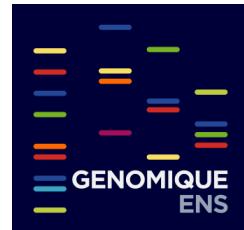


Formation à l'utilisation d'un séquenceur Minlon

Session expérimentale



L'équipe de la plateforme génomique de l'IBENS



Catherine
Senamaud-
Beaufort



Corinne
Blugeon



Morgane
Thomas-
Chollier



Stéphane
Le Crom



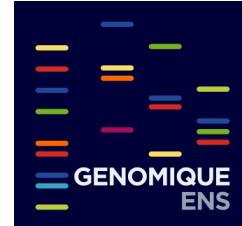
Laurent
Jourdren



Sophie
Lemoine

- Le personnel est composé de deux expérimentateurs et de deux bioinformaticiens statutaires

Evolution technologique



Microarrays
(1998 - 2013)

Illumina HiSeq 1500
2nd generation sequencer
(2011 - 2016)

Illumina NextSeq 500
2nd generation sequencer
(Since 2015)

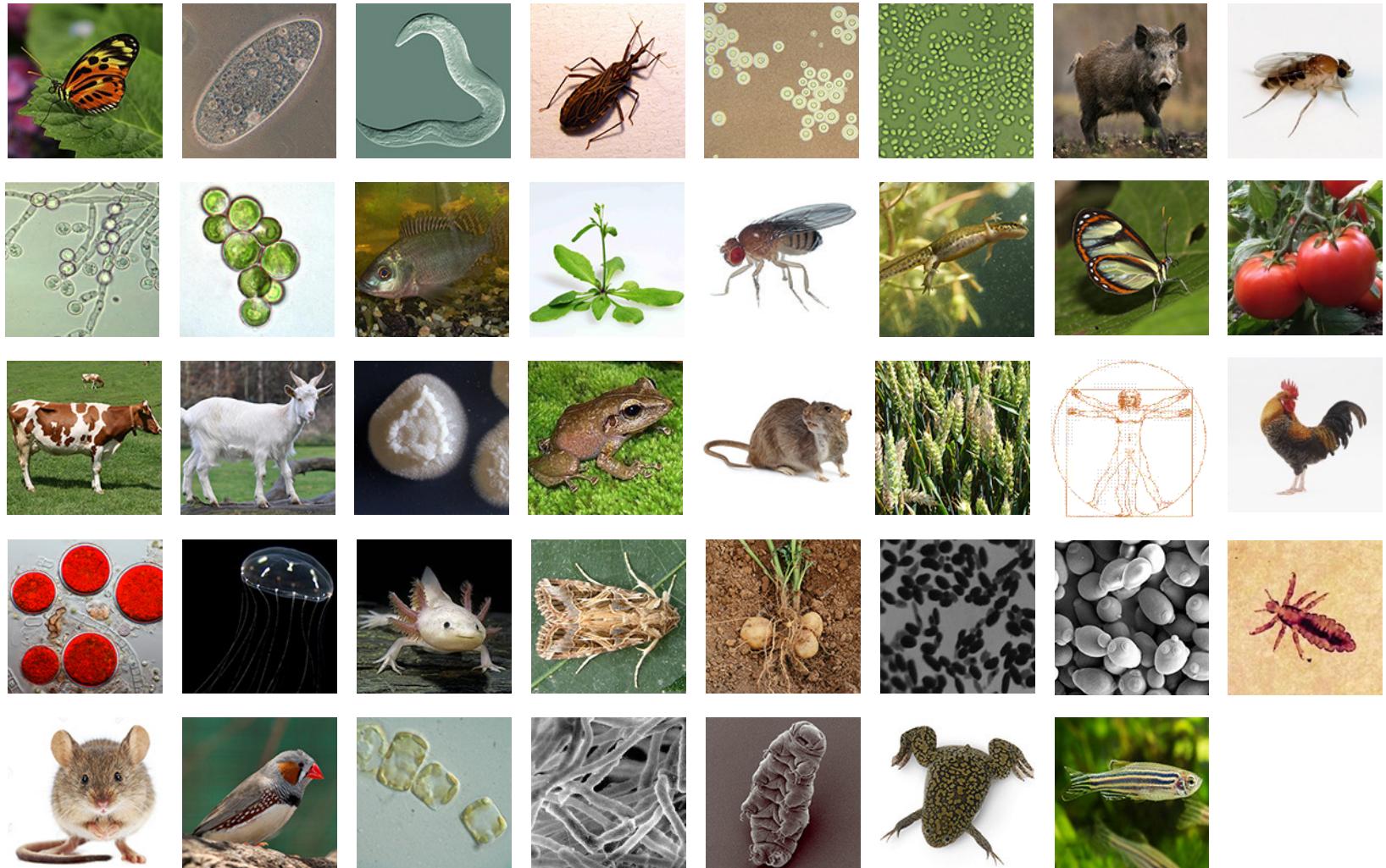
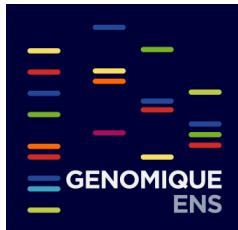
Nanopore MinION
3rd generation sequencer
(Since 2016)



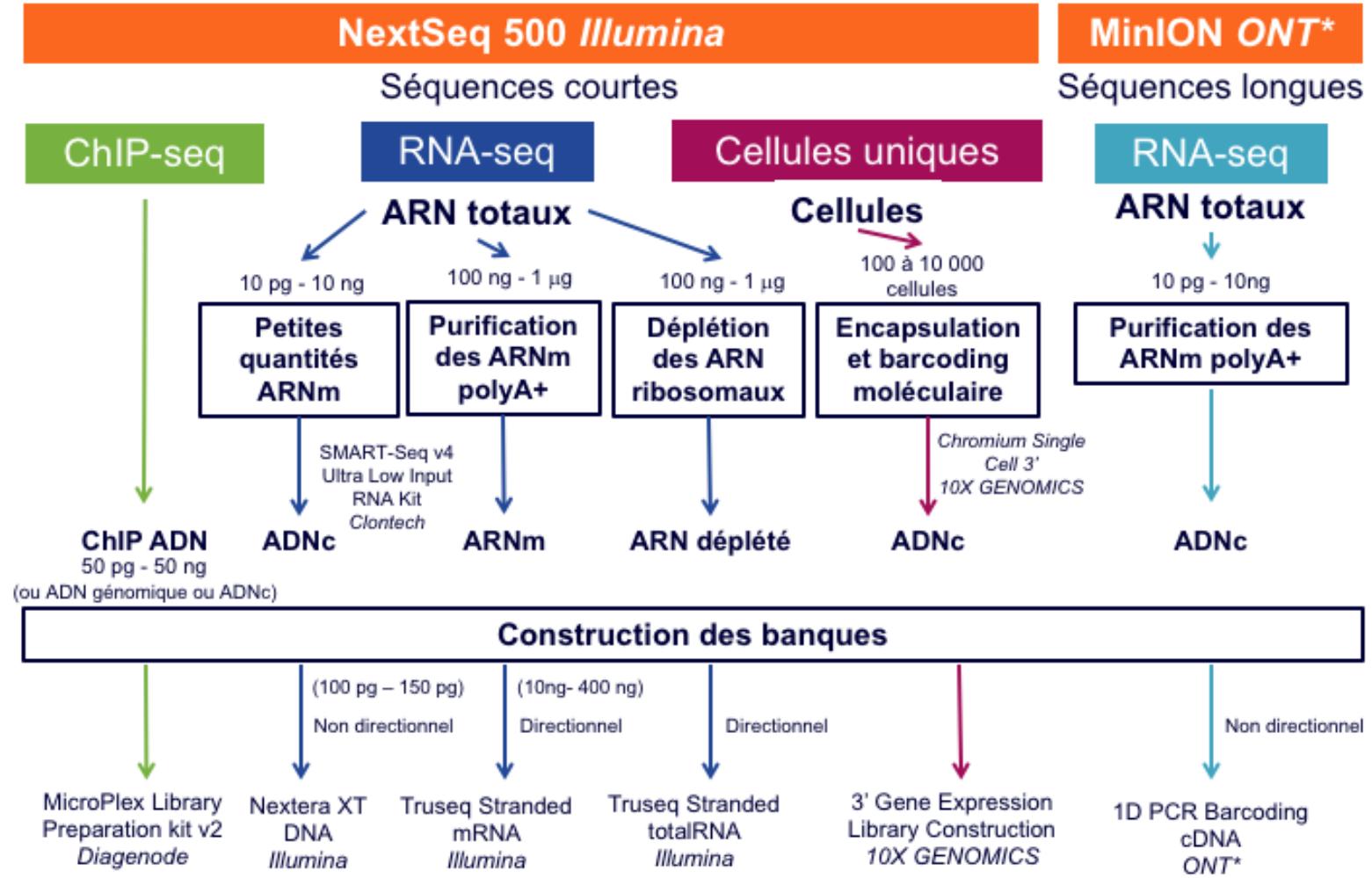
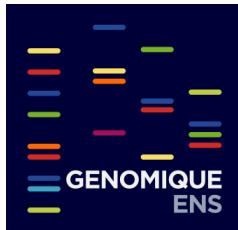
Illumina NextSeq 2000
(since 2021)

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

Chez les organismes eucaryotes

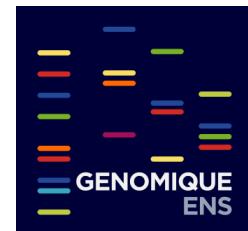


Spécialistes en génomique fonctionnelle



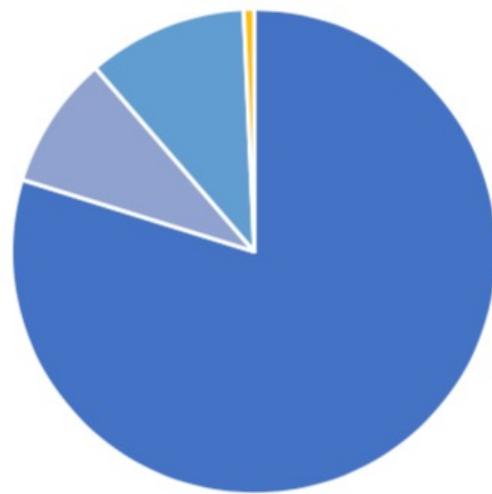
*Oxford Nanopore Technologies

Ouvert à 60% aux laboratoires extérieurs

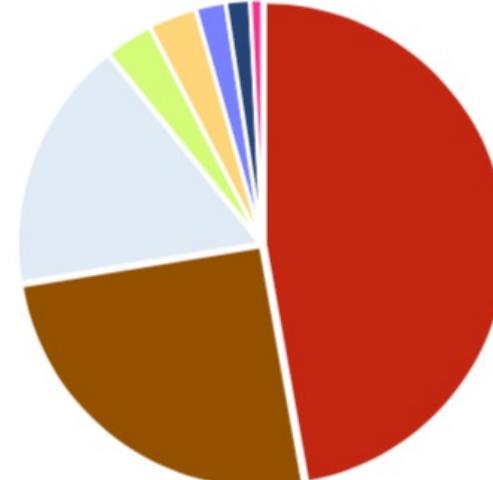


■ Nous travaillons principalement avec des laboratoires de la région parisienne (89 %).

Générale



En Île-de-France

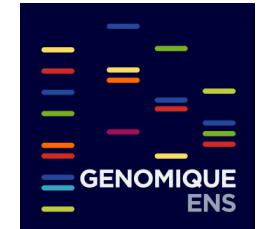


■ Paris ■ Île-de-France ■ Province ■ Étranger

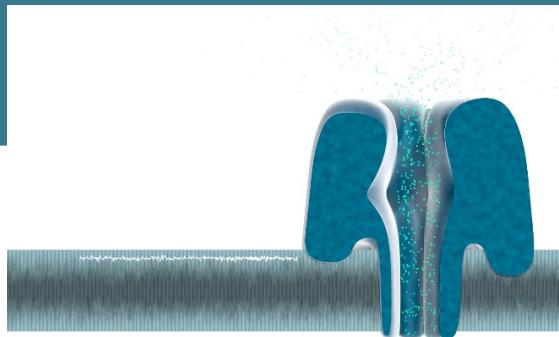
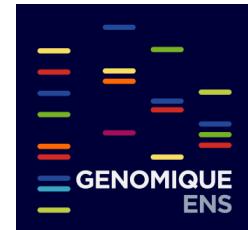
■ PSL
■ Université de Paris
■ Université Paris-Saclay
■ IFPEN
■ Sorbonne Université
■ INRA IdF
■ Université Paris Est Créteil
■ Institut Pasteur

Distribution de l'origine des projets sur la période 2015-2019

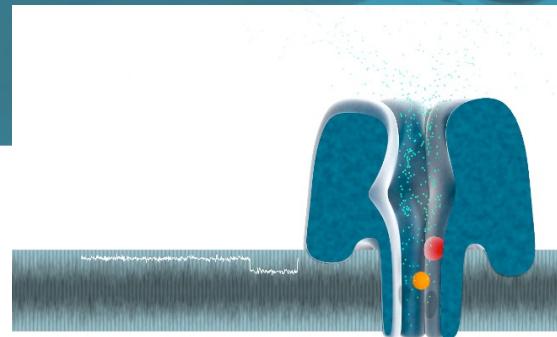
Tour de table



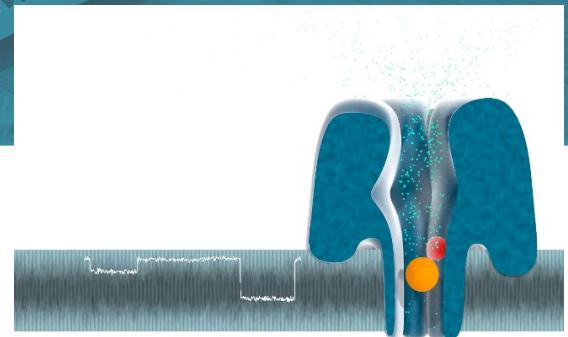
Comment fonctionne les pores



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



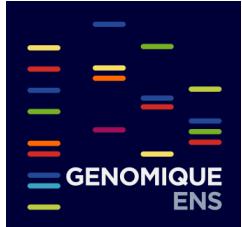
An ionic current is passed through the nanopore by setting a voltage across this membrane.



