

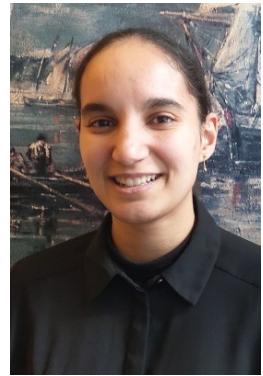


Formation à l'utilisation d'un séquenceur Minlon

Session expérimentale



L'équipe de la plateforme génomique de l'IBENS



Médine
Benchouaia

Catherine
Senamaud-
Beaufort

Corinne
Blugeon

Morgane
Thomas-
Chollier

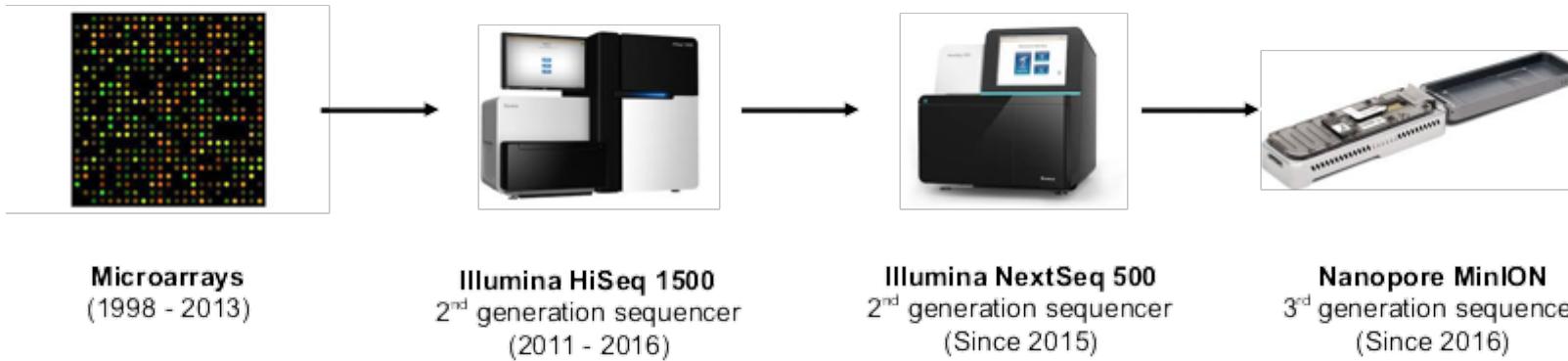
Stéphane
Le Crom

Laurent
Jourdren

Sophie
Lemoine

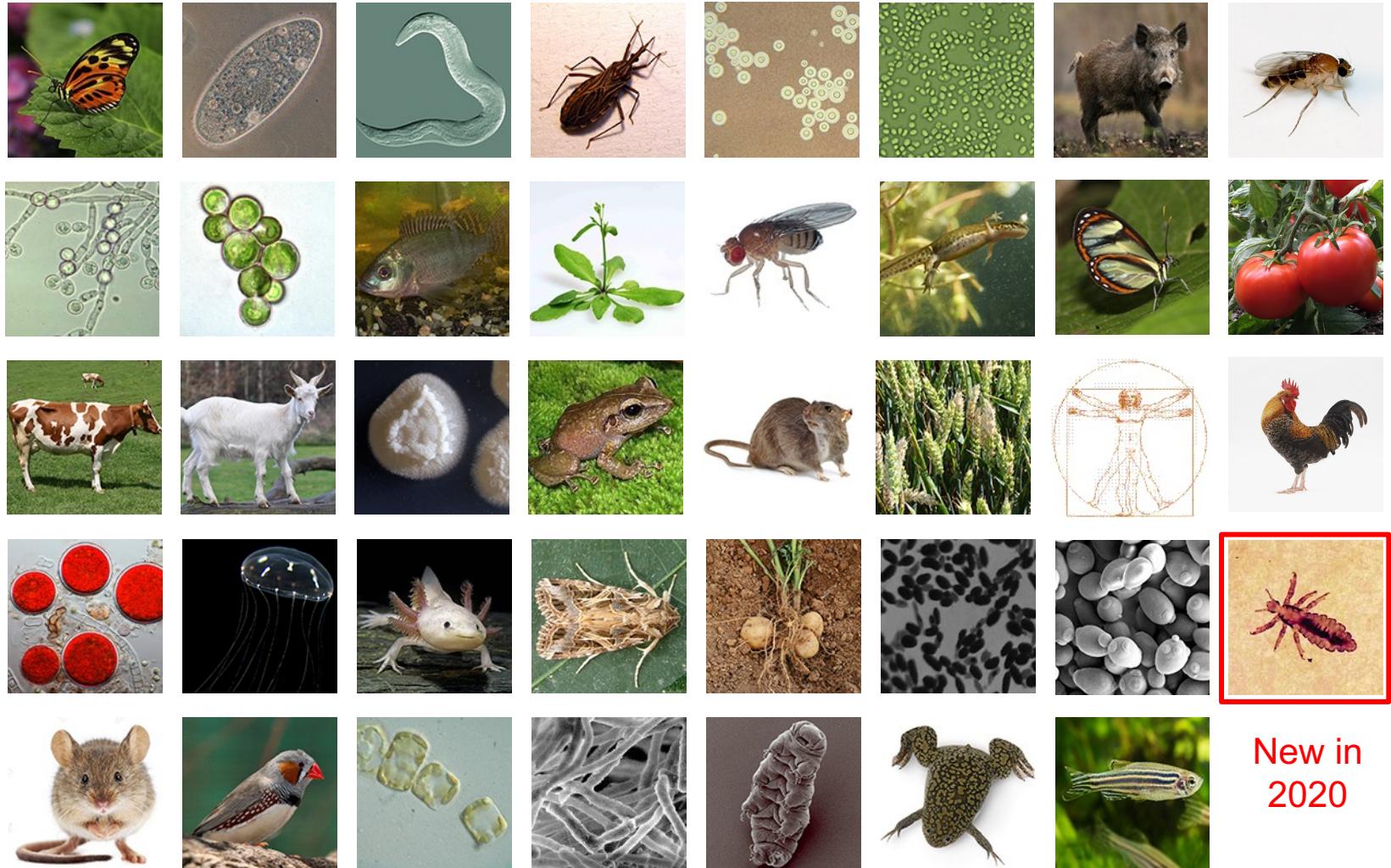
■ Le personnel est composé de trois expérimentateurs et de deux bioinformaticiens

Evolution technologique



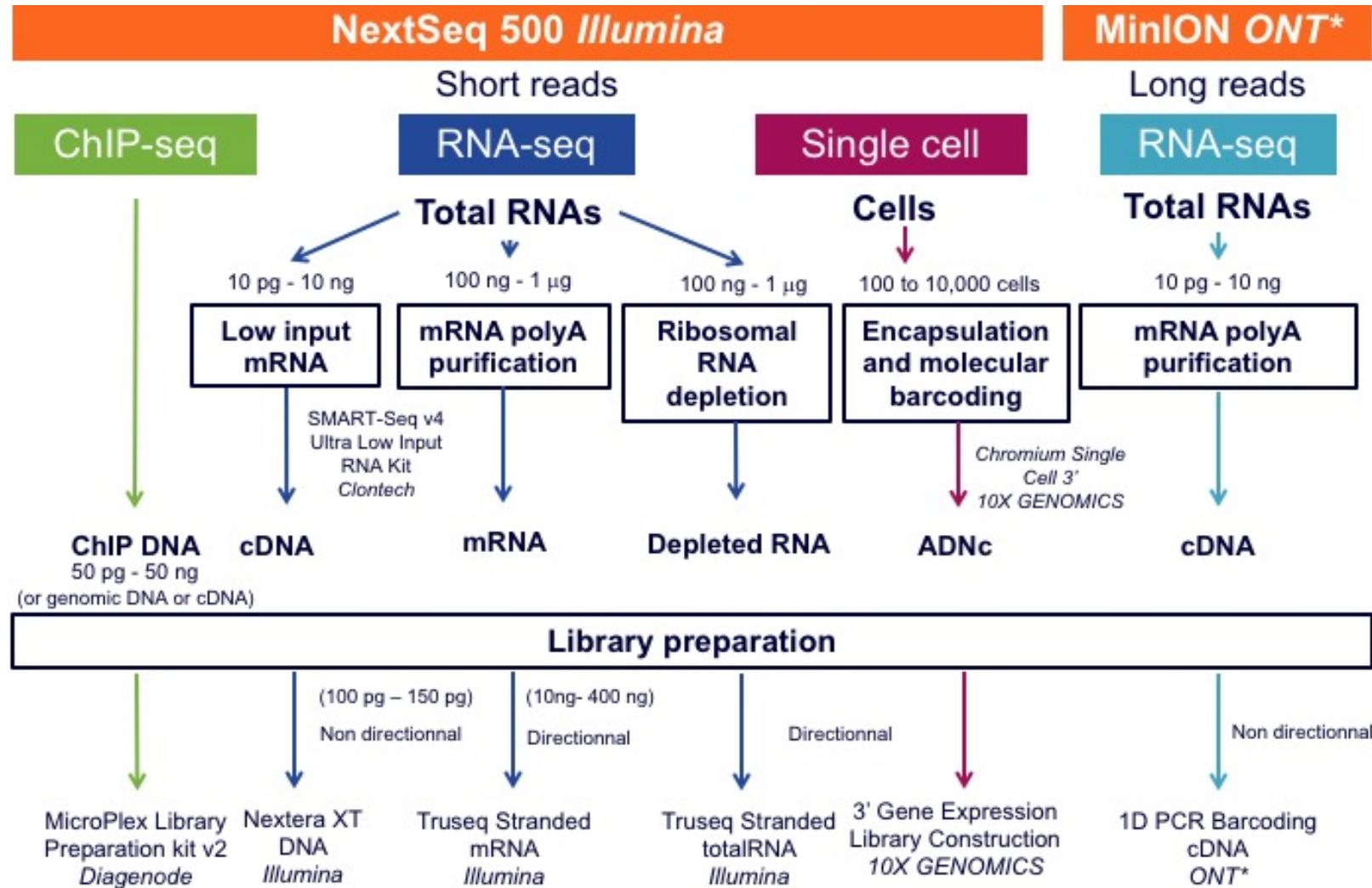
- La plateforme existe depuis 1999 et a suivi le changement des technologies notamment en transcriptomique

Spécialisée sur les organismes eucaryotes



New in
2020

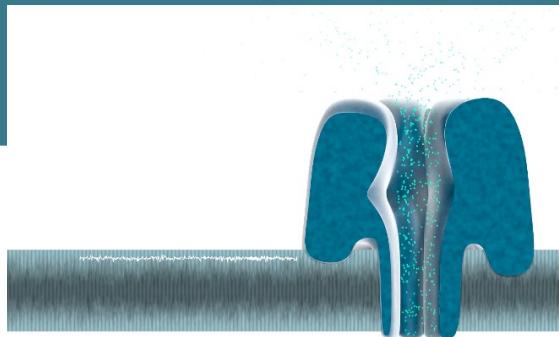
Nous concentrons nos efforts en génomique fonctionnelle



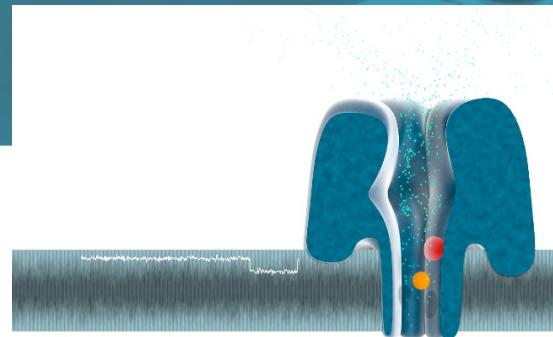
Tour de table



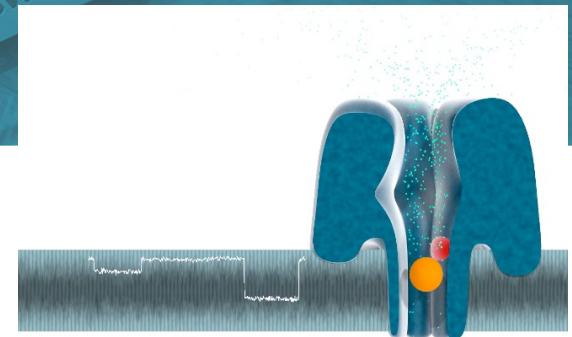
Comment fonctionne les pores



A protein nanopore is set in an electrically resistant synthetic polymer membrane.



An ionic current is passed through the nanopore by setting a voltage across this membrane.



