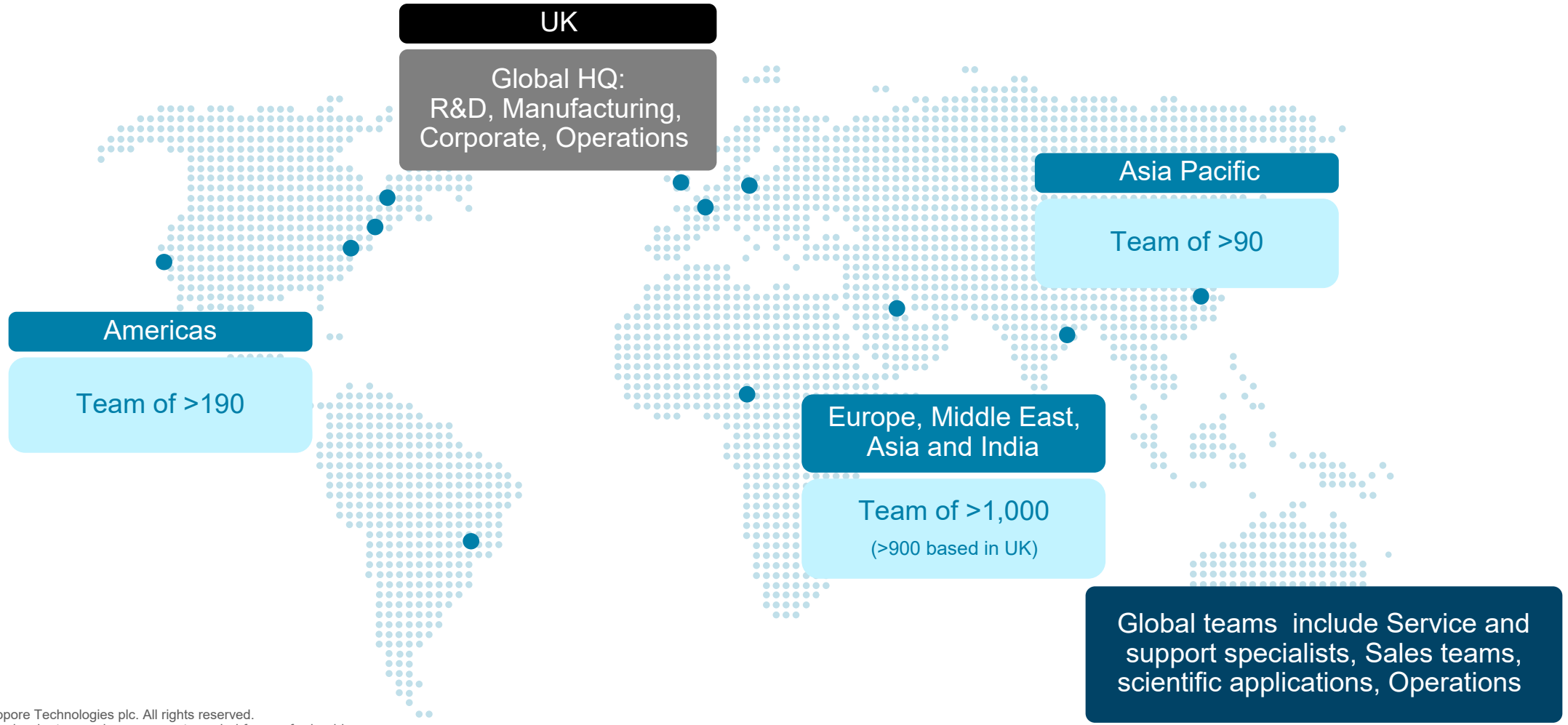
Oxford Nanopore Technologies

GENERATE ULTRA-RICH DATA FOR ANSWERS WITH IMPACT

Ji Young Kim
1st Phile Korea technology
Application TechSupport Manager

A UK-headquartered company with an international footprint



Nanopore sequencing: An end-to-end platform

Comprehensive solutions for library preparation, sequencing and data analysis

Platform technology: Flexibility and control at every stage

Prepare

- ✓ Output optimised
- ✓ Speed optimised
- ✓ Manual & Automated

Sequence

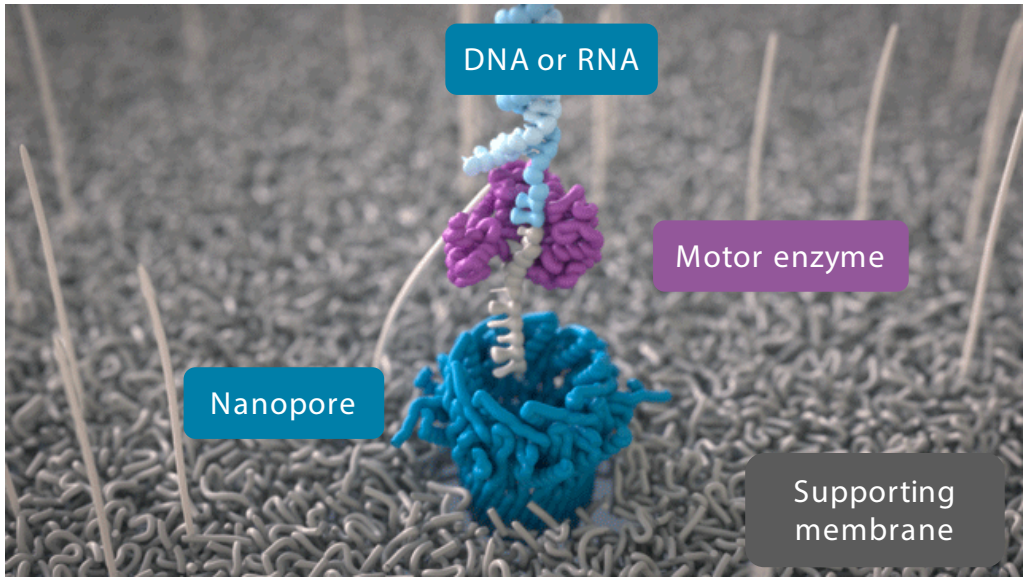
- ✓ Field devices
- ✓ Lab devices
- ✓ Low & high output platforms (low $ / test & low $ / Gb)

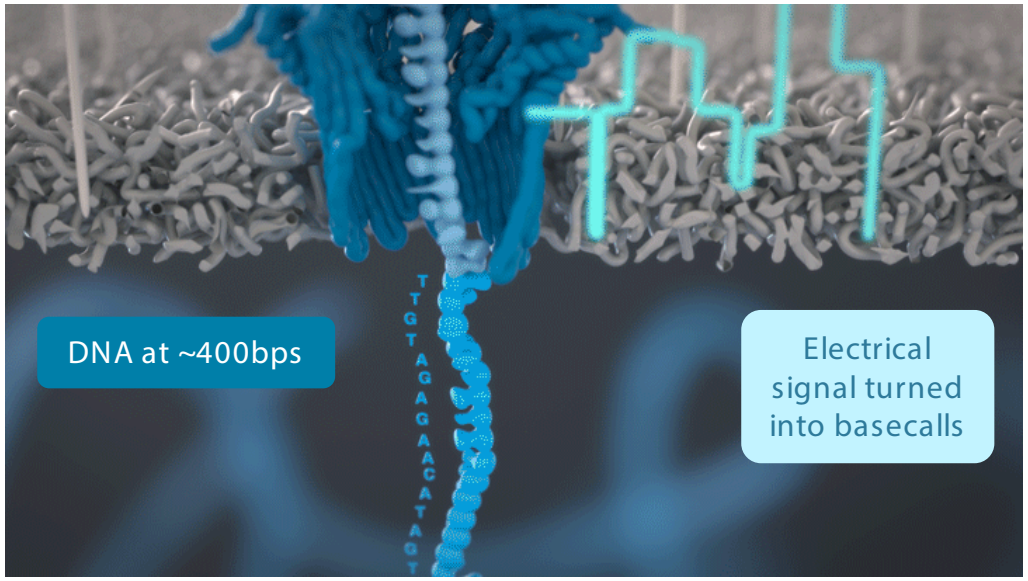
Analyse

- ✓ Accessible
- ✓ Scalable
- ✓ Versatile

Nanopore sequencing: how it works

An adapted DNA/RNA strand is passed through a nanopore, an electrical signal is interpreted into sequence data



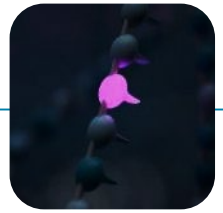
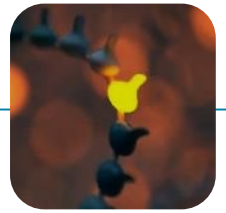


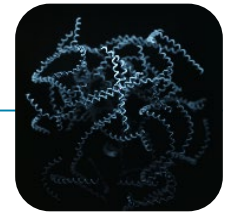
Features	Direct sequencing of DNA and RNA	PCR free, no amplification bias	Read length-agnostic	Real-time analysis	Chemistry on bespoke electronics
Benefits	Richer information including epigenetics	Simpler workflows, Richer information including epigenetics	One platform for any sample; see the true scope in your biology	Rapid results Intelligent analyses e.g. adaptive sampling	Scalable, from small to large formats Low-cost

ONE sequencing platform

SNP & INDEL | Methylation | Structural Variation | Phasing | Assembly

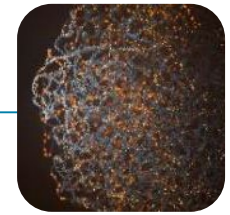
Full biology

From any read length

..from 20 bases to over 4 million bases…



Theoretical max*

Full biology

50Gb

MinION

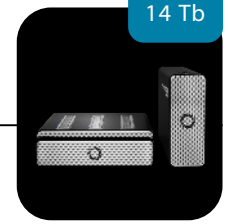
250Gb

GridION

580Gb

PromethION

7 Tb - 14 Tb

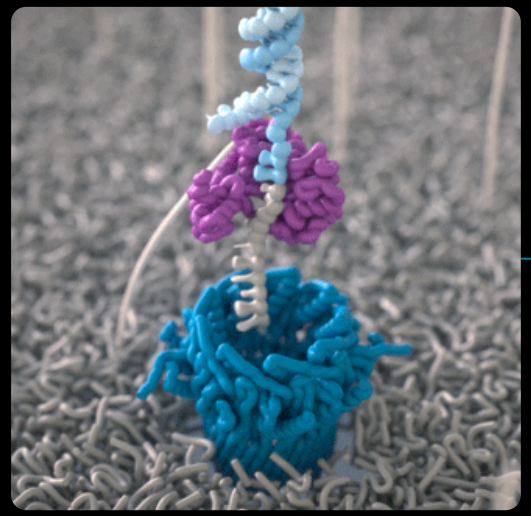


With nanopore unique features

Simple workflows | Real-time analysis | Upgrades included

*Theoretical max output when system is run for 72 hours at 400 bases / second. Outputs may vary according to library type, run conditions, etc.

