

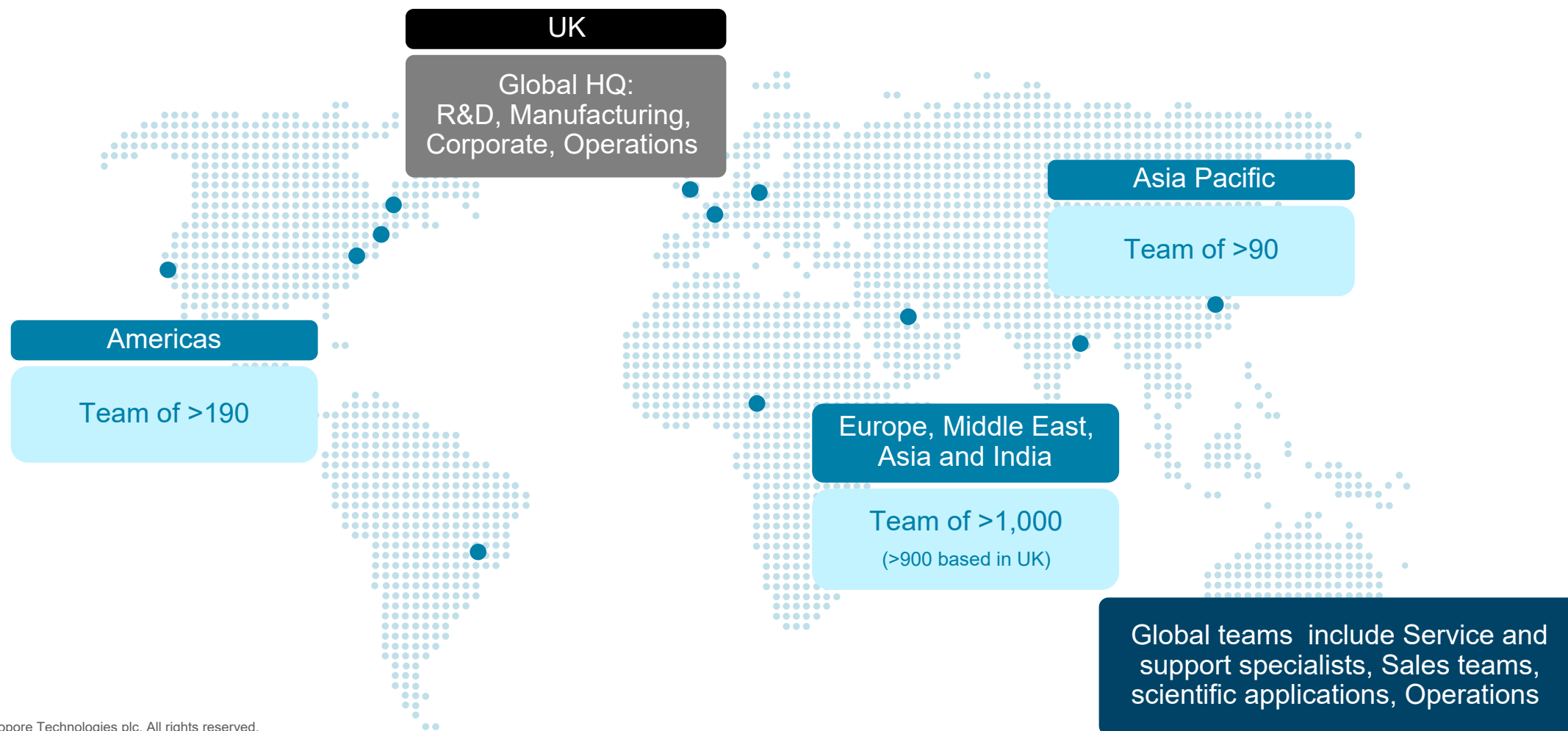
GENERATE **ULTRA-RICH** **DATA** FOR ANSWERS WITH IMPACT

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)

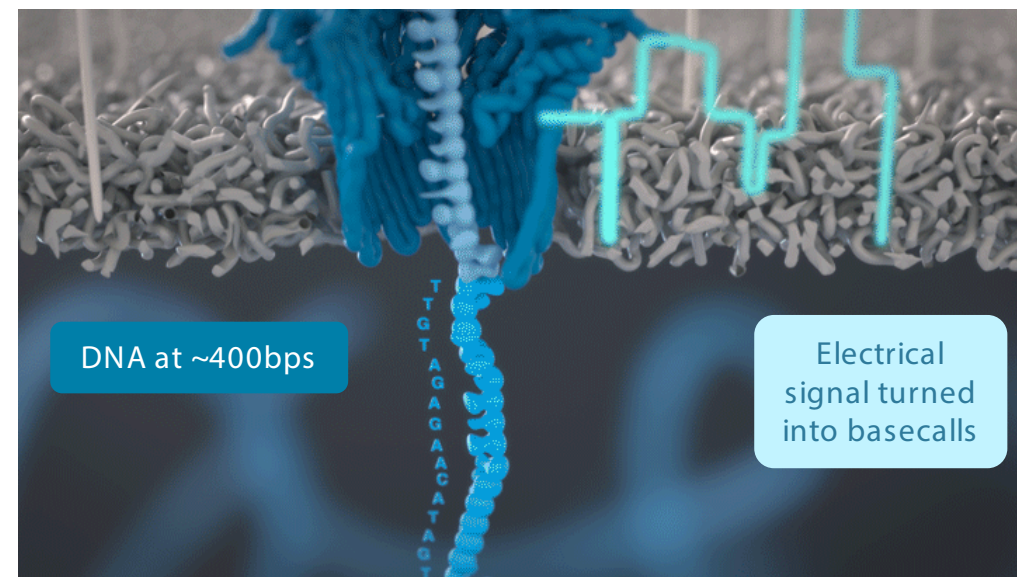
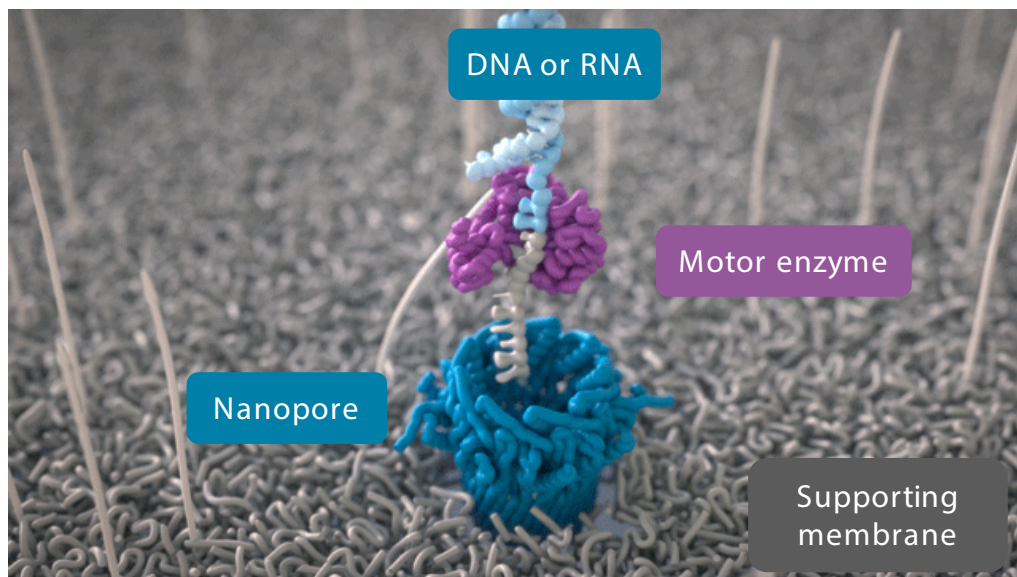
Analyse

- ✓ 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

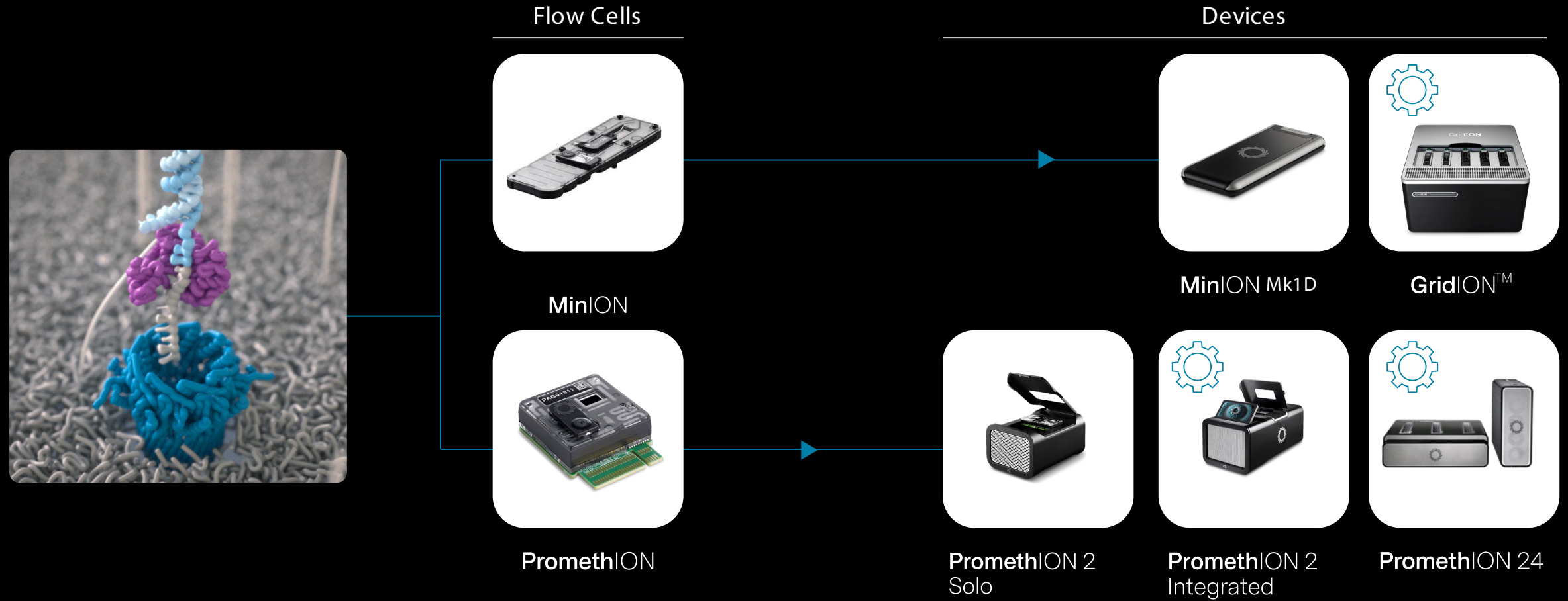
One platform for any
sample; see the true scope
in your biology