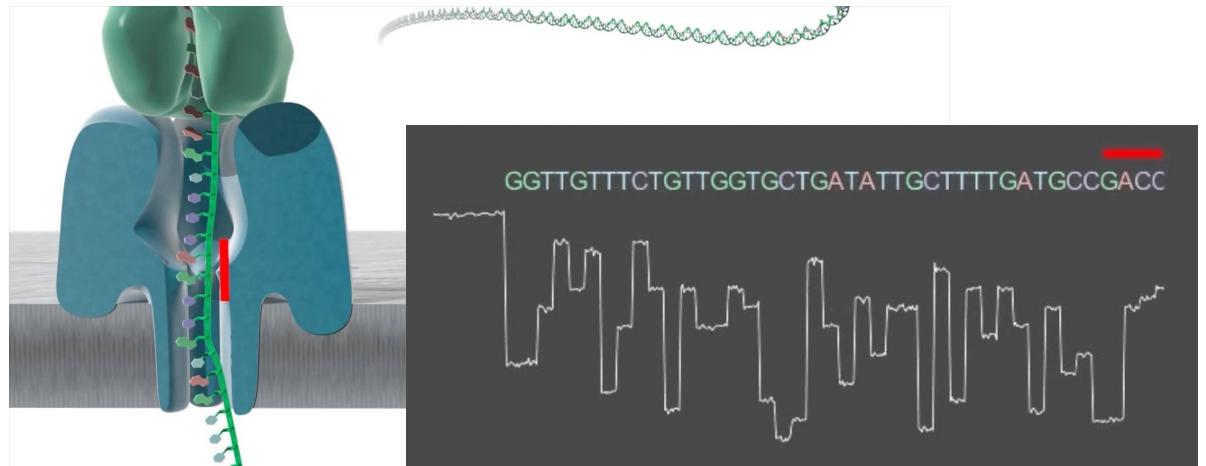
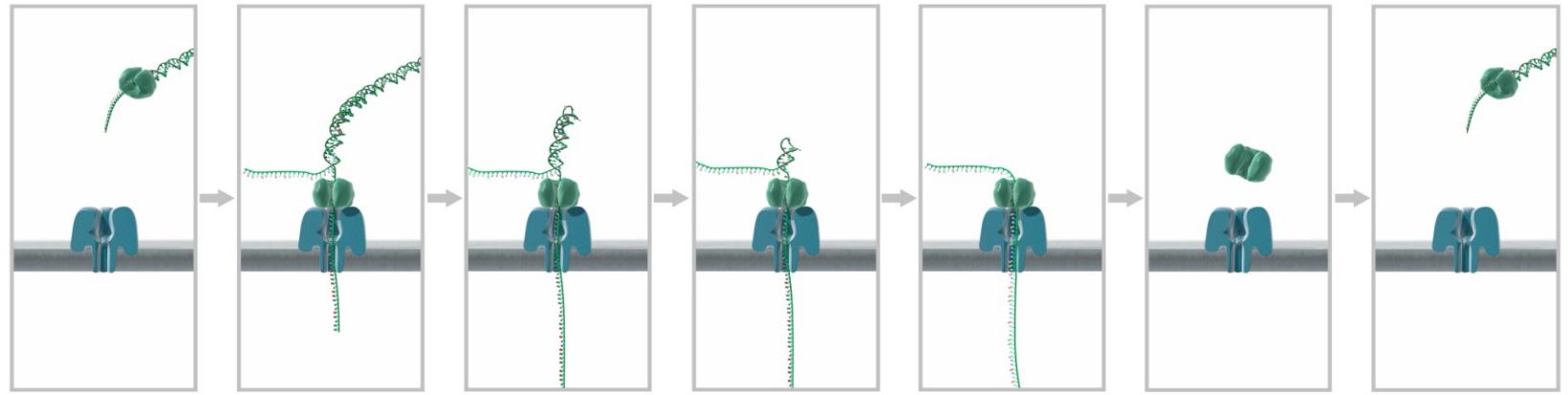
If an analyte passes through the pore or near it, this event creates a characteristic disruption in current.

<https://nanoporetech.com/how-it-works>

Séquencer avec des pores

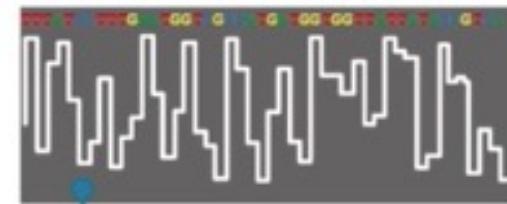
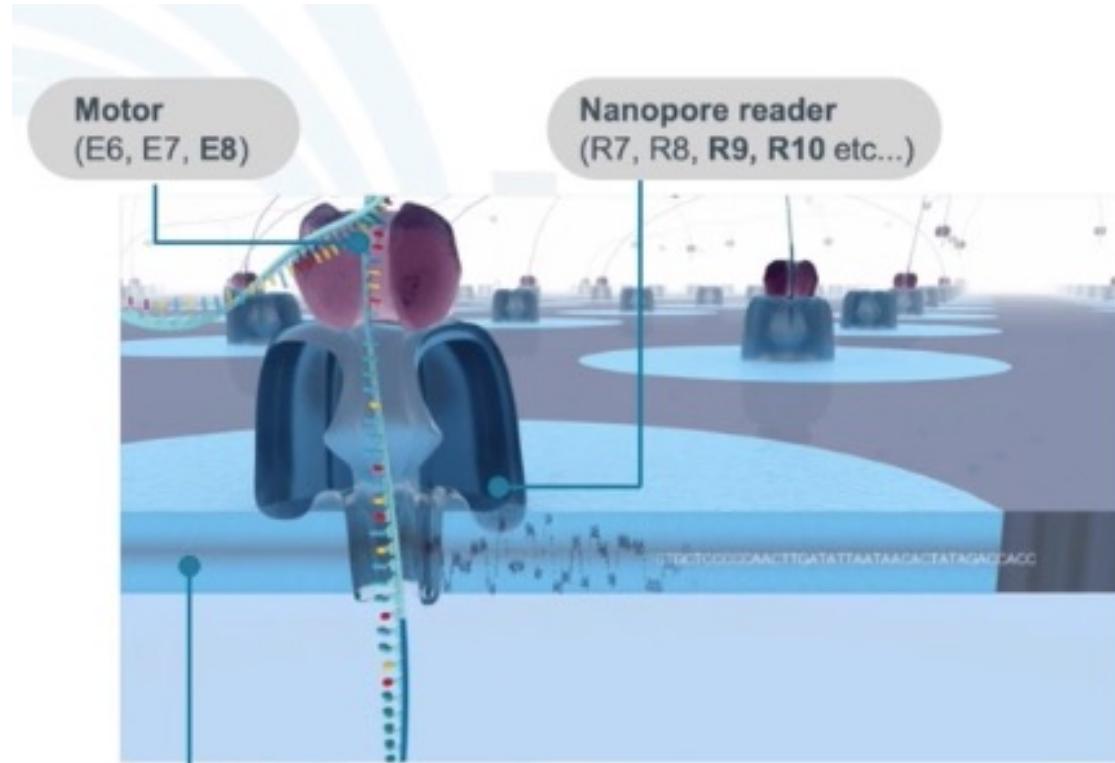


■ A strand of DNA is passed through a nanopore helped with a **motor protein** (helicase).



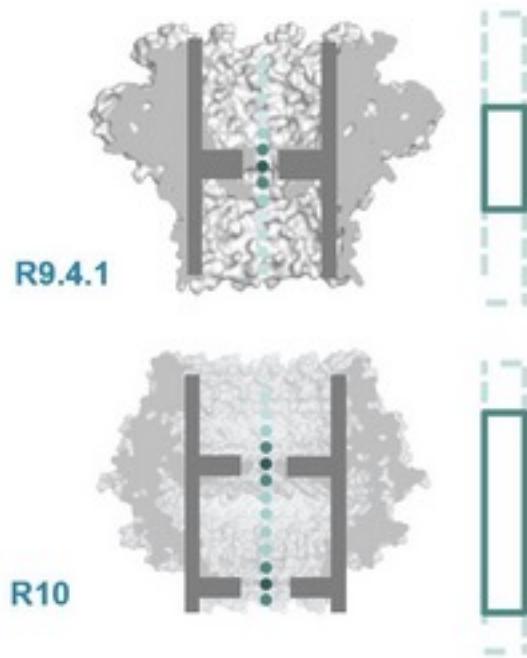
<https://nanoporetech.com/>

Un pore



Run conditions
(Salt, fuel, script, temperature...)

Pore R10



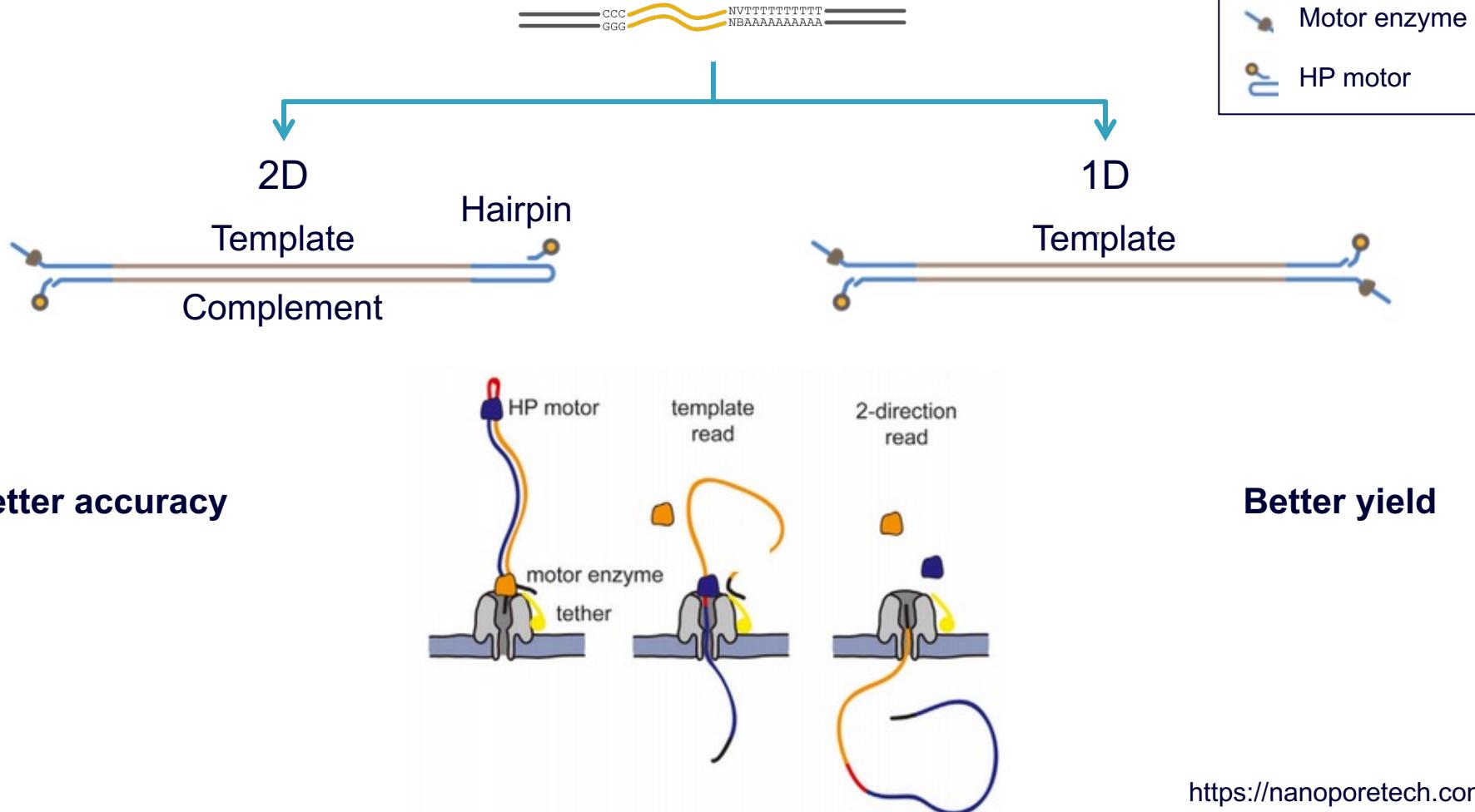
R10 is a new series of nanopore containing a long or dual reader enabling improved resolution of homopolymer signam