One core technology at any scale



Flow Cells

Devices

MinION

PromethION

MinION Mk1D

GridION™

PromethION 2 Solo

PromethION 2 Integrated

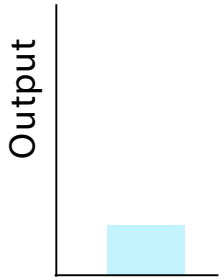
PromethION 24

= compute on board

It starts with a flow cell

A single chemistry delivered across increasing number of nanopores

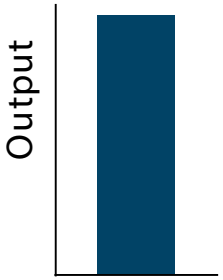
MinION



Choose MinION™

- ~35 Gb of data Output
- Multiplex small genomes
- Low-pass sequencing of larger genomes

PromethION

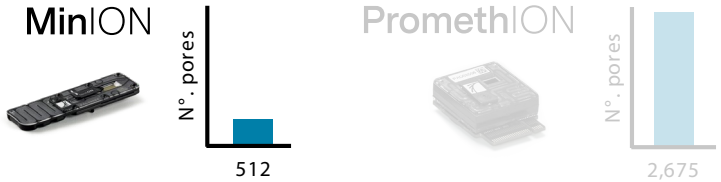


Choose PromethION™

- Generate hundreds of Gigabases of data
- Sequence large genomes to high coverage

MinION Flow Cells

Flexible and versatile for multiple applications



15-35 Gb native gDNA reads (read N50 ~25 kb)

Multiplexed sequencing of up to 96 samples with PCR or PCR-free barcoding

Highly accurate full-length plasmid sequencing

Wash and re-use for multiple successive libraries with Flow Cell Wash Kit

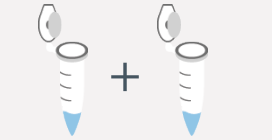
10-20 Gb ultra-long native DNA reads (>50 kb read N50)

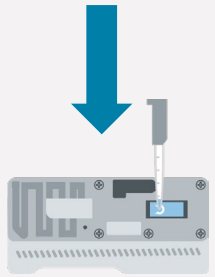
Plus: direct methylation detection, direct RNA sequencing, read lengths from short (20 bases) to ultra-long (>4 million bases), …

MinION Mk1D: real-time sequencing in diverse environment

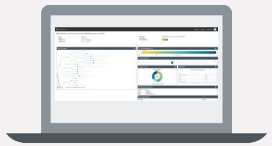
Sequencing of anything, anywhere by anyone in real-time

Simple workflow





Real-time analysis



Find out more: nanoporetech.com/products/minion

Status LEDs provide visual feedback of run progress

Lid blocks out light during sequencing

Improved thermal regulation allows for operation across a broader range of temperatures

Consumable flow cell contains sensing chemistry , nanopore, and electronics

USB-C Port powers device and passes data to PC

Mk1D IT specification

Important - Only USB-C ports are supported to connect your MinION Mk1D to your computer. Ensure the computer you purchase has a USB-C port available. USB-A to USB-C adapters or USB-A to USB-C cables are not supported. USB-A ports typically cannot deliver enough power for the MinION Mk1D and may compromise sequencing performance.

Component	Windows, Linux	macOS
Operating system	Windows 10/11, Ubuntu 20.04/22.04 LTS	macOS
Peripheral	USB Type-C (USB 2.0 speeds or greater)	USB Type-C (USB 2.0 speeds or greater)
Memory	16 GB or higher	16 GB or higher
GPU	NVIDIA RTX 4070 or higher	Apple M3 Max
CPU	Intel or AMD Processor with at least 4 cores/8 threads	Apple M3 Max
Storage	1 TB SSD or greater	1 TB SSD or greater

We recommend internal solid-state storage for MinKNOW installation as well as data output/acquisition. Solid-state drives are much faster than traditional hard drives and are able to keep up with the flow of data generated during a sequencing run.

Example laptops meeting spec (non exhaustive list)

Razer blade 18 (RTX 4070 or greater)

Apple MacBook Pro 14” M3 Max

Benchmarked to run all different basecalling models of over a 72 hour run

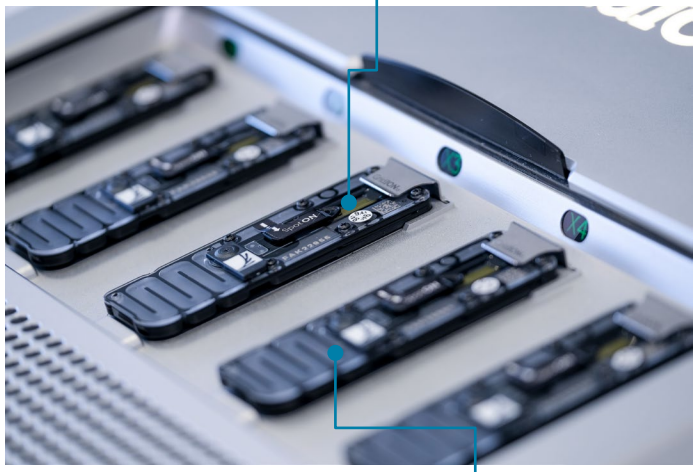
GridION Mk1

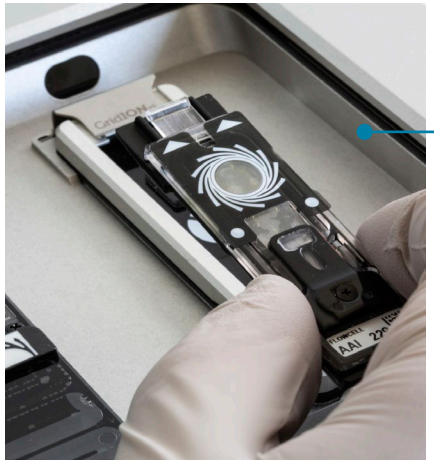
Self-contained, on-demand benchtop sequencing device

- Runs up to five MinION Cell (allows optimisation)
- Individually addressable flow cells: multiple users may start and stop experiments as needed
- High end compute:
  V100 GPU: 122 TFLOP
  64 GB RAM, 4 TB SSD

Onboard compute: simple installation, designed for real-time analysis

Sample added to flow cell here. DNA/RNA/cDNA from any sample, for any application





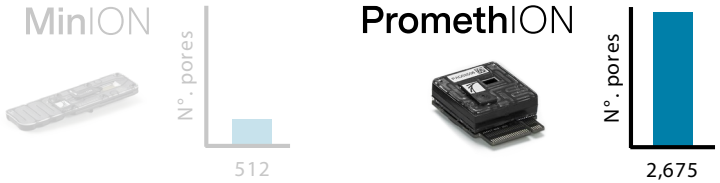
Flexible: runs up to 5 MinION or Flongle Flow Cells at any one time

Modular/on-demand: use as many flow cells as required, for as long as is needed; MinION and/or Flongle

Find out more : nanoporetech.com /products/ gridion

The PromethION flow cells

High output for multiple applications



100-200 Gb native gDNA reads (read N50 ~25 kb)

Multiplexed sequencing of up to 96 samples with PCR or PCR-free barcoding

>200 Gb on metagenome samples (read N50 ~10kb)

>100 million cDNA reads for isoform-level analysis

50-100 Gb ultra-long native DNA reads
(>50 kb read N50)

Plus: direct methylation detection, chromatin conformation capture with Pore-C, direct RNA sequencing, read lengths from short (20 bases) to ultra long (>4 million bases), …

The PromethION 2 devices

PromethION 2 Solo (P2 Solo)

Adapt existing resource for high-output nanopore sequencing

H 152 x W110 x D 87mm

Component	Specification
Integrated compute	N/A – Run from GridION or computer/laptop
Number of flow cell positions	2
Power requirements	100-240 V 50/60 Hz
OS	Compatible with Windows, Linux, MacOS

PromethION 2 Integrated (P2i)

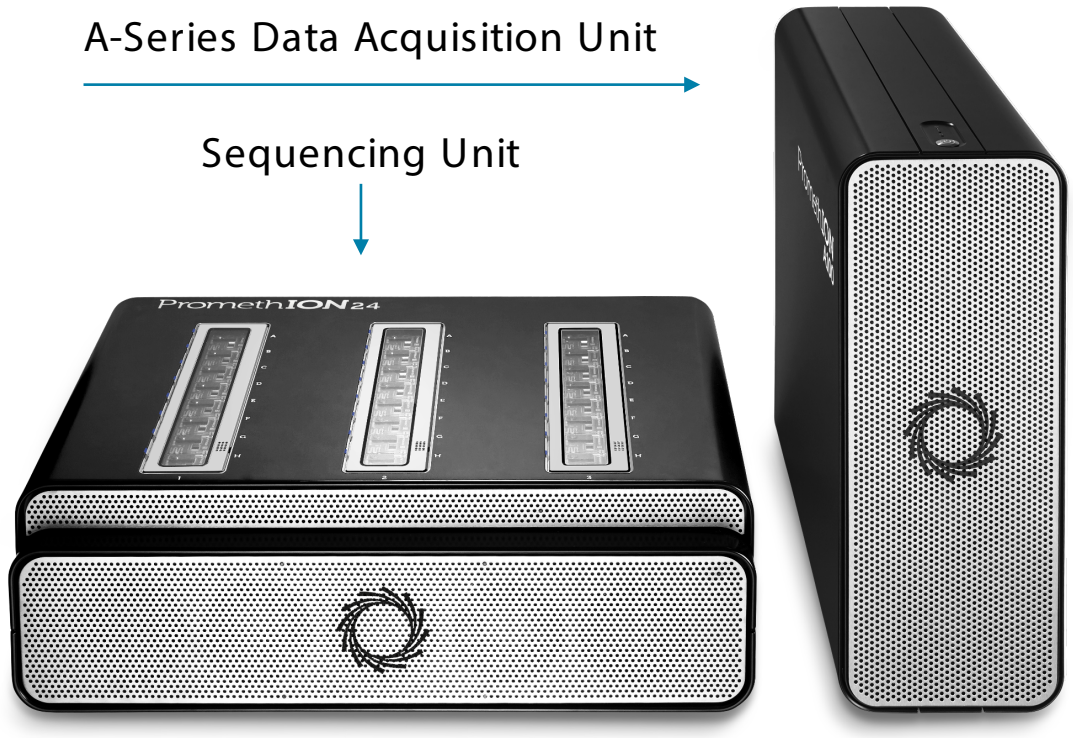
Plug-and-play, self-contained device for high-output nanopore sequencing

H 152 x W110 x D 87mm

Component	Specification
Integrated compute	1x Ampere GPU, Intel CPU
Number of flow cell positions	2
Power requirements	TBD
OS	Ubuntu

PromethION™ 24

High-throughput, high-output, flexible sequencing on your benchtop



H 190 x W 590 x D 430 mm, 28 kg

H 440 x W 178 x D 470mm, 25 kg

Component	Specification
Integrated compute	4x NVIDIA Ampere GPU, Intel CPU, 60 TB SSD, 512 GB RAM
No. flow cell positions	24
Power requirements	Supply voltage: 100-240v (50/60Hz) Maximum power: 2200 W
OS	Ubuntu 24.04

Unrivalled insight into human research

- Real-time sequencing and basecalling – methylation included
- Genome sequencing at scale, compatible with automation
- Medium to high-throughput DNA/RNA project capabilities

Flow cell warranty & return

Simple process

Warranty of fully released products

MinION: use within 12 weeks. QC before use ≥ 800 pores
PromethION: use within use within 12 weeks. QC before use ≥ 5,000 pores
We will replace flow cells that do not pass the flow cell check

Return process

1. Washing (optional)
2. Packing
3. Completing returns form

The packaging serve as a returns pouch and instructions are available in the link below:
https://community.nanoporetech.com/support/returns/flow_cells