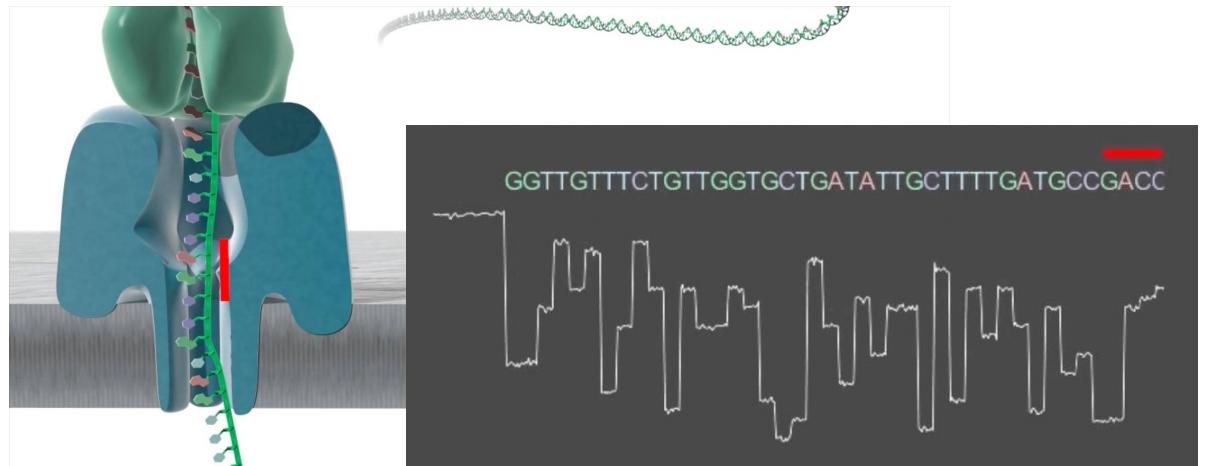
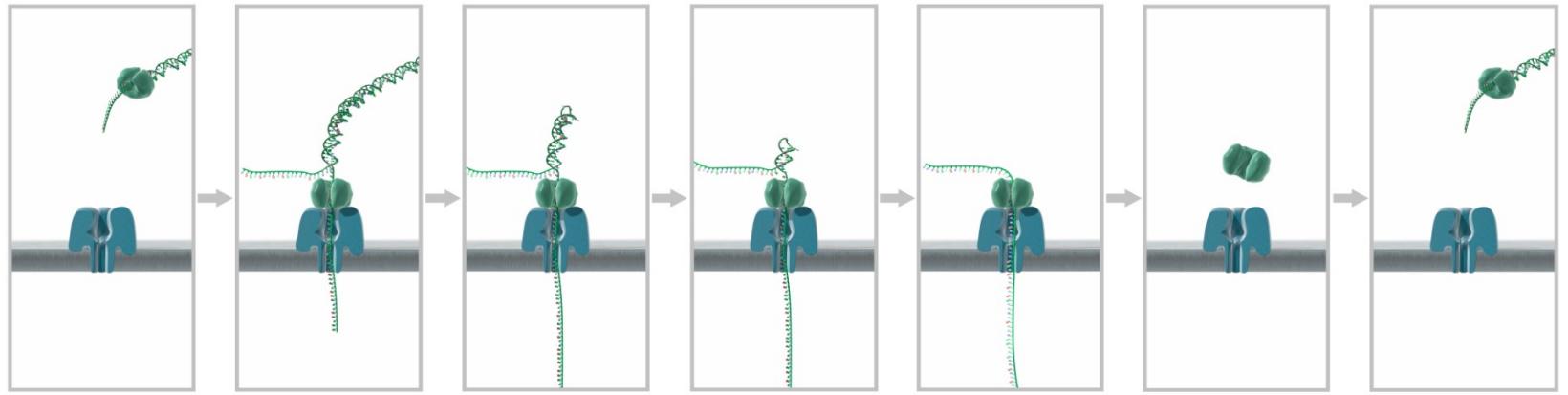
If an analyte passes through the pore or near it, this event creates a characteristic disruption in current.

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

Séquencer avec des pores

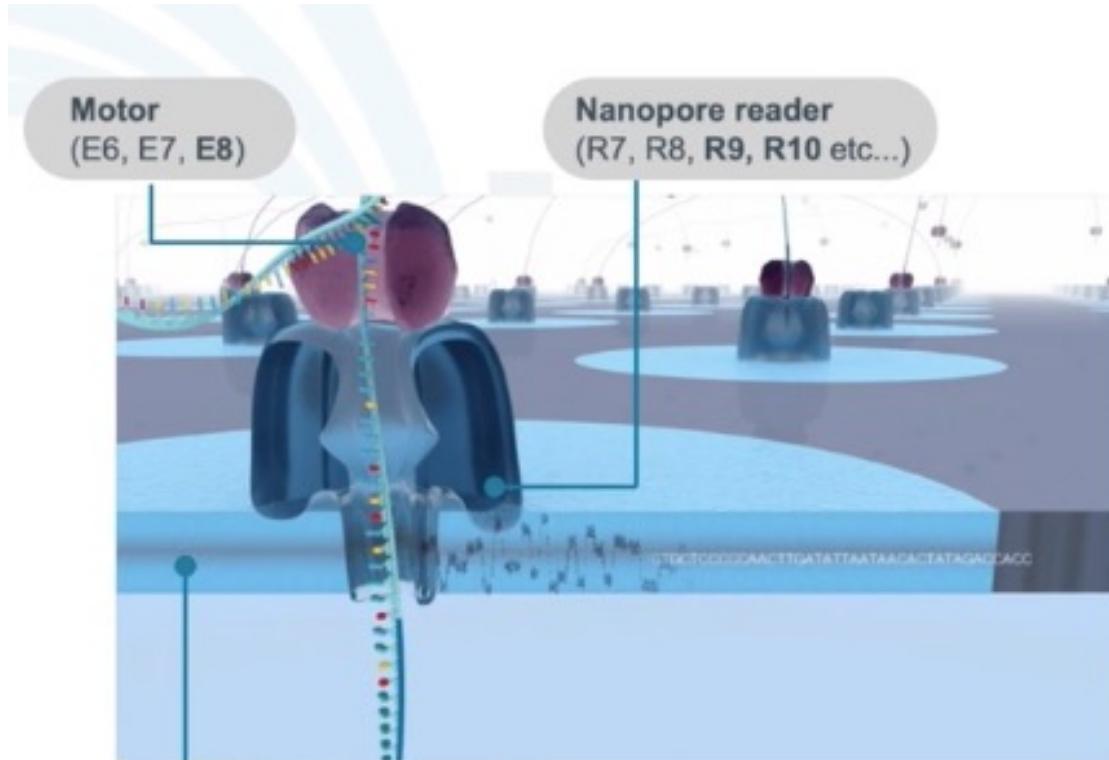
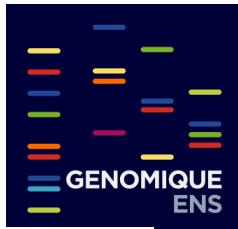


A strand of DNA is passed through a nanopore helped with a **motor protein** (helicase).

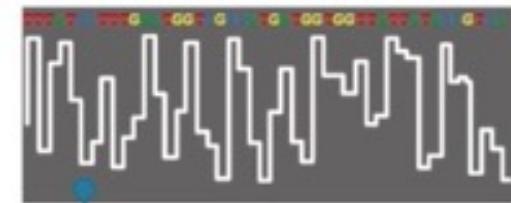


<https://nanoporetech.com/>

Un pore



Nanopore reader
(R7, R8, R9, R10 etc...)

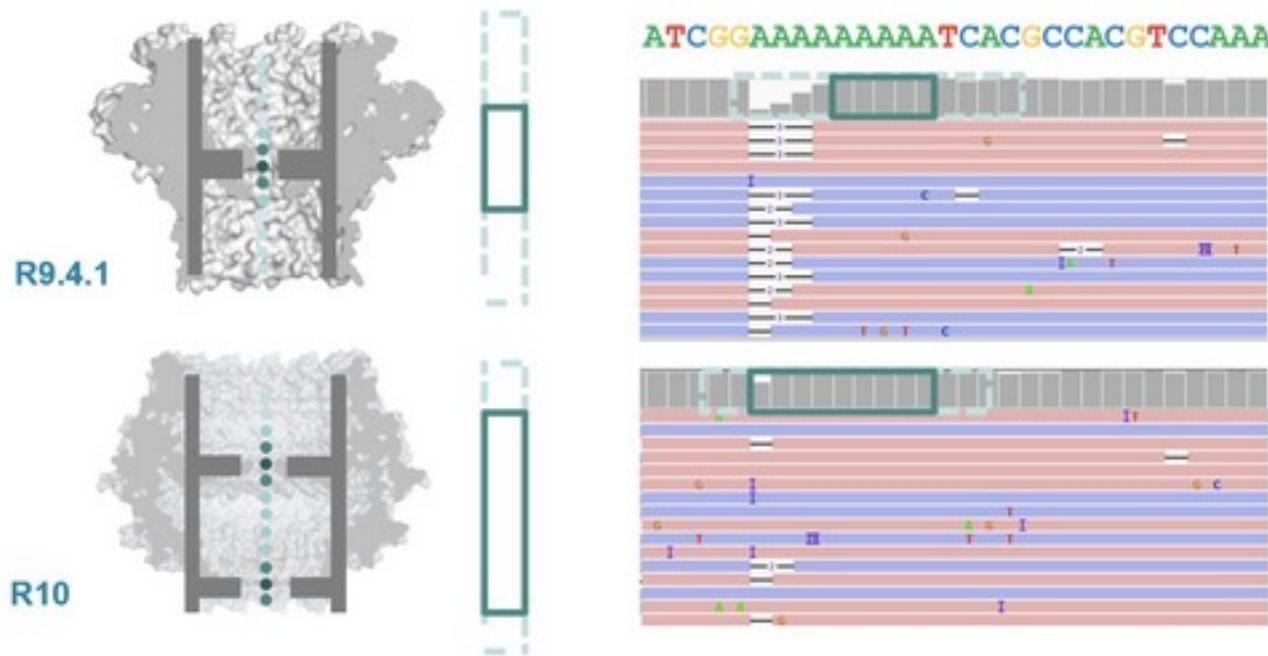
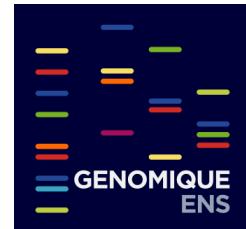


Algorithm
(RNN)

DNA passes through
nanopore at 400+
bases per second

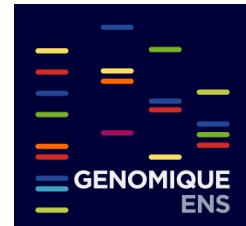
Run conditions
(Salt, fuel, script, temperature...)

Pore R10



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

R10.4.1 disponible au Q3 2022. la R9 va s'arrêter en fin d'année 2022

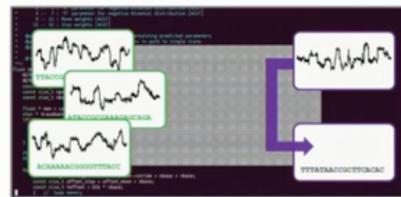
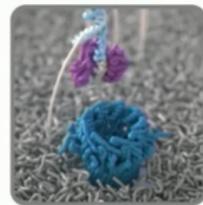


Nanopore Introduction

Nanopore accuracy recap (Nov 2021)

Sequencing chemistry tuned with latest base callers

- "Q20+", kit 12 release upgrades enzyme motor
- Refined motor "E8.1" – better movement quality, 250 bps
- Combine with latest base callers for high accuracy
- Works with standard R9.4.1 flowcells and R10.4
- Hitting > Q20 raw read, single pass accuracy (Simplex)



R9.4.1 and R10.4 chemistries > Q20 Simplex



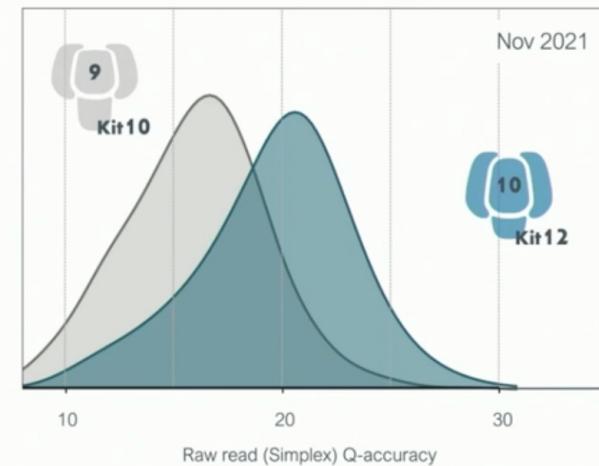
=

R9.4.1 nanopore
E8.0 motor



=

R10.4 nanopore
E8.1 motor



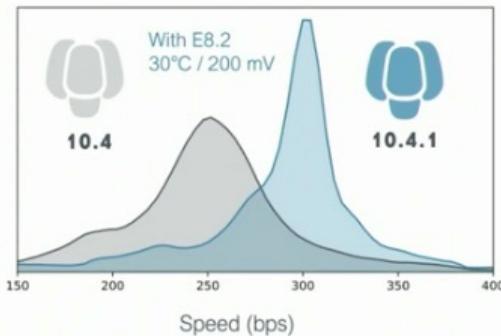
Oxford
NANOPORE
Technologies

Nanopore Accuracy

Chemistry update - motor and pore improvements

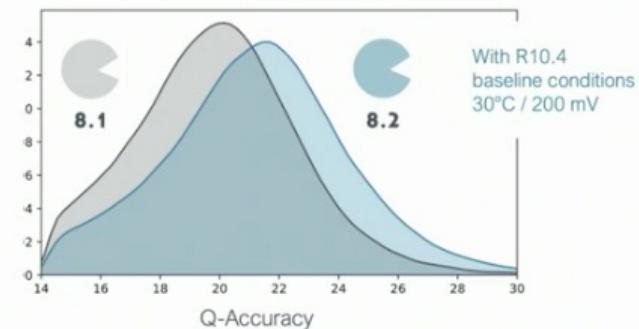
New pore: R10.4.1

- Improved pore designs tune enzyme-pore docking
- Faster speeds (~250-420 bps)
 - Yield much higher output compared to current Q20 chemistry
- Tighter speed distributions
 - Helps to reduce errors

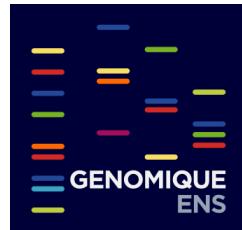


New motor: E8.2

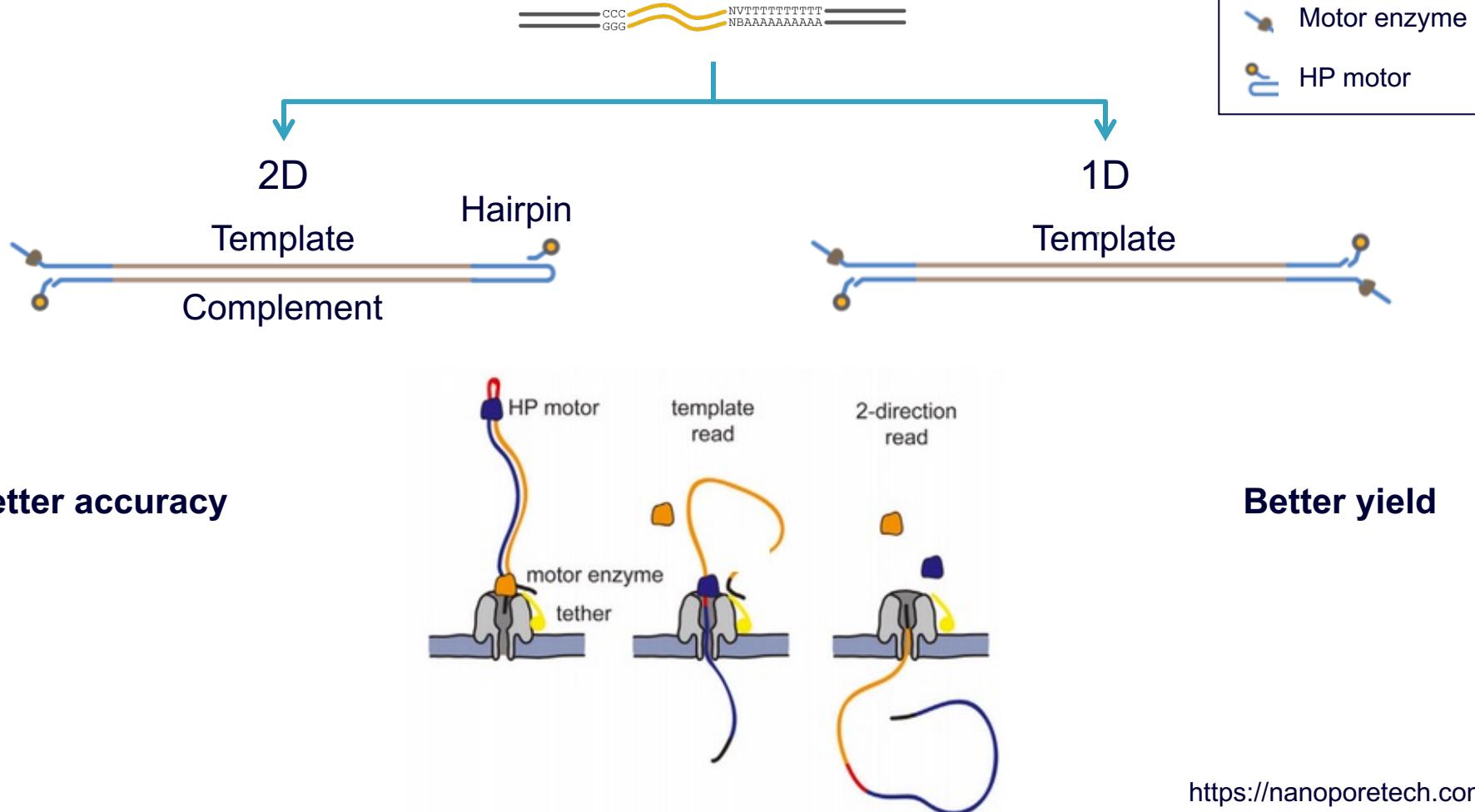
- Improved movement properties with more consistent movement
 - Better defined levels
 - Fewer mis-steps
 - Improved accuracy
- ~Halved error rates.