Enable for ligation kit only

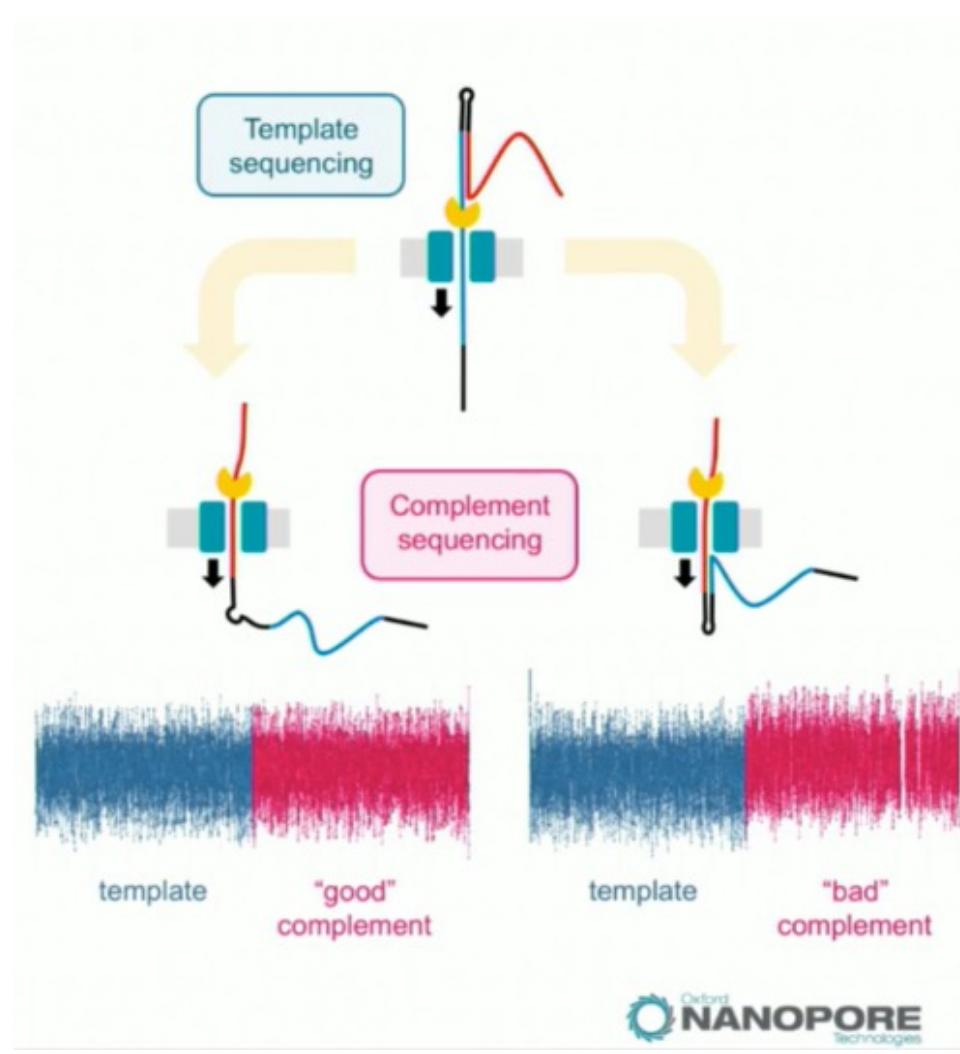
cDNA library construction



Full length cDNA from total RNA
Poly A selection, non directional



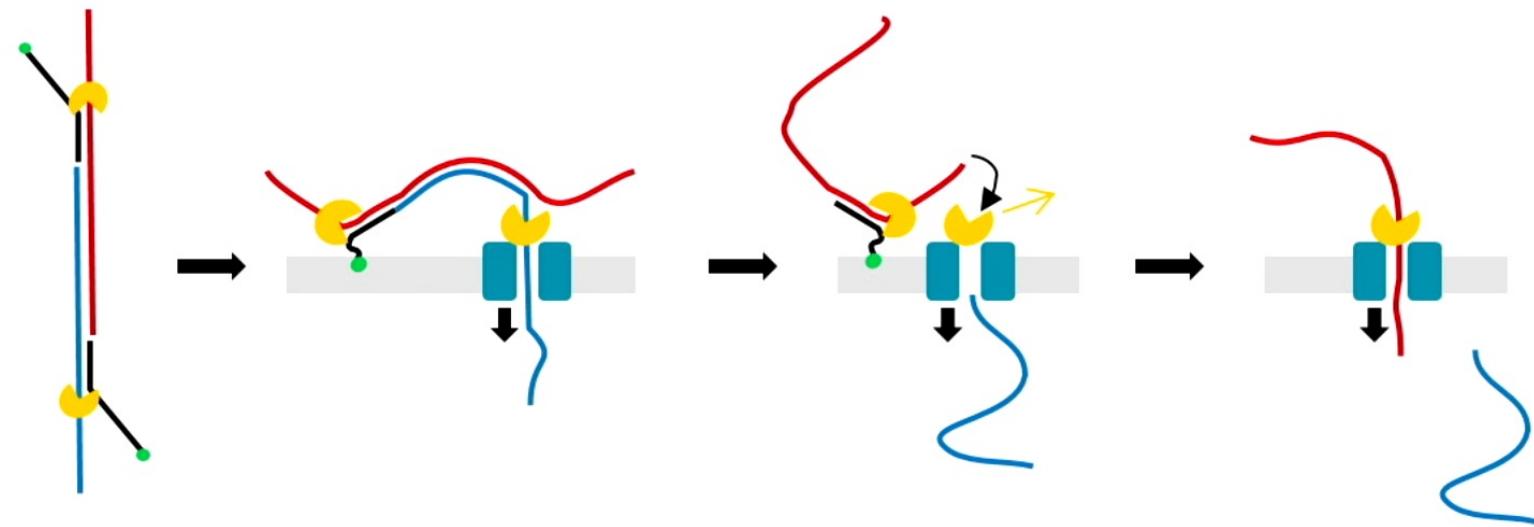
Problème du 2D



Evolution du 2D



= $1D^2$



© Copyright 2016 Oxford Nanopore Technologies

- Specific adapters, that has a higher affinity to the pore, encourages the complement strand to immediately follow the template strand.

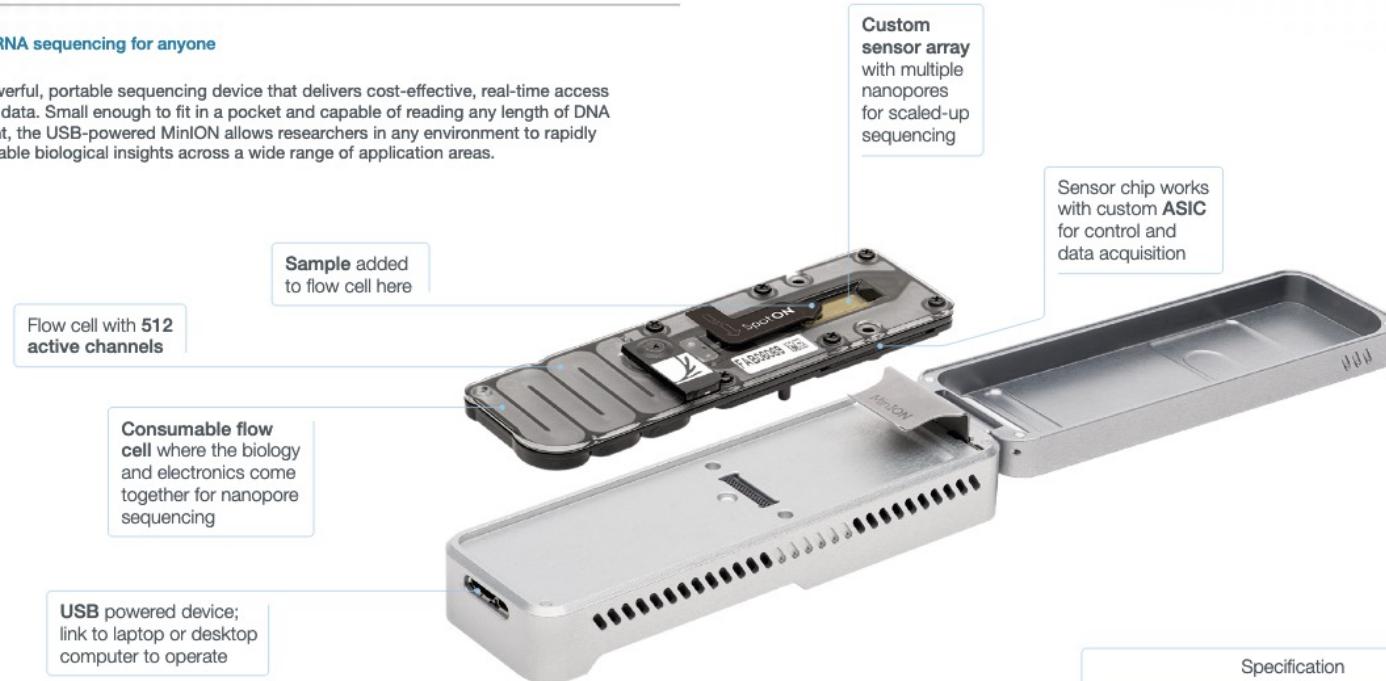
Instruments disponibles



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.

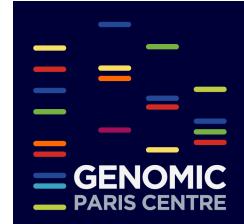


Specification	
Weight	87 g (103 g with flow cell)



Order now store.nanoporetech.com/devices

MinION Mk1c



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: Bluetooth and Wi-Fi enabled — upload and share your data, wherever you are

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

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



Data files are written to an **onboard, high-capacity SSD**; data can then be transferred to your own system

Specification

Weight
420 g

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



Order now store.nanoporetech.com/devices

GridION Mk1



GridION Mk1

High-throughput, benchtop system with integrated compute module

With the capacity to run five flow cells either concurrently or individually, GridION provides busy labs and service providers with cost-efficient, on-demand access to the advantages of real-time nanopore sequencing. Integrated, high-performance data processing alleviates the need for complex IT infrastructure.

Up to 2,560 active channels
can be sequencing at one time on the GridION

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

Onboard data analysis offering real-time local analysis



Sample added to flow cell here

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



Service provider certification is available for the GridION

Specification

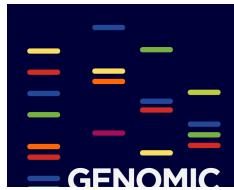
Weight
11 kg

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



Order now store.nanoporetech.com/devices

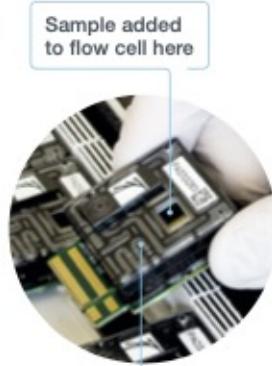
PromethION



PromethION 24 and PromethION 48

High-throughput, high-sample number benchtop systems

PromethION devices deliver flexible, high-yield, benchtop sequencing ideal for large-scale projects and high-throughput laboratories. Up to 24 (PromethION 24) or 48 (PromethION 48) high-capacity flow cells can be run either simultaneously or individually, delivering on-demand access to terabases of sequencing data at your desired read length — from short to ultra long (e.g. >2 Mb). Integrated, high-performance compute allows real-time base calling and onward analysis for rapid access to results.



Sample added to flow cell here

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

Each flow cell comprises up to 3,000 active channels



Service provider certification is available for the PromethION



Sequencing module

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



Compute module

PromethION 48 can deliver over 7 Tb of data in a single run

Specification

	Weight	Size
Sequencing module:	28 kg	W 590 mm H 190 mm D 430 mm
Compute module:	25 kg	W 178 mm H 440 mm D 470 mm

Order now store.nanoporetech.com/devices

Comparaison instrumentation



	Flongle	MinION Mk1B	MinION Mk1C	GridION Mk1	PromethION 24	PromethION
Read length		Nanopores read the length of DNA presented to them. Longest read so far: > 4 Mb.				
Max yield per flow cell (run on control samples; internal data)	2 Gb	44 Gb	44 Gb	44 Gb	242 Gb	242 Gb
Number of flow cells per device	1	1	1	5	24	48
Max yield per device (run on control samples; internal data)	2 Gb	44 Gb	44 Gb	220 Gb	5 Tb	10 Tb
Best in field yield per flow cell (yields will vary according to sample and preparation methods)	1 - 1.8 Gb	42 Gb	42 Gb	42 Gb	245 Gb	245 Gb
System access	From \$1,460	From \$1,000	From \$4,900	From \$49,995	From \$195,455	From \$285,455

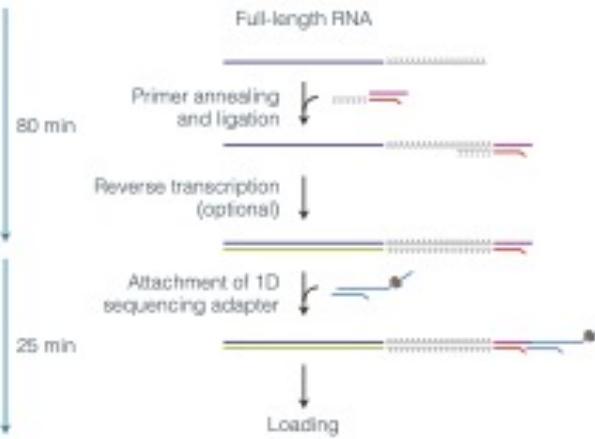
Préparation des librairies ARN



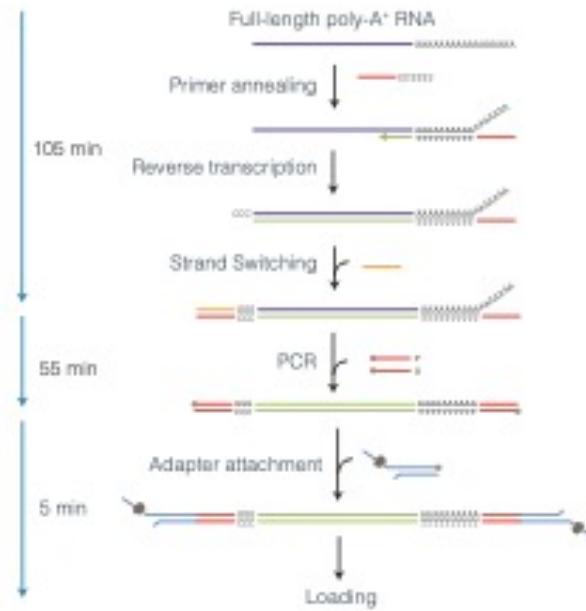
For sequencing the RNA molecule directly

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

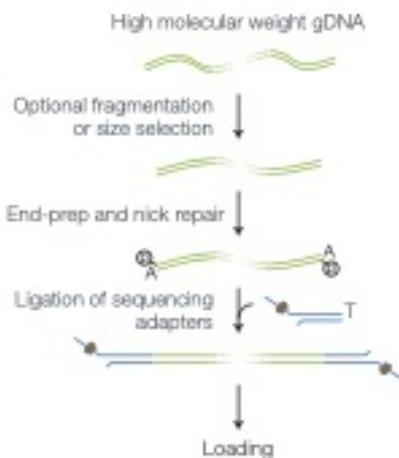
Préparation des librairies ADN



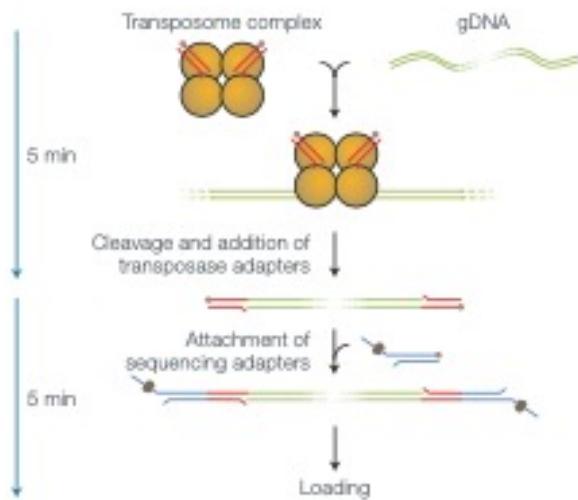
For maximum throughput

For minimal preparation time

Ligation Sequencing Kit



Rapid Sequencing Kit with transposase



- DNA ends are repaired and dA-tailed
- Sequencing adapters are ligated onto the prepared ends
- Fragment lengths can be controlled by fragmentation or size selection