Rapid results
Intelligent analyses
e.g. adaptive sampling

Scalable, from small to large
formats Low-cost



One core technology at any scale

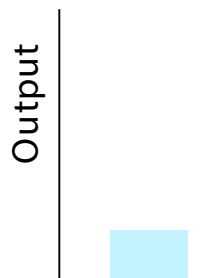




It starts with a flow cell

A single chemistry delivered across increasing number of nanopores

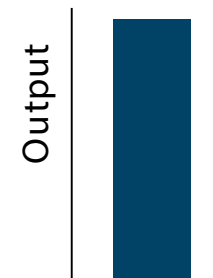
MinION



Choose MinION™

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

PromethION



Choose PromethION™

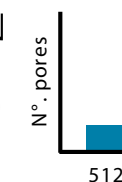
- ✓ Generate hundreds of Gigabases of data
- ✓ Sequence large genomes to high coverage



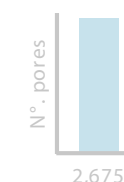
MinION Flow Cells

Flexible and versatile for multiple applications

MinION



PromethION



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

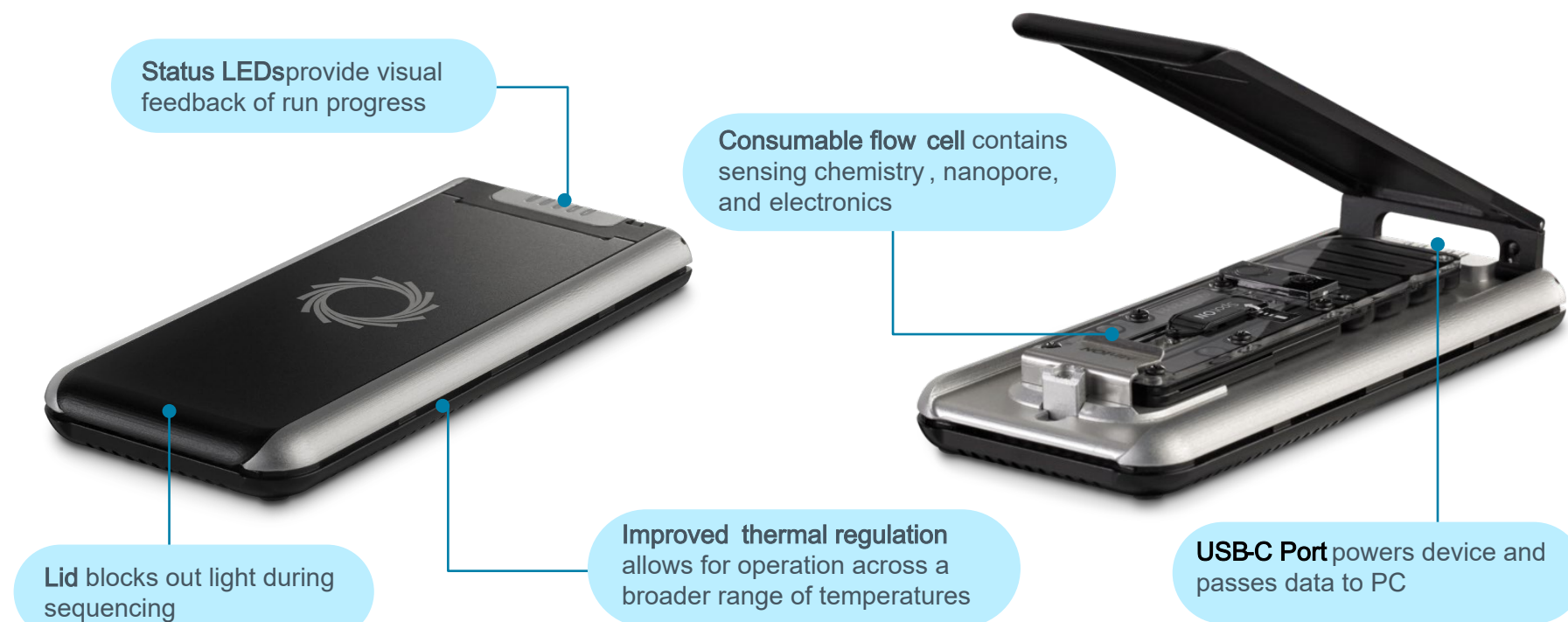
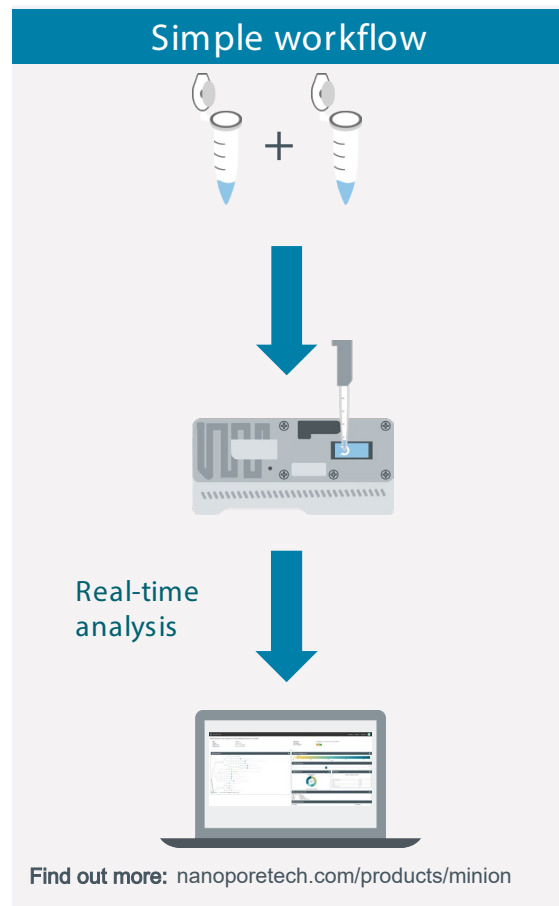
10-20 Gb ultra-long
native DNA reads
(>50 kb read N50)

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





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

Find out more : [nanoporetech.com /products/ gridion](https://nanoporetech.com/products/gridion)

