, 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



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

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.

	Kit Selection Find your kit below ut	ing the filter buttons		
Kit	Sample type	PCR-Free PCR PCR-Free	Multiplexing	Control
	SQK-LRK001 SQK LSK108			
	SQK-LSK109 SQK-RAD003 SQK-RAD004			
	SQK-REK001 VSK-VEK001			
Custom Script Custom script is CP+	VSK-VMK001 VSK-VSK001			

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:

- 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

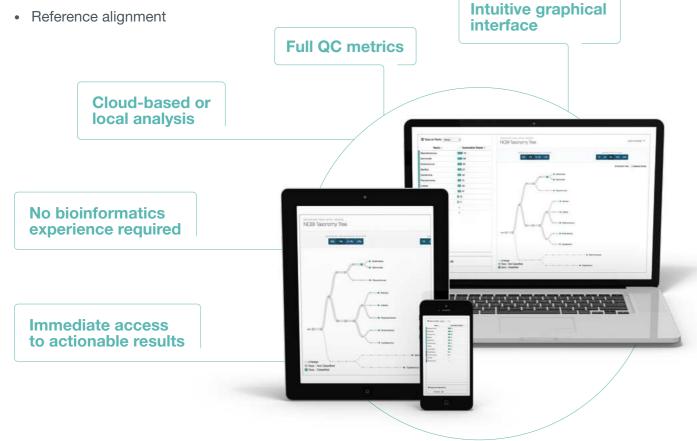
# Simplified analysis with EPI2ME Labs

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



Rapid and portable, single-tube sample preparation



6.

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### **SmidgION**<sup>™</sup>

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

















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