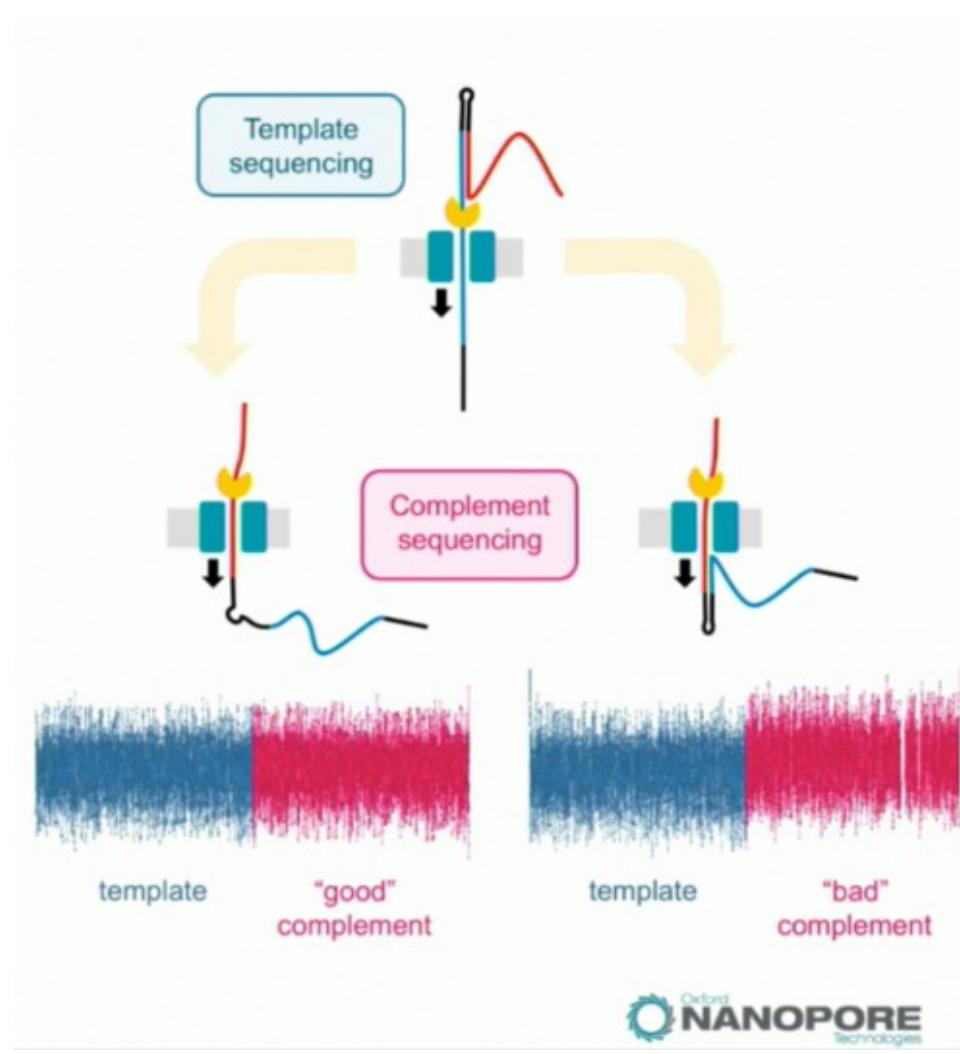
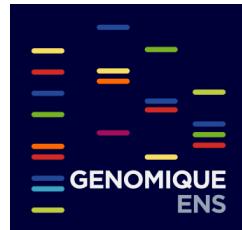
cDNA library construction



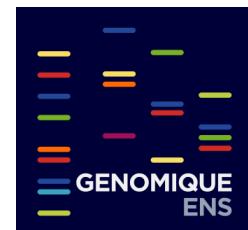
Full length cDNA from total RNA
Poly A selection, non directional



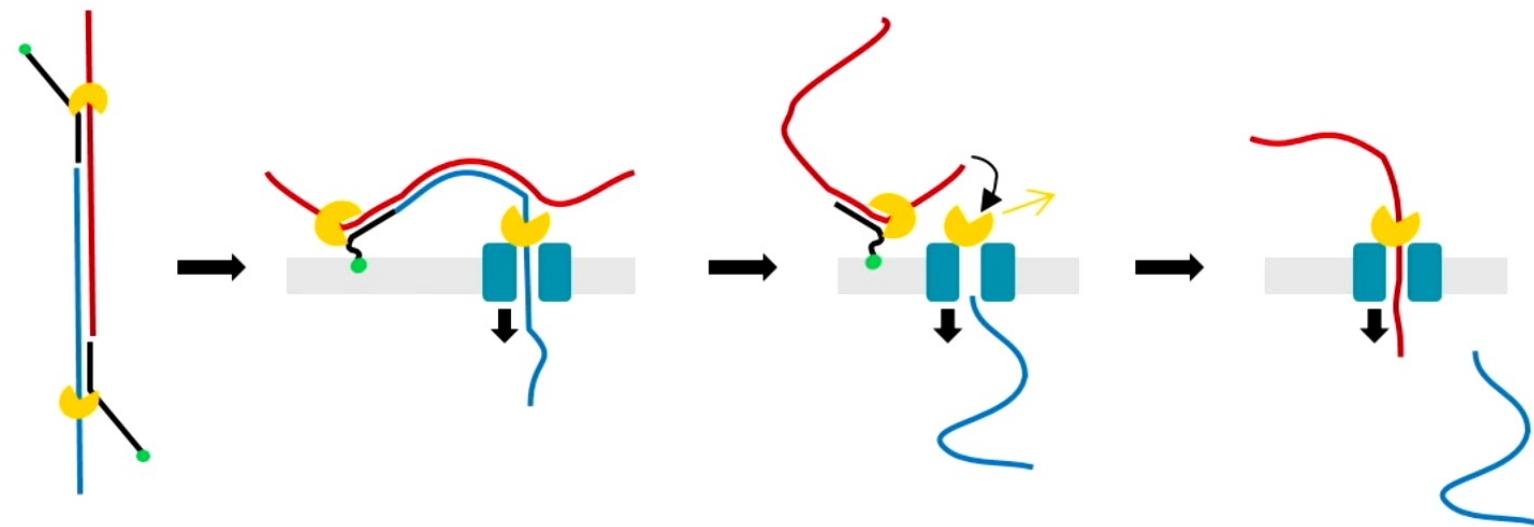
Problème du 2D



Evolution du 2D



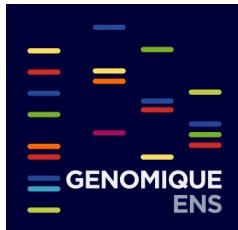
= duplex



© 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**.

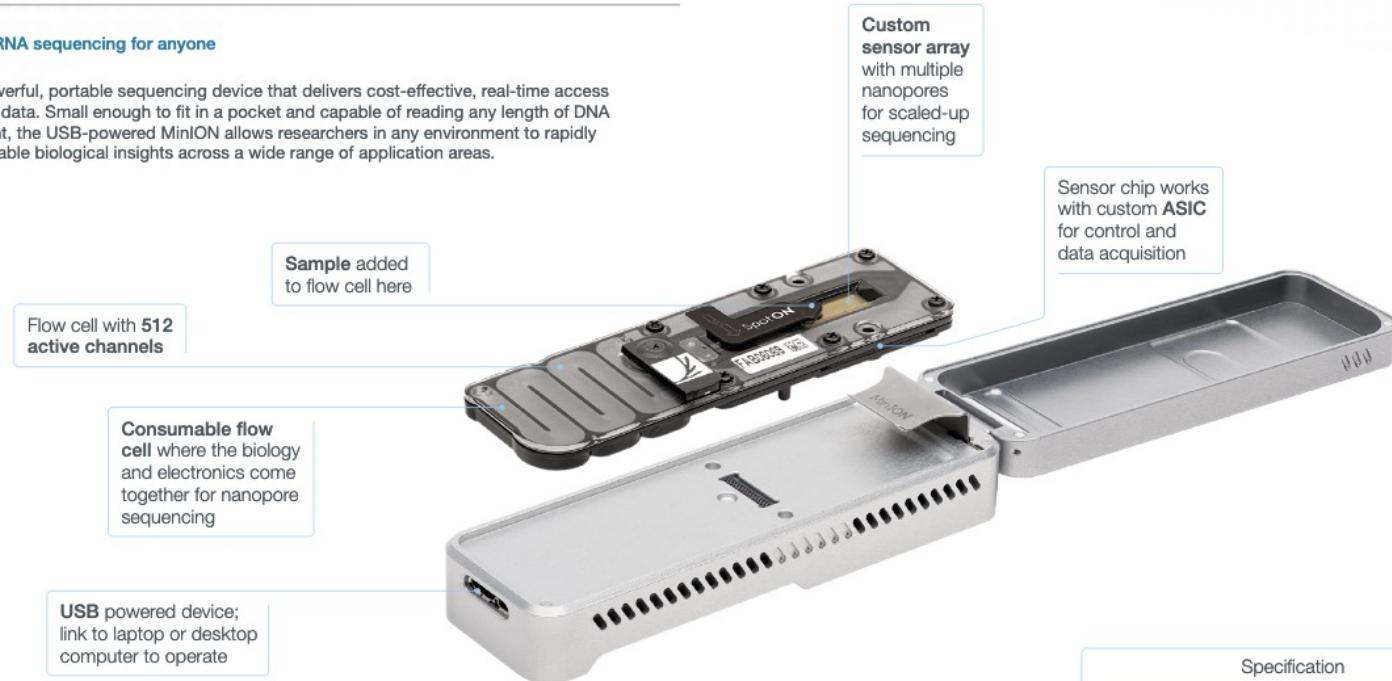
Instruments disponibles



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.

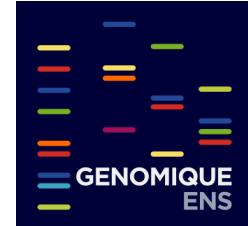


Specification	
Weight	87 g (103 g with flow cell)



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

Use **Flongle** for smaller tests and analyses, or **MinION Flow Cells** for tens of gigabases of data

Specification

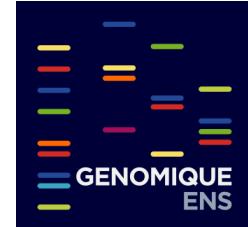
Weight
420 g

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



Service provider certification is available for the GridION



Sample added to flow cell here

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

Specification

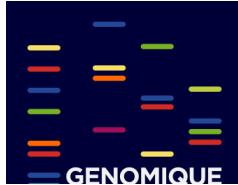
Weight
11 kg

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



[Order now](#) store.nanoporetech.com/devices

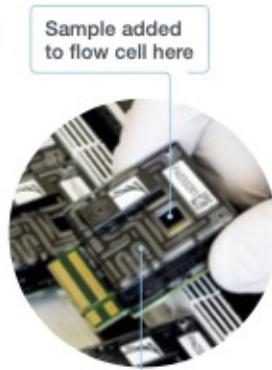
PromethION



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

PromethION™ “P2”

Powerful flowcells, small device



Prototype devices in Q2 2022

Pre-order in store today: P2: \$59,955
Consumable pricing will enable human WGS for under \$1,000



Two PromethION flowcells, integrated GPU

- Based on existing flow cell design
- Two individually addressable prom flow cells
- Integrated with powerful compute
- Ideal for studying large genomes or transcriptomics
- Easier to place in automation solutions
- Available as a starter pack or CapEx

Device specifications

Processor	16 CPU cores
GPU	Latest Ampere GPU
Storage	16 TB SSD
RAM	64 GB RAM

PromethION “P2 solo”

Powerful flow cells, small device – up to 550-600Gb/run*

Two PromethION flowcells, connects to existing compute

- Based on existing flow cell design
- Two individually addressable prom flow cells
- Small versatile instrument to couple to existing GPUs/storage
- Ideal for studying large genomes or transcriptomics
- Easier to place in automation solutions
- Available as a starter pack or CapEx

Pre-order in store today

P2 Solo: \$10,455

Consumable pricing will enable human WGS for under \$1,000

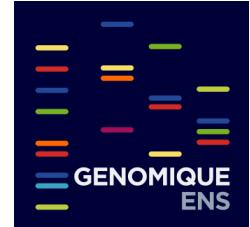


Early Access devices ship early Q2 2022

Four chamber flowcells in development

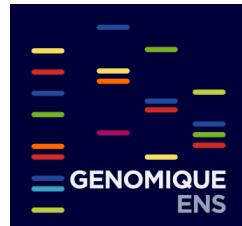


Comparaison instrumentation



	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

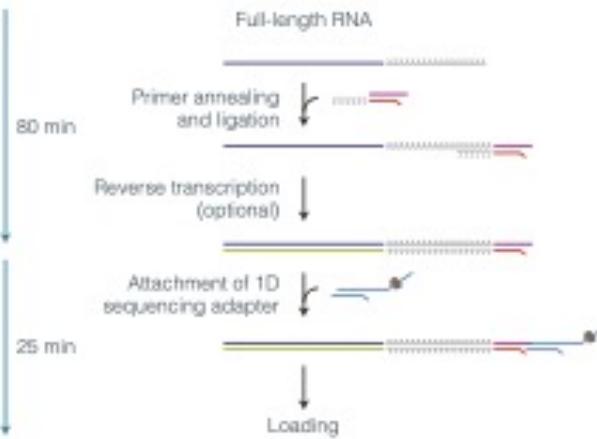
Préparation des librairies ARN



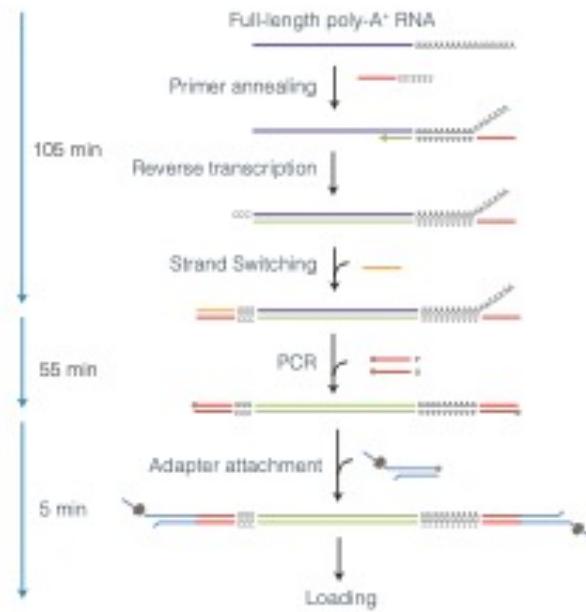
For sequencing the RNA molecule directly