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

The PromethION flow cells

High output for multiple applications

MinION



PromethION



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



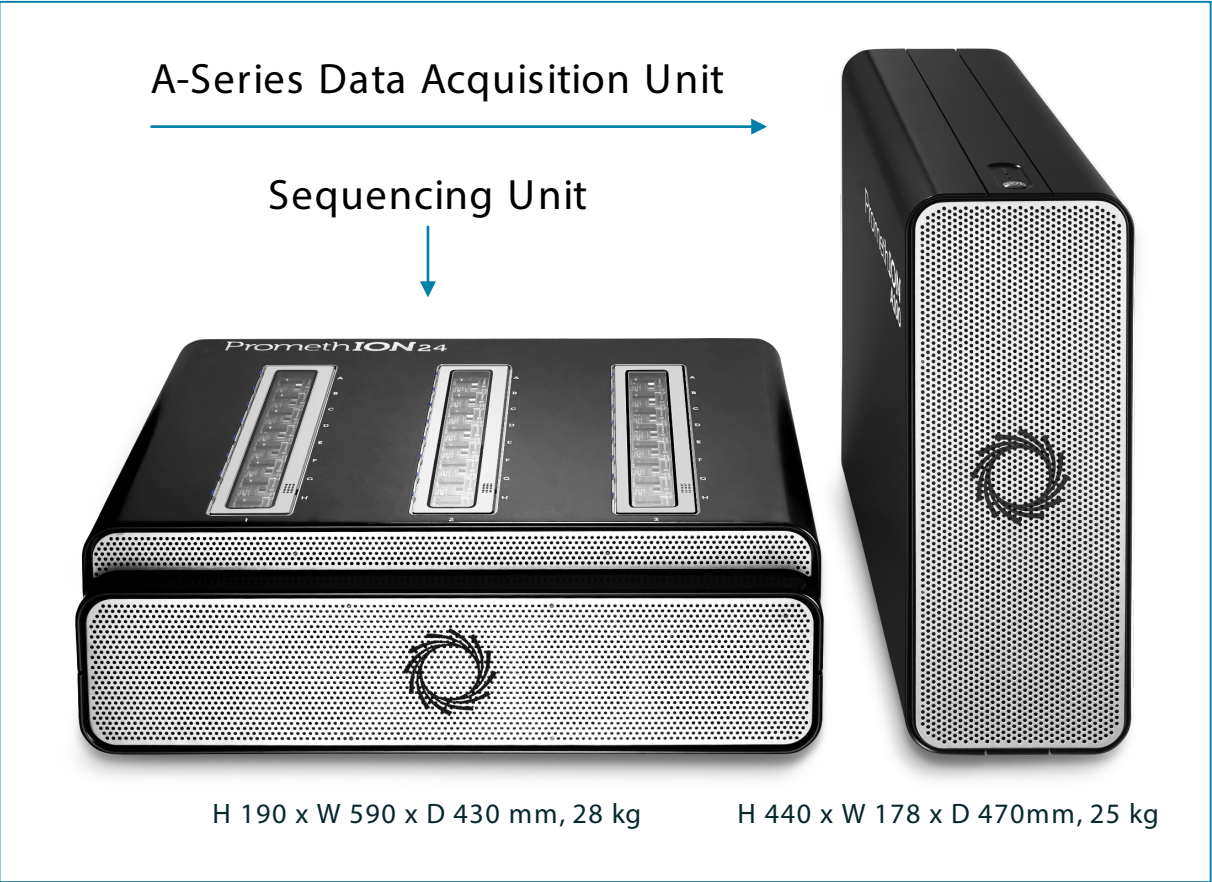
H 152 x W110 x D 87mm

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



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 $\geq 5,000$ 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



Nanopore sequencing chemistry

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

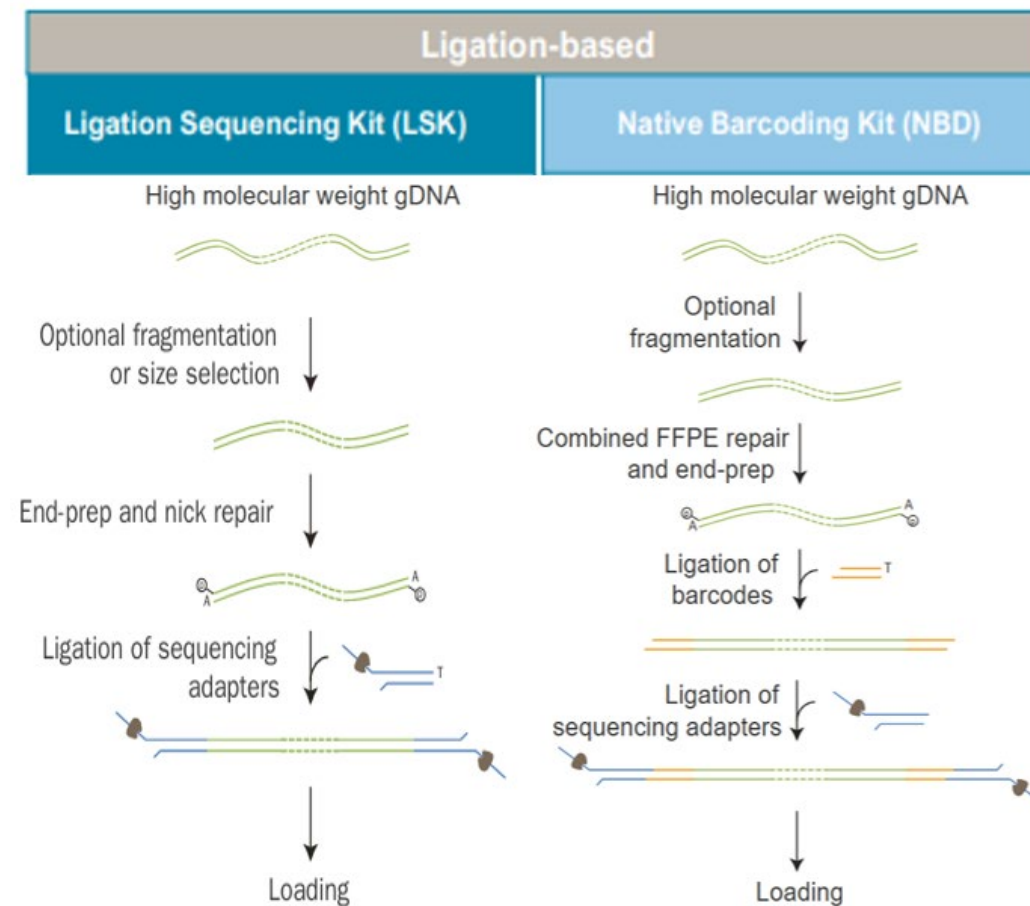
	DNA					RNA	
	Output optimised	Speed optimised	Ultra-long reads optimised	Low input optimised	Targeted sequencing	Direct RNA	cDNA
	Ligation Sequencing Kit	Rapid Sequencing Kit	Ultra-Long DNA Sequencing Kit	Rapid PCR Barcoding Kit	16S Barcoding Kit	Direct RNA Sequencing Kit	cDNA-PCR Sequencing Kit
Preparation 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	<i>In development</i>	cDNA-PCR Barcoding Kit 24 plex
Typical output	●●●	●●○	●●○	●●○	●●○	20M reads	>100M reads
Adaptive sampling	✓	✓	✓	✓		<i>In development</i>	<i>In development</i>
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





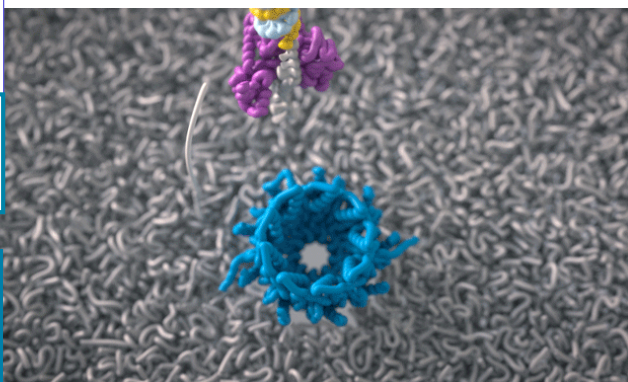
Targeted Sequencing : Adaptive Sampling

Adaptive Sampling

1	chr1	2228695	2310119	SKI	28.67	61424
2	chr1	2556366	2563829	THFRSF14	11.35	7463
3	chr1	3049211	3438421	PRDM14	30.67	369410
4	chr1	6185020	6189612	RPL22	26.96	14592
5	chr1	6785324	7769706	CANTAL	33.13	984382
6	chr1	11106535	11262507	MTOR	31.63	155972
7	chr1	13704855	13825079	PRDM2	36.52	120224
8	chr1	15492270	15524295	CASF9	30.93	32025
9	chr1	15847864	15940460	SPEN	32.57	92596
10	chr1	17018722	17084170	SOXB	30.83	35448
11	chr1	17539035	17697849	ARHGEF10L	32.24	158034
12	chr1	18631006	18736138	PAX7	31.2	105132
13	chr1	23557518	23559794	ID3	22.95	1876
14	chr1	23551455	23640568	MDJ2	33.43	59073
15	chr1	26696033	26782110	ARID1A	34.14	86077
16	chr1	32251239	32286165	LCK	36.92	34926
17	chr1	35182552	35193148	SFEQ	34.06	10216
18	chr1	36224416	36305357	THRAP3	37.17	80941
19	chr1	36466043	36483278	CSF3R	26.94	17235
20	chr1	38595426	38902013	MYCL	26.67	6507
21	chr1	43337849	43352772	NPL	34.28	14923
22	chr1	45329242	45340388	MUTYH	27.67	11146

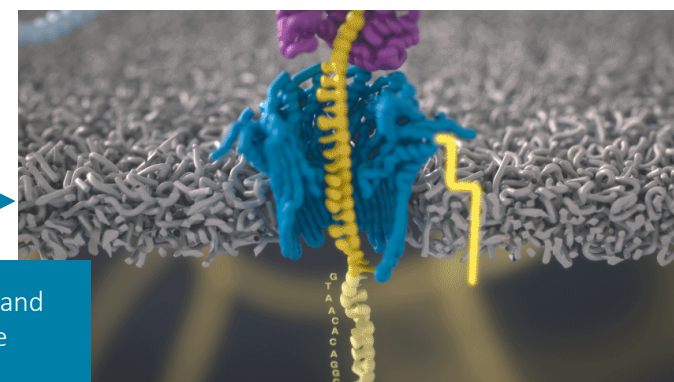
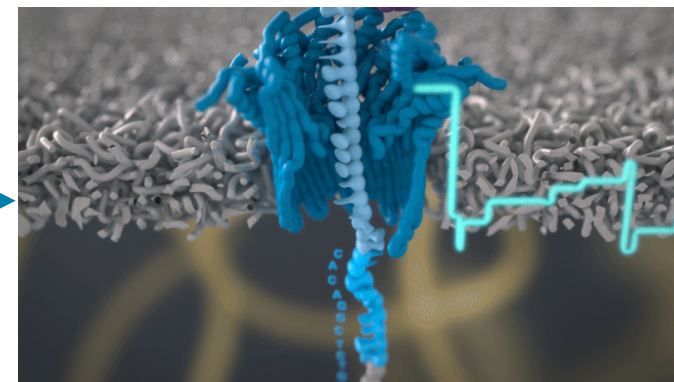
Upload reference file
to MinKNOW