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

Retour d'expérience



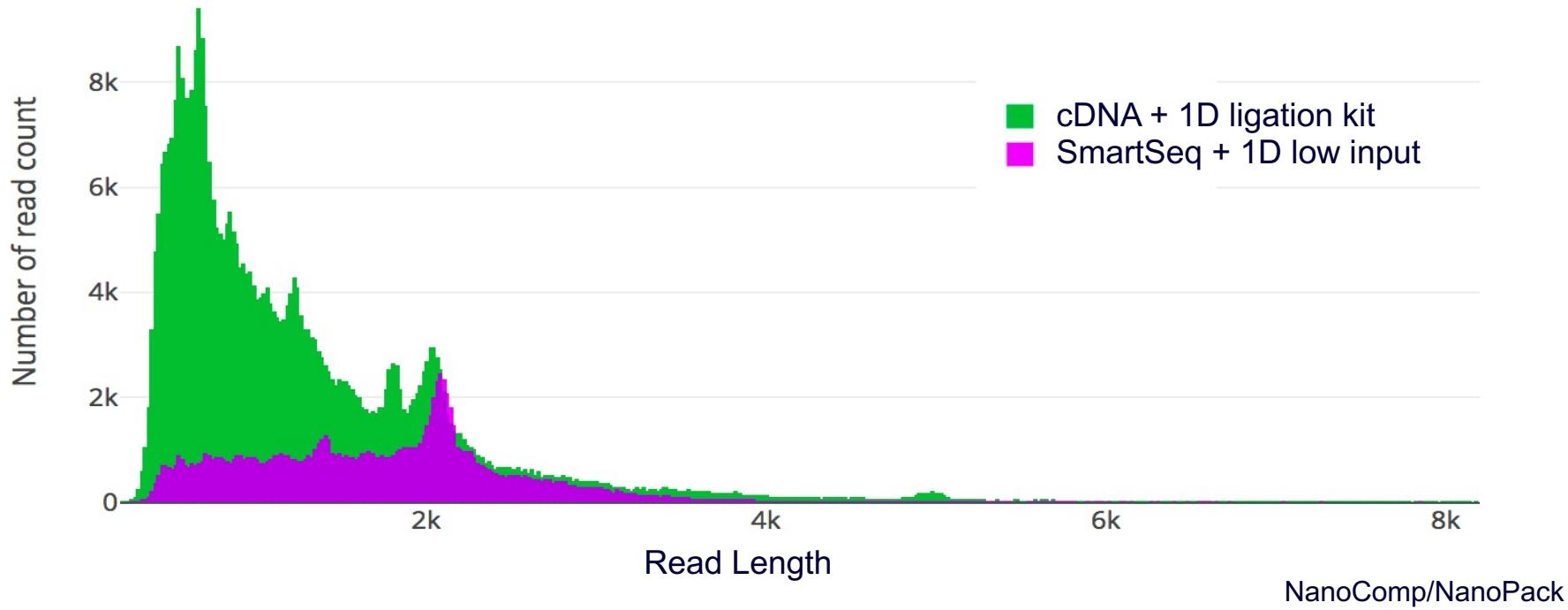
Reducing the amount of starting material



We compared **two protocols**:

- 100 ng Total RNA: cDNA (ONT)
+ 1D ligation kit (ONT);
- 1 ng Total RNA: SmartSeq (Clontech)
+ 1D low input (ONT).

Protocol	Mean read length	Read number
cDNA + Ligation kit	1.2 kb	5 millions
SmartSeq + Low input	1.9 kb	2 millions



We combined SmartSeq with 1D ligation kit

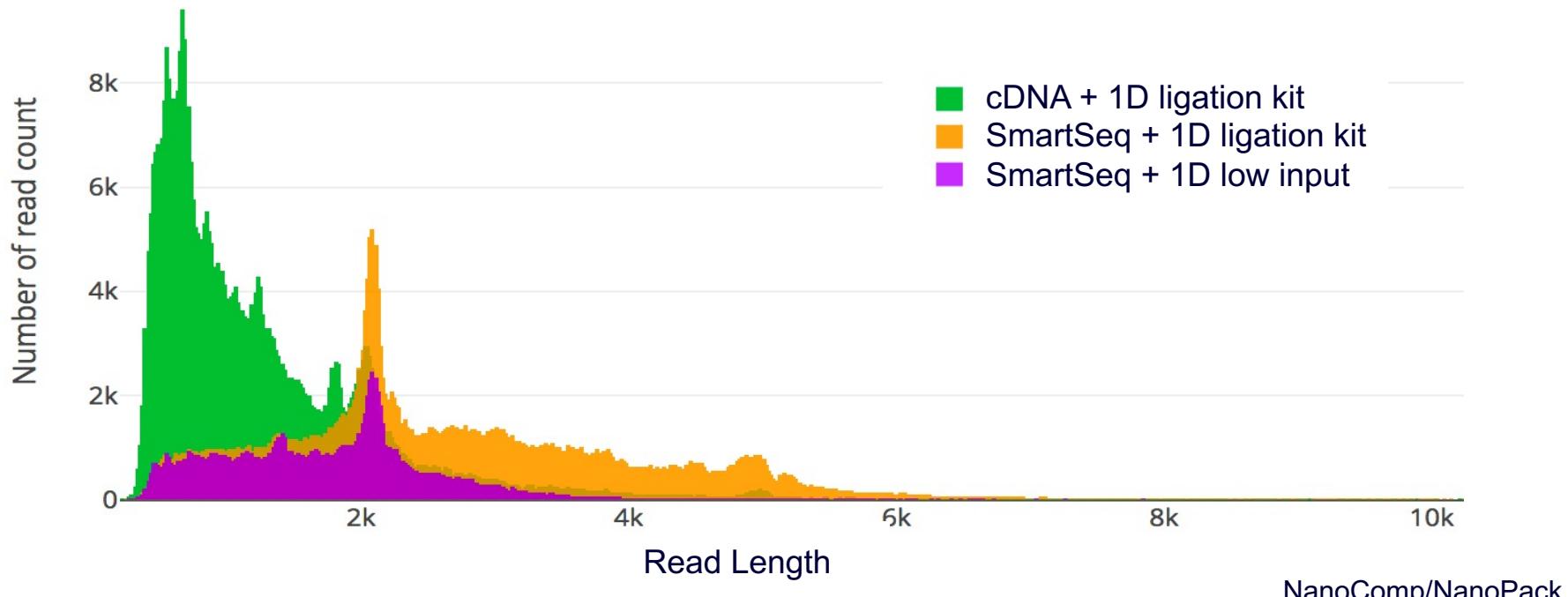


— We used:

- **10 ng of total RNA;**
- SmartSeq v4 (Clontech);
- 1D ligation kit LSK108 (ONT).

Protocol	cDNA	Low input	SmartSeq
Mean	1.2 kb	1.9 kb	2.6 kb
Max	34.6 kb	11 kb	42.3 kb

— We got **3 millions reads** and more than **50%** of reads are **longer than 2 kb**.

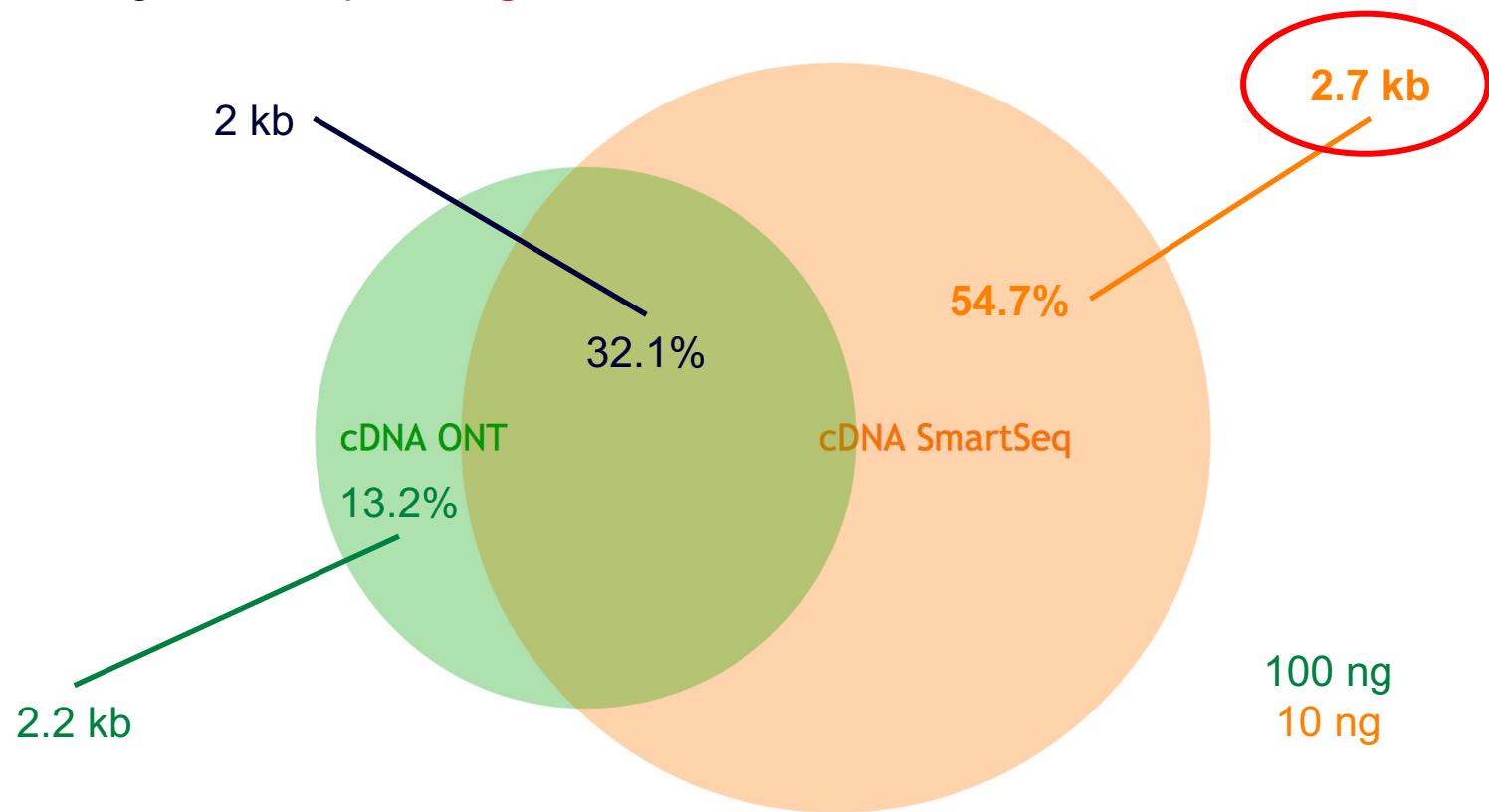


We obtained more differentially expressed genes



— DGE results (adjusted P-value < 0.01):

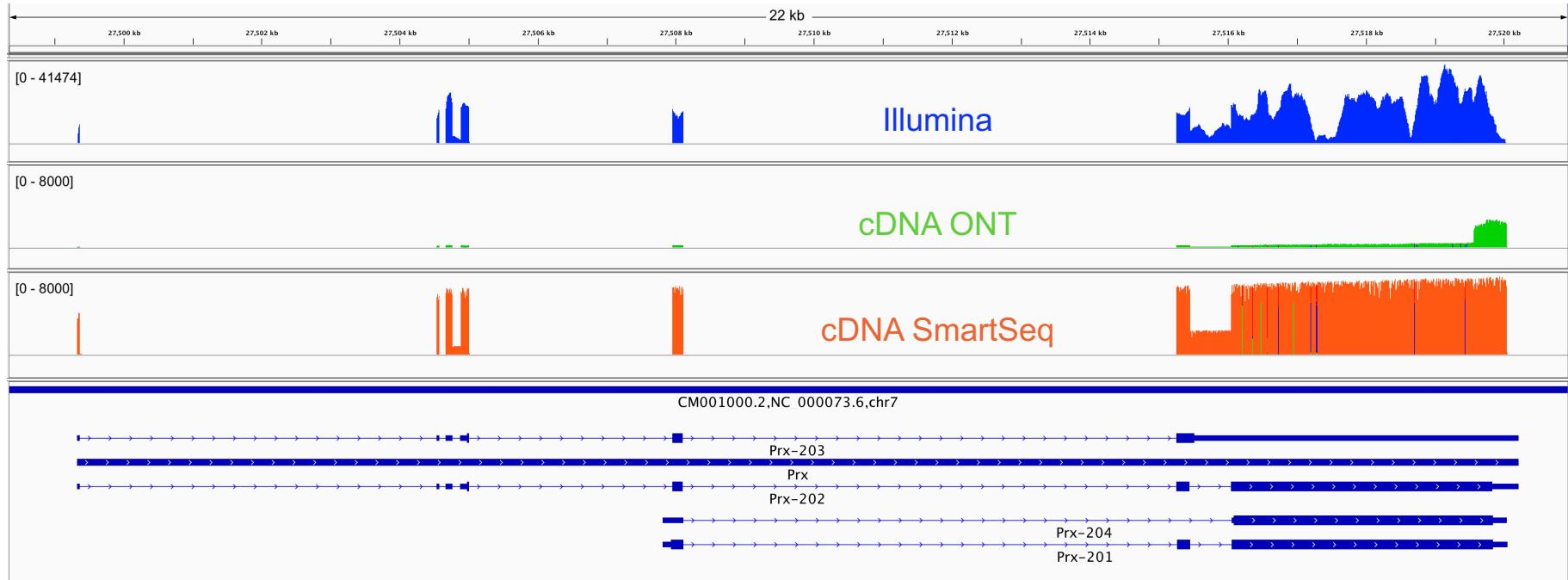
- 100 ng cDNA: 640 genes;
- 10 ng SmartSeq: **1,230 genes.**



And homogenous coverage along transcript



- Example with **long transcripts** like Periaxin (4,5 kb)



- Combining SmartSeq with ONT's ligation protocol allows to **sequence longer transcripts** with a **higher 5'-end coverage**.
- R&D: Single Cell long read