For full-length transcript analysis with high throughput

Direct RNA Sequencing Kit



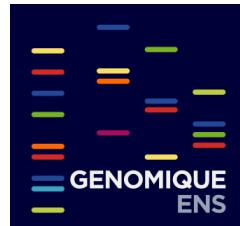
PCR-cDNA Sequencing Kit



- 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

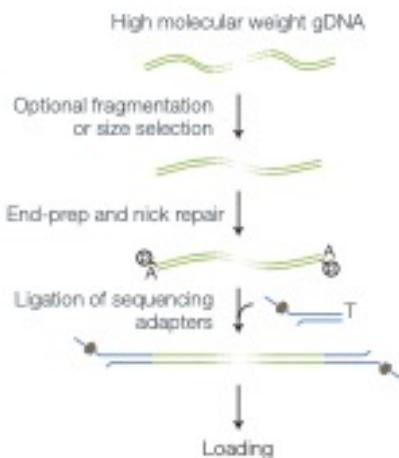
Préparation des librairies ADN



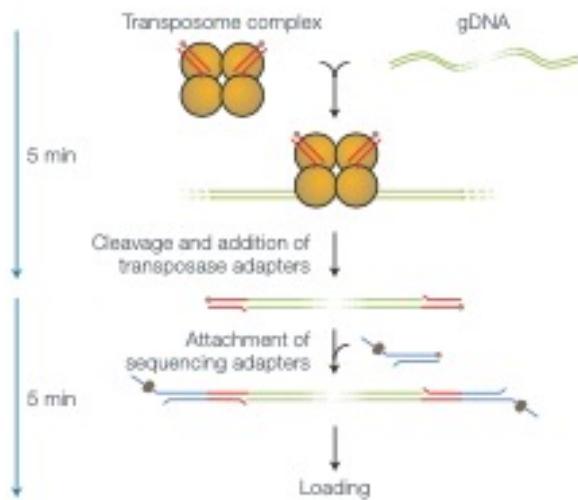
For maximum throughput

For minimal preparation time

Ligation Sequencing Kit



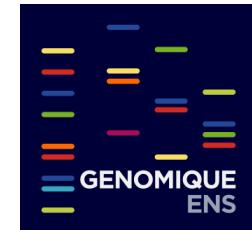
Rapid Sequencing Kit with transposase



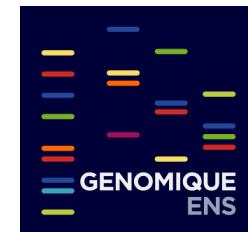
- DNA ends are repaired and dA-tailed
- Sequencing adapters are ligated onto the prepared ends
- Fragment lengths can be controlled by fragmentation or size selection

- 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



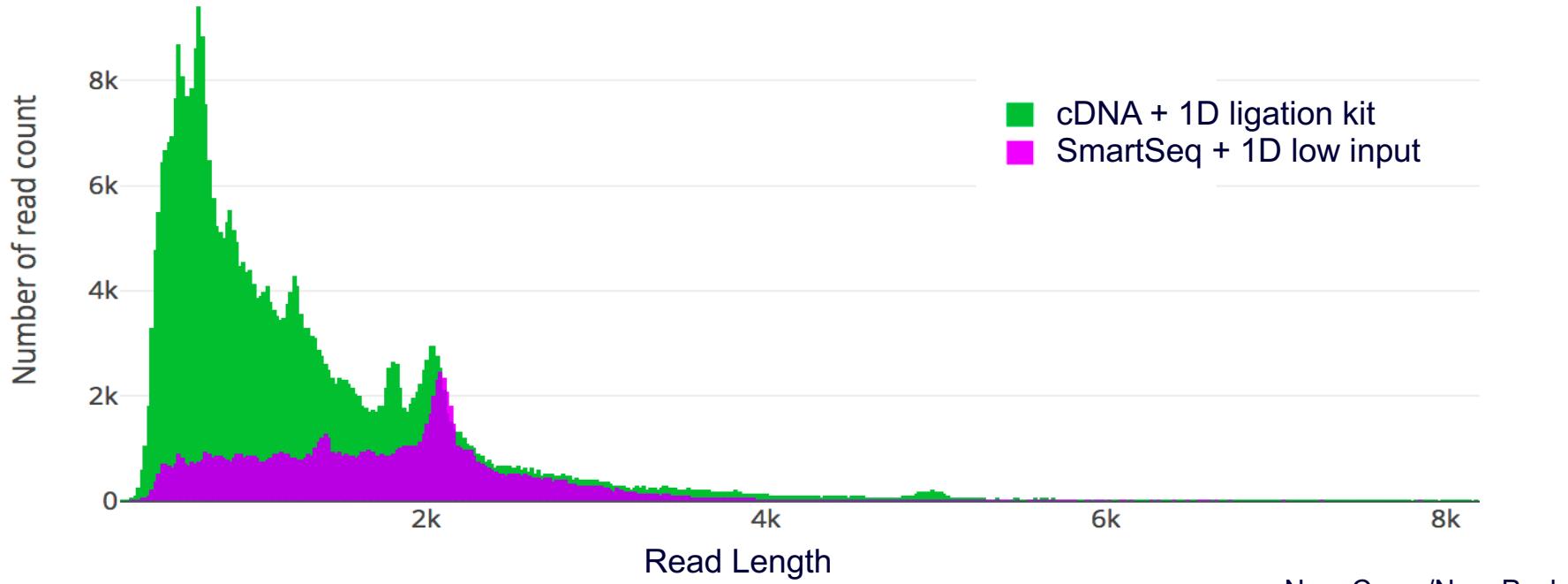
Reducing the amount of starting material



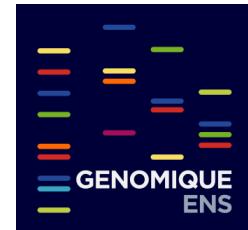
We compared **two protocols**:

- 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

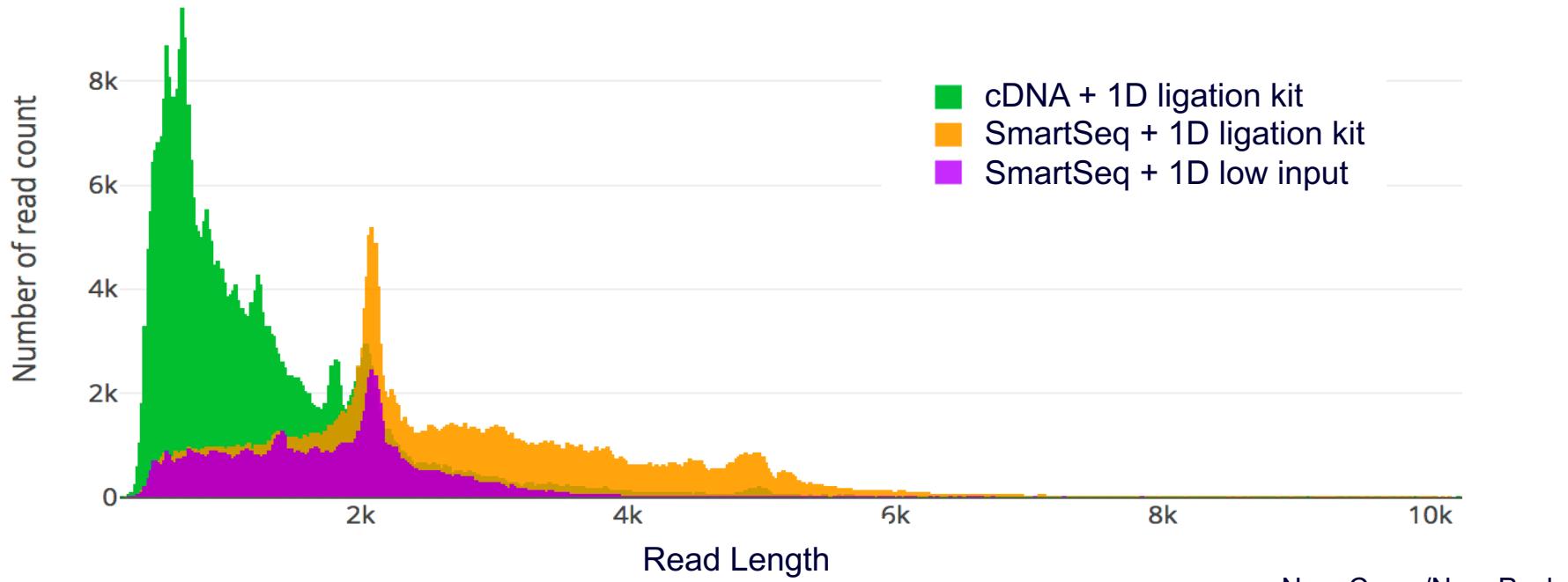


— We used:

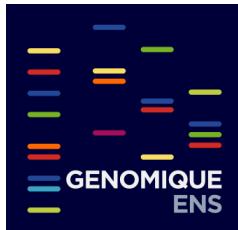
- **10 ng of total RNA;**
- SmartSeq v4 (Clontech);
- 1D ligation kit LSK108 (ONT).

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**.

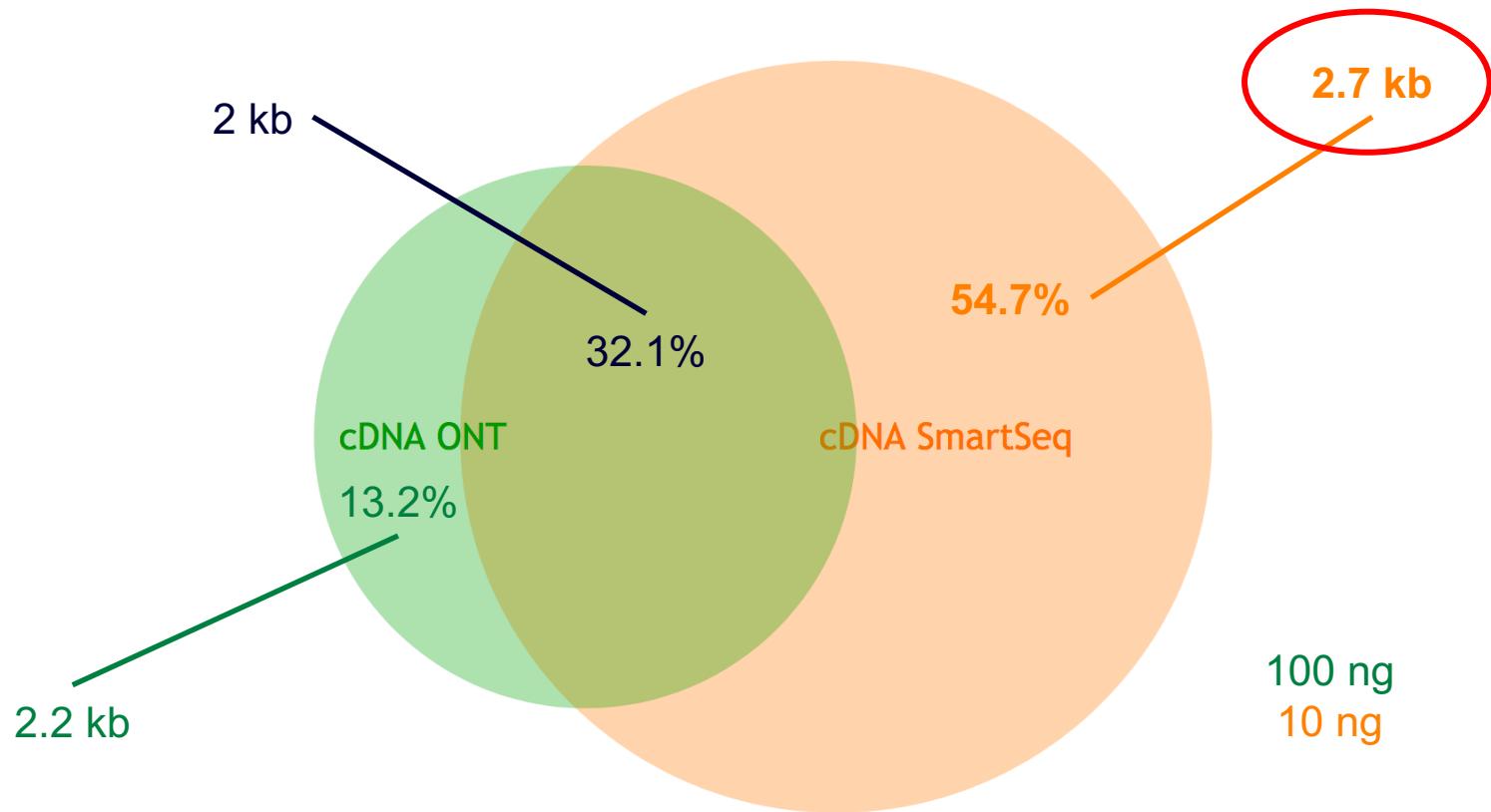


We obtained more differentially expressed genes



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

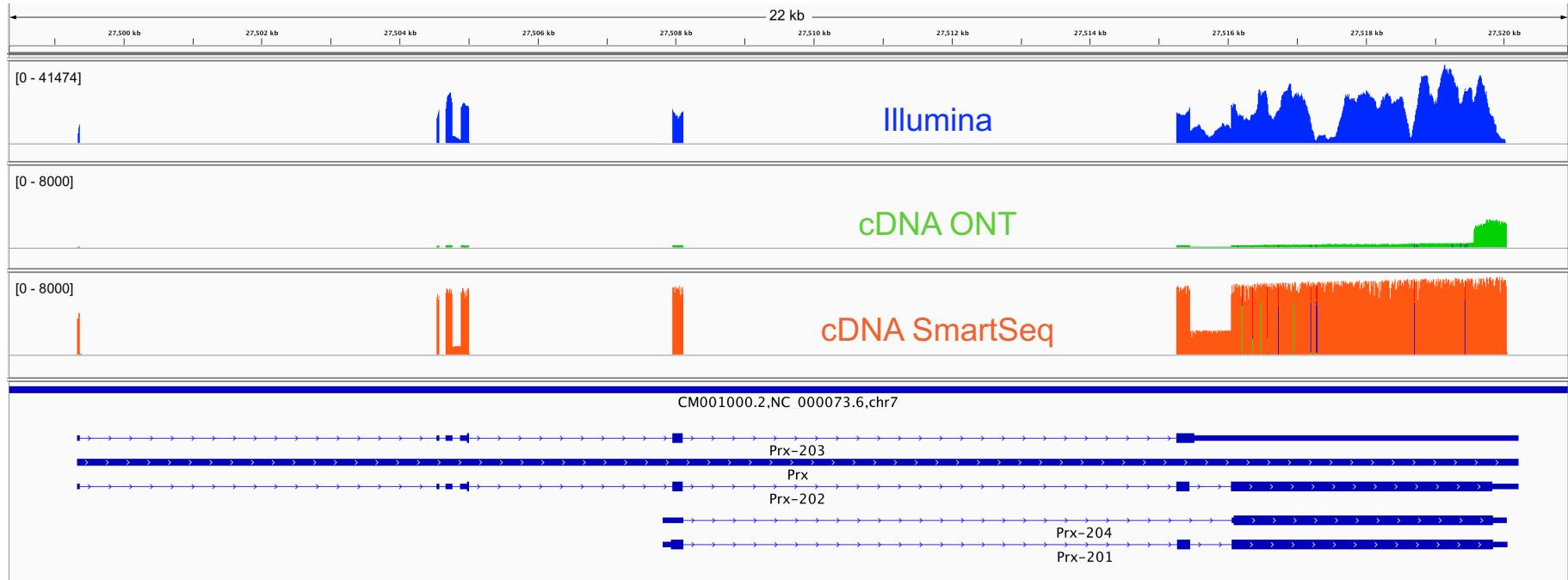
- 100 ng cDNA: 640 genes;
- 10 ng SmartSeq: **1,230 genes.**