Introduction to library preparation kit

Oxford NANOPORE Technologies

Nanopore sequencing chemistry

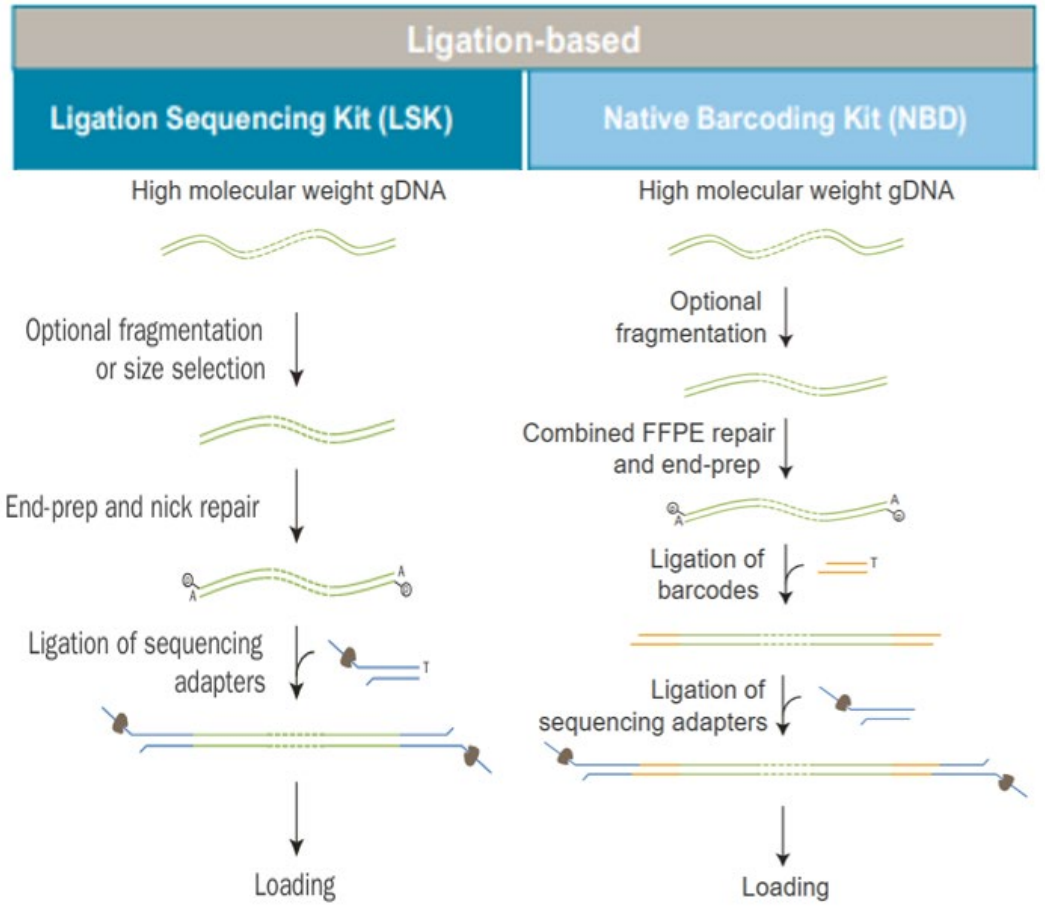
Library preparation: converting sample into a format compatible with nanopre sequencing

	DNA					RNA	
	Output optimised	Speed optimised	Ultra-long reads optimised	Low input optimised	Targeted sequencing	Direct RNA	cDNA
	Ligation Sequencing Kit	Rapid Sequencing Kit	Ultra-Long DNA Sequencing Kit	Rapid PCR Barcoding Kit	16S Barcoding Kit	Direct RNA Sequencing Kit	cDNA-PCR Sequencing Kit
Preparation time	60 mins	10 mins	200* mins + 1xO/N incubation	15 mins + PCR	25 mins + PCR	135 mins	~225 mins + PCR
Input recommendation	~1000 ng gDNA 100 – 200 fmol amplicons	~100 ng	6M cells	1 – 5 ng	10 ng	300 ng poly(A)+ RNA or 1 µg total RNA	10 ng poly(A)+ RNA or 500 ng total RNA
Fragmentation	Optional	Transposase-based	Transposase-based	Transposase-based	-	-	-
Amplification	No	No	No	Yes	Yes	Optional reverse transcription	Yes
Barcode options	Native Barcoding Kits 24 & 96 plex	Rapid Barcoding Kits 24 & 96 plex	-	24 plex	24 plex	*In development*	cDNA-PCR Barcoding Kit 24 plex
Typical output	●●●	●●○	●●○	●●○	●●○	20M reads	>100M reads
Adaptive sampling	✓	✓	✓	✓		*In development*	*In development*
Methylation included	✓	✓	✓			✓	
Highlights	• Base modifications preserved and included • Automatable workflows on various liquid handlers, and XL kits enable production-scale sequencing			Ideal for low input		Base modifications preserved and included with the Direct RNA Sequencing Kit	

Ligation sequencing kit V14

Ligation Sequencing kit V14 (SQK-LSK114)

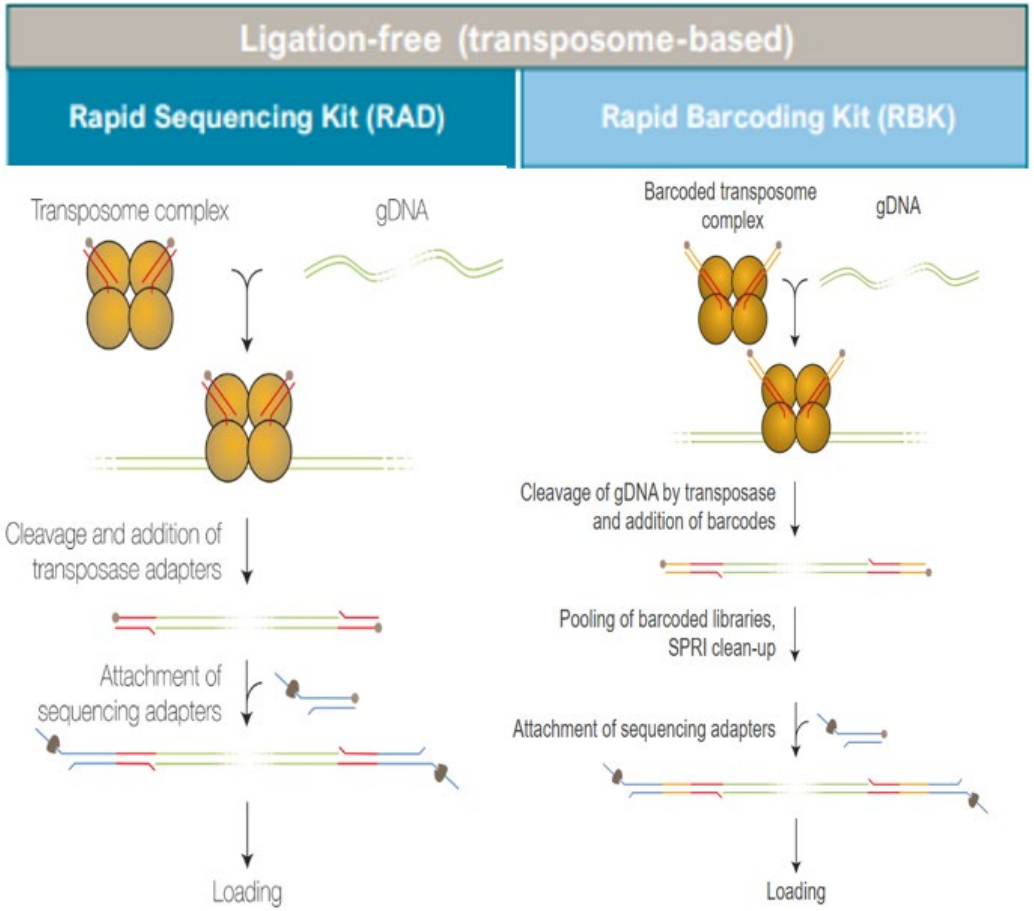
- Ligation Sequencing kit, Native Barcoding kit (24 or 96)
- Prep is simple and straightforward, ~140 mins
- gDNA, amplicons
- PCR-free
- Number of preps : 6 reaction



Rapid Sequencing kit V14

Rapid Sequencing kit (SQK-RSK114)

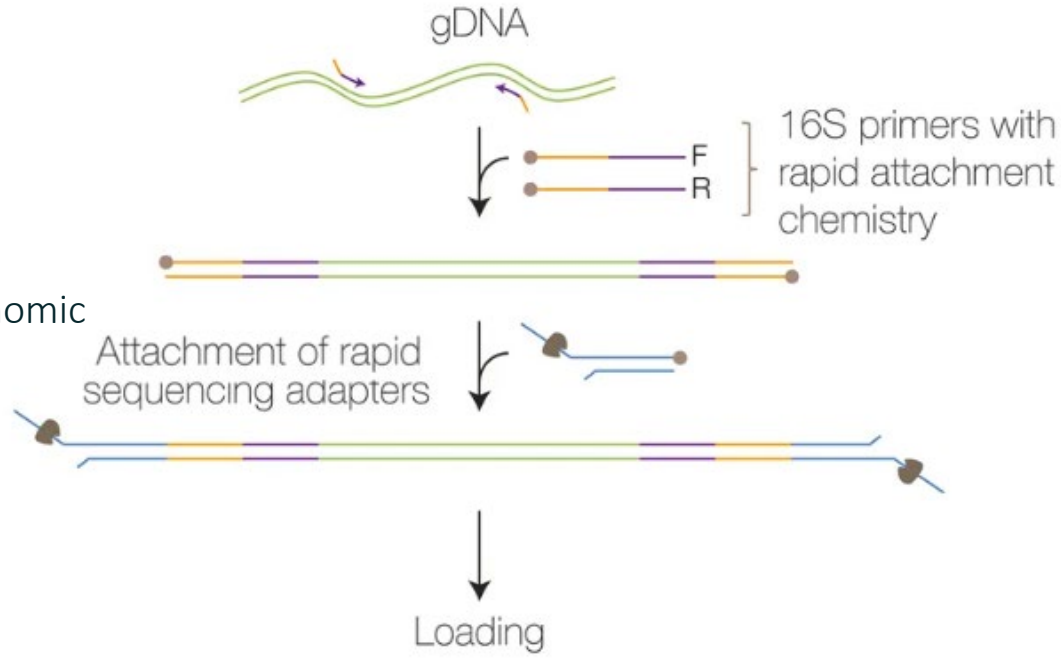
- Rapid Sequencing kit, Rapid Barcoding kit (24 or 96)
- Transposome-based
- Skip the end repair step
- Perform cleavage and adapter ligation concurrently
- gDNA, amplicons
- PCR-free
- Number of preps : 6 reaction



Targeted Sequencing : 16S rRNA sequencing

16S barcoding kit (SQK-16S114.24)

- 16S barcoding kit 24 V14 (SQK-16S114.24)
- Used for sequencing the 16S rRNA gene region in metagenomic analysis.
- 16S Gene Region: Approximately 1.5 kb
- Includes 24 barcodes
- sequencing the entire region from V1 to V9