RUN MinION



Le nanopore sensor ou capteur

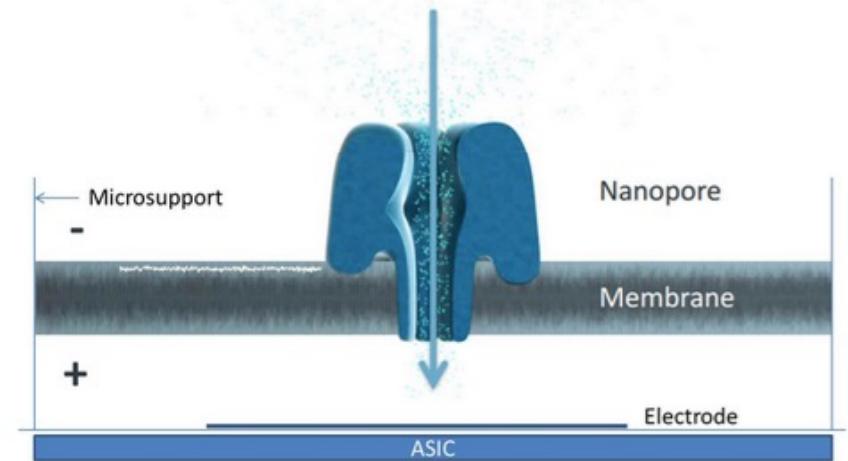


Composant du nanopore sensor:

- membrane
- Pore
- électrode
- circuit intégré: chanel ASIC

(Application-Specific Integrated Circuit)

Chaque électrode est connecté à un canal sur le circuit intégré (ASIC: Application-Specific Integrated Circuit) qui contrôle et mesure le signal du pore



Un logiciel contrôle le nanopore sensor, récupère et traite le signal du pore

Lien entre le nanopre sensor et le circuit ASIC

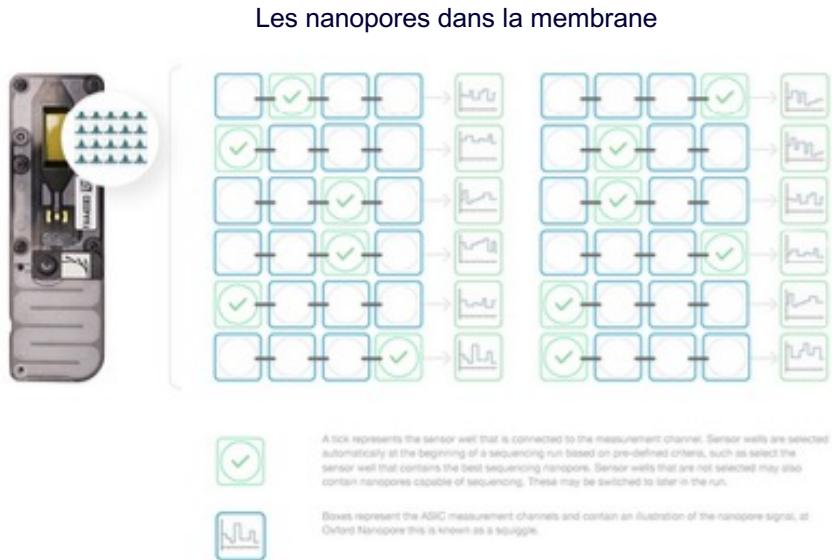
(Application-Specific Integrated Circuit)



La Flow Cell du MinION contient 2048 nanopore sensor dans des puits

Ils sont connectés à un circuit intégré de 512 canaux. Chaque canal du circuit intégré sélectionne 4 nanopore sensor

Un scan est fait au début de chaque expérience et l'ASIC choisi le meilleur nanopore sensor disponible



Les nanopore sensor non sélectionnés restent disponibles et peuvent être utilisés plus tard pendant le run

L'ASIC peut contrôler d'autres conditions que la mesure du signal comme le voltage et la capacité du pore. Ceci permet d'inverser le potentiel et tenter d'expulser un analyte qui bloquerait le pore comme des impuretés ou une structure secondaire complexe

Evolution du voltage durant un run de séquençage

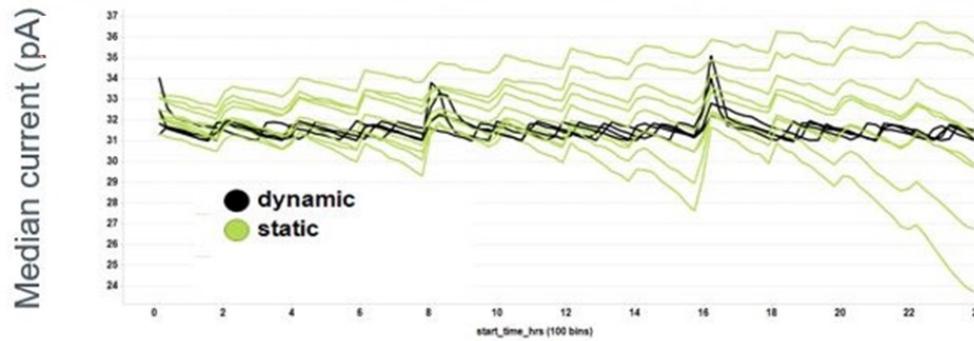
Durant le séquençage, le voltage change. Le démarrage se fait avec un voltage de -180mV qui est optimal pour la chimie R9.4. Le voltage dérive durant le run à cause de l'épuisement des réactifs rédox.

Afin de garder le voltage constant, le logiciel MinKNOW permet un « Dynamic Voltage Control » qui contrôle le signal brut et ajuste le voltage si besoin.

Ce système est plus stable dans le temps que le système précédent appelé « Static Voltage Control

DYNAMIC VOLTAGE CONTROL:

Tracks the median current range of strands and adjusts voltage as needed



Results in stable run conditions throughout the entire course of the experiment

Adjusting the starting potential

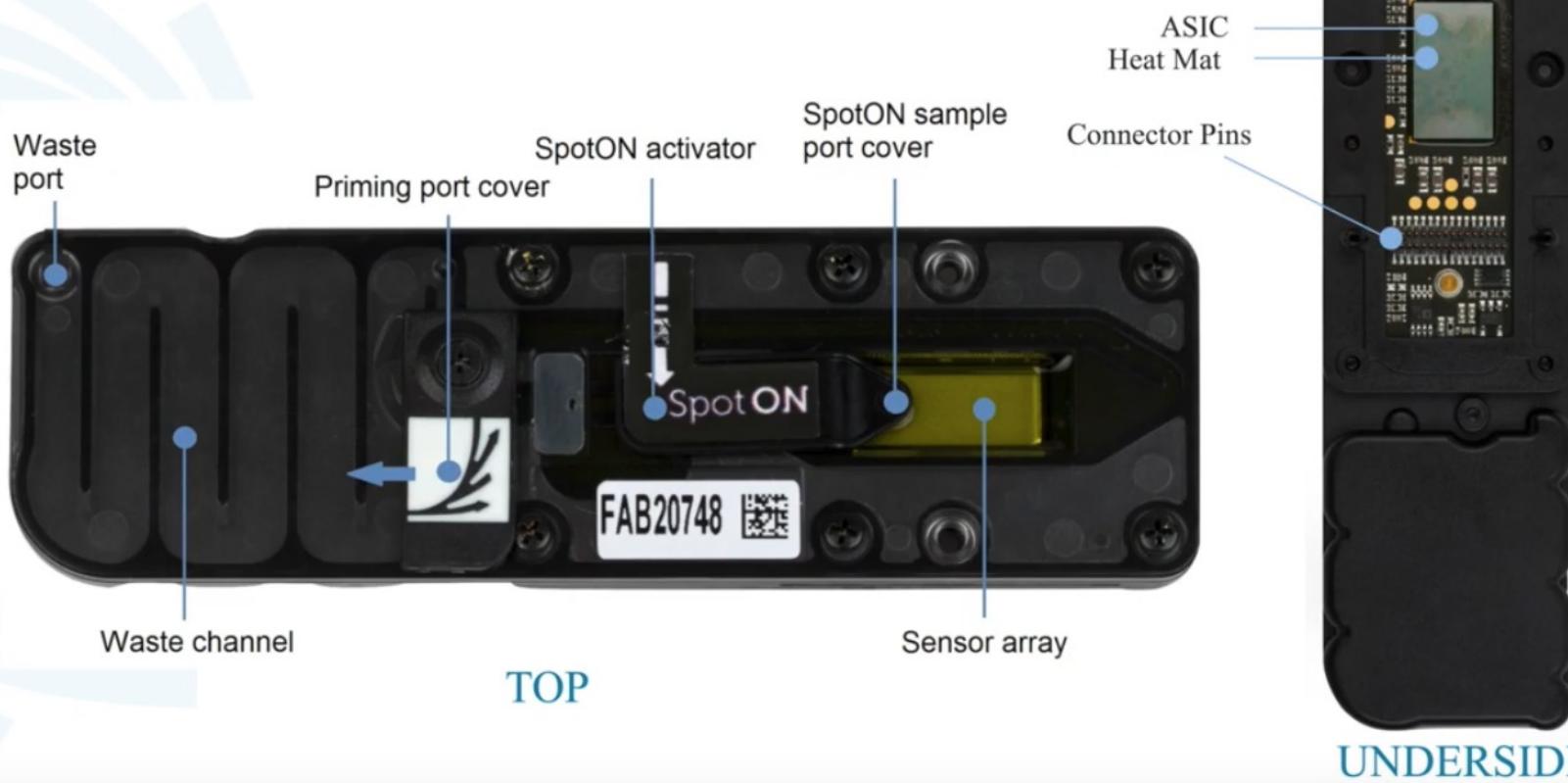
If a flow cell is re-used (e.g. after running an experiment and washing the sample out with the Flow Cell Wash Kit), the common voltage will be lower than -180 mV due to the voltage drift. The exact voltage value will depend on the length of the experiment. To account for drift, the starting voltage has to be adjusted for the next run on the same flow cell using the following scheme:

Total previous runtime (hours)	Voltage to set (mV) for MinION Mk 1B, Flongle, or GridION	Voltage to set (mV) for PromethION
12	-190	-180
24	-210	-200
36	-230	-220
48 or more	-250	-240

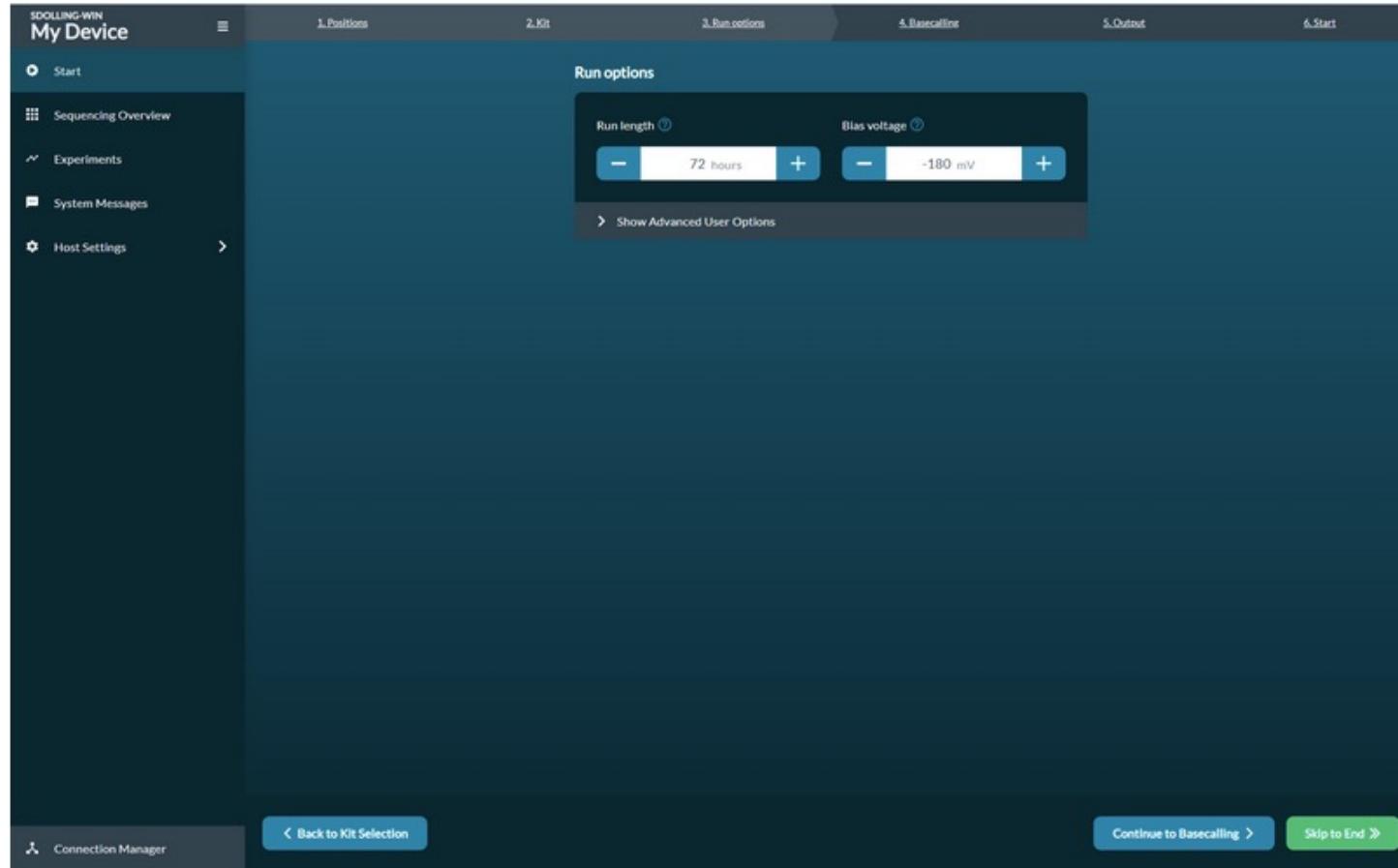
Flow Cell



MinION FLOW CELL COMPONENTS?