And homogenous coverage along transcript

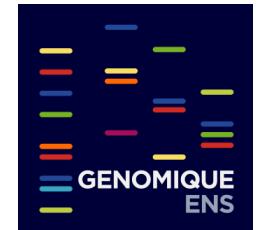


- Example with **long transcripts** like Periaxin (4,5 kb)

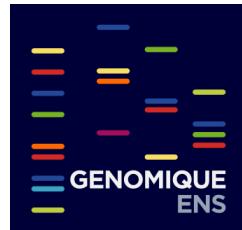


- Combining SmartSeq with ONT's ligation protocol allows to **sequence longer transcripts with a higher 5'-end coverage**.
- R&D: Single Cell long read

RUN MinION



Le nanopore sensor ou capteur

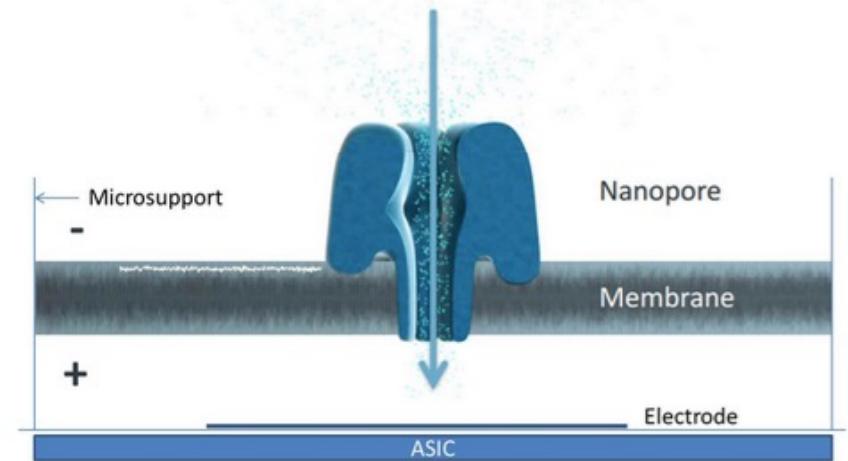


Composant du nanopore sensor:

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

(Application-Specific Integrated Circuit)

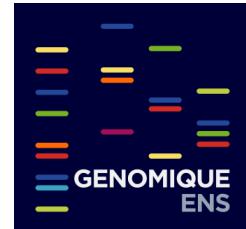
Chaque électrode est connecté à un canal sur le circuit intégré (ASIC: Application-Specific Integrated Circuit) qui contrôle et mesure le signal du pore



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

Lien entre le nanopre sensor et le circuit ASIC

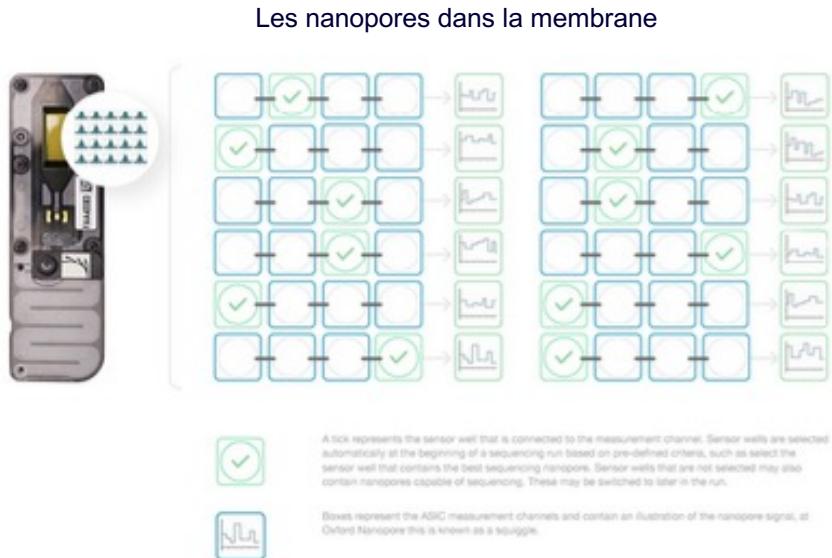
(Application-Specific Integrated Circuit)



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

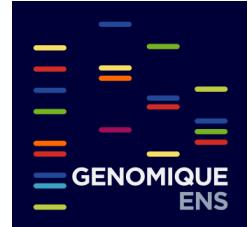
Ils sont connectés à un circuit intégré de 512 canaux. Chaque canal du circuit intégré sélectionne 4 nanopore sensor

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

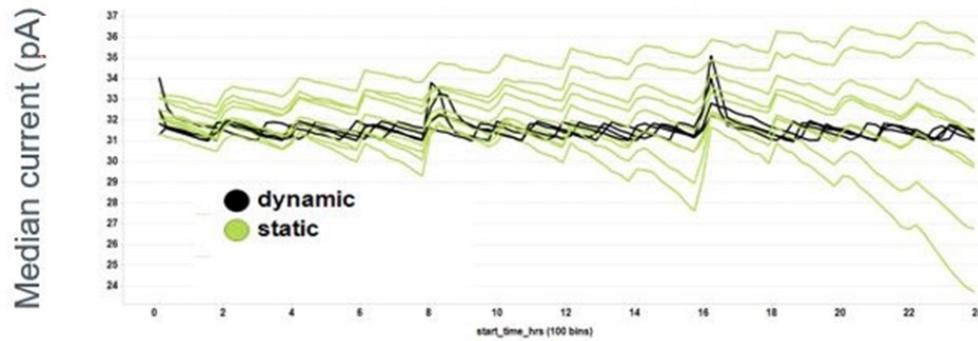
Durant le séquençage, le voltage change. Le démarrage se fait avec un voltage de -180mV qui est optimal pour la chimie R9.4. Le voltage dérive durant le run à cause de l'épuisement des réactifs rédox.

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.

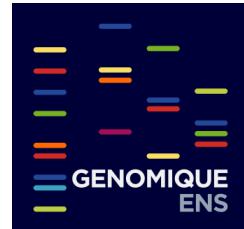
Ce système est plus stable dans le temps que le système précédent appelé « Static Voltage Control

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

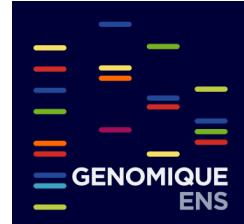


Adjusting the starting potential

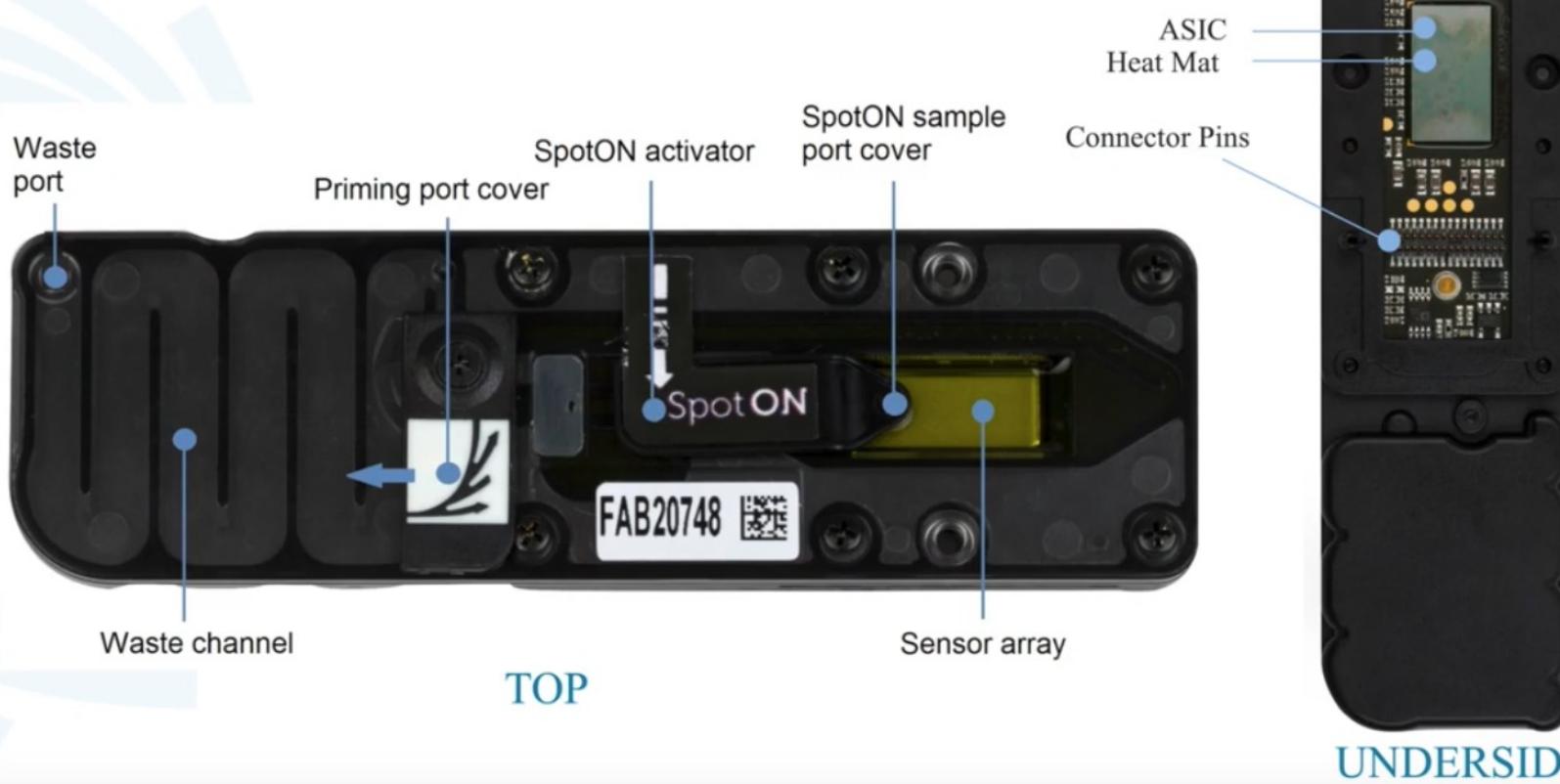
If a flow cell is re-used (e.g. after running an experiment and washing the sample out with the Flow Cell Wash Kit), the common voltage will be lower than -180 mV due to the voltage drift. The exact voltage value will depend on the length of the experiment. To account for drift, the starting voltage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

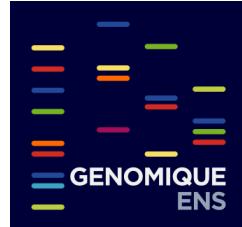
Flow Cell



MinION FLOW CELL COMPONENTS?



© Copyright 2017 Oxford Nanopore Technologies | 3

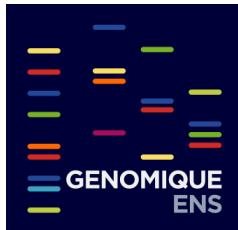


Select **Start Sequencing** of the homepage and navigate to **Run Options** to alter the bias voltage by selecting and typing or using the + and – options to the appropriate voltage.

The screenshot shows the MinKNOW software interface with the following details:

- Header:** SDOLLING-WIN, My Device, Start (radio button selected).
- Left Sidebar:** Sequencing Overview, Experiments (expanded), System Messages, Host Settings.
- Top Navigation:** 1. Positions, 2. Kit, 3. Run options (selected), 4. Basecalling, 5. Output, 6. Start.
- Run options section:** Run length (72 hours), Bias voltage (-180 mV). Buttons for increasing (+) and decreasing (-) both values are present.
- Advanced Options:** Show Advanced User Options (button).
- Bottom Buttons:** Connection Manager, Back to Kit Selection, Continue to Basecalling >, Skip to End >.

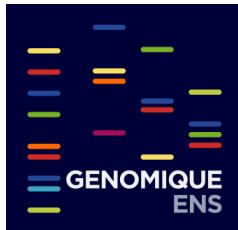
Pore occupancy



- 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.



Duty time plots



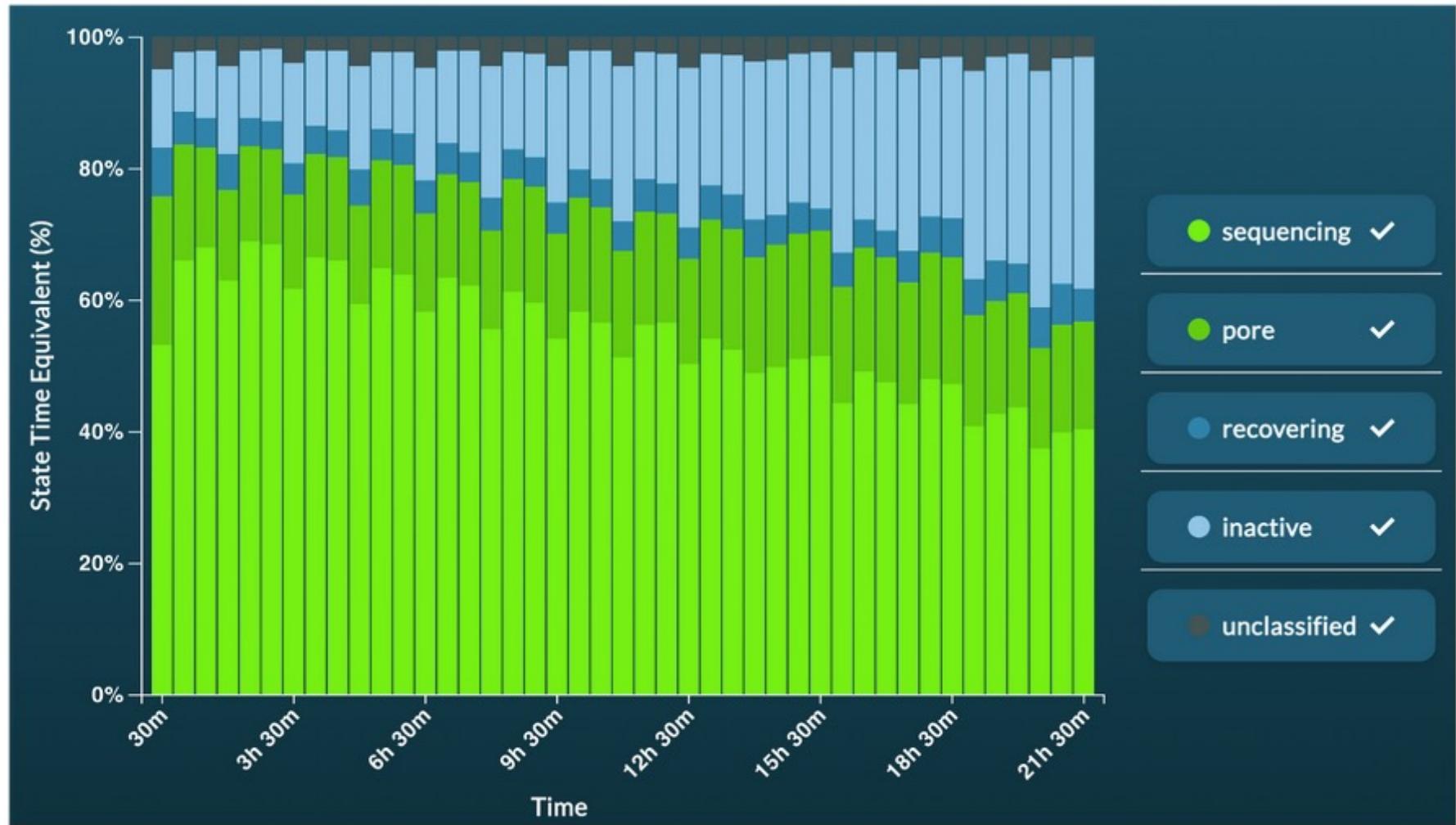
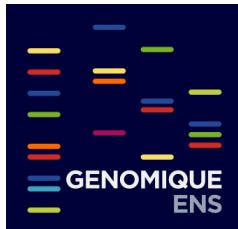
The duty time plot summarises the channel states over time.