Targeted Sequencing : Adaptive Sampling

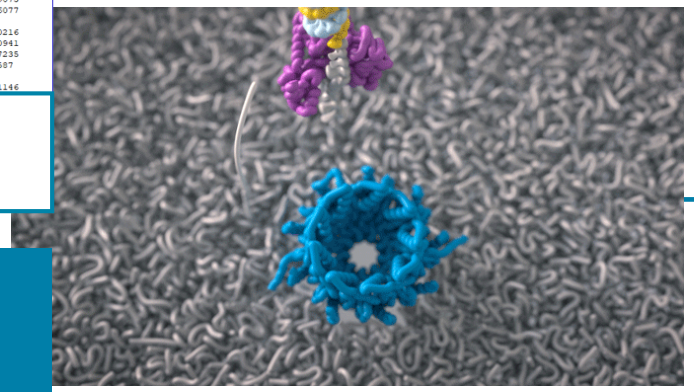
Adaptive Sampling

nanopore_adaptive_seq_cosmic.bed

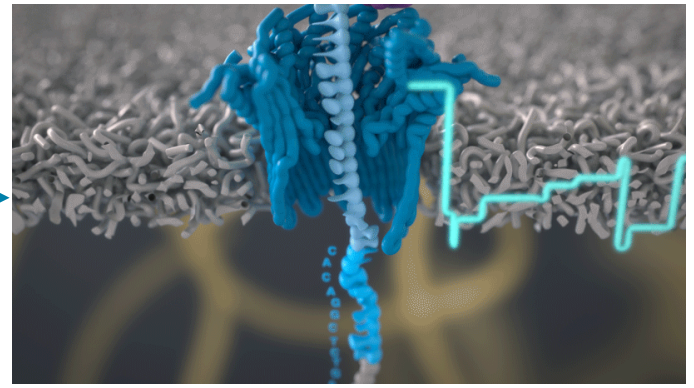
1	chr1	2228695	2310119	SKI	28.67	81424
2	chr1	2556366	2563829	TNFRSF14	11.35	7463
3	chr1	3069211	3438621	PRDM16	30.67	369410
4	chr1	6185020	6199612	RPL22	26.96	14592
5	chr1	6785324	7769706	CAMTA1	33.13	984382
6	chr1	11106535	11262507	MTOR	31.63	155972
7	chr1	13704855	13825079	PRDM2	36.52	120224
8	chr1	15492270	15524295	CASP9	30.93	32025
9	chr1	15847864	15940460	SPEN	32.57	92596
10	chr1	17018722	17054170	SDHB	30.83	35448
11	chr1	17539835	17697869	ARHGEF10L	32.24	158034
12	chr1	18631006	18736138	PAX7	31.2	105132
13	chr1	23557918	23559794	ID3	22.95	1876
14	chr1	23581495	23640568	MDS2	33.43	59073
15	chr1	26696033	26782110	ARID1A	34.14	86077
16	chr1	32251239	32286165	LCK	36.92	34926
17	chr1	35182932	35193148	SFPQ	34.06	10216
18	chr1	36224616	36305357	THRAP3	37.17	80941
19	chr1	36466043	36483278	CSF3R	26.94	17235
20	chr1	39895426	39902013	MYCL	26.67	6587
21	chr1	43337849	43352772	MPL	34.28	14923
22	chr1	45329242	45340388	MUTYH	27.97	11146

Upload reference file to MinKNOW

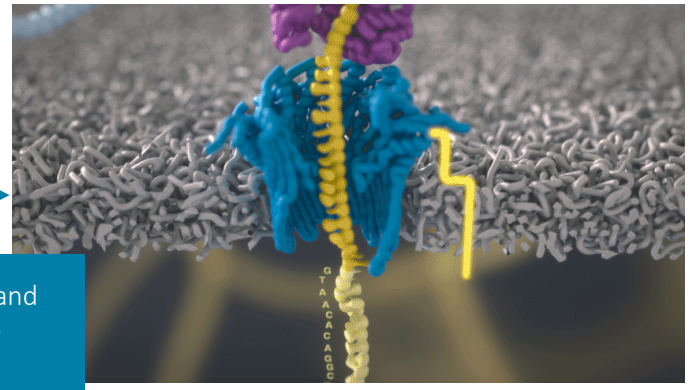
Strand approaches nanopore and sequencing starts



Real-time base calling and alignment



Region of interest? Strand allowed to continue sequencing



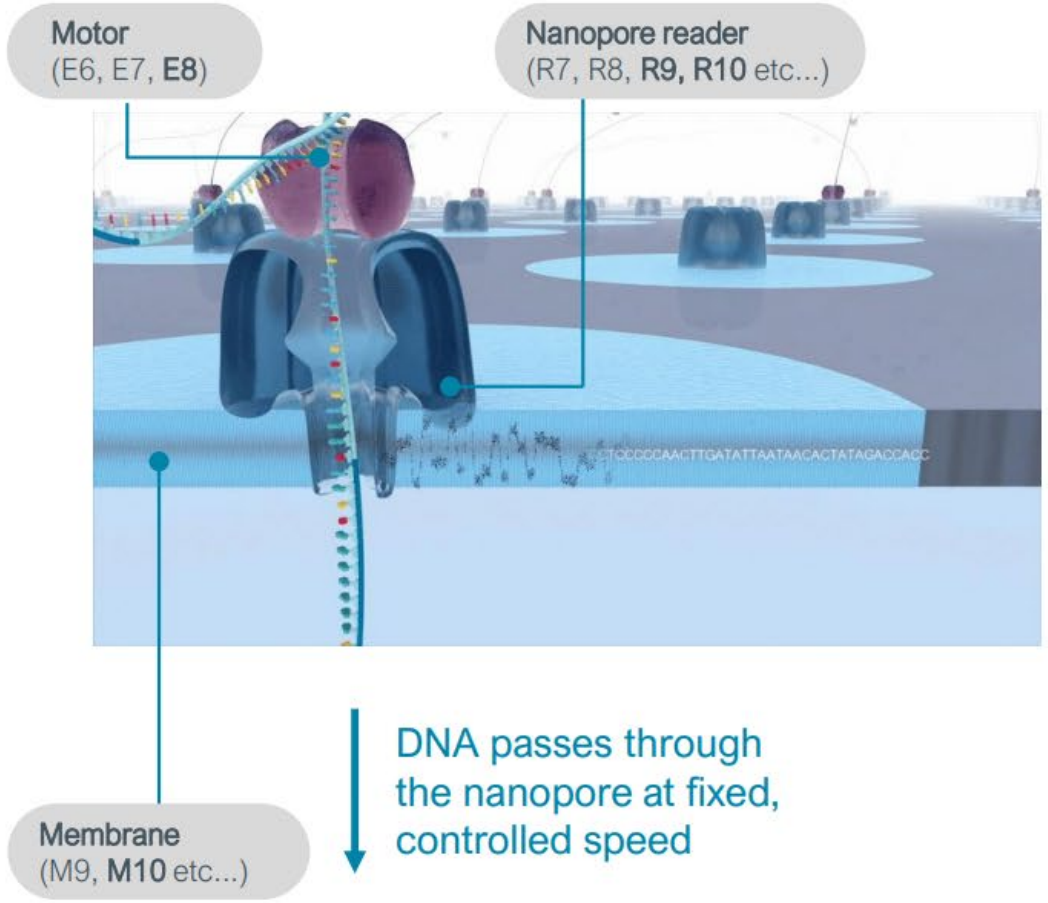
Oxford NANOPORE Technologies

Preparing libraries

Oxford NANOPORE Technologies

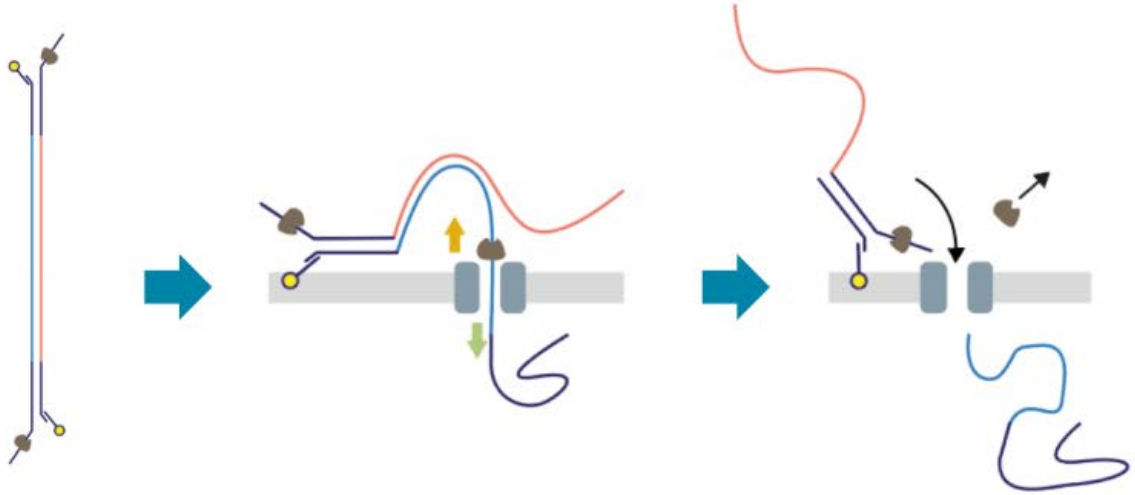
Nanopore sequencing chemistry

Library preparation: converting sample into a format compatible with nanopore sequencing



Library prep for nanopore sequencing

- Attachment of "sequencing adapter"
- Motor protein is pre-bound to adapter
- Adapter facilitates tethering
- Both strands can be sequenced



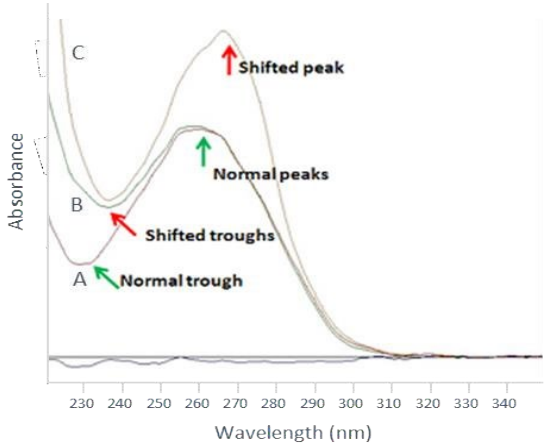
Sample QC

Quantification: Qubit fluorimeter

Purity: Nanodrop

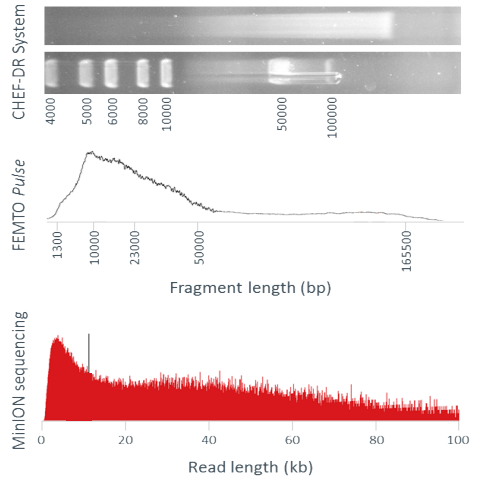
OD measurement of pure DNA :

- A260/A280 = ~1.8
- A260/A230 = ~2.0-2.2



Quality: Standard gel-based analysis, Agilent Bioanalyzer/Tapestation

- Fragment length size measurement is an essential factor for accurate DNA quantification.