Select **Start Sequencing** of the homepage and navigate to **Run Options** to alter the bias voltage by selecting and typing or using the + and – options to the appropriate voltage.



The screenshot shows the MinNOW software interface. On the left, there is a sidebar with the following items:

- SDOLLING-WIN
- My Device** (highlighted)
- Start (radio button selected)
- Sequencing Overview
- Experiments
- System Messages
- Host Settings

The main area has a breadcrumb navigation: 1. Positions → 2. Kit → 3. Run options → 4. Basecalling → 5. Output → 6. Start.

The central part of the screen is titled "Run options". It contains two input fields:

- "Run length" set to "72 hours" with a minus sign, a plus sign, and a tooltip icon.
- "Bias voltage" set to "-180 mV" with a minus sign, a plus sign, and a tooltip icon.

Below these fields is a link "Show Advanced User Options". At the bottom of the screen are three buttons: "Connection Manager", "< Back to Kit Selection", "Continue to Basecalling >", and "Skip to End >".

Pore occupancy



- A good library will be indicated by a higher proportion of light green channels in **Sequencing** than are in **Pore**. The combination of Sequencing and Pore indicates the number of active pores at any point in time. A low proportion of Sequencing channels will reduce the throughput of the run.
- **Recovering** indicates channels that may become available for sequencing again. A high proportion of this may indicate additional clean up steps are required during your library preparation.
- **Inactive** indicates channels that are no longer available for sequencing. A high proportion of these as soon as the run begins may indicate an osmotic imbalance.
- **Unclassified** are channels that have not yet been assigned one of the above classifications.



Pore occupancy



Clicking on the **Show Detailed** button reveals a more detailed array of channel states:

- **Strand:** the channel is in strand
- **Adapter:** the channel has just captured a new strand
- **Single pore:** the channel appears to show a single pore. Available for sequencing
- **Unavailable:** the channel appears to show a pore that is currently unavailable for sequencing
- **Active feedback:** the channel is reversing the current flow to eject the analyte
- **No pore from scan:** the [Mux scan](#)① has not detected a pore in the well
- **Out of range 2:** current level is between 10 and 9999 pA. Currently unavailable for sequencing
- **Possible multiple:** the channel appears to show more than one pore. Unavailable for sequencing
- **Saturated:** the channel has switched off due to current levels exceeding hardware limitations
- **Out of range 1:** current level is between -5 and -9999 pA. Currently unavailable for sequencing
- **Zero:** Current level is between -5 and 10 pA. Currently unavailable for sequencing



Duty time plots



The duty time plot summarises the channel states over time.

Each bar shows the sum of all channel activity in a particular amount of time. This time bucket defaults to 1 minute, and scales to 5 minutes automatically after reaching 48 buckets. However, bucket size can be adjusted in the "Bucket size" box in **Display Settings**.

The graph populates over time, and can be used as a way to assess the quality of your sequencing experiment, and make an early decision whether to continue with the experiment or to stop the run.



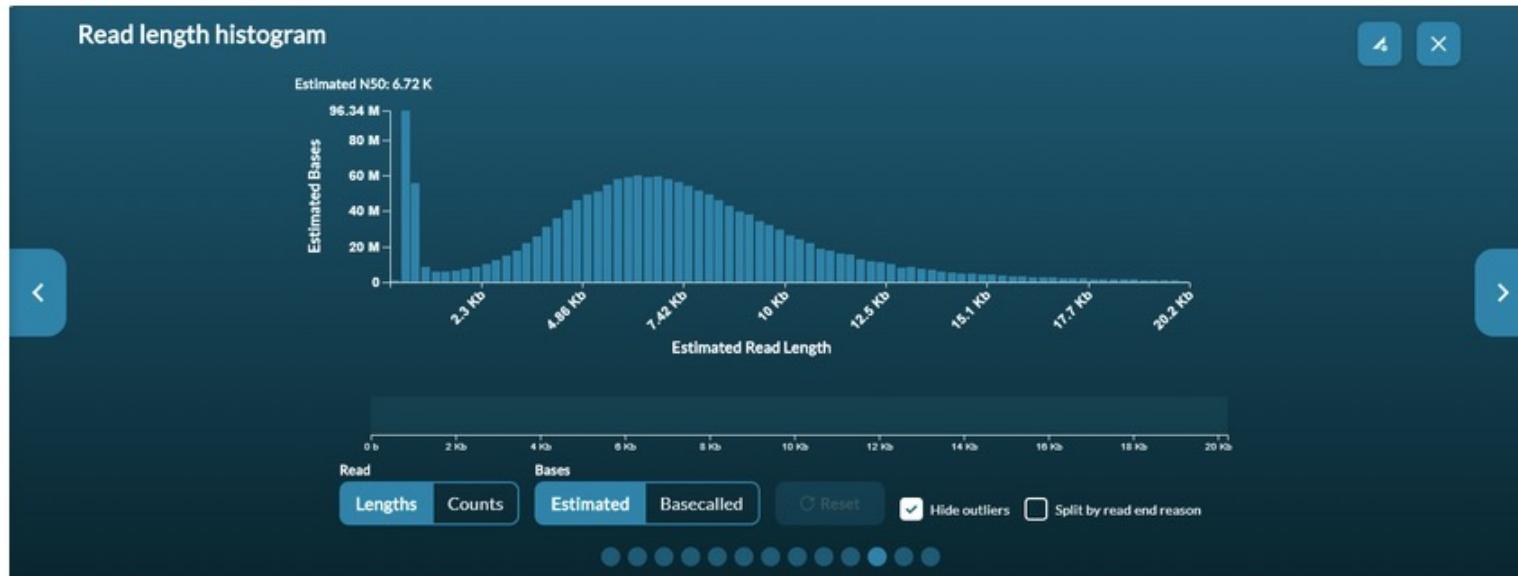
Read length histogram



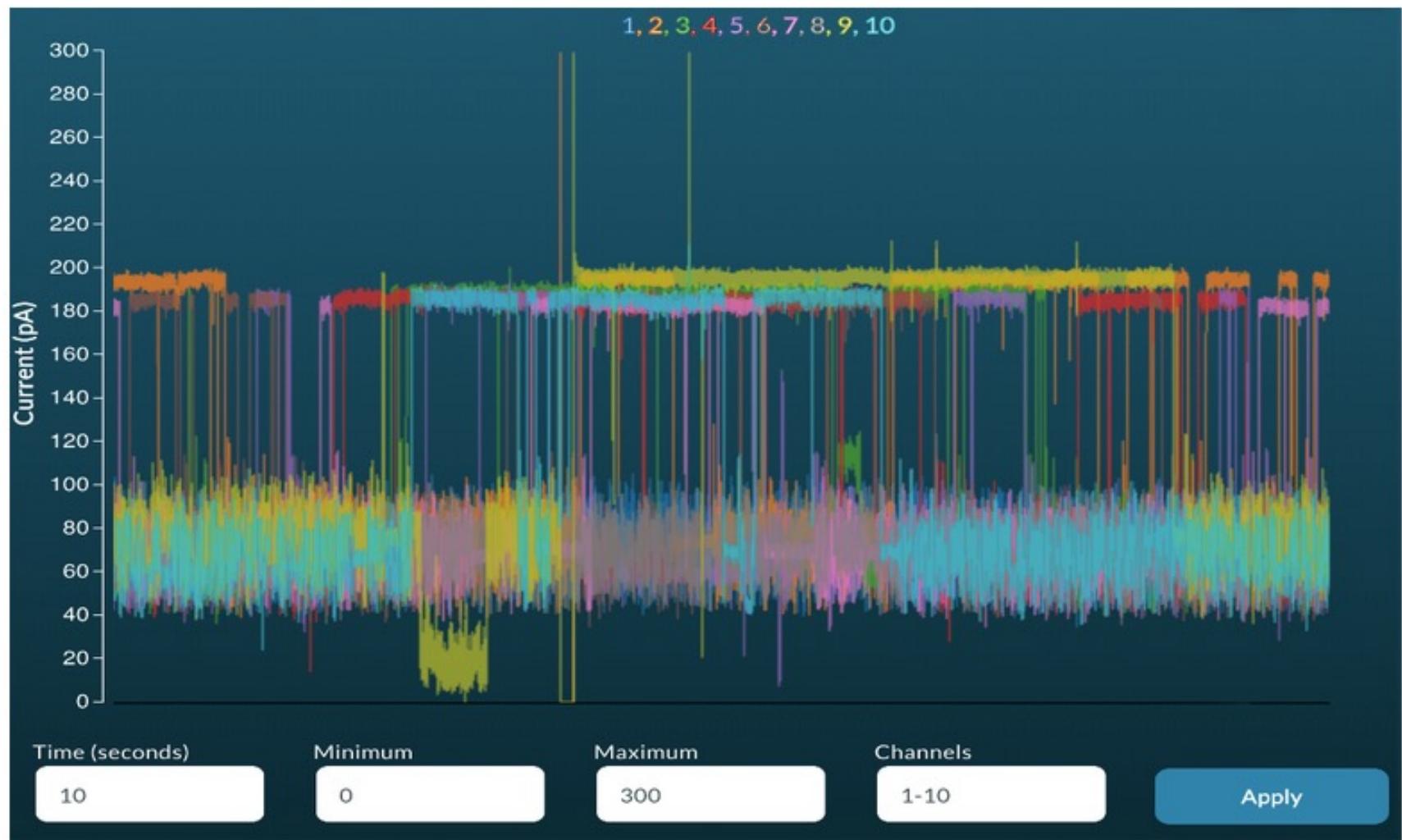
There are two cumulative histograms that can be toggled between in MinNOW. These are:

1. Read count - this shows the number of reads vs read length. This enables the user to understand how the read lengths vary in number and size.
2. Read length - this shows the total number of bases vs the read length.

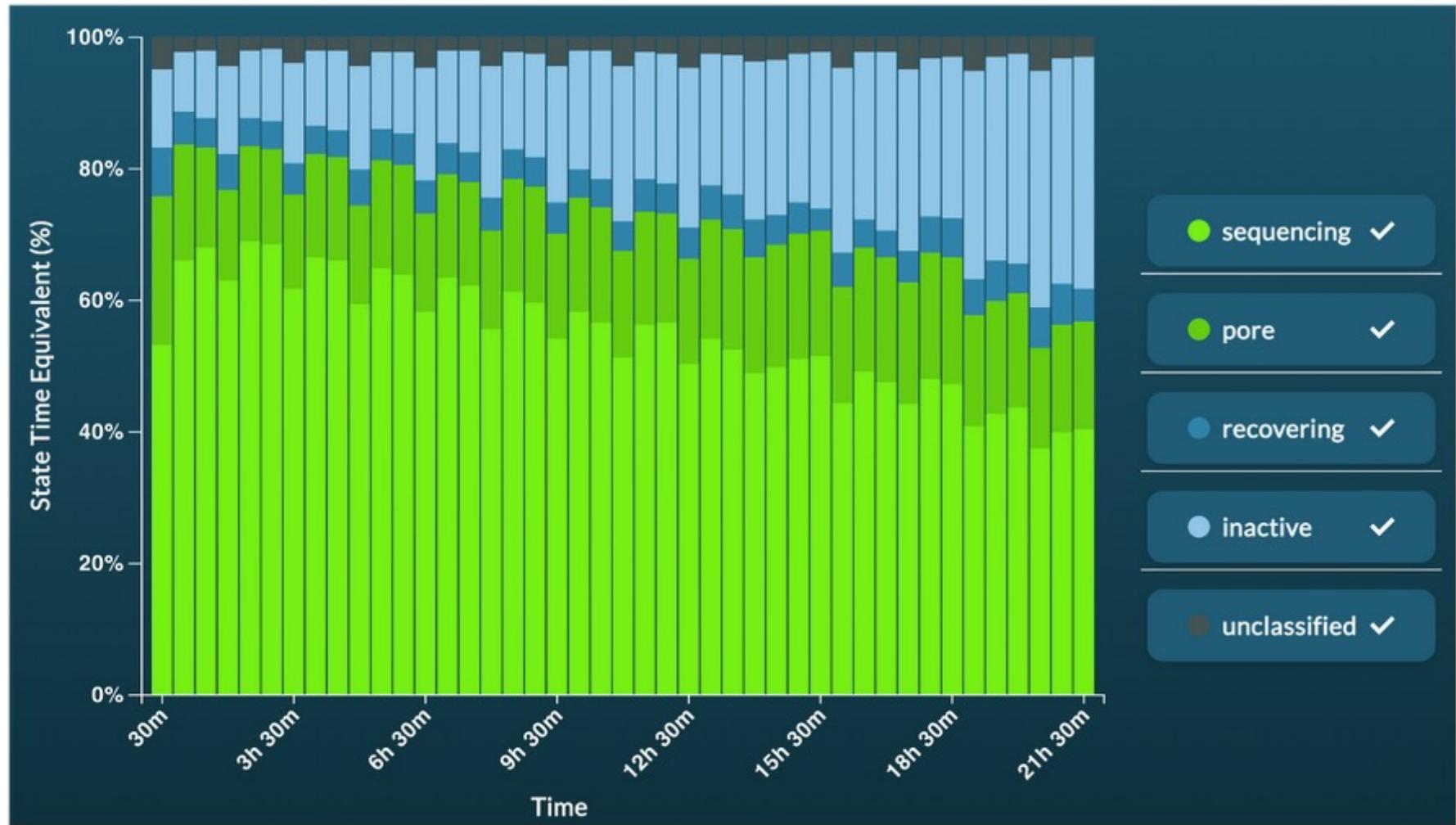
The N50 value is presented (only for the whole set of passed reads) in the top left corner of the histogram.