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**.

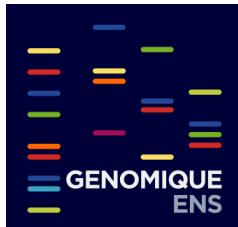
The graph populates over time, and can be used as a way to assess the quality of your sequencing experiment, and make an early decision whether to continue with the experiment or to stop the run.



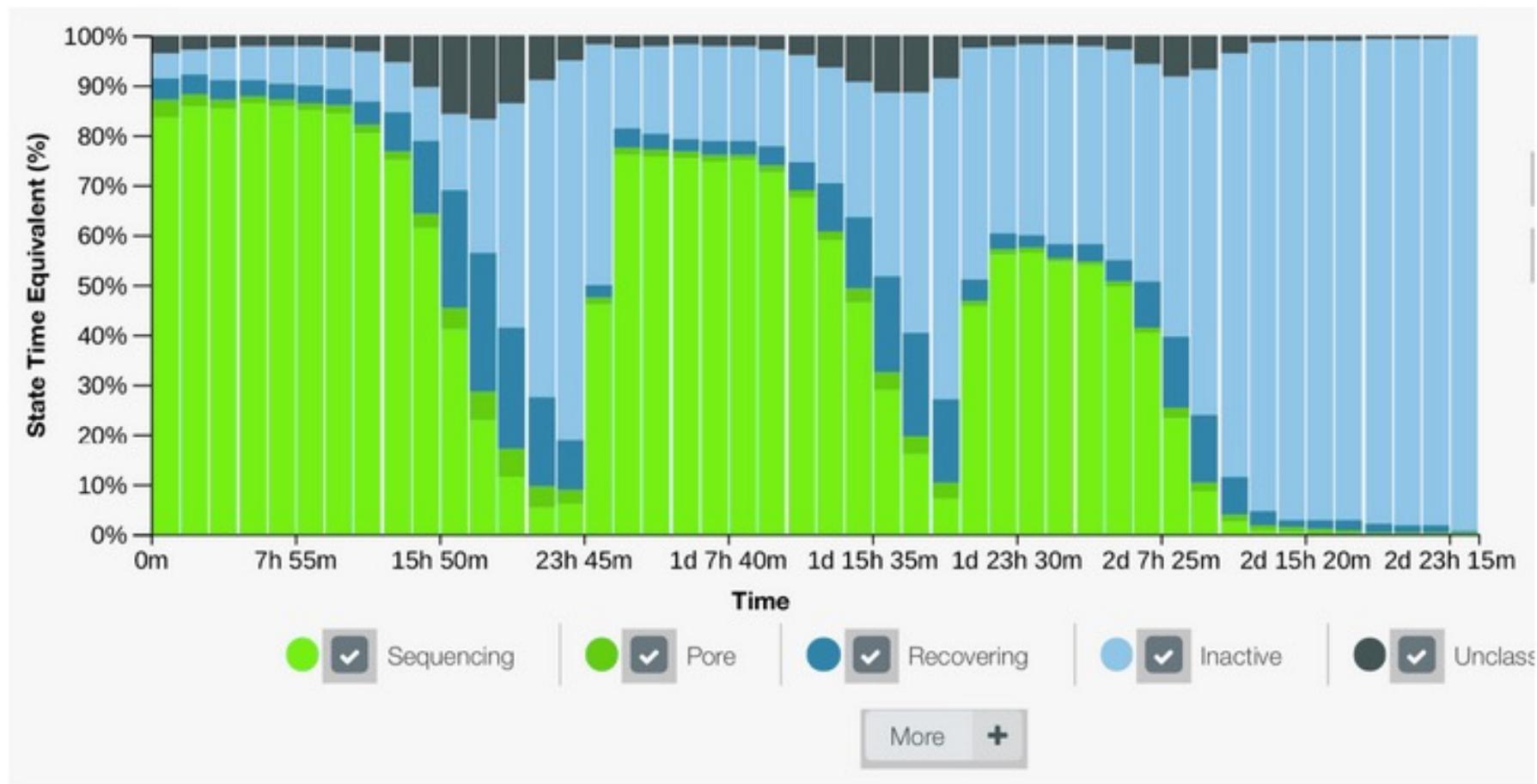
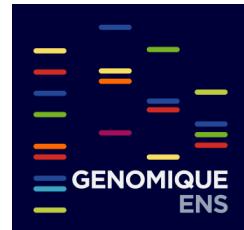
Duty Time Run RNA



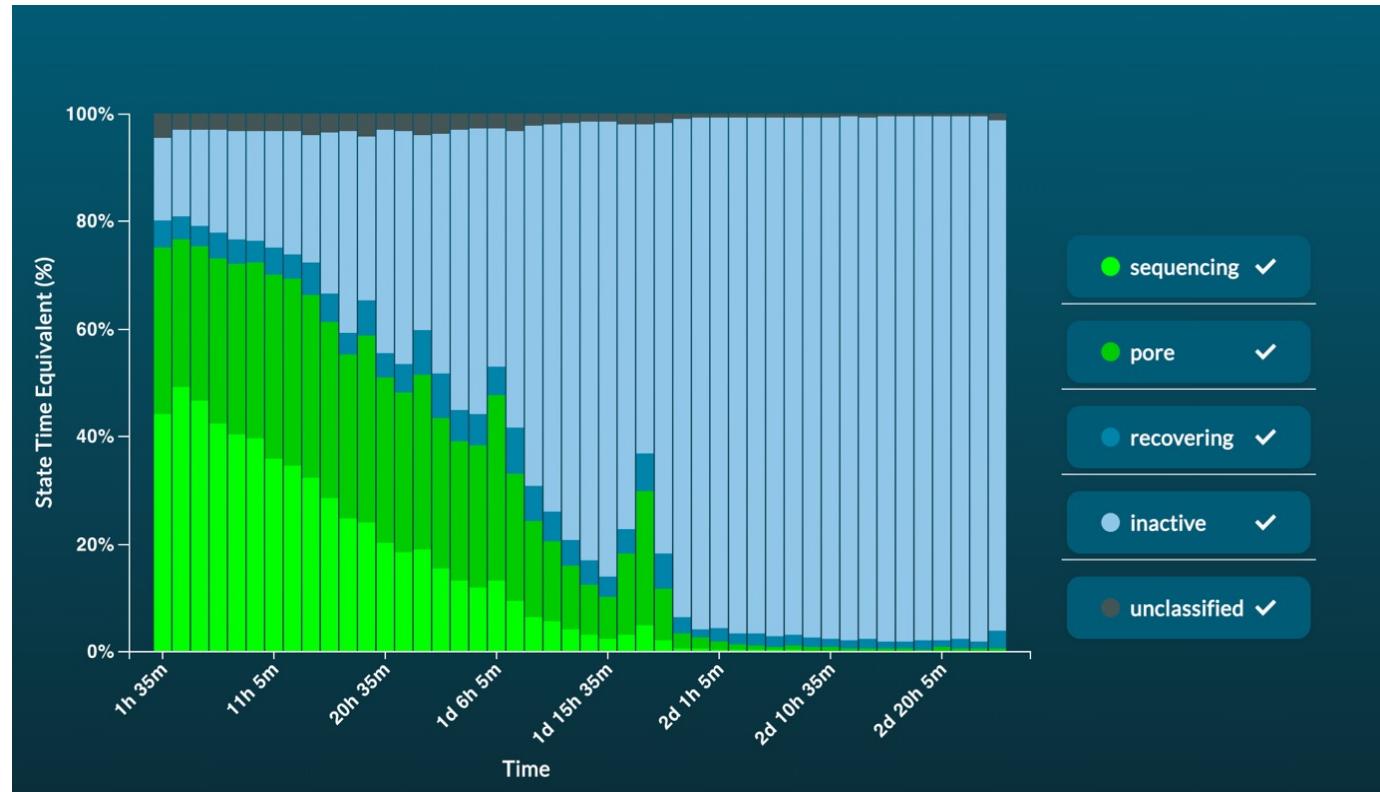
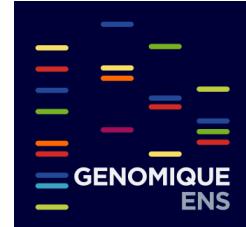
Re dépôt



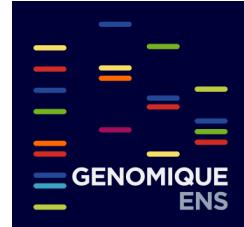
Troubleshooting



Troubleshooting



Dépôt sur Flow Cell



■ cDNA: d'après notre expérience

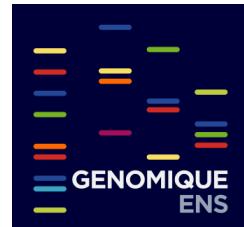
20 à 40 fmol sur du MinION- 4 à 10 M read
220 fmol sur du Promethion-150 M read

■ DNA: recommandations d'ONT Ligation sequencing gDNA - whole genome amplification (SQK-LSK112)
5-10 fmol sur du MinION-