Library storage

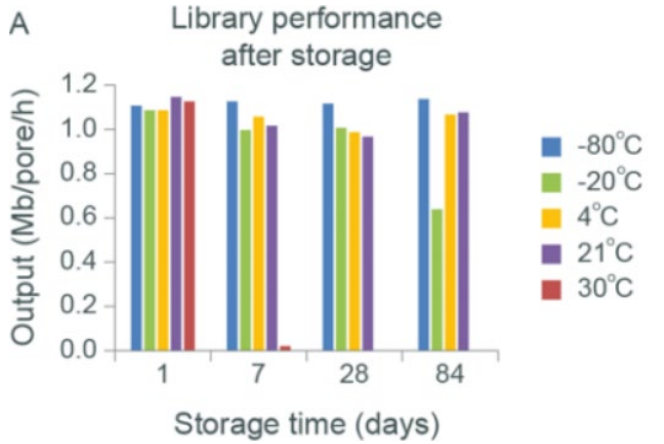
- The final step of the DNA library preparation is eluting the prepared library from the Elution buffer, and the library can be stored after this step

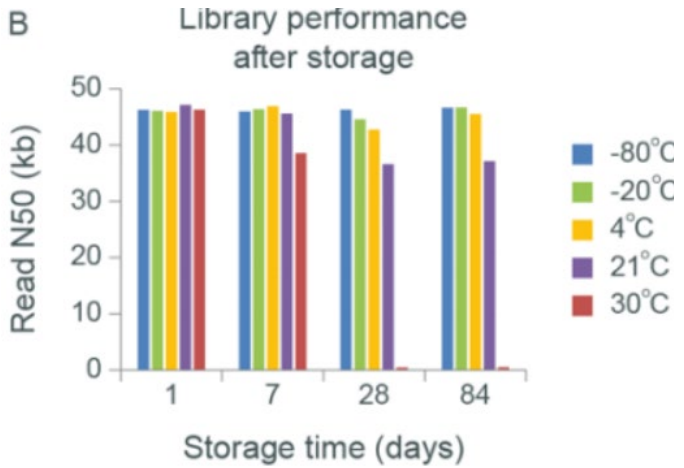
- For short-term storage or library re-use

  : Store at 4°C in an Eppendorf DNA LoBind tube

- For single-use or long-term storage (more than 3 months)

  : Store at -80°C





Flow cell Light Shield

Maximizing the output

- The light shield is included with the flow cell, and attaching it **increases sequencing output by approximately three times** .
- To achieve maximum data output, it's essential to attach the light shield to the flow cell before starting sequencing.

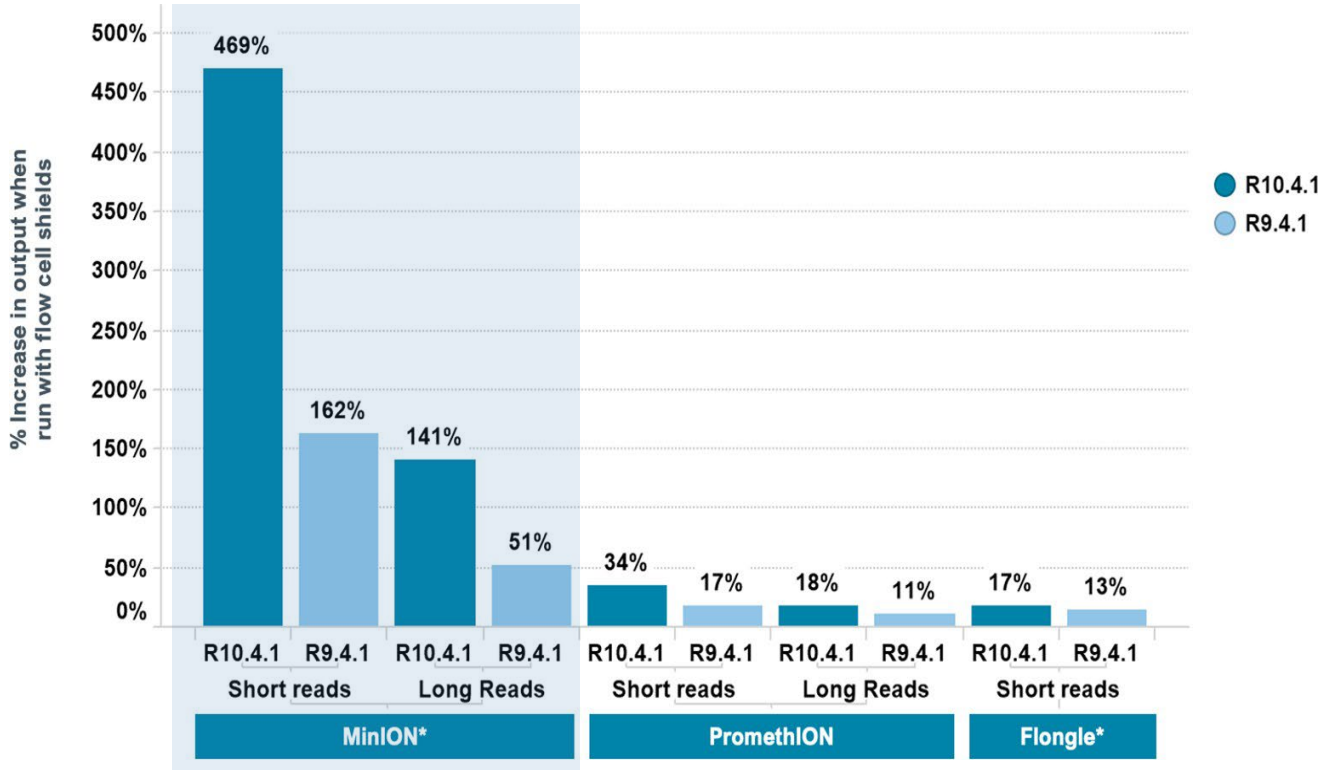
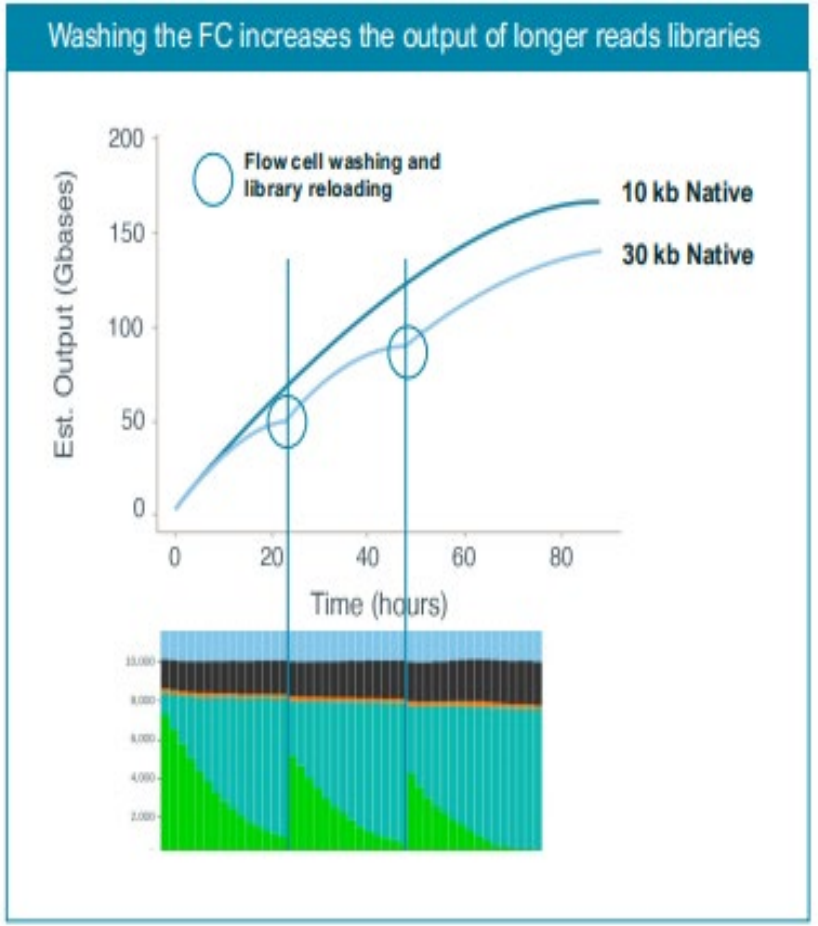


*Figure: Example % Increase in output from flow cells where the array is shielded from light during sequencing in internal experiments. R10.4.1 and flow cells with short fragment libraries observe the most benefit to running in the dark. Short reads = 200bp amplicon. Long reads = 30Kb N50 human native DNA. Samples prepped with SQK-LSK114 (R10.4.1) or SQK-LSK110 (R9.4.1) reagents. Shielding of light with flow cell light shields. *MinION and Flongle Flow Cells were run on a GridION device.*

Flow Cell Wash Kit (EXP-WSH004)

Maximizing flow cell usage

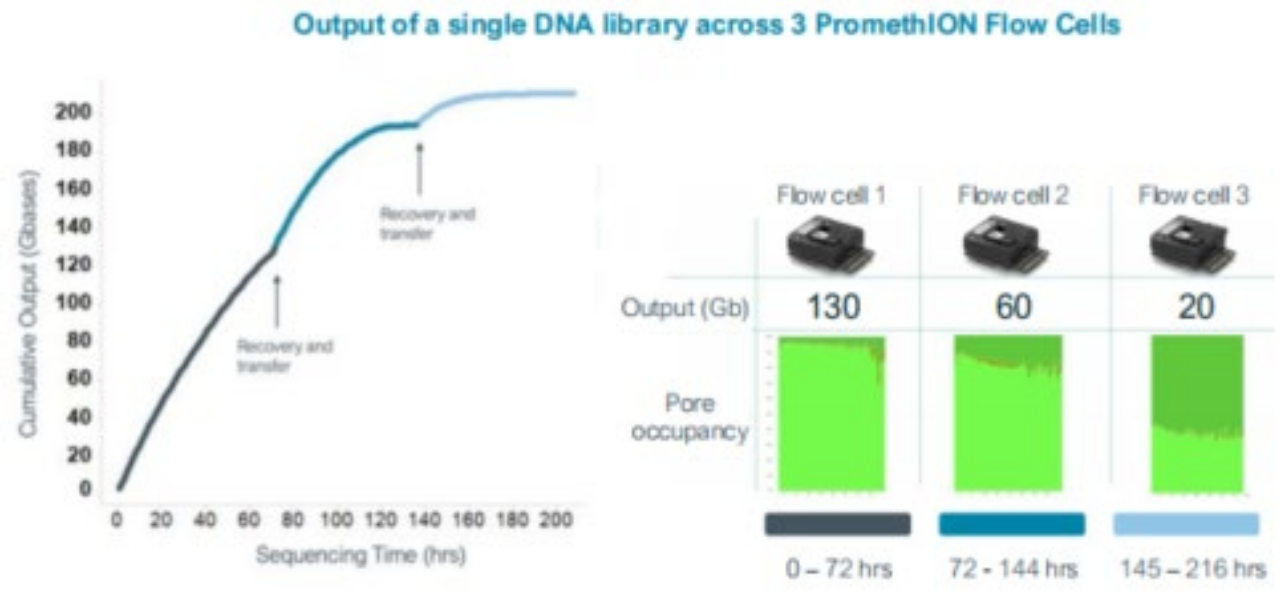
- Removes up to 99.9% of template molecules from previous library
- Uses a nuclease to digest library strands only DNA
- A fresh library can be loaded and sequenced on the same flow cel;
- The number of flow cells: 6



Recover DNA library and reuse it

Maximizing the data output with one library

- Run – Recover library – Run on a new flow cell
- Used when the samples available for sequencing are limited.
- Applicable when an issue occurs during sequencing or flow cell loading.
- Can be applied to both MinION and PromethION flow cells.
- Applicable to all libraries generated with Oxford Nanopore Technologies' DNA library preparation kits.



