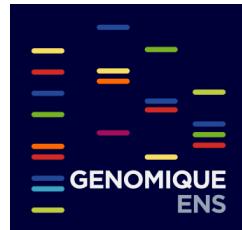


# Formation à l'utilisation d'un séquenceur Minlon et Promethlon

Session expérimentale



# L'équipe de la plateforme GenomiqueENS



<https://genomique.biologie.ens.fr>  
genomique@bio.ens.psl.eu    Genomique\_ENS

## Experimentation



Catherine  
Senameaud-  
Beaufort

Corinne  
Blugeon

Tiphaine  
Marvillet

Oumy  
Seydi



Ali  
Hamraoui

Laurent  
Jourdren

Sophie  
Lemoine

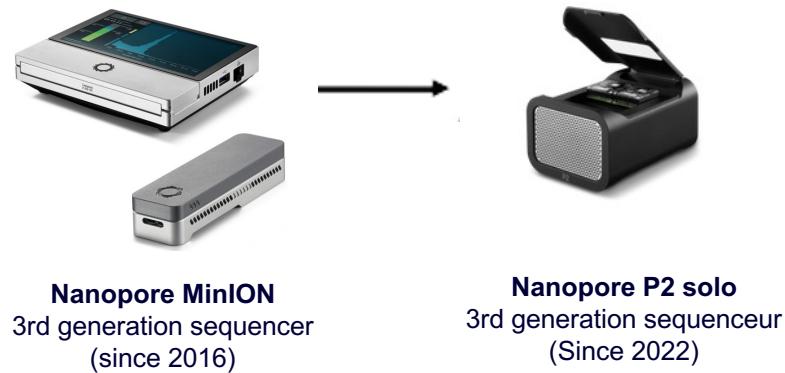
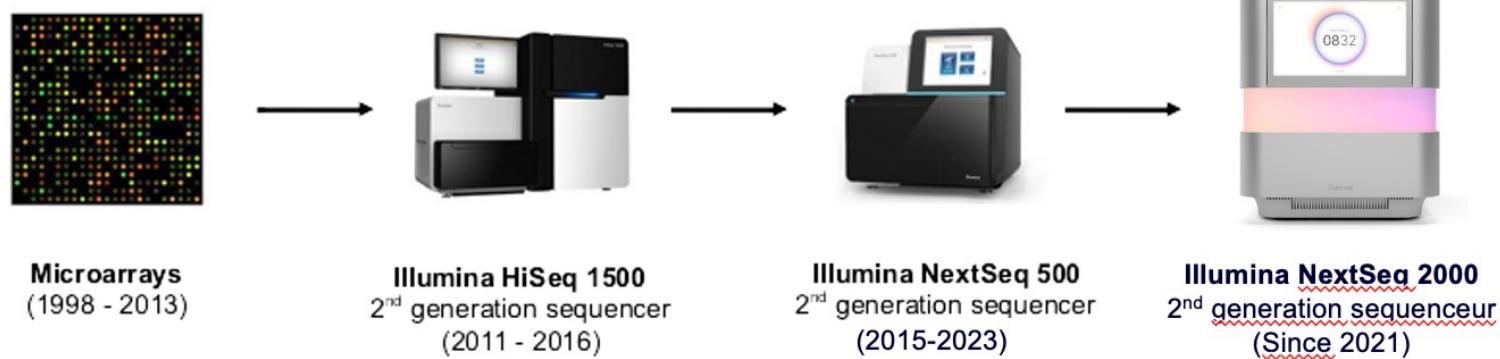
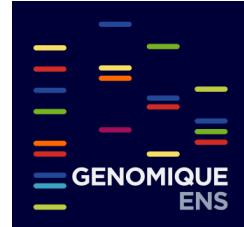
Salomé  
Brunon

Morgane  
Thomas-  
Chollier



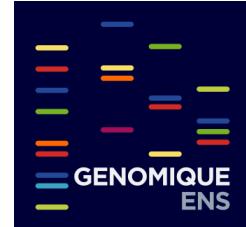
Stéphane  
Le Crom

# Evolution technologique



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

# Nos instruments



- = **Quality & Quantity check RNA/DNA:** Fragment Analyzer, Nanodrop OneC, QuBit3.0  
=> Training for autonomous users

## Sequencing