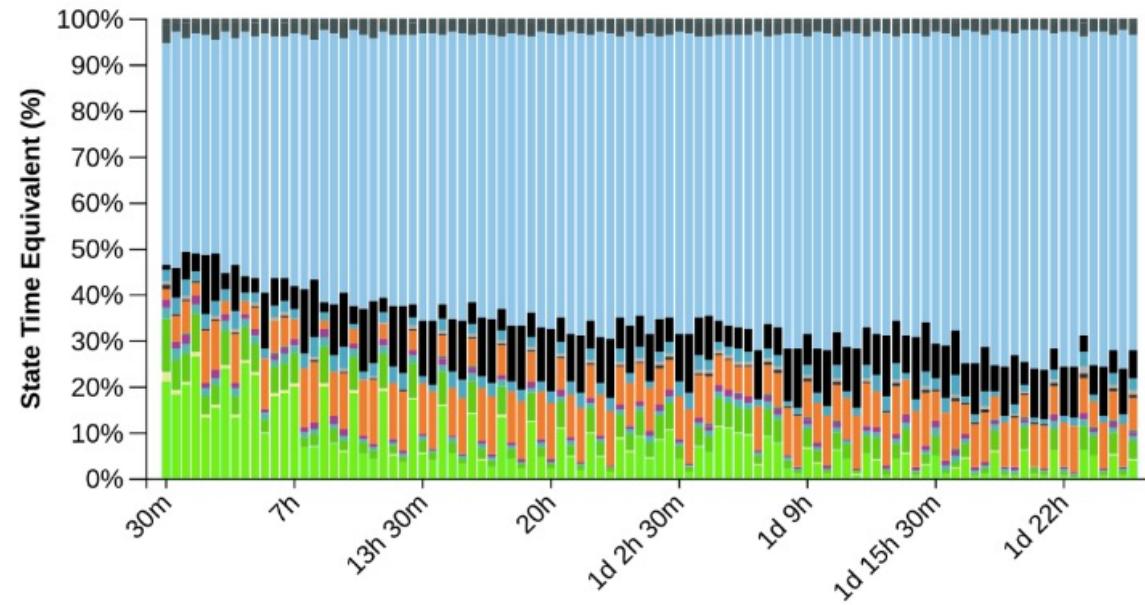


Large proportion of recovering pores.
Contaminants or too much librairie

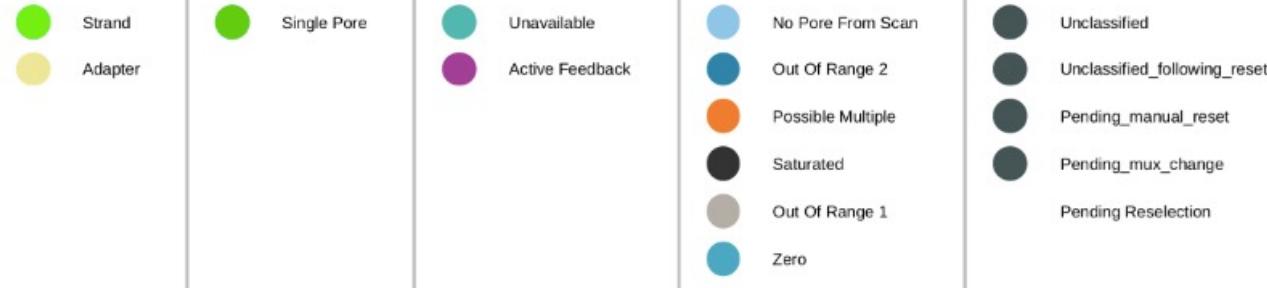
Troubleshooting



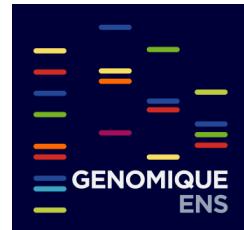
Duty time Categorised



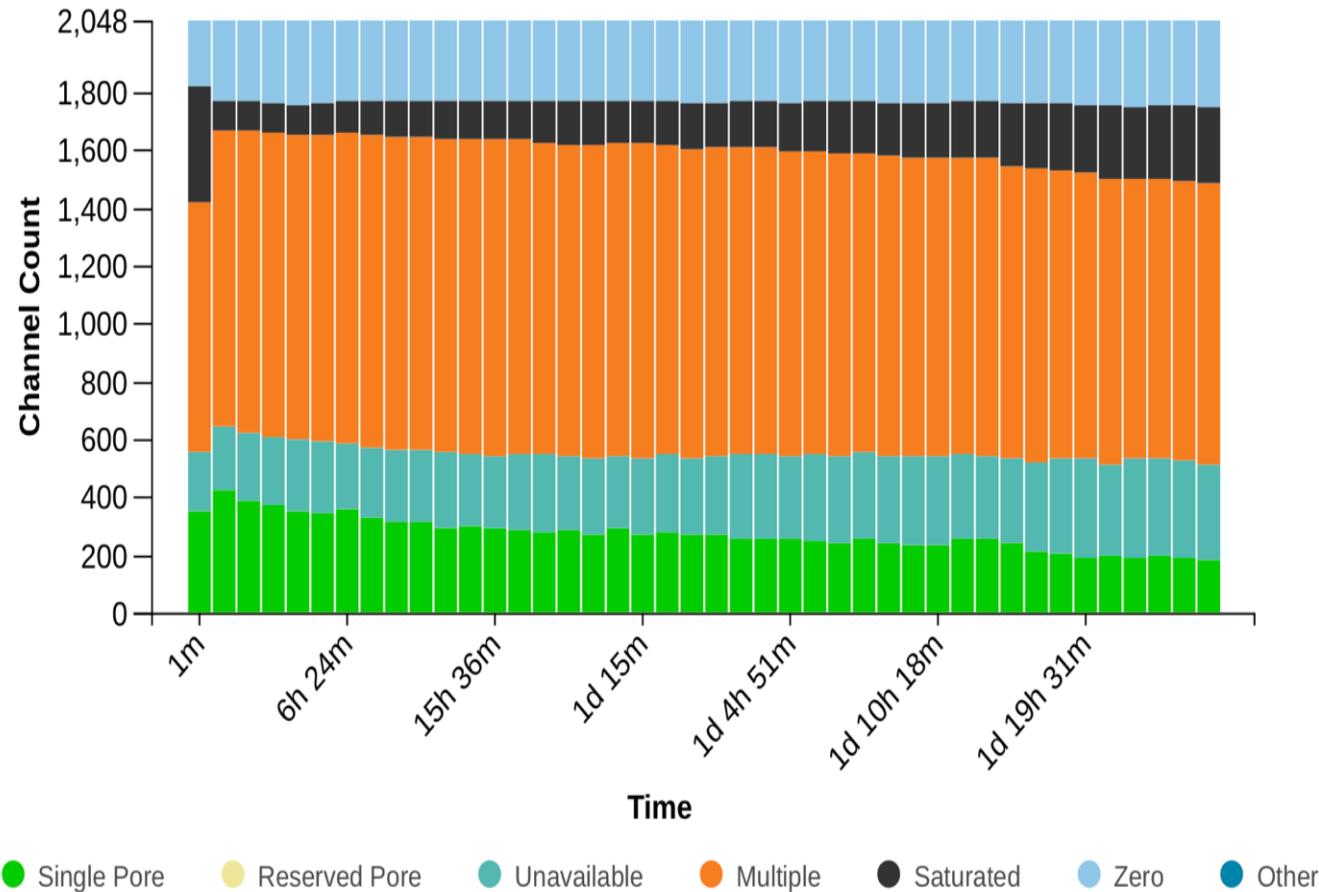
Time



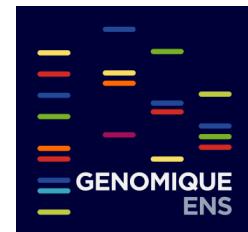
Troubleshooting



Mux Scan Categorised

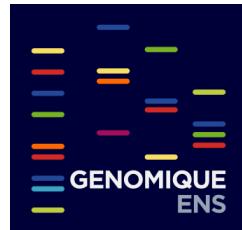


Troubleshooting

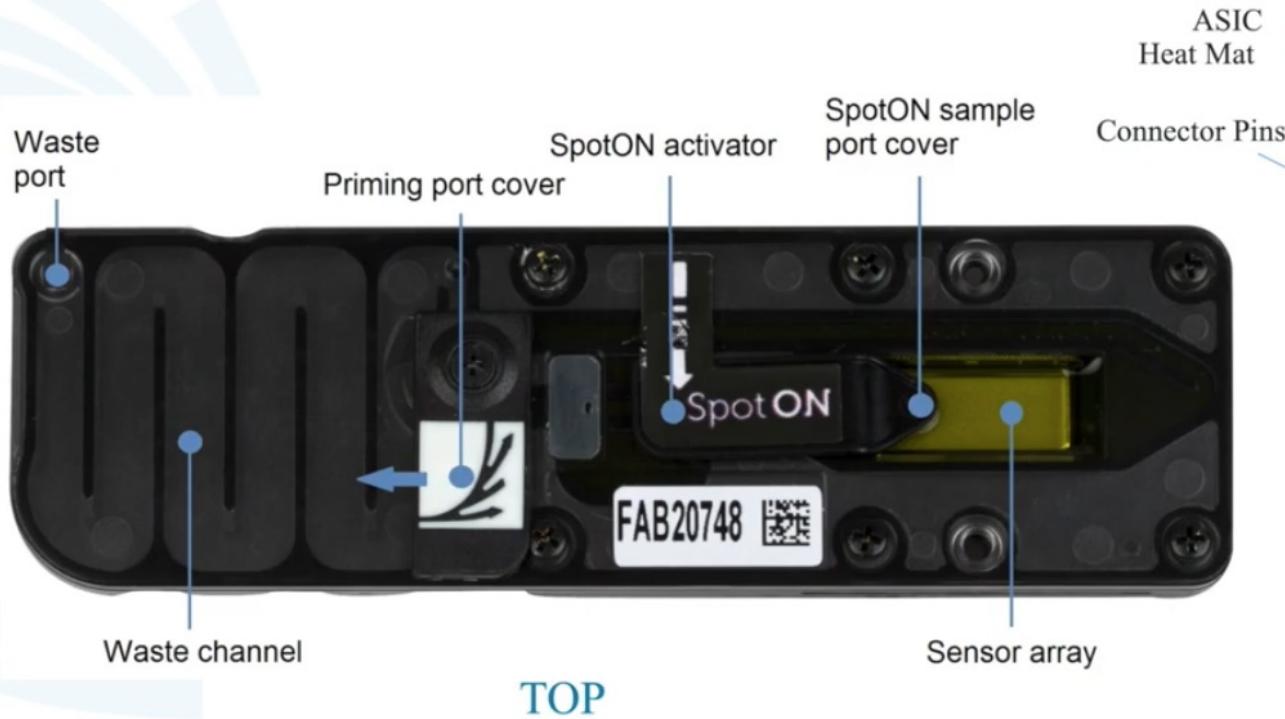


https://community.nanoporetech.com/protocols/experiment-companion-minknow/v/mke_1013_v1_revbl_11apr2016/troubleshooting-your-run-from-the-duty-time-plots

Flow Cell du MinION

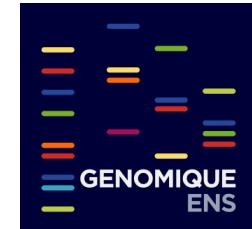


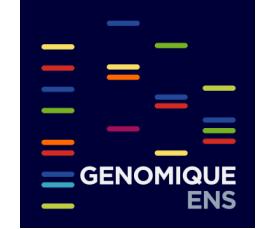
MinION FLOW CELL COMPONENTS?



UNDERSIDE

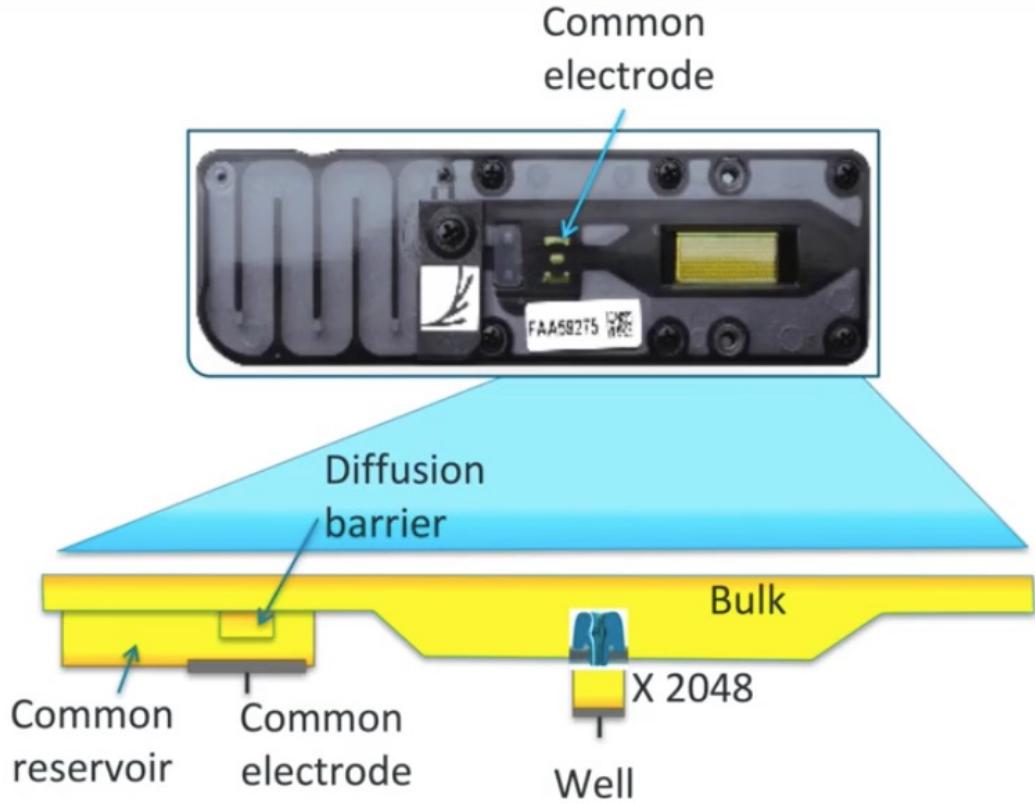
Dépôt sur Flow Cell





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



Library prep

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

Y-adapter

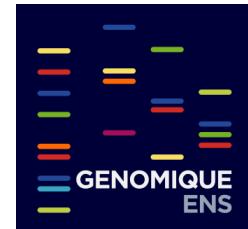


Y-adapter



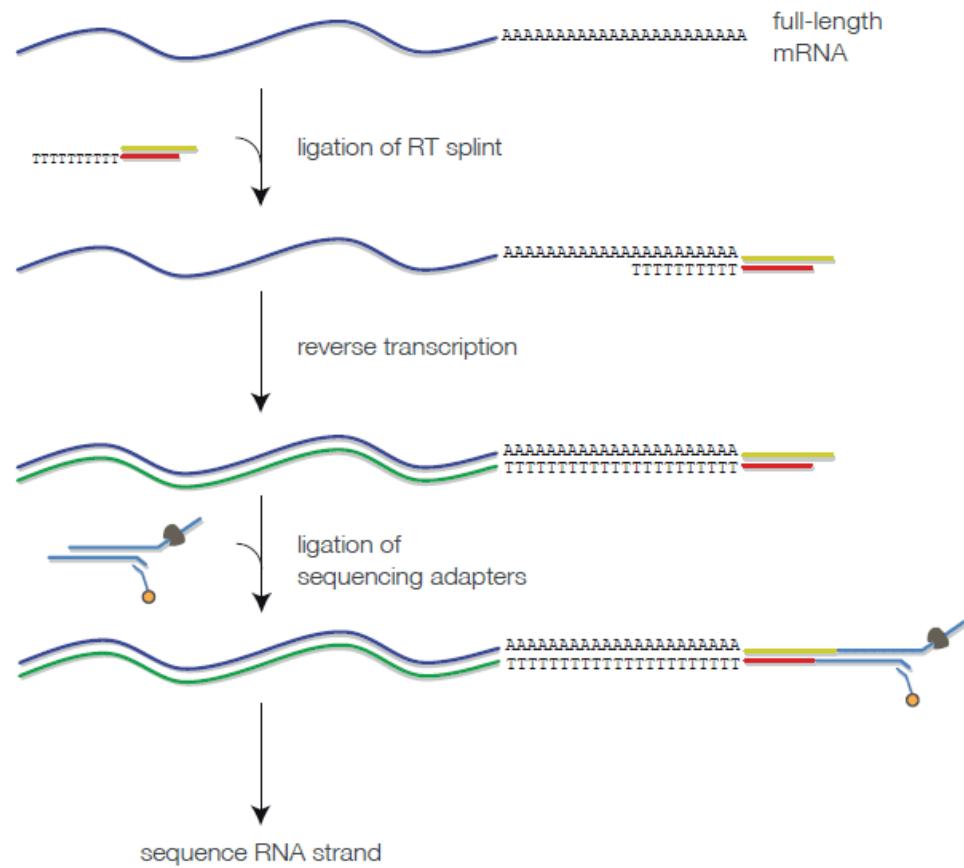
Library DNA

Direct RNA sequencing

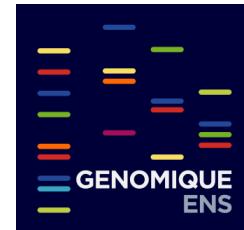


Native RNA strand is sequenced:

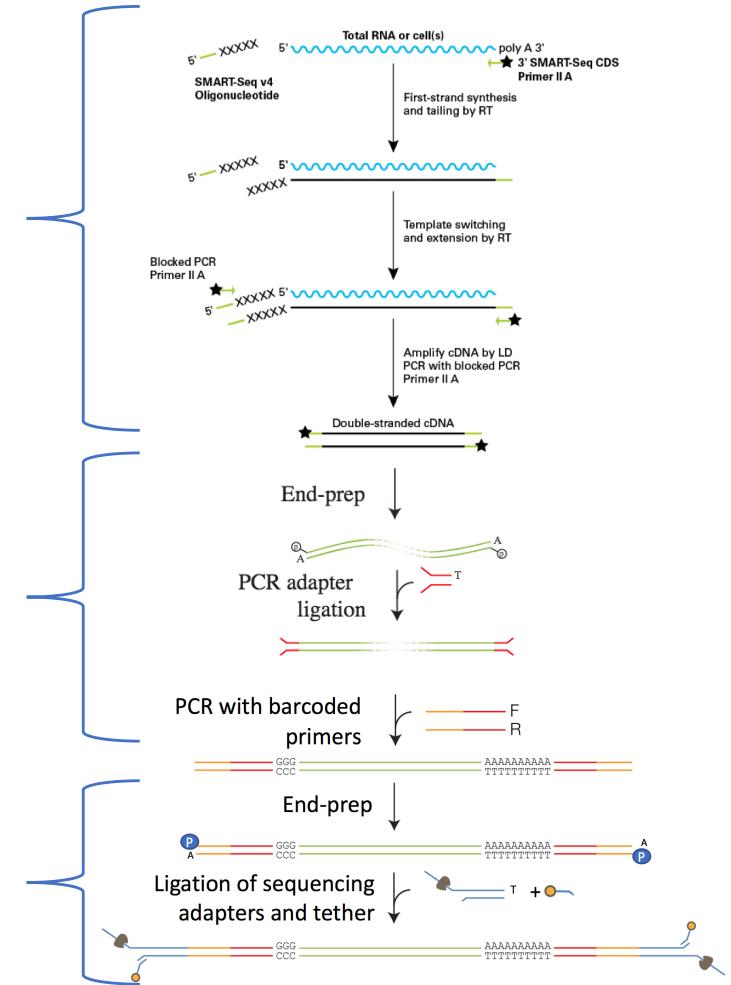
- No PCR biases;
- Modified bases detection (example methylation);
- 500 ng polyA+ RNA required.



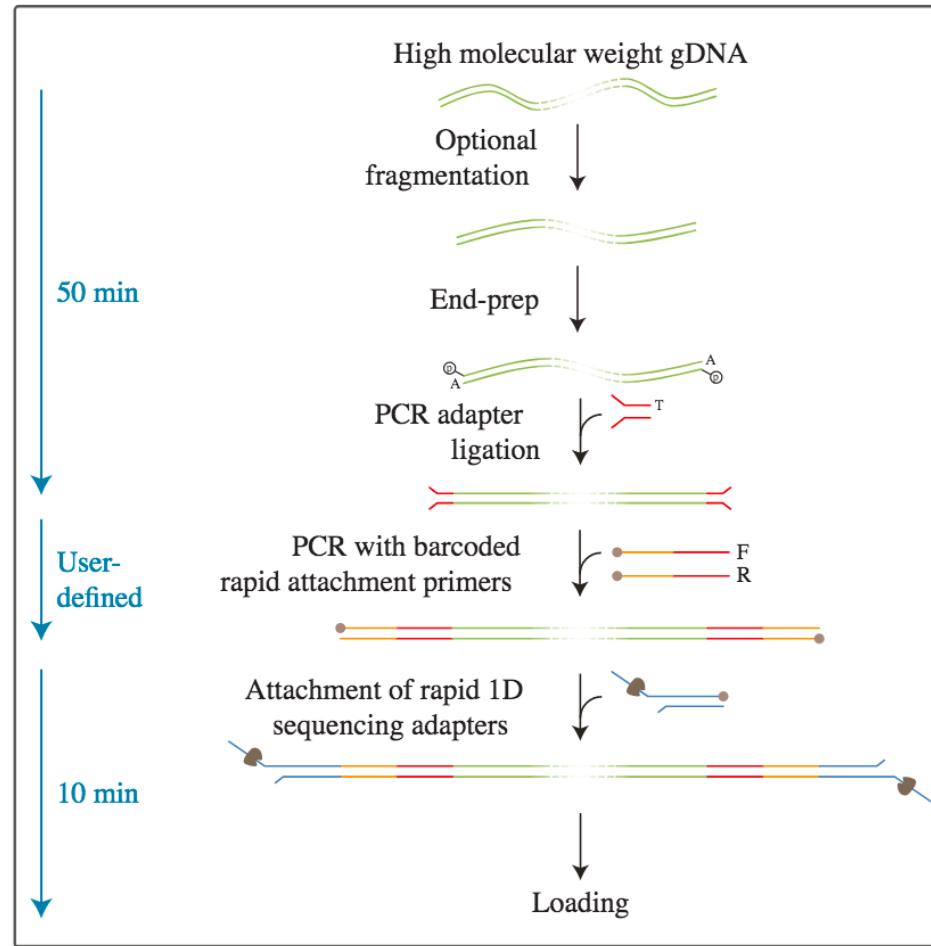
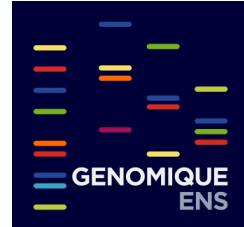
SmartLengthMinION_A2018