Strand approaches
nanopore and
sequencing starts



Real-time base calling
and alignment

Region of interest? Strand
allowed to continue
sequencing



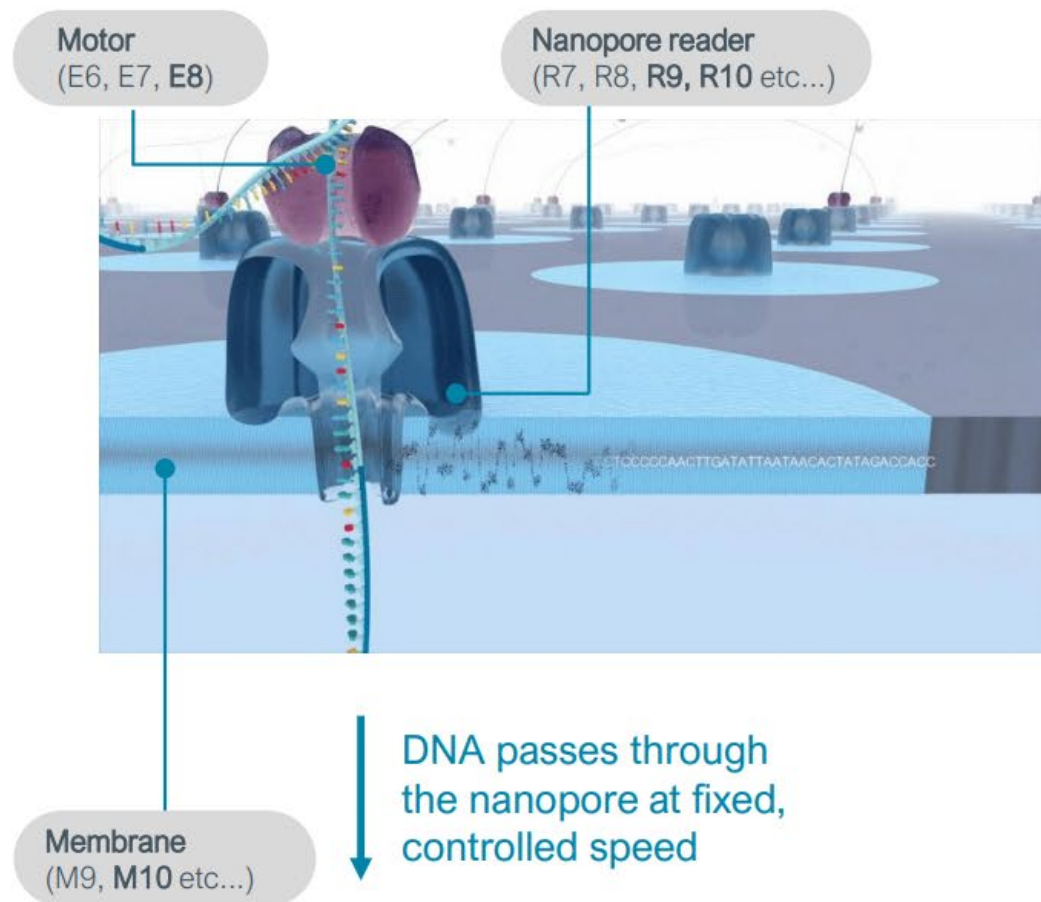


Preparing libraries



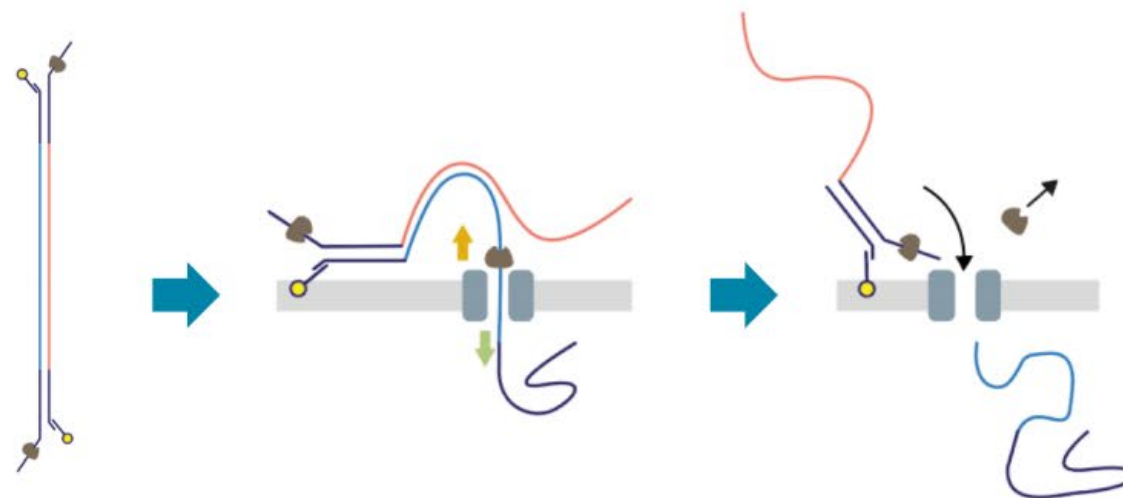
Nanopore sequencing chemistry

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

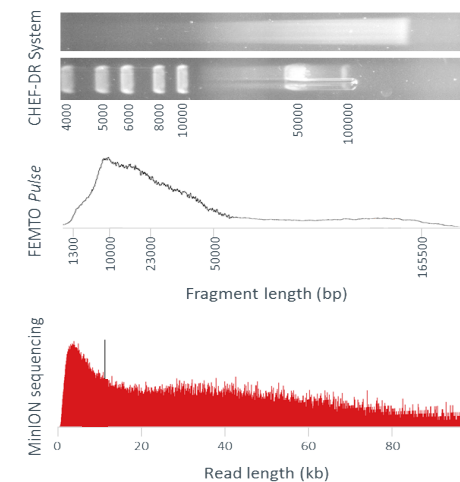
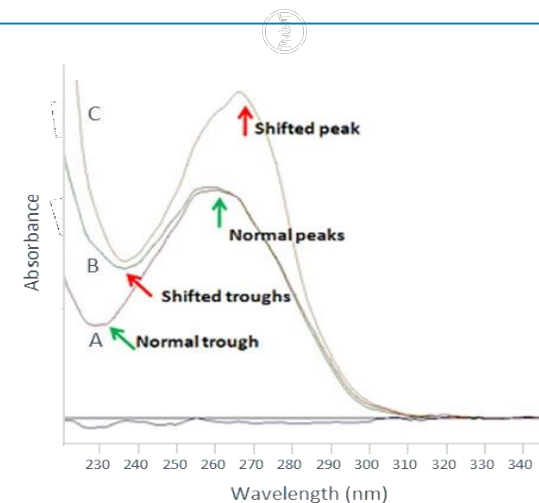
Purity: Nanodrop

OD measurement of pure DNA :

- $A_{260}/A_{280} = \sim 1.8$
- $A_{260}/A_{230} = \sim 2.0-2.2$

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

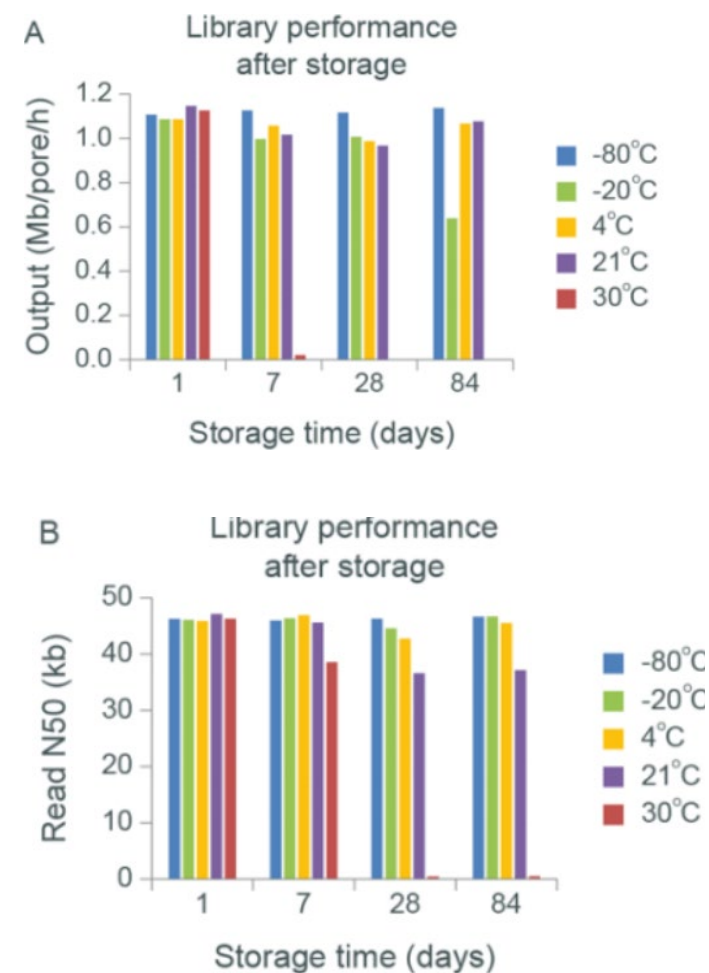
- Fragment length size measurement is an essential factor for accurate DNA quantification.





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.