Introduction of Software : MinKNOW

Oxford NANOPORE Technologies

MinKNOW software

Device control → Data collection → Live basecalling → Analysis

Key functions

1. Hardware check

- Checking for any issues in the operation of the software and device

2. Flow cell check

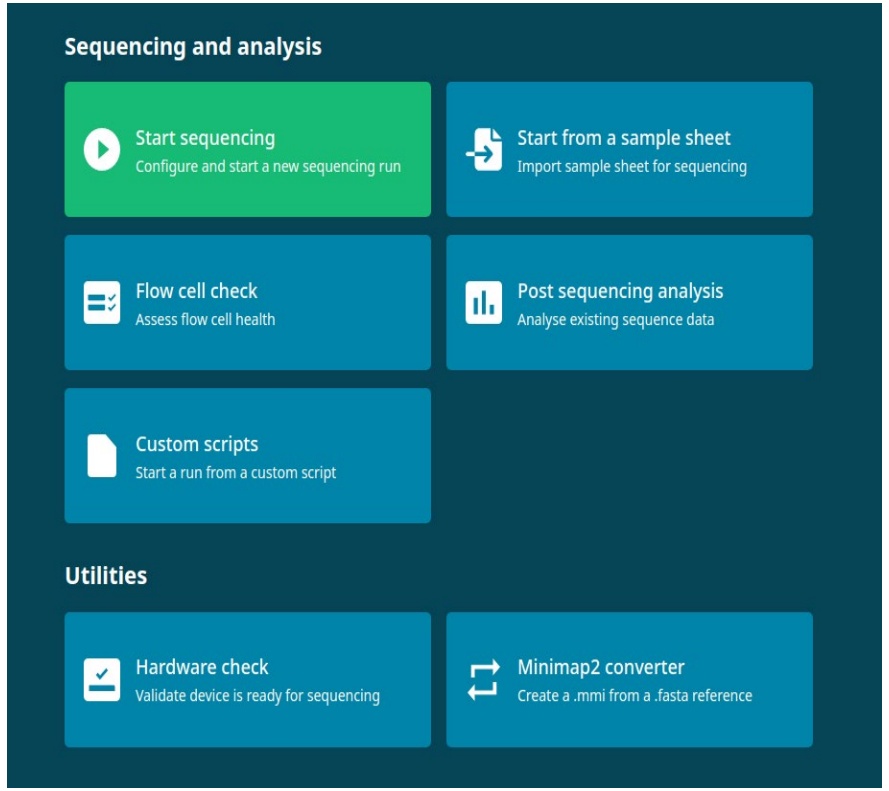
- Checking the membrane integrity and number of pores in the flow cell

3. Start sequencing

- Initiates the steps and start of the sequencing run

4. Analysis

- Performs post-run basecalling, demultiplexing, and alignment

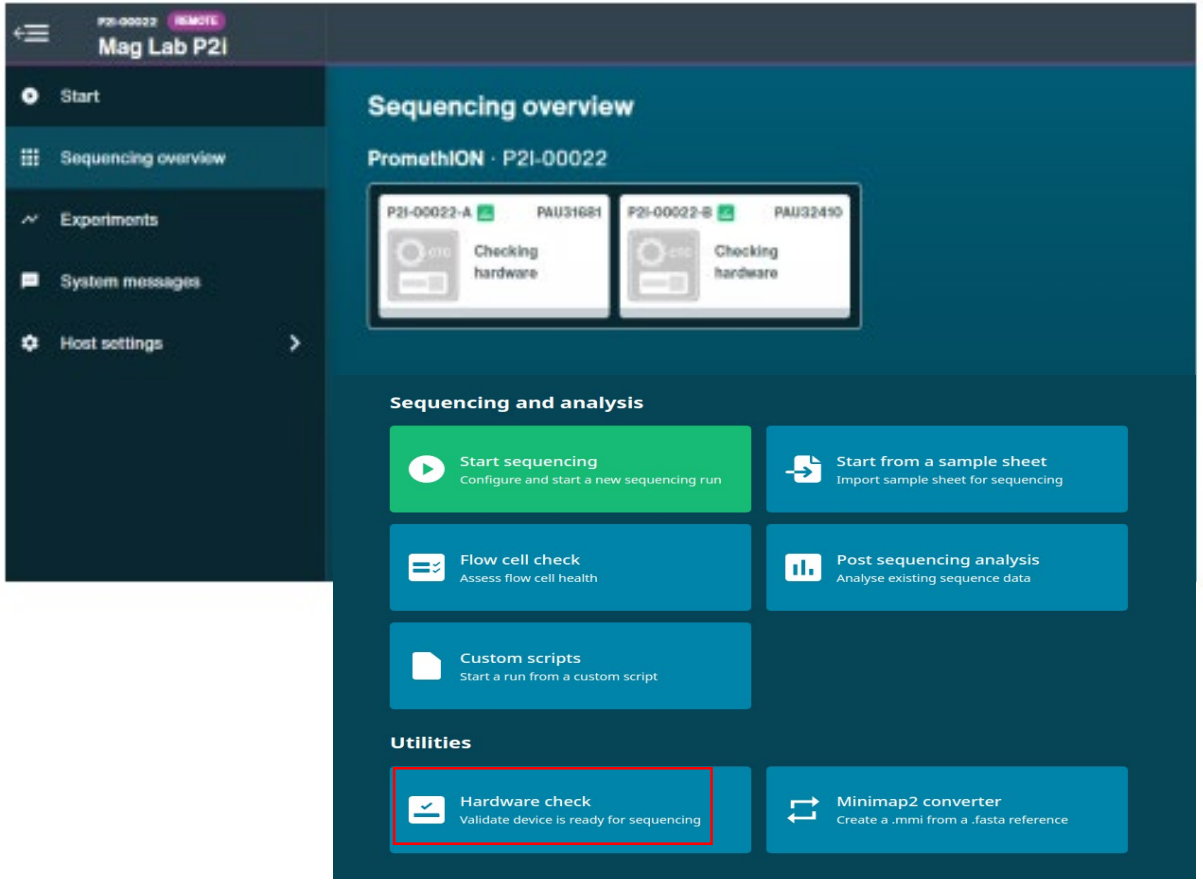


Hardware check

Checking for any issues in the operation of the MinKNOW software and device

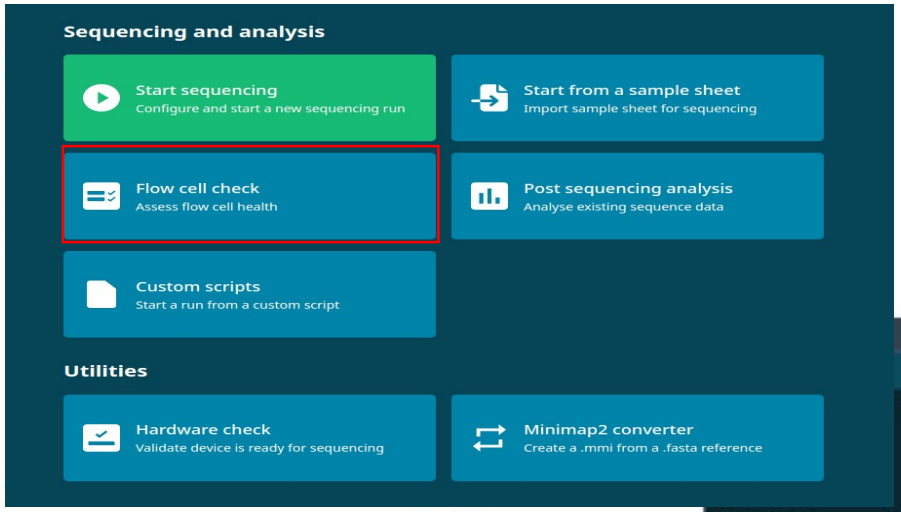
Processing Hardware check

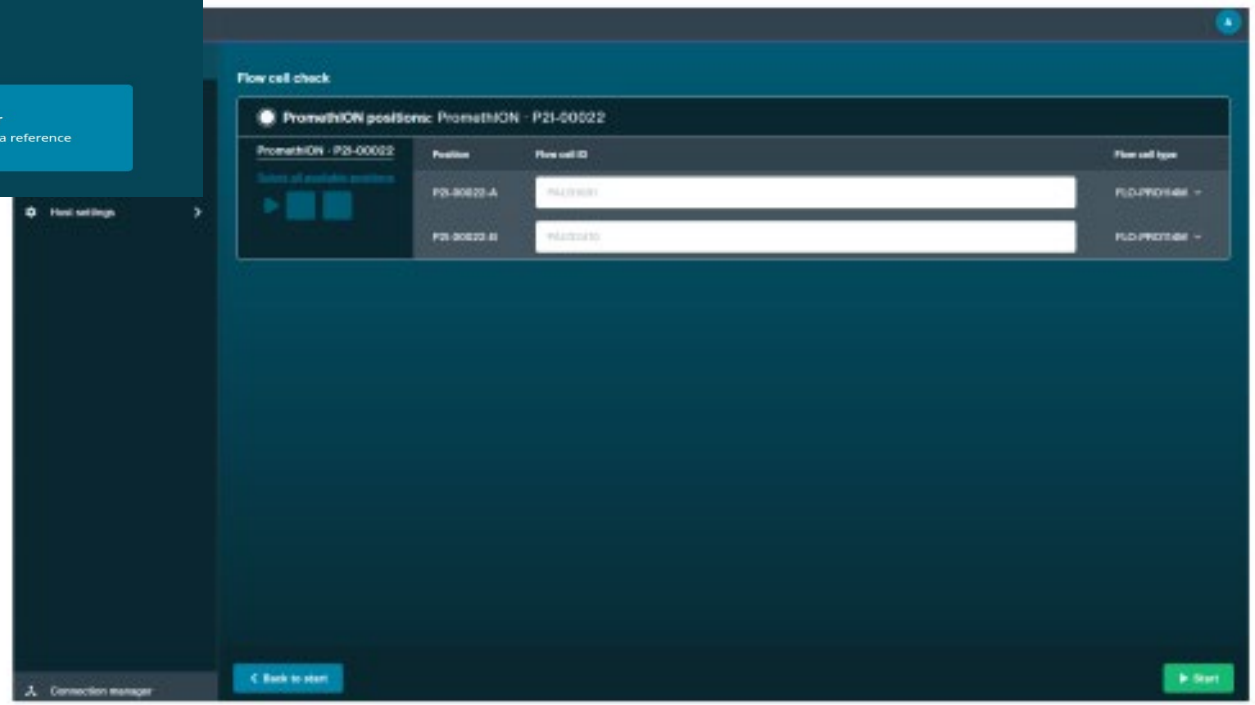
1. Mount CTC (Configuration Test Cell) onto the device
2. Click the “Hardware check” icon
3. Check whether the CTC is recognized at each position of the device
4. Click the “Start” button
5. Wait about one minute
6. Check a green check mark (v) appears on the position – “Hardware check passed”



Flow cell check

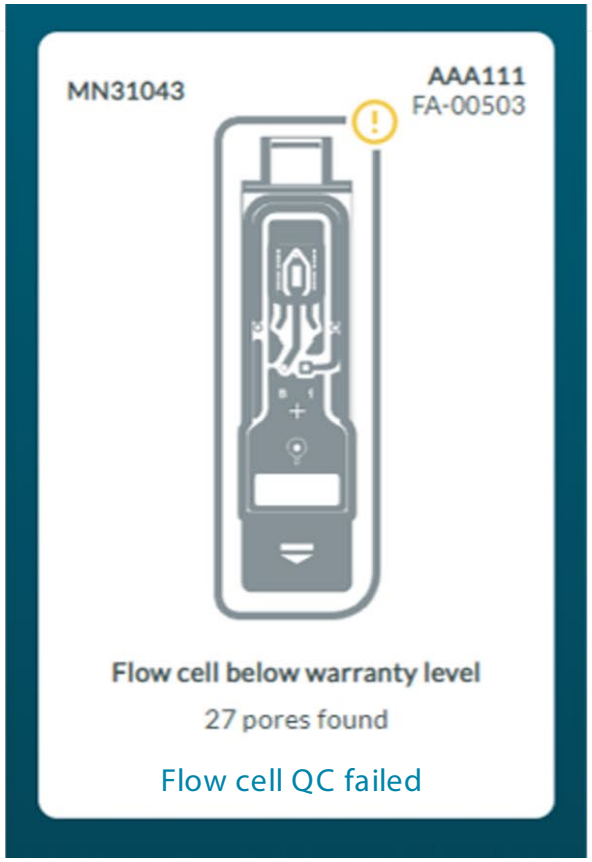
Checking the membrane integrity and number of pores in the flow cell