Voltage dans les pores



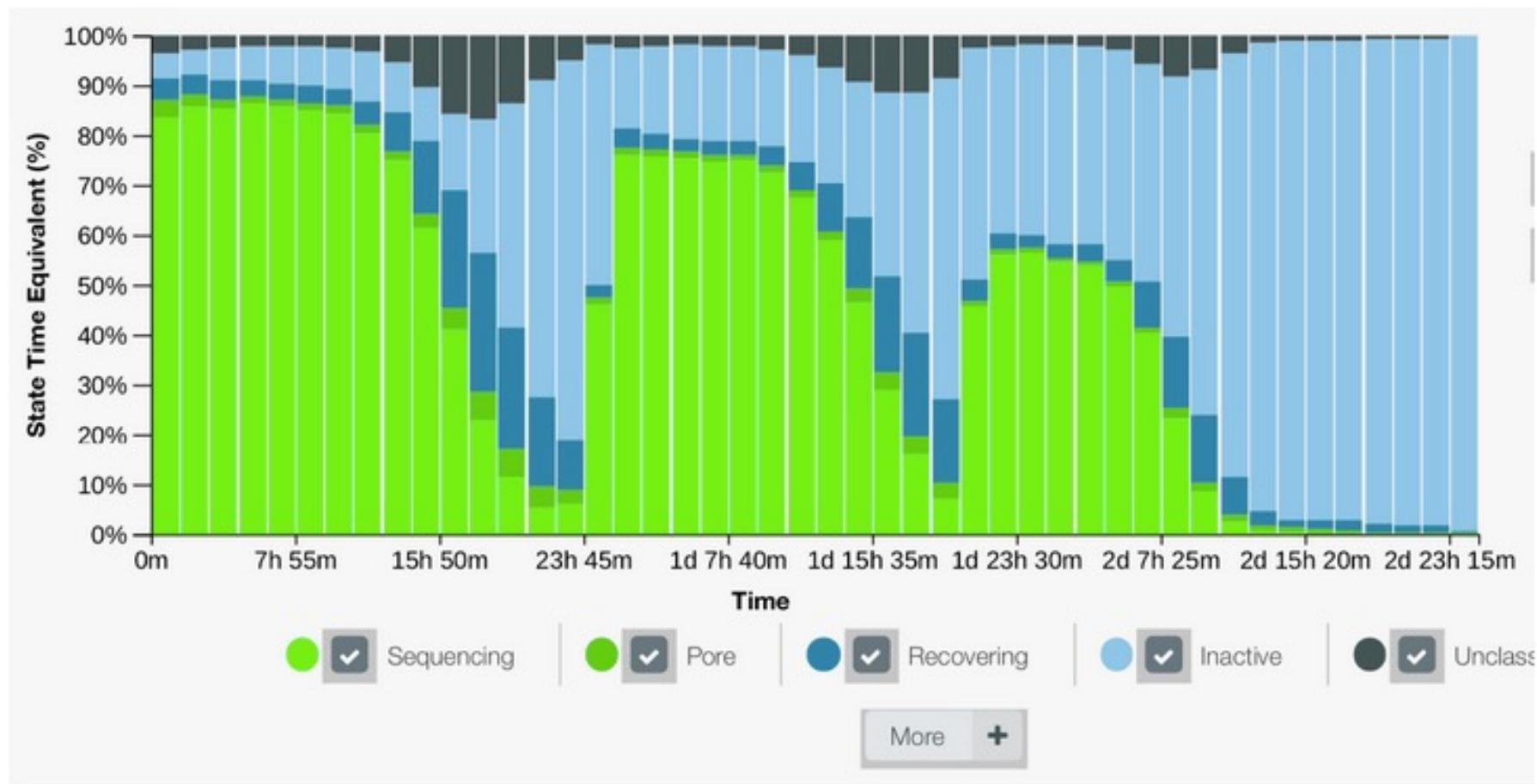
Duty Time Run RNA



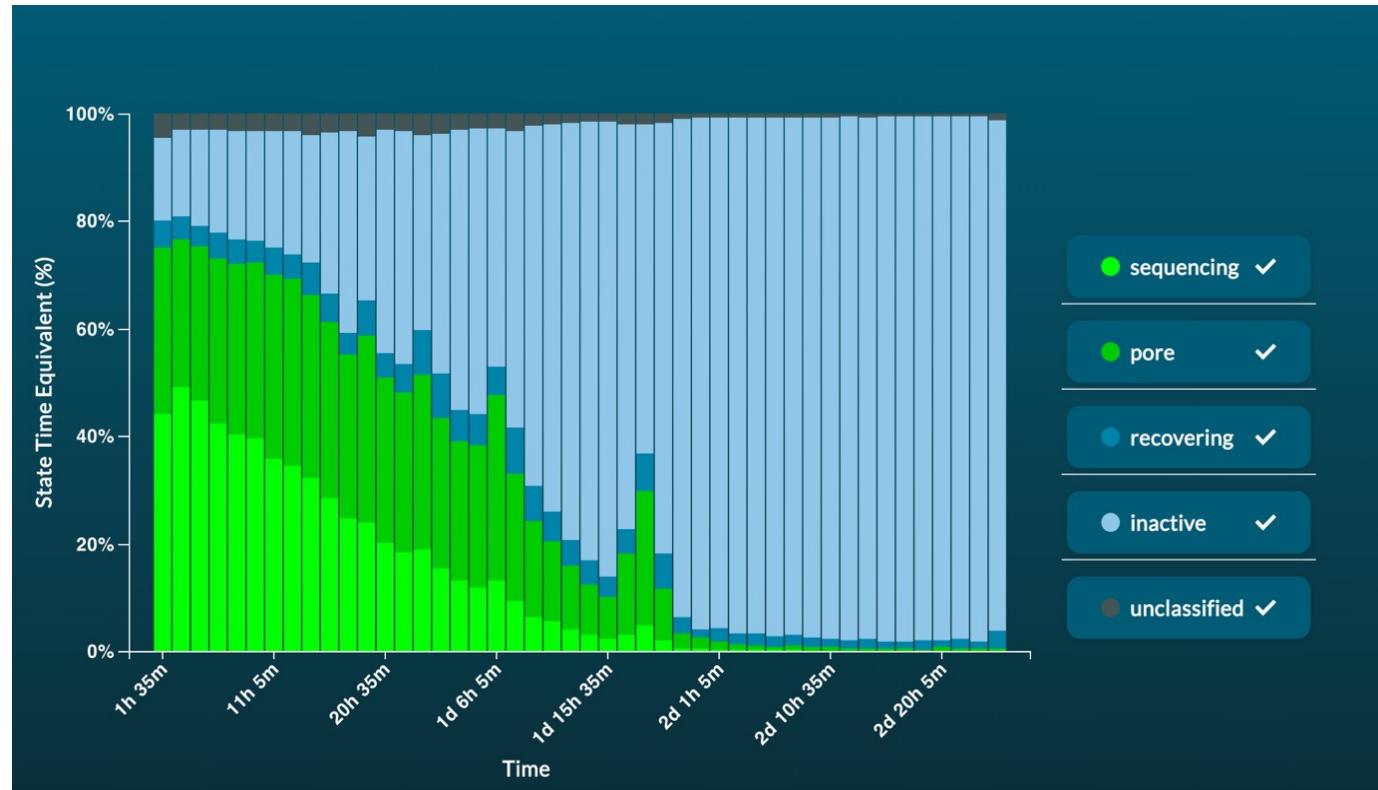
Re dépôt



Troubleshooting



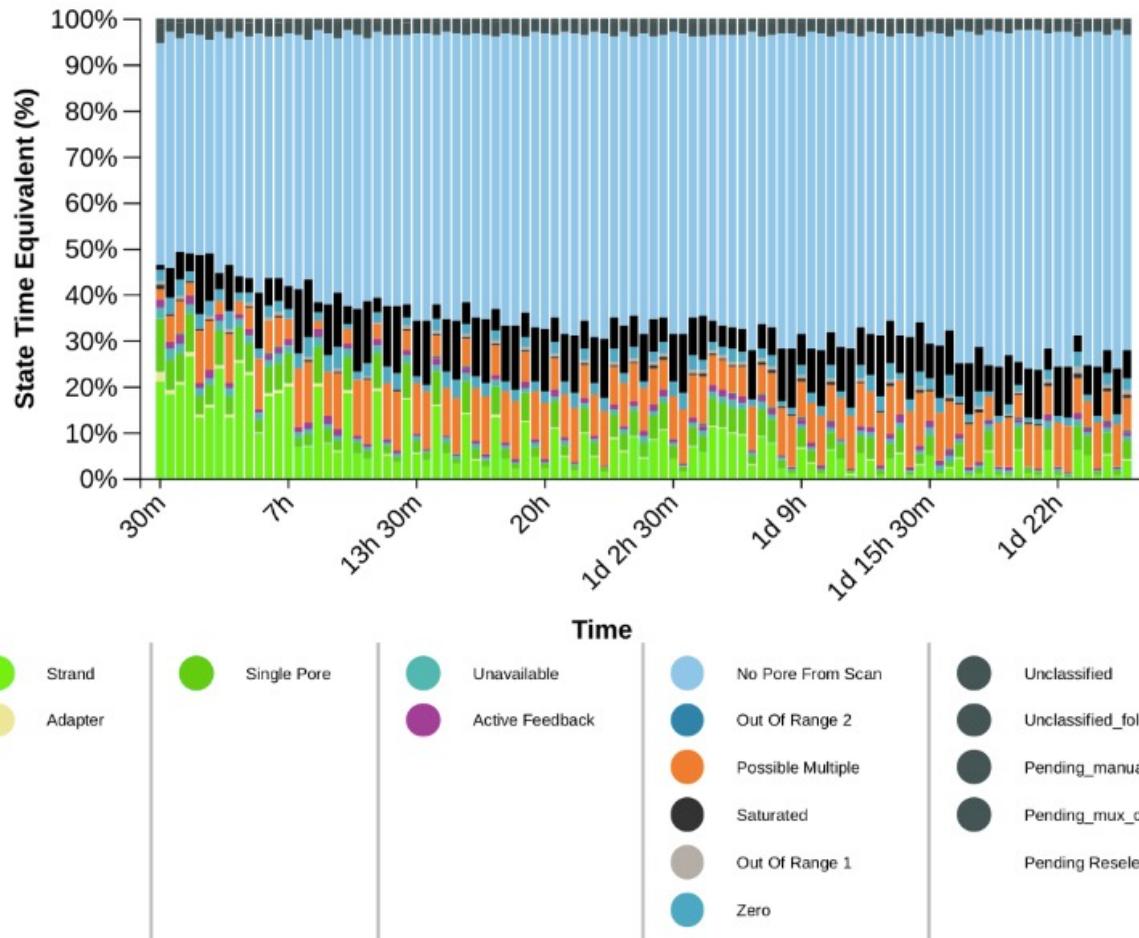
Troubleshooting



Troubleshooting



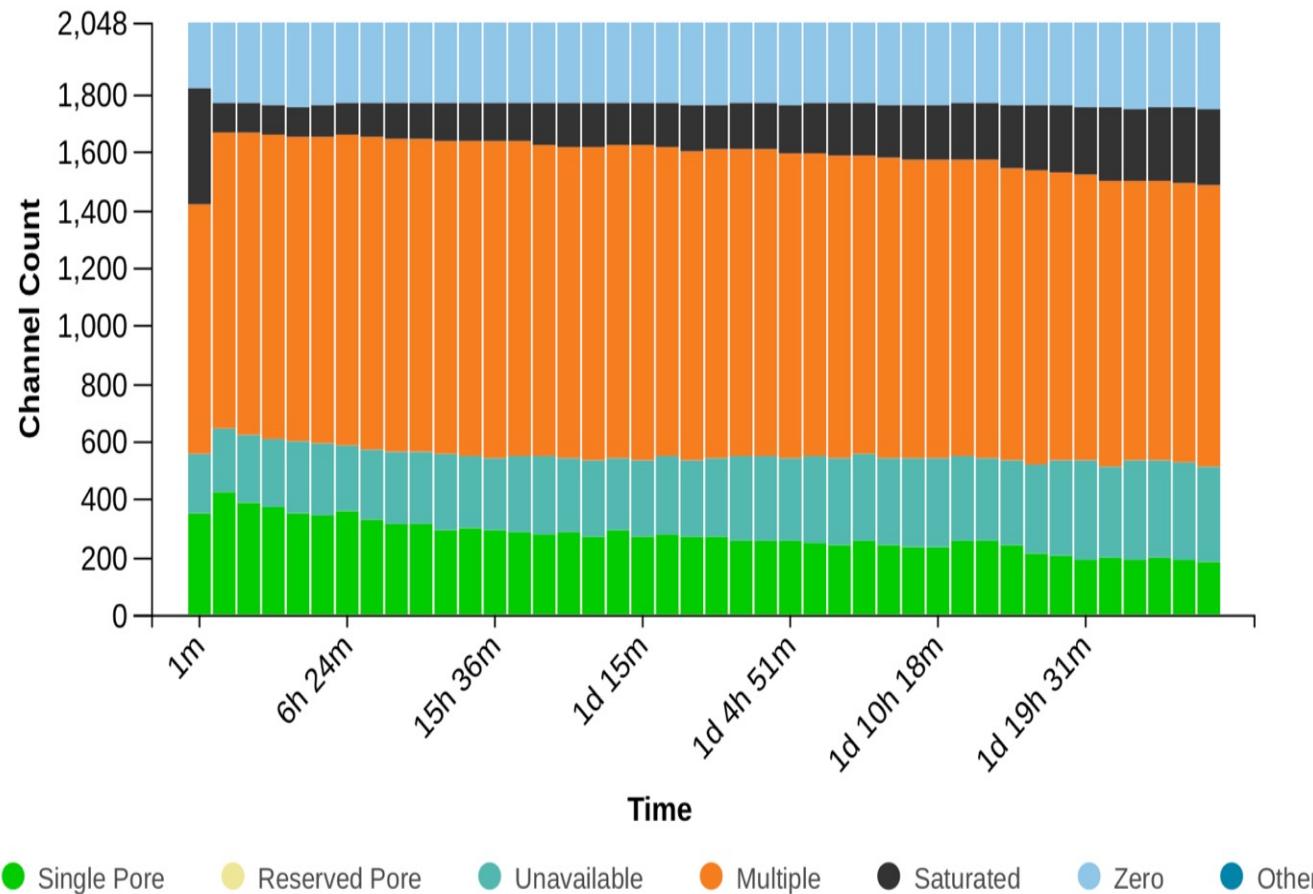
Duty time Categorised



Troubleshooting



Mux Scan Categorised



Troubleshooting



https://community.nanoporetech.com/protocols/experiment-companion-minknow/v/mke_1013_v1_revbl_11apr2016/troubleshooting-your-run-from-the-duty-time-plots

- Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FB) at RT.
- Mix the Sequencing Buffer (SQB), Flush Tether (FLT) and Flush Buffer (FB) tubes by vortexing and spin down at RT.
- Open the MinION Mk1B lid and slide the flow cell under the clip.
- Slide the priming port cover clockwise to open the priming port.