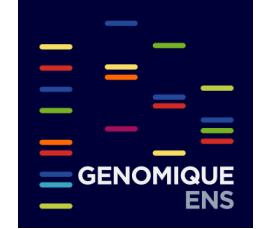


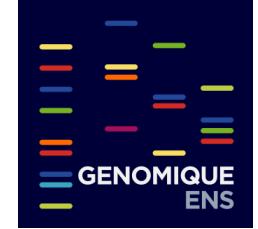
Kit	Etapes	Quantité Requise
SmartSeq v4 clontech	1er brin cDNA	10 ng d'ARN
	2 nd brin cDNA	
	PCR 9 cycles	Minimum 5 ng
PCR Barcoding kit - ONT	End prep	5 à 10 ng pour la PCR
	Ligation Barcode adapter (BCA)	
	PCR 18 cycles	2 à 3 µg post PCR
Ligation Sequencing kit 108 - ONT	Multiplexage	1 pmol poolé dans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



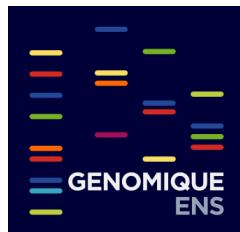
SQK-PBK004 PCR Barcoding Kit



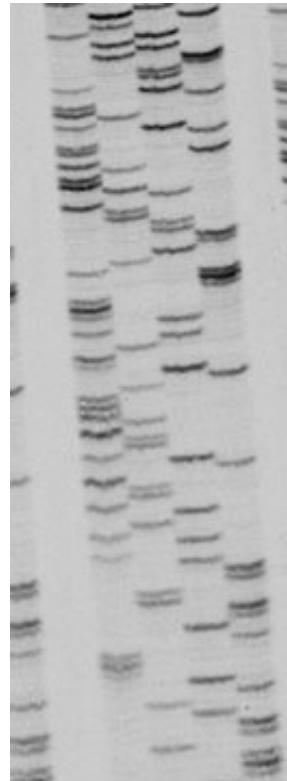




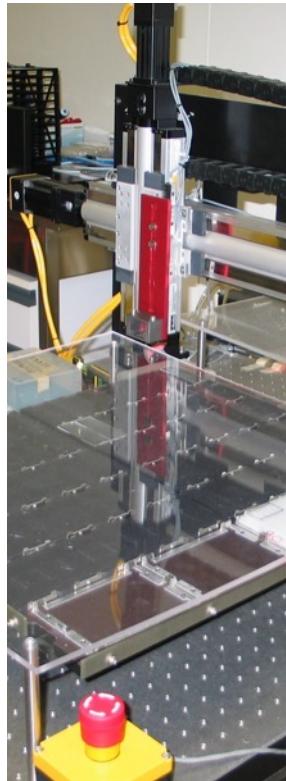
20 ans de génomique à l'ENS



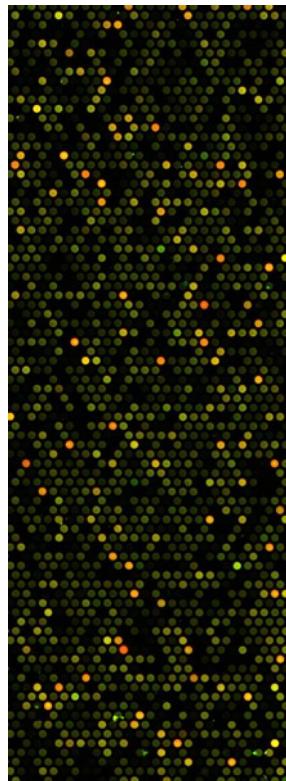
1996



1999



2005



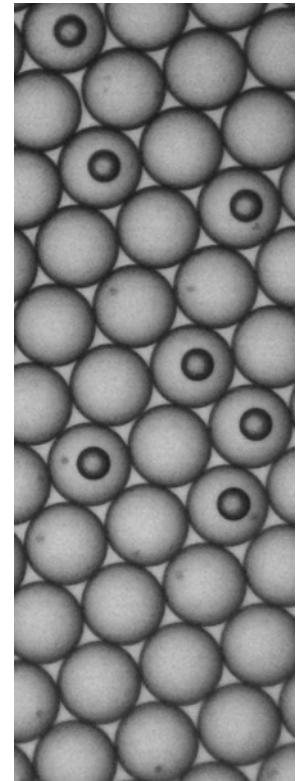
2010



2016



2017



Projet
génome de
la levure

Puces à
ADN maison

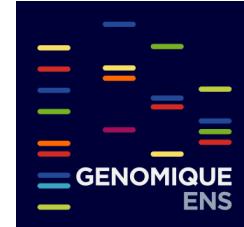
Puces
transcriptome

Séquençage
à haut débit

Séquençage
nanopore

Cellules
uniques

Une équipe équilibrée entre expérimentalistes et bioinformaticiens – 4,8 TP



Morgane THOMAS-CHOLLIER - MC ENS
Stéphane LE CROM – PU SU
Responsables scientifiques



Laurent JOURDREN - IR CNRS 100%
Responsable du pôle bioinformatique



Corinne BLUGEON - IE CNRS 100%
Responsable du pôle expérimental



Sophie LEMOINE
IE CNRS 80%



Bioinformatique

Recrutement en 2022
CDD AI ENS 100%

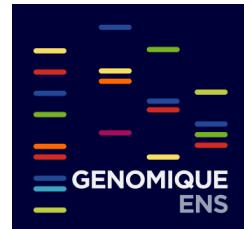


Catherine SENAMAUD-BEAUFORT
AI Inserm 100%

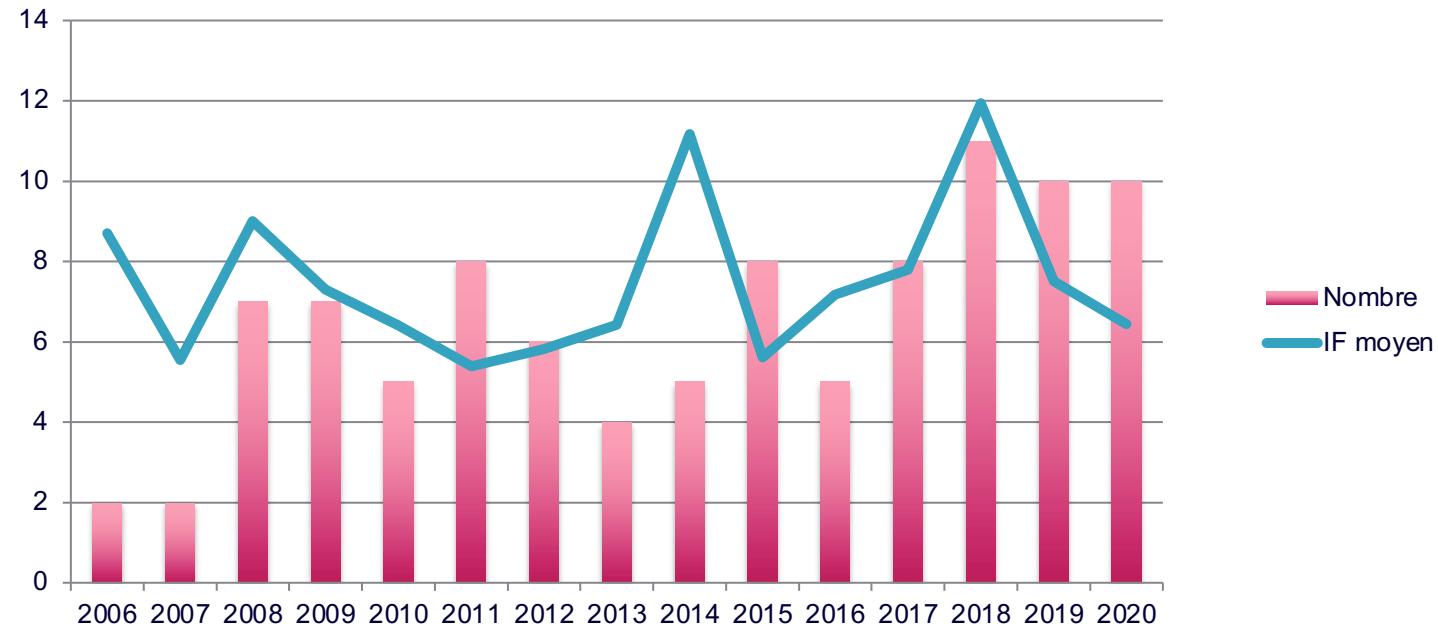


Expérimentation

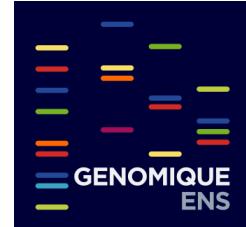
Publications



- Nous travaillons sur un **mode de partenariat collaboratif** avec nos utilisateurs, nous permettant de participer à **44 publications** sur les **5 dernières années**.



Nos dernières réalisations



Expérimental

Premier NextSeq 2000 installé en France

- Mise en service octobre 2021



Transcriptomique Nanopore

- Petites quantités : SmartSeq full-length cDNA
- ARN direct
- Épitranscriptomique m6A

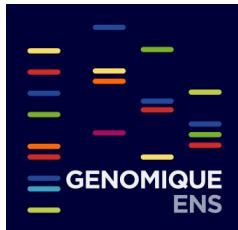


Bioinformatique

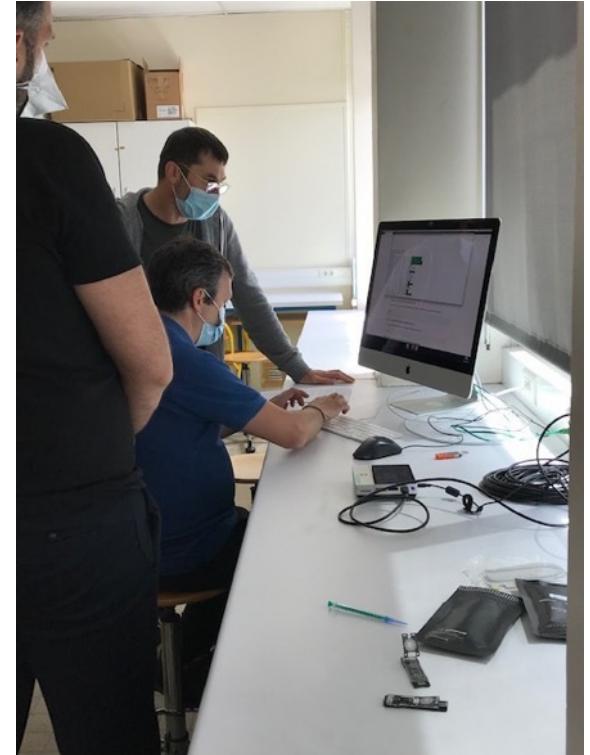
ToulligQC 2

- QC spécifique pour séquençage Nanopore
- Présentation orale à JOBIM 2021 / session organisée par l'IFB

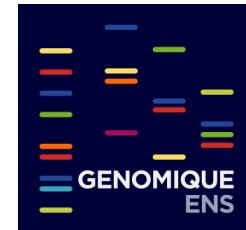
Création d'une formation MinION



- Volet expérimental et bioinformatique sur 2 dernières journées chacune.
- 3 sessions réalisées en présentiel depuis le mois de mars 2021.



Les développements futurs



Transcriptomique en cellule unique

scRNA-seq long-read Nanopore

- Protocole ScNaUmi-seq + Sicelore
- Contacts avec Kevin Lebrigand (UCAGenomix)
- Collaboration avec le CNRGH pour le séquençage à très grande profondeur

scRNA-seq cellules fixées

- Tests sur ver marin et tardigrade

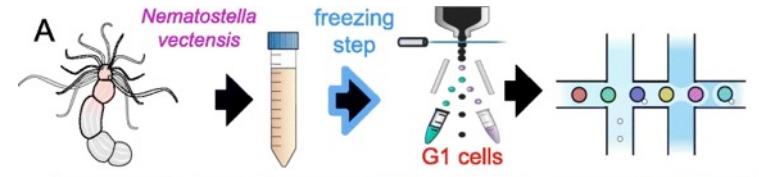
Bioinformatique

Réorientation des lectures Nanopores

- Intégré à notre pipeline RNA-seq Eoulsan

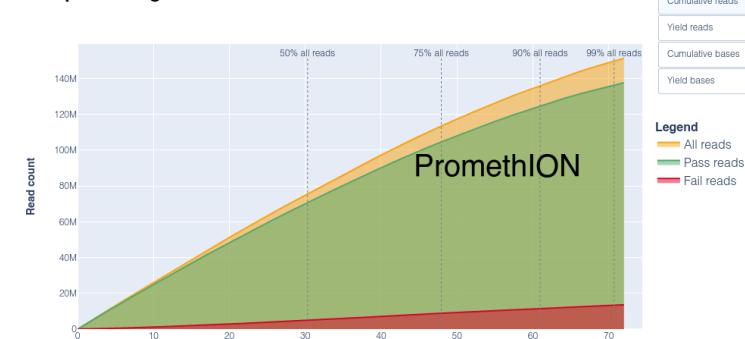
Détection et quantification d'isoformes

- Développement d'un pipeline d'analyse RNA-seq long-read

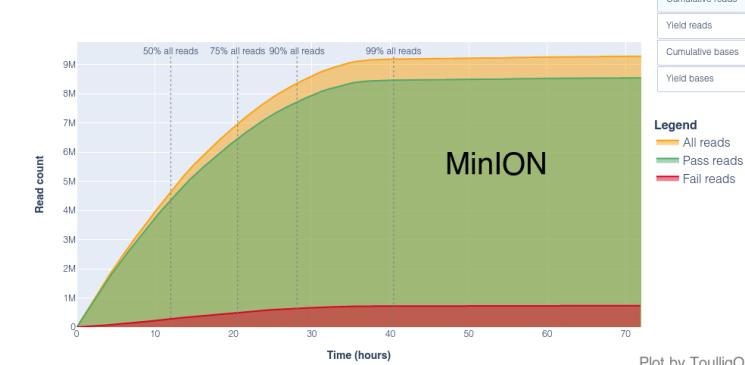


ACME dissociation + FACS + 10X Genomics

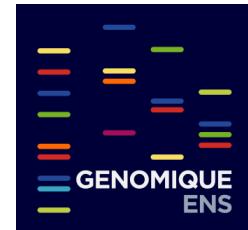
Yield plot through time ⓘ



Yield plot through time ⓘ



Nouveaux tarifs RNA-seq Illumina



Nouveaux protocoles banques RNA-seq

+ flowcell haut débit P3 NextSeq 2000

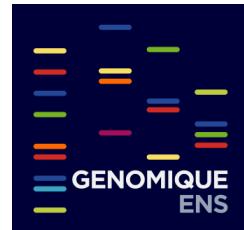
+ QC et bioinfo incluse

= **baisse de jusqu'à 50% du coût du RNA-seq par échantillon**

Grâce à l'aide en fonctionnement de l'IBENS nous allons proposer **20 % de réduction sur nos tarifs Illumina aux équipes internes**



Nous avons rejoint le « noyau central » de France Génomique



Conditions :

1. IBI SA et certification NFX50-900
2. Au moins 5 ETP dont 3 permanents
3. 1 ETP dédié à la R&D
4. Diffuser les développements de FG
5. Remonter les indicateurs en temps réel

Intérêt :

- Participer à une R&D de pointe en génomique
- Bénéficier des financements de développements de France Génomique