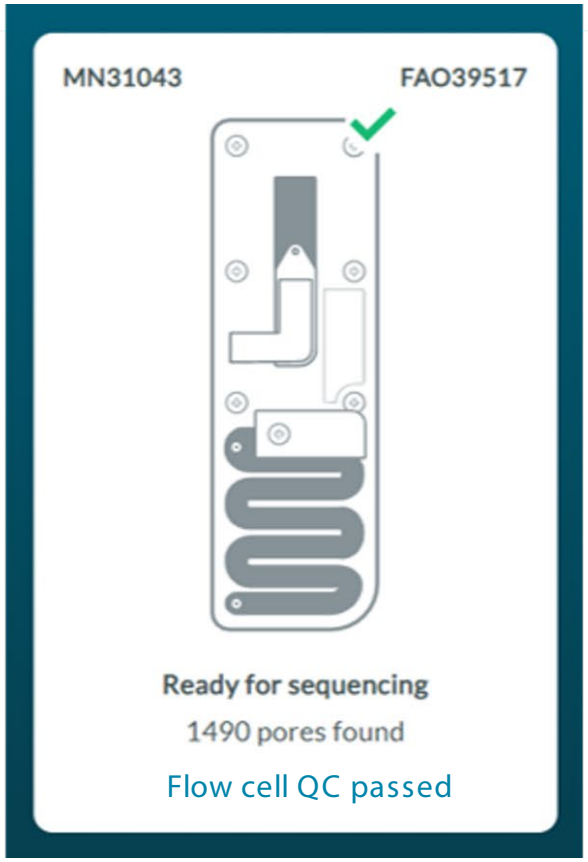


Flow cell check

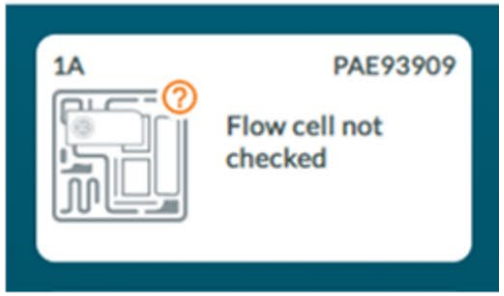
Checking the membrane integrity and number of pores in the flow cell



Flow cell QC failed



Flow cell QC passed



Not performing Flow cell QC (restart Flow cell Check)

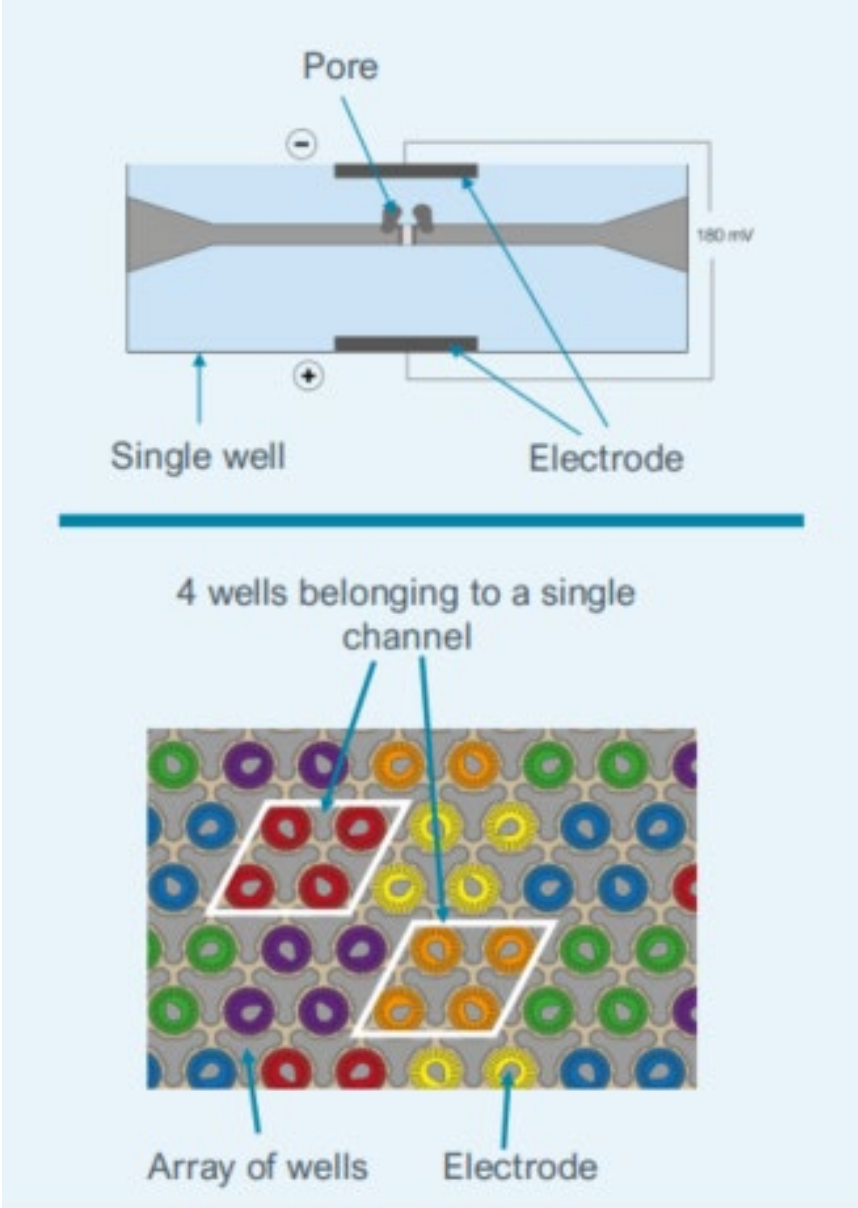
Meaning of Pore Scan and flow cell check

Mux scan

- Flow cell consists of wells and electrodes
- Each well has its own electrode and an individual nanopore
- In MinION flow cell, it is composed of 512 nanopore channels - 2,048 wells.
- In PromethION flow cell, it is composed of 2675 nanopore channels - 10,700 wells

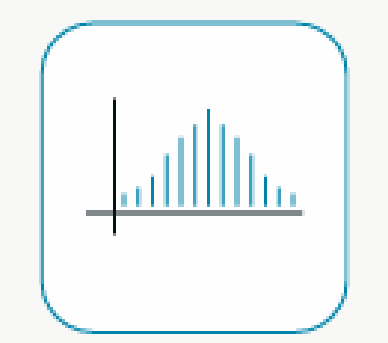
Pore scan

- During sequencing, the current generated in each channel is recorded individually, and each well is tested to select the one well (pore) that will first perform sequencing.
- All sequencing scripts start with a Pore scan



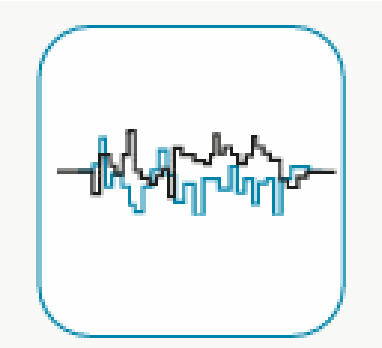
MinKNOW : Overview

Our devices software: from basecalling to reporting



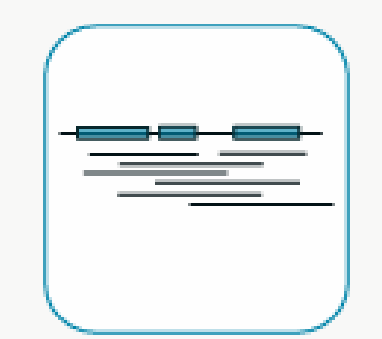
Real time QC

- Real-time quality control of the run
  - Stop and resume as needed



Basecalling

- Real-time basecalling
  - Choose desired accuracy
- Methylation calling



Analyse your data

- Seamless Analysis in real time including
  - Enrichment or depletion with Adaptive sampling
  - Alignment
  - Barcode demultiplexing