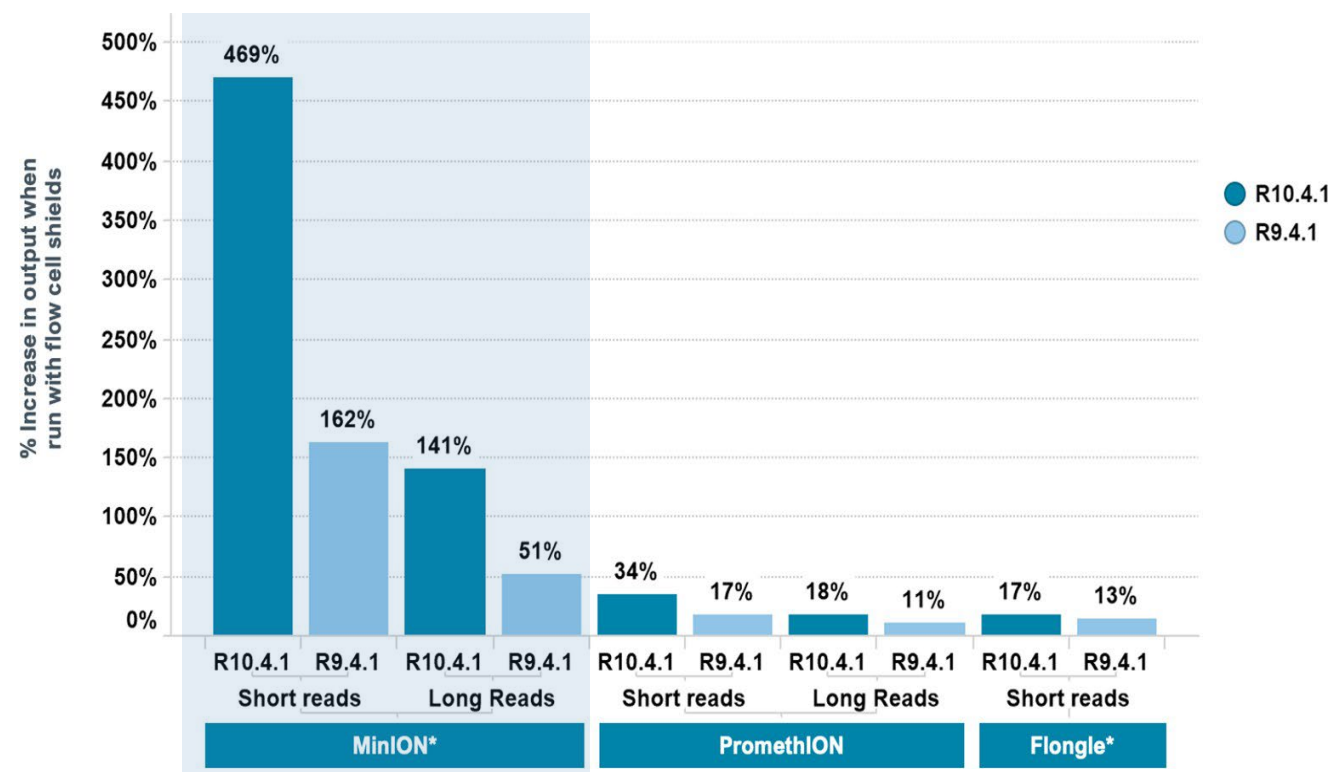


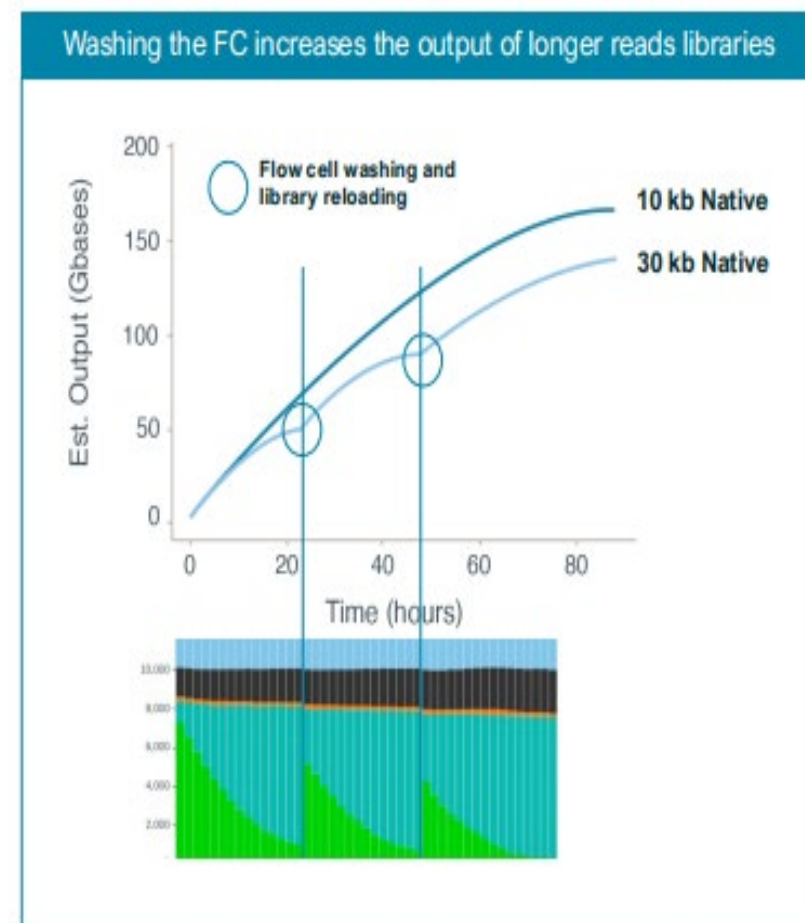
Figure: Example % Increase in output from flow cells where the array is shielded from light during sequencing in internal experiments. R10.4.1 and flow cells with short fragment libraries observe the most benefit to running in the dark. Short reads = 200bp amplicon. Long reads = 30Kb N50 human native 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 cell;
- The number of flow cells: 6

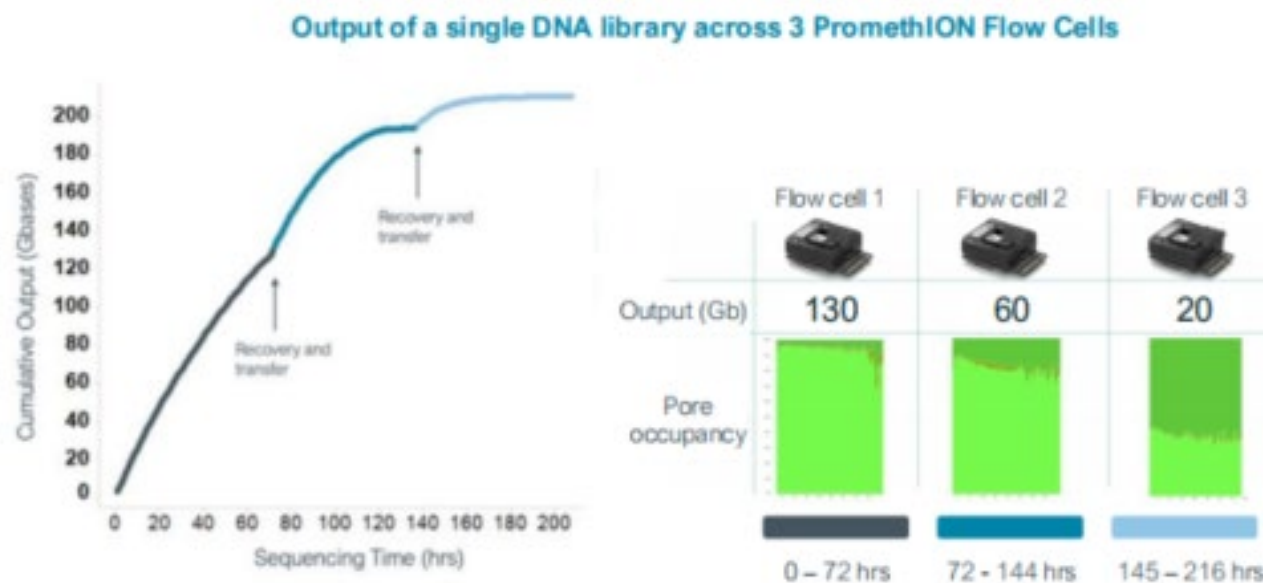




Recover DNA library and reuse it

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.