IMPORTANT

- Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles (a few µl):

- Set a P1000 pipette to 200 µl
- Insert the tip into the priming port
- Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip

- To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FB), and mix by vortexing at RT.
- Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below.
- Thoroughly mix the contents of the Loading Beads (LB) tubes by vortexing.

IMPORTANT

- The Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

Dépôt sur Flow Cell



In a new tube, prepare the library for loading as follows:

- 34 µl Sequencing Buffer (SQB)
- 25.5 µl Loading Beads (LB), mixed immediately before use
- 15.5 µl Nuclease-free water

Complete the flow cell priming:

- Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
- Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.

- Mix the prepared library gently by pipetting up and down just prior to loading.
- Add 75 µl of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.
- Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION Mk1B lid.

Ending the experiment

- After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Wash Kit instructions and store the washed flow cell at 2-8°C, OR
- Follow the returns procedure by washing out the flow cell ready to send back to Oxford Nanopore.

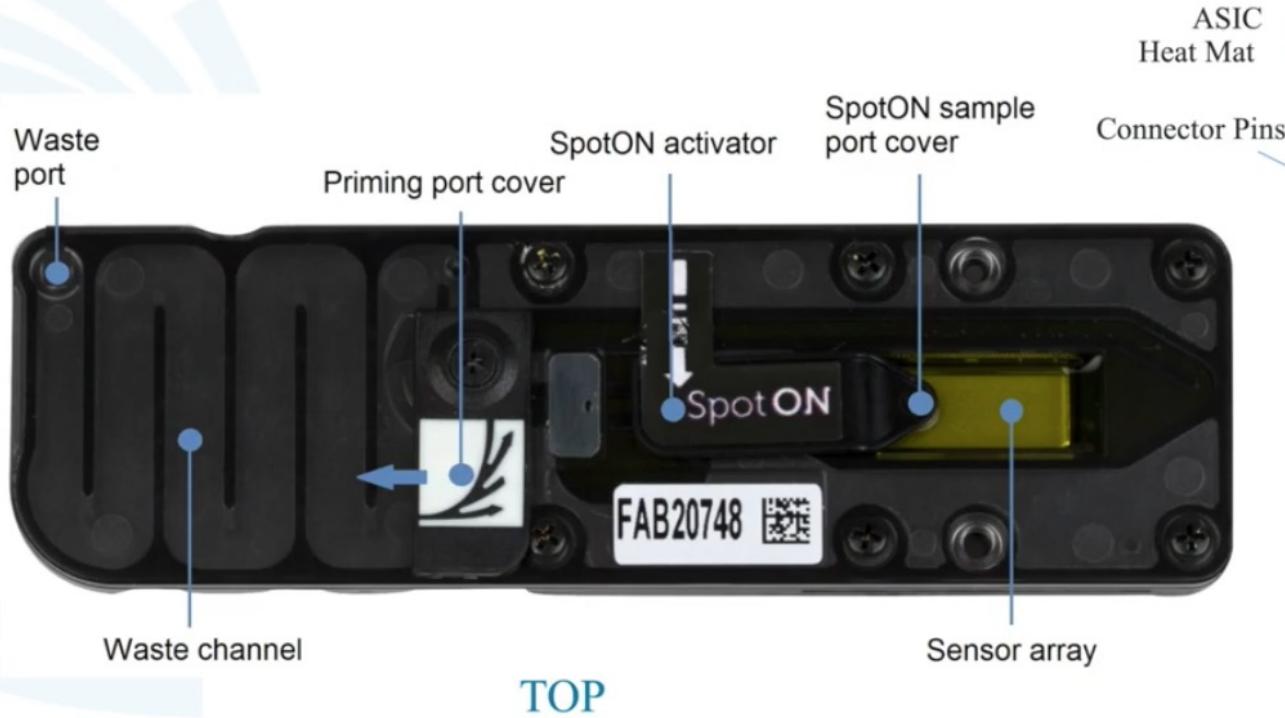
IMPORTANT

- If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in the online version of this protocol.

Flow Cell du MinION



MinION FLOW CELL COMPONENTS?





Library prep

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

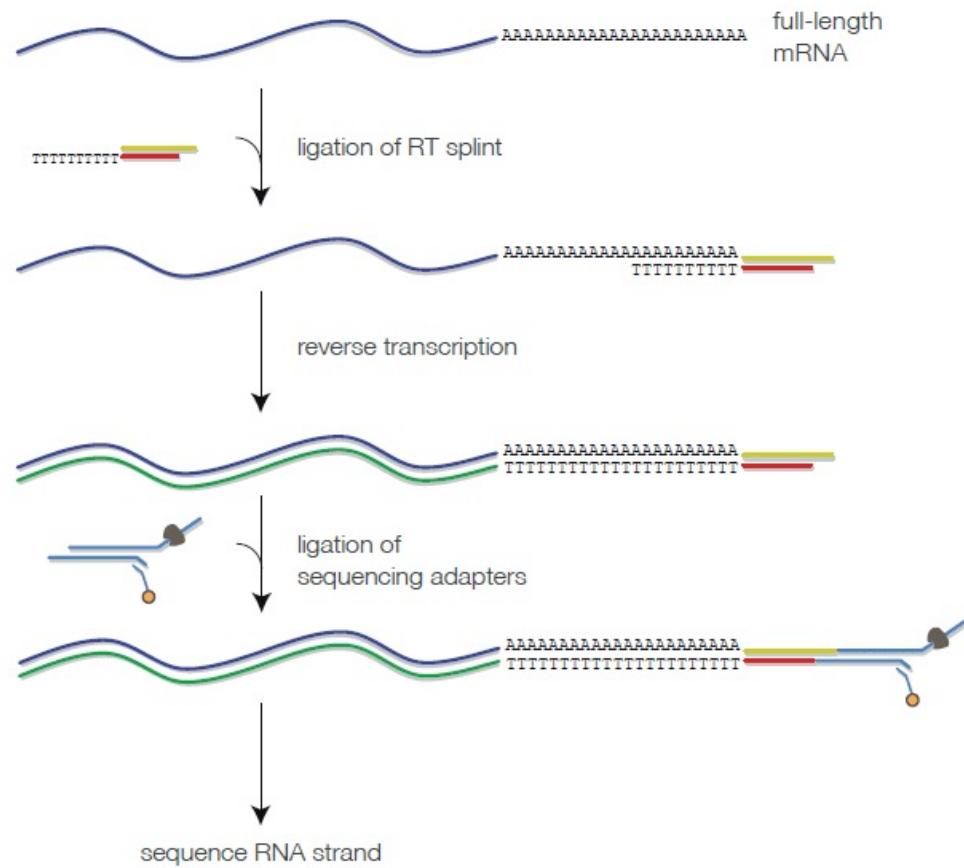


Direct RNA sequencing



Native RNA strand is sequenced:

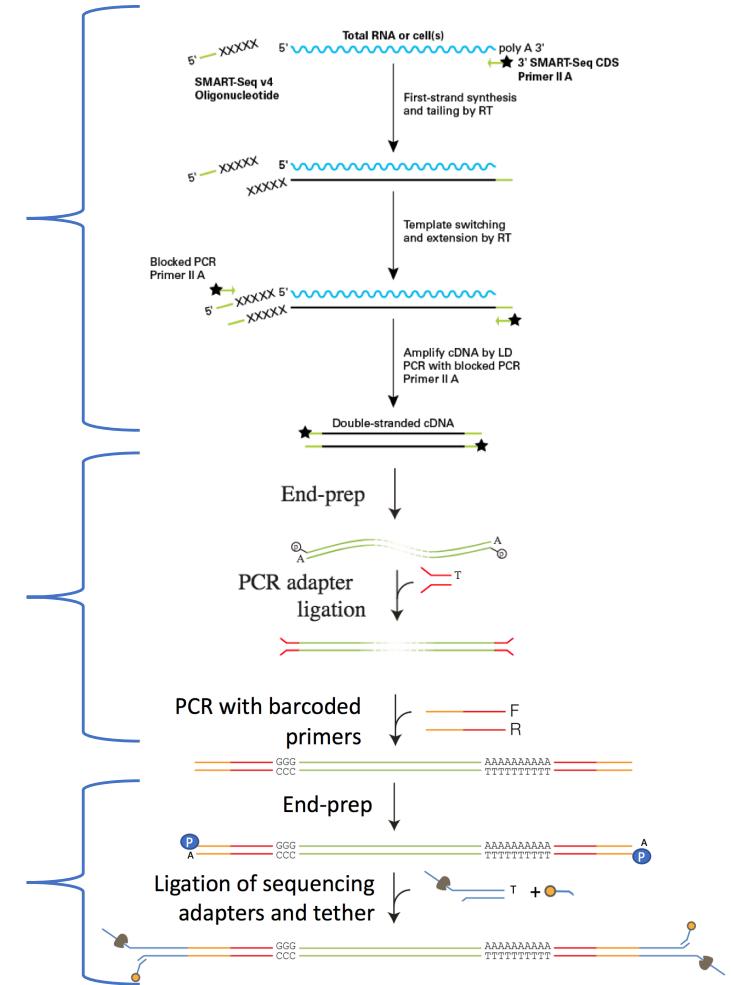
- No PCR biases;
- Modified bases detection (example methylation);
- 500 ng polyA+ RNA required.



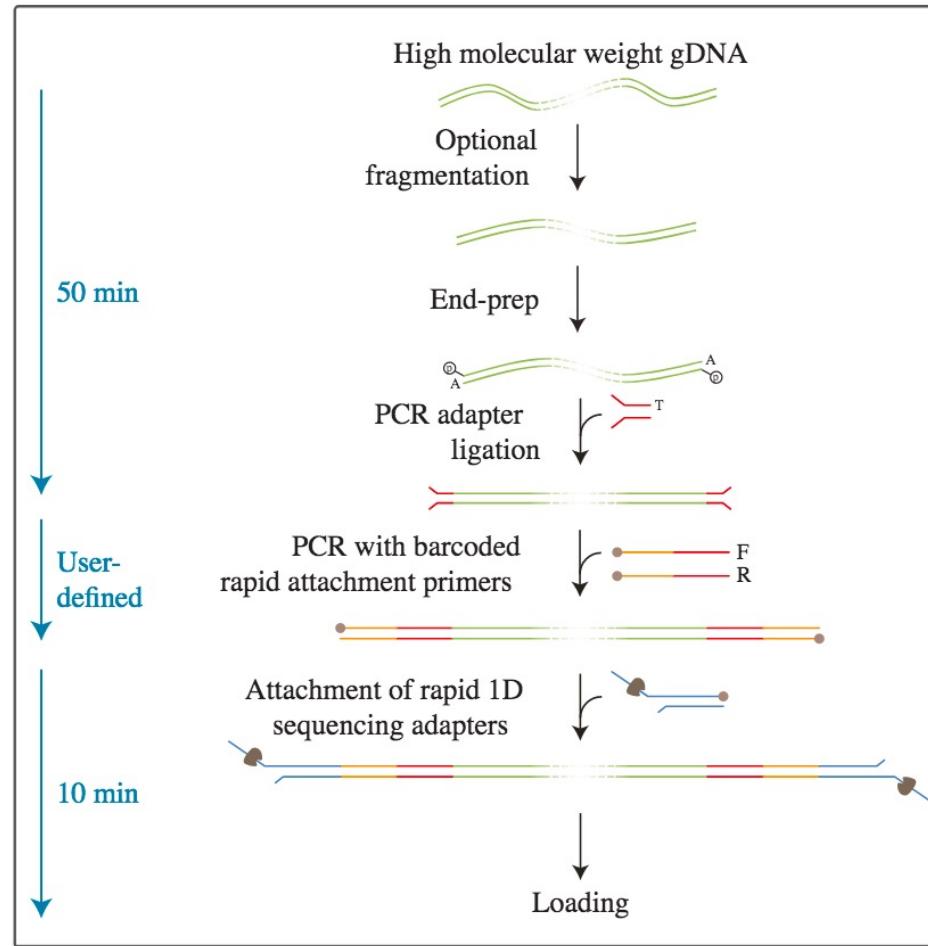
SmartLengthMinION_A2018



Kit	Etapes	Quantité Requise
SmartSeq v4 clontech	1er brin cDNA	10 ng d'ARN
	2 nd brin cDNA	
	PCR 9 cycles	Minimum 5 ng
PCR Barcoding kit - ONT	End prep	5 à 10 ng pour la PCR
	Ligation Barcode adapter (BCA)	
	PCR 18 cycles	2 à 3 µg post PCR
Ligation Sequencing kit 108 - ONT	Multiplexage	1 pmol poolé dans 50µL
	End repair	
	Ligation des adapters de séquençage	Dépôt : 0,3-0,4 pmol



SQK-PBK004 PCR Barcoding Kit



FLOW CELL

- Three main compartments
 - Common Reservoir
 - Bulk Buffer
 - Buffer in Wells
- Shipped with storage buffer in all 3 compartments.
- Separation allows change in running buffer without changing electrochemistry