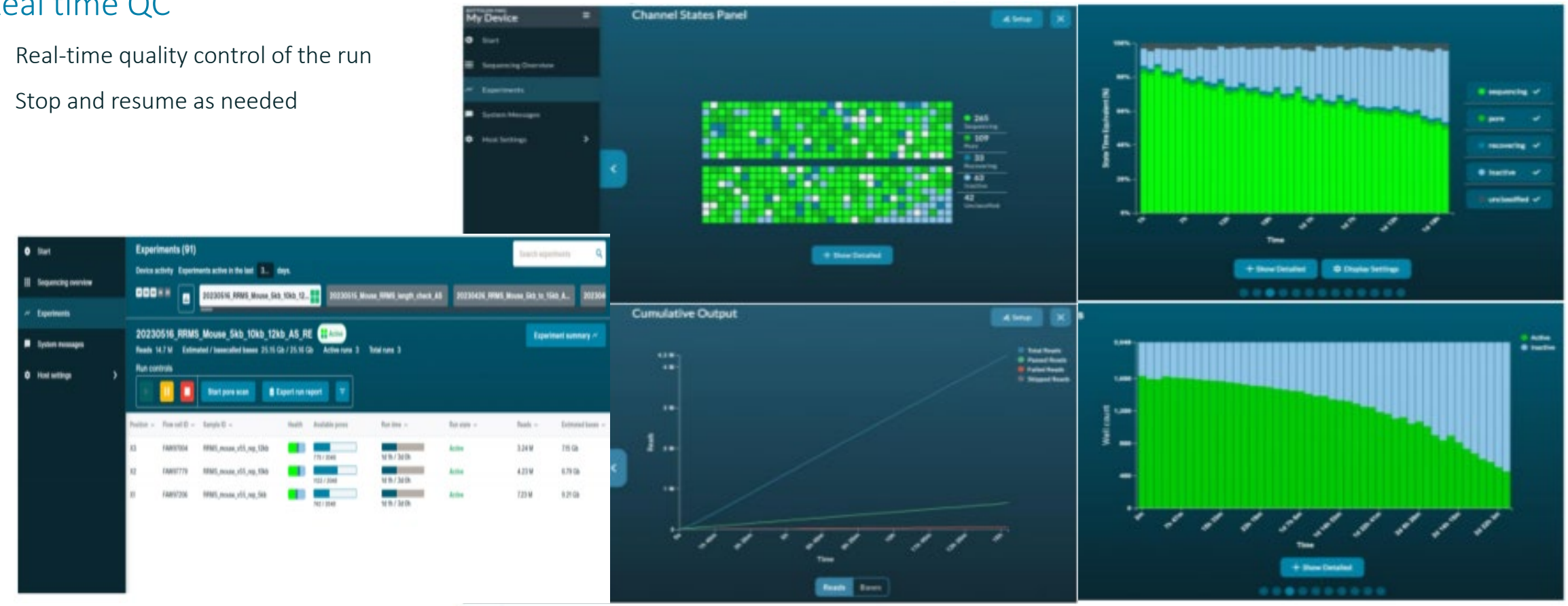
Generate automatic report

- QC reports of each run

MinKNOW : Real time QC

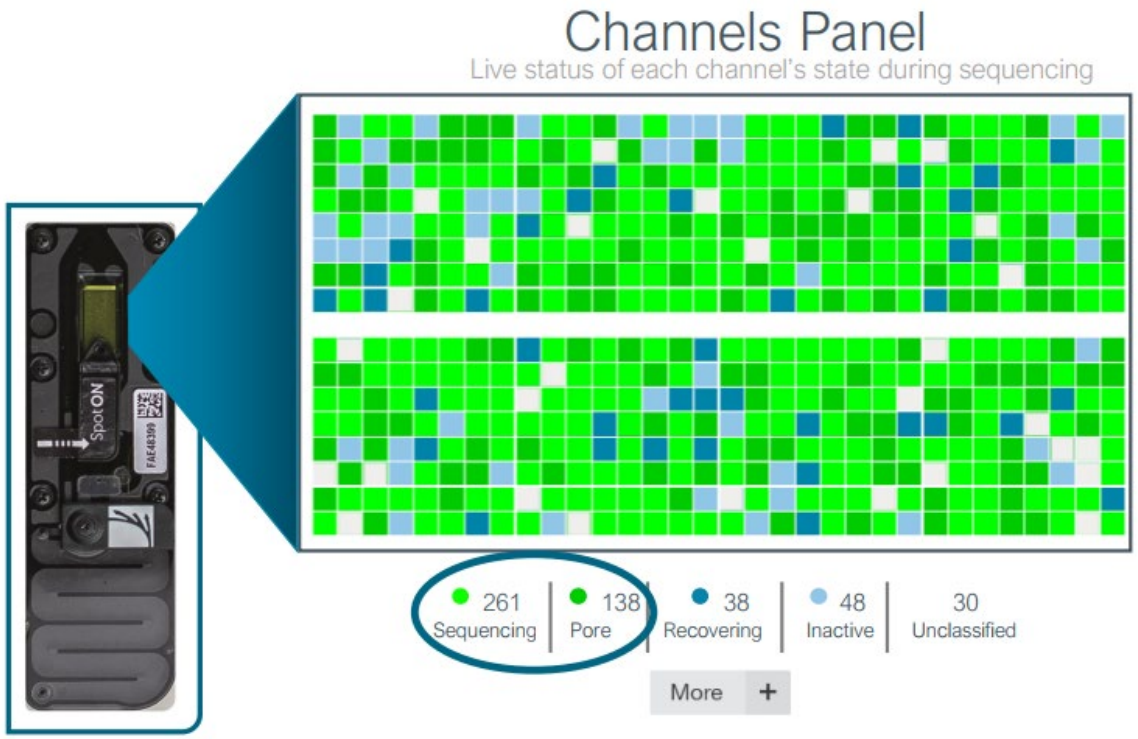
Real time QC

- Real-time quality control of the run
- Stop and resume as needed

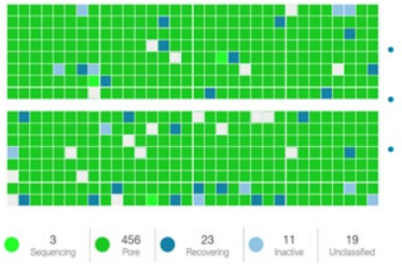


MinKNOW: Monitoring a sequencing run

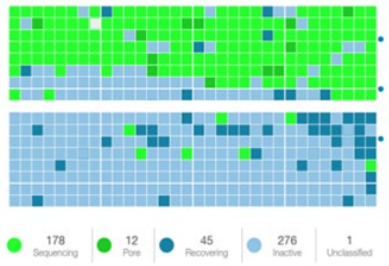
Available real time quality control of the sequencing run



- High ratio of strand to open pore
- Small amount of recovering and inactive
- Successful library preparation



- Very low ratio of strand to open pore
- Small number of recovering and inactive
- Indicates low input amount of DNA or poor attachment of sequencing adapters



- High number of inactive channels
- Clear spatial pattern
- Indicates an air bubble has been introduced onto the sensor array

MinKNOW : Basecalling

Data formats

Oxford Nanopore format — POD5 (or FAST5)

- Contains raw signal data and read metadata, permitting re-basecalling
- Large in size
- Optional

Standard format — FASTQ

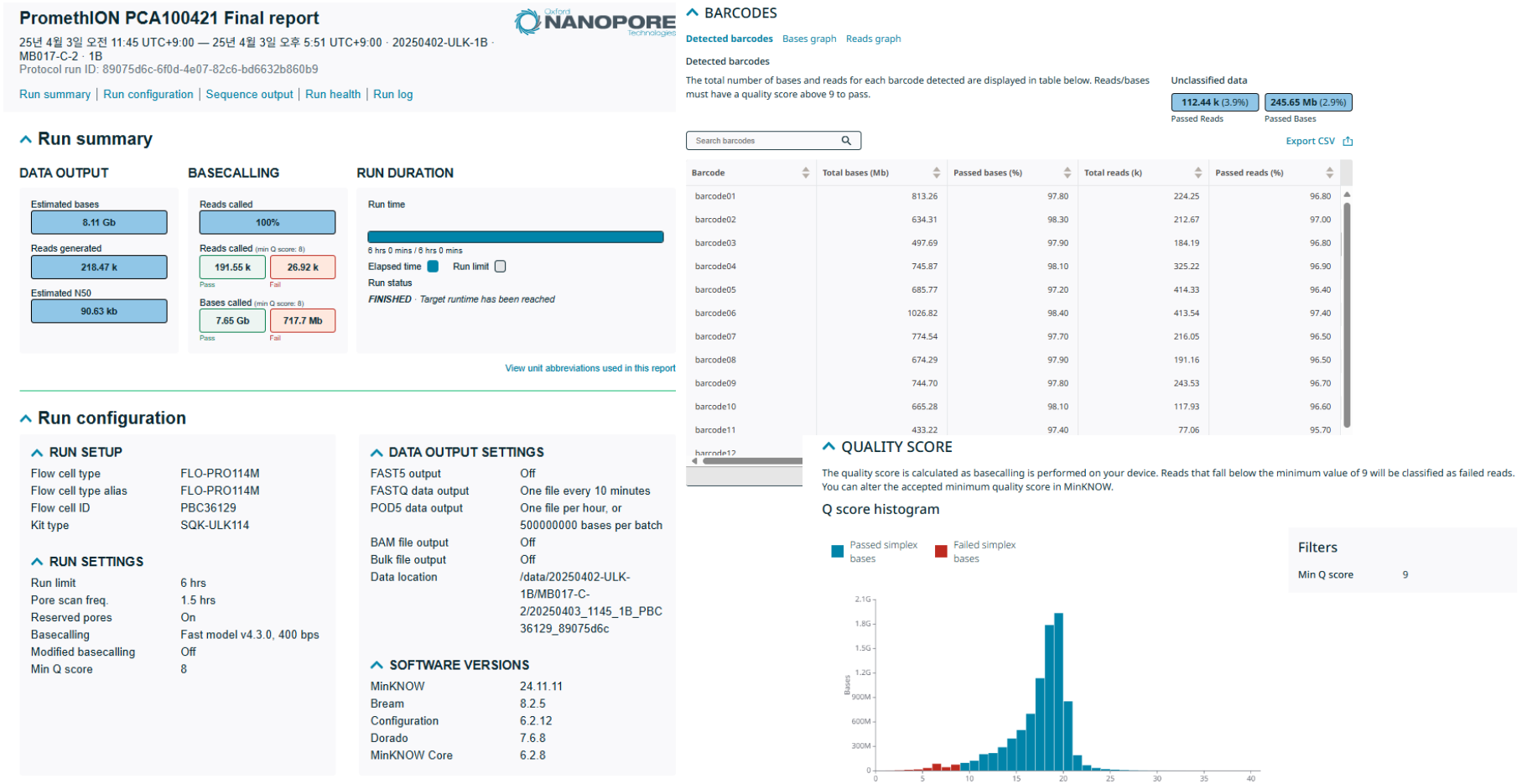
- Standard format for basecalled sequence data with per-read quality scores
- Text-based file format, small in size
- Default

Standard format — BAM

- Standard sequence alignment format
- Compressed text-based format, small in size
- Optional / default for modified bases

MinKNOW: Sequencing run report

Summary of the sequencing run



PromethION PCA100421 Final report

25년 4월 3일 오전 11:45 UTC+9:00 — 25년 4월 3일 오후 5:51 UTC+9:00 · 20250402-ULK-1B · MB017-C-2 · 1B
Protocol run ID: 89075d6c-6f0d-4e07-82c6-bd6632b860b9

Run summary | Run configuration | Sequence output | Run health | Run log

Run summary

DATA OUTPUT
Estimated bases: 8.11 Gb
Reads generated: 218.47 k
Estimated N50: 90.63 kb

BASECALLING
Reads called: 100%
Reads called (min Q score: 8): 191.55 k (Pass) / 26.92 k (Fail)
Bases called (min Q score: 8): 7.65 Gb (Pass) / 717.7 Mb (Fail)

RUN DURATION
Run time: 6 hrs 0 mins / 6 hrs 0 mins
Elapsed time | Run limit
Run status: *FINISHED* · *Target runtime has been reached*

View unit abbreviations used in this report

Run configuration

RUN SETUP

Flow cell type	FLO-PRO114M
Flow cell type alias	FLO-PRO114M
Flow cell ID	PBC36129
Kit type	SQK-ULK114

RUN SETTINGS

Run limit	6 hrs
Pore scan freq.	1.5 hrs
Reserved pores	On
Basecalling	Fast model v4.3.0, 400 bps
Modified basecalling	Off
Min Q score	8

DATA OUTPUT SETTINGS

FAST5 output	Off
FASTQ data output	One file every 10 minutes
POD5 data output	One file per hour, or 500000000 bases per batch
BAM file output	Off
Bulk file output	Off
Data location	/data/20250402-ULK-1B/MB017-C-2/20250403_1145_1B_PBC36129_89075d6c

SOFTWARE VERSIONS

MinKNOW	24.11.11
Bream	8.2.5
Configuration	6.2.12
Dorado	7.6.8
MinKNOW Core	6.2.8

BARCODES

Detected barcodes | Bases graph | Reads graph

Detected barcodes
The total number of bases and reads for each barcode detected are displayed in table below. Reads/bases must have a quality score above 9 to pass.

Unclassified data: 112.44 k (3.9%) Passed Reads; 245.65 Mb (2.9%) Passed Bases

Search barcodes | Export CSV

Barcode	Total bases (Mb)	Passed bases (%)	Total reads (k)	Passed reads (%)
barcode01	813.26	97.80	224.25	96.80
barcode02	634.31	98.30	212.67	97.00
barcode03	497.69	97.90	184.19	96.80
barcode04	745.87	98.10	325.22	96.90
barcode05	685.77	97.20	414.33	96.40
barcode06	1026.82	98.40	413.54	97.40
barcode07	774.54	97.70	216.05	96.50
barcode08	674.29	97.90	191.16	96.50
barcode09	744.70	97.80	243.53	96.70
barcode10	665.28	98.10	117.93	96.60
barcode11	433.22	97.40	77.06	95.70
barcode12				

QUALITY SCORE

The quality score is calculated as basecalling is performed on your device. Reads that fall below the minimum value of 9 will be classified as failed reads. You can alter the accepted minimum quality score in MinKNOW.

Q score histogram

Passed simplex bases | Failed simplex bases

Filters

Min Q score	9

Oxford Nanopore Technologies

Thank you