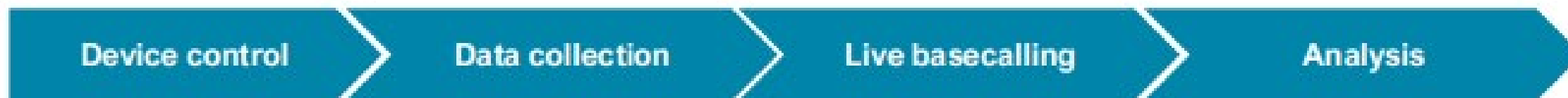




Introduction of Software : MinKNOW



MinKNOW software



Key functions

1. Hardware check

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

2. Flow cell check

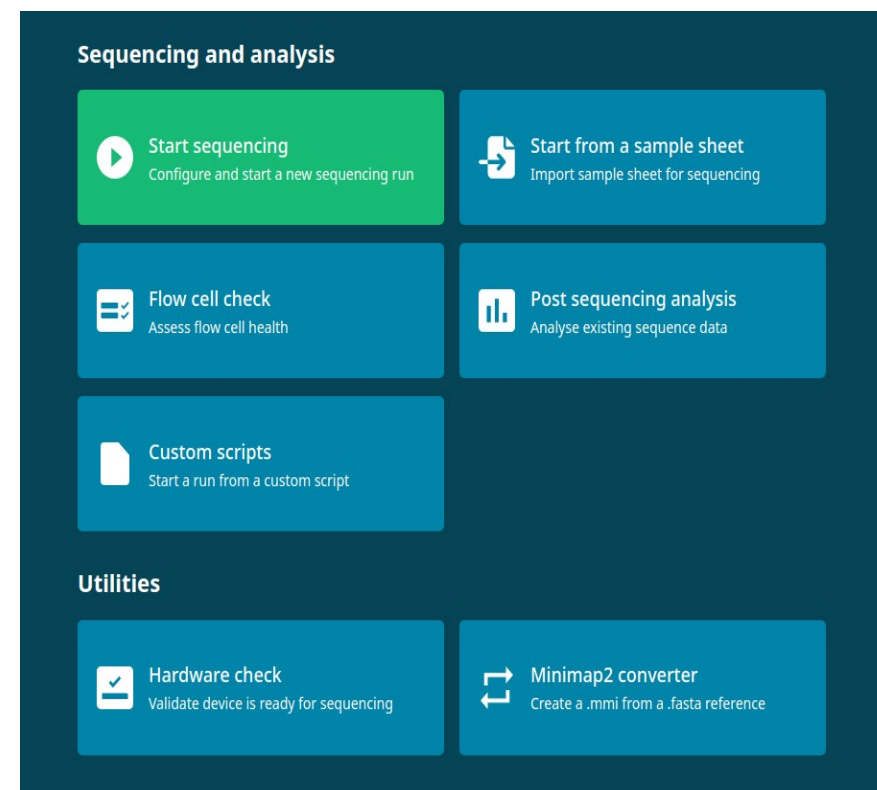
- Checking the membrane integrity and number of pores in the flow cell

3. Start sequencing

- Initiates the steps and start of the sequencing run

4. Analysis

- Performs post-run basecalling, demultiplexing, and alignment



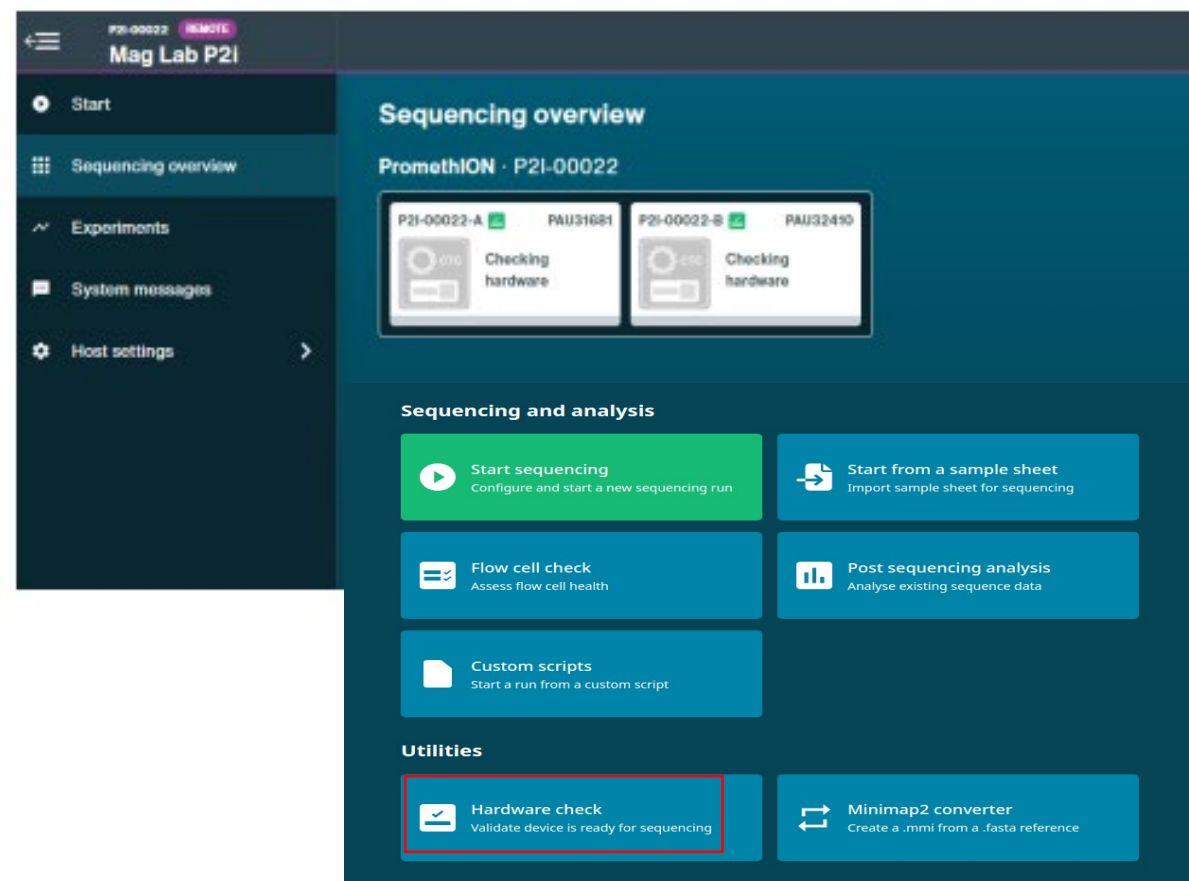


Hardware check

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

Processing Hardware check

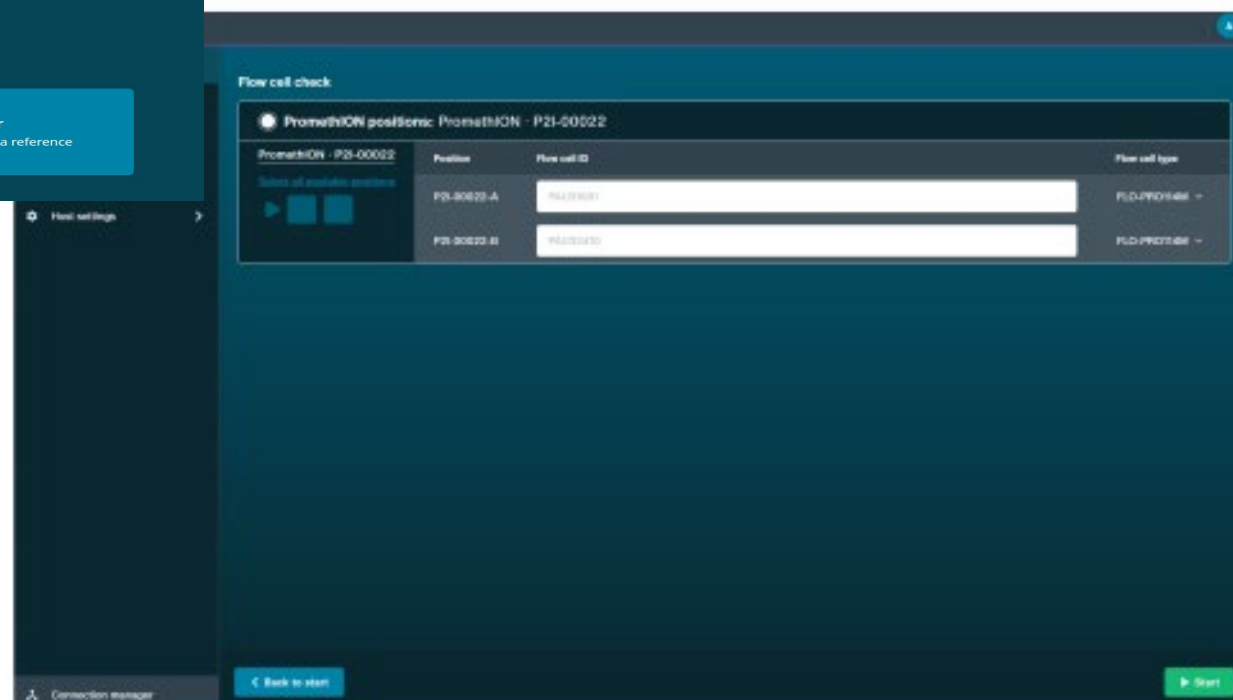
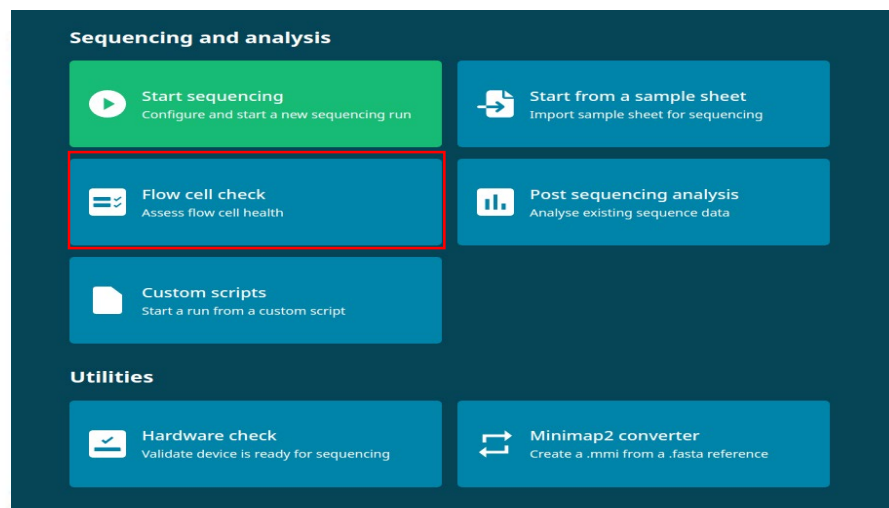
1. Mount CTC (Configuration Test Cell) onto the device
2. Click the “Hardware check” icon
3. Check whether the CTC is recognized at each position of the device
4. Click the “Start” button
5. Wait about one minute
6. Check a green check mark (v) appears on the position – “Hardware check passed”





Flow cell check

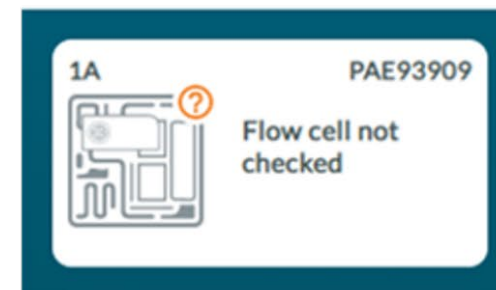
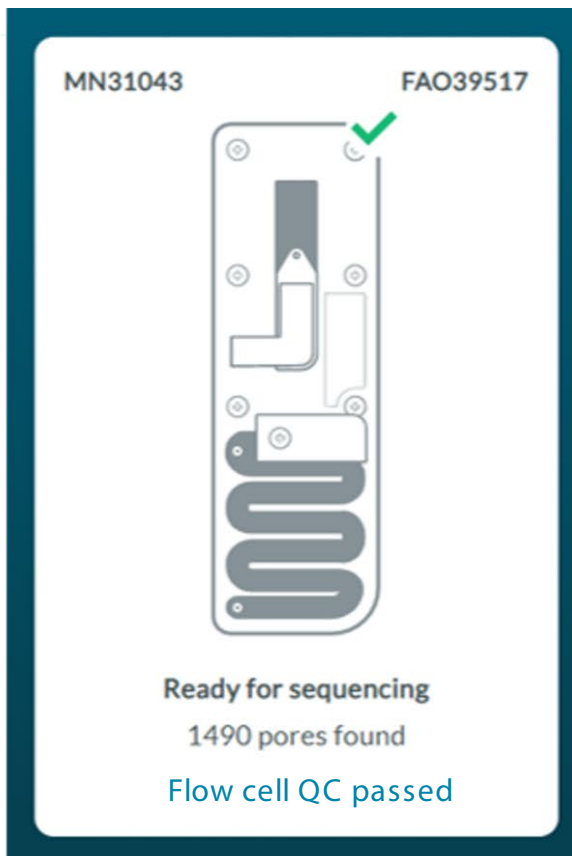
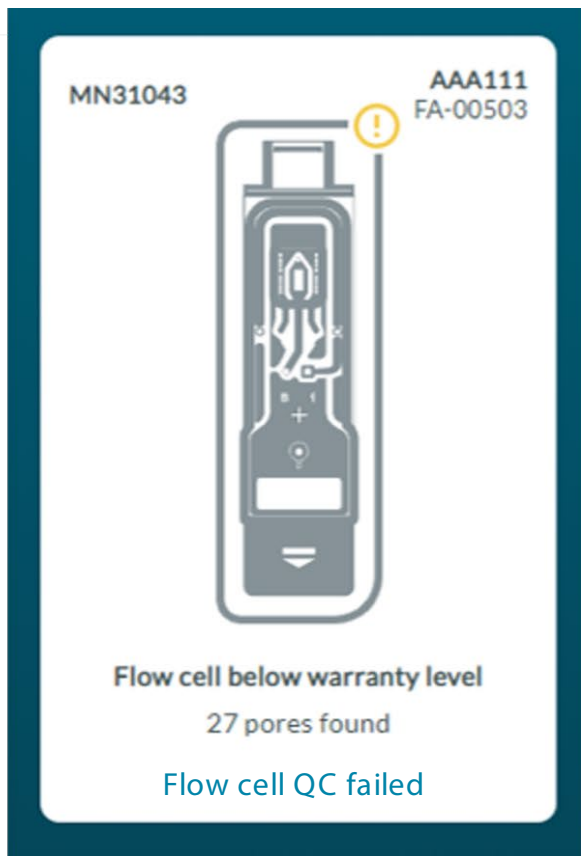
Checking the membrane integrity and number of pores in the flow cell





Flow cell check

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



Not performing Flow cell QC (restart Flow cell Check)



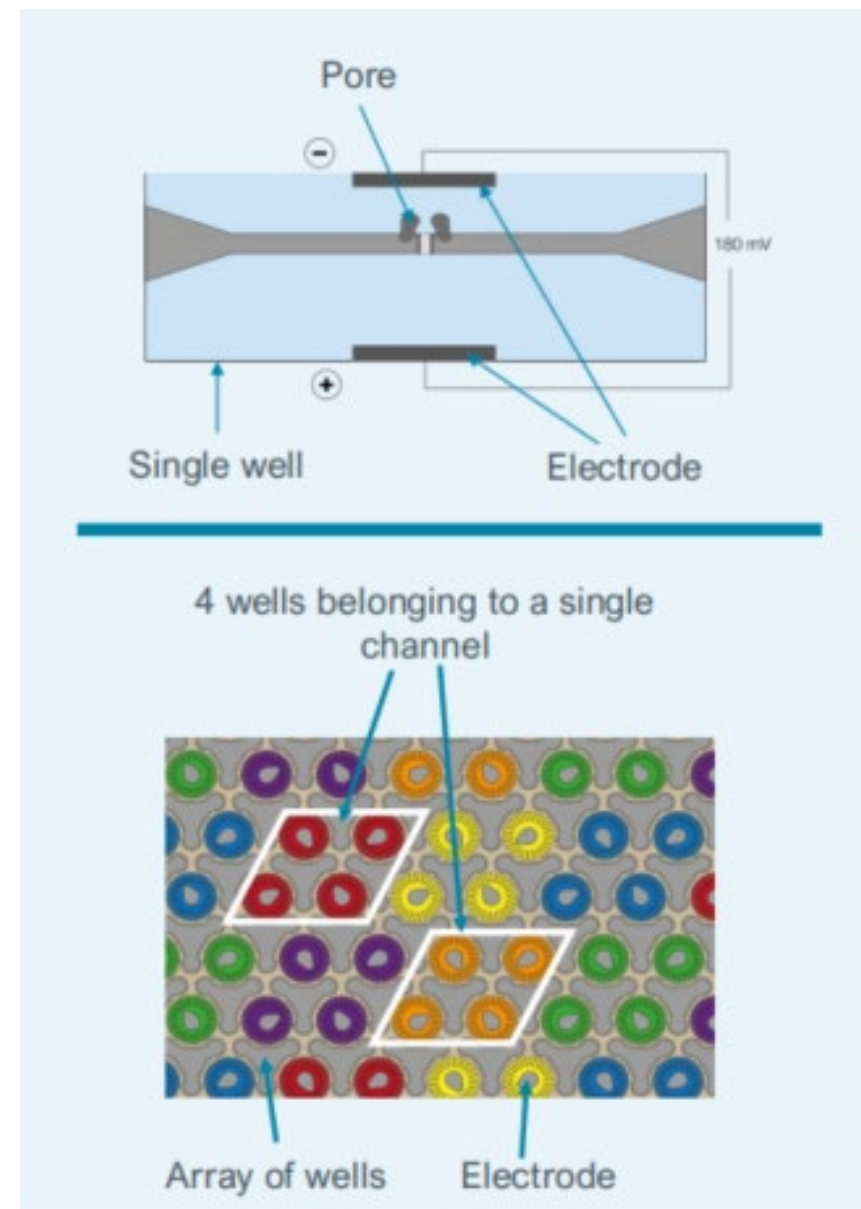
Meaning of Pore Scan and flow cell check

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

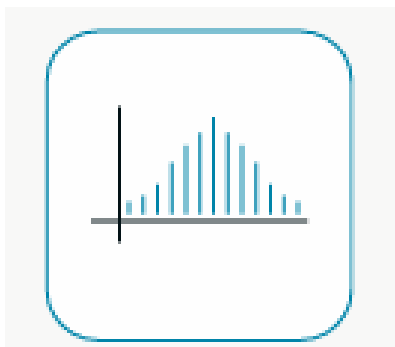
- During sequencing, the current generated in each channel is recorded individually, and each well is tested to select the one well (pore) that will first perform sequencing.
- All sequencing scripts start with a Pore scan





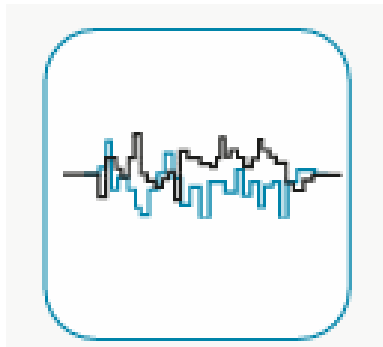
MinKNOW : Overview

Our devices software: from basecalling to reporting



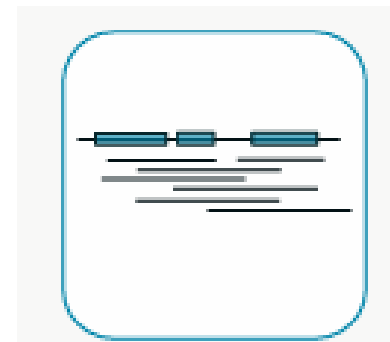
Real time QC

- 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

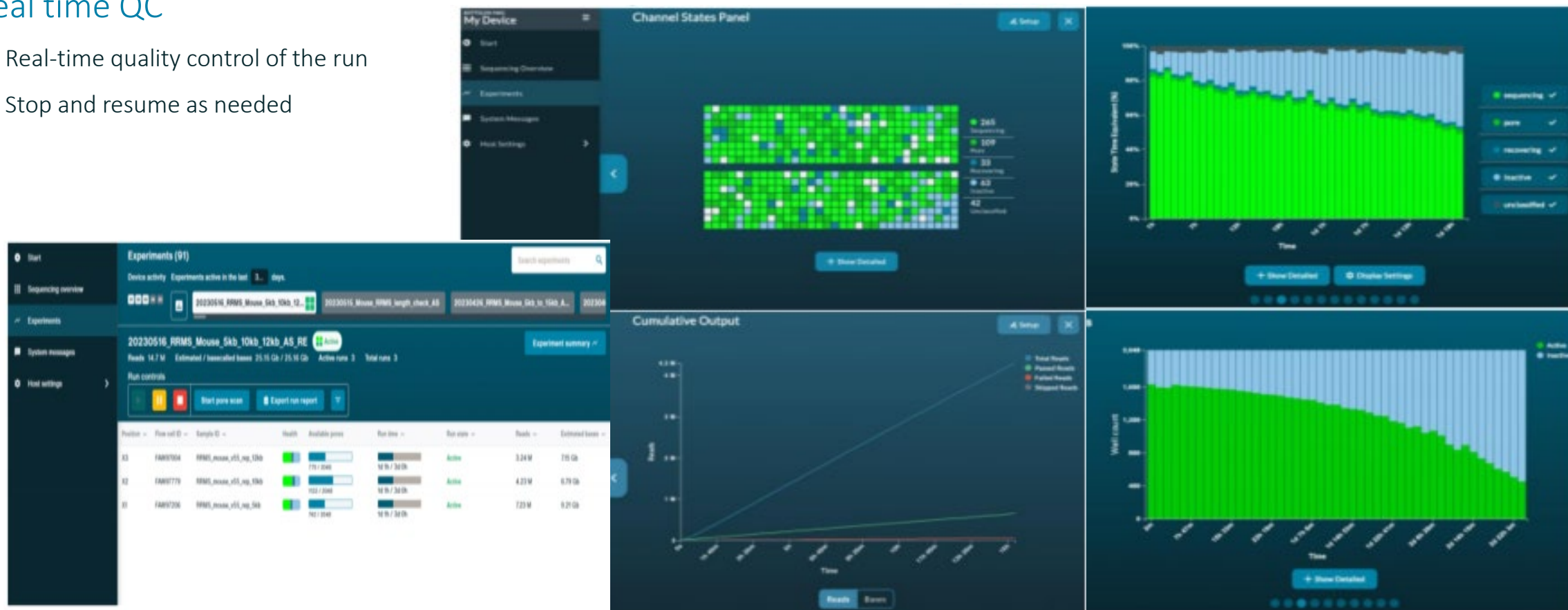
- QC reports of each run



MinKNOW : Real time QC

Real time QC

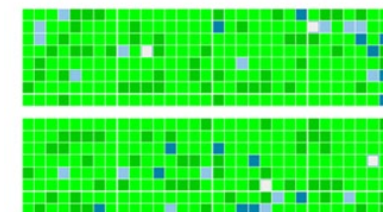
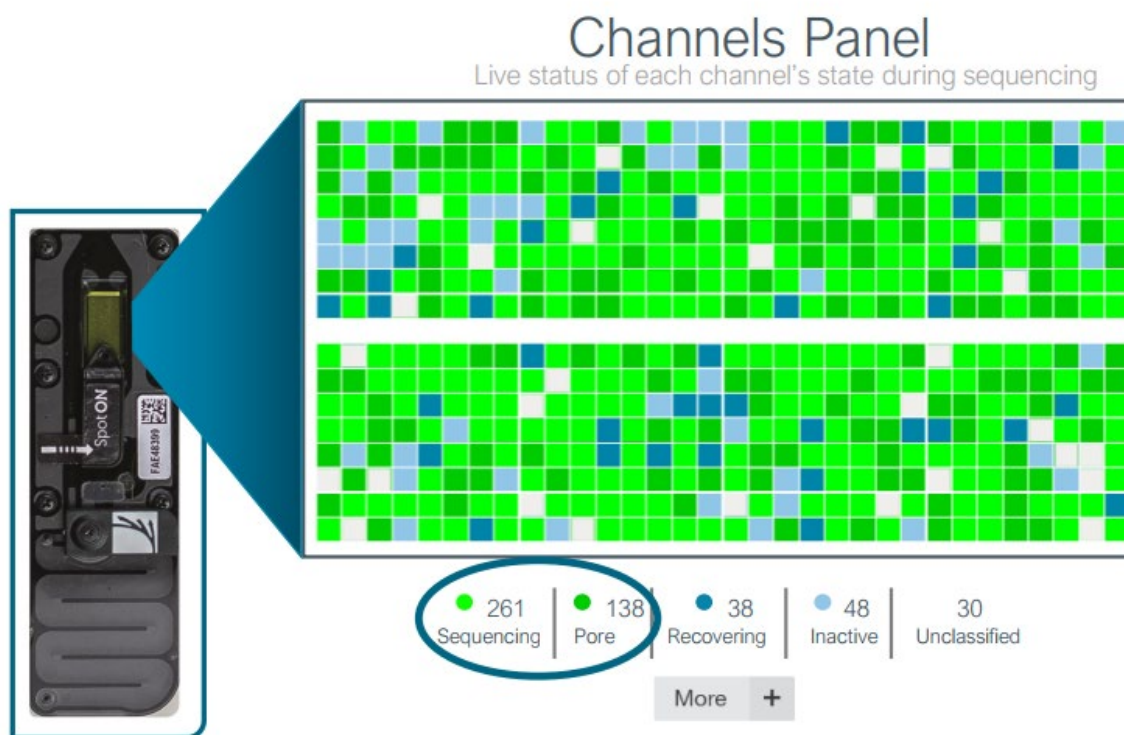
- Real-time quality control of the run
- Stop and resume as needed





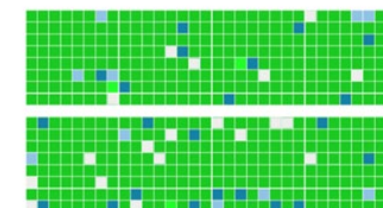
MinKNOW: Monitoring a sequencing run

Available real time quality control of the sequencing run



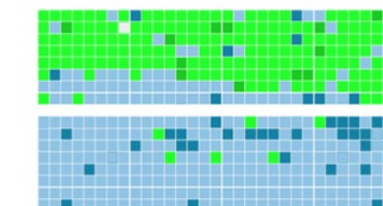
- High ratio of strand to open pore
- Small amount of recovering and inactive
- Successful library preparation

378 Sequencing 96 Pore 15 Recovering 18 Inactive 5 Unclassified



- 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

3 Sequencing 456 Pore 23 Recovering 11 Inactive 19 Unclassified



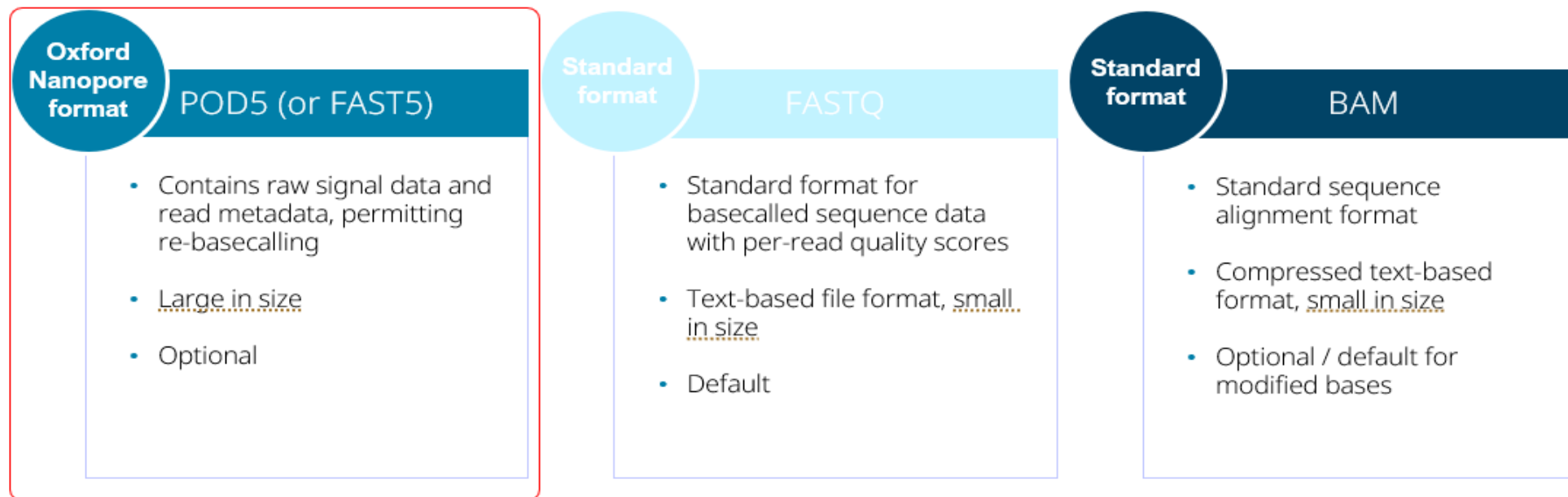
- High number of inactive channels
- Clear spatial pattern
- Indicates an air bubble has been introduced onto the sensor array

178 Sequencing 12 Pore 45 Recovering 276 Inactive 1 Unclassified



MinKNOW : Basecalling

Data formats



Thank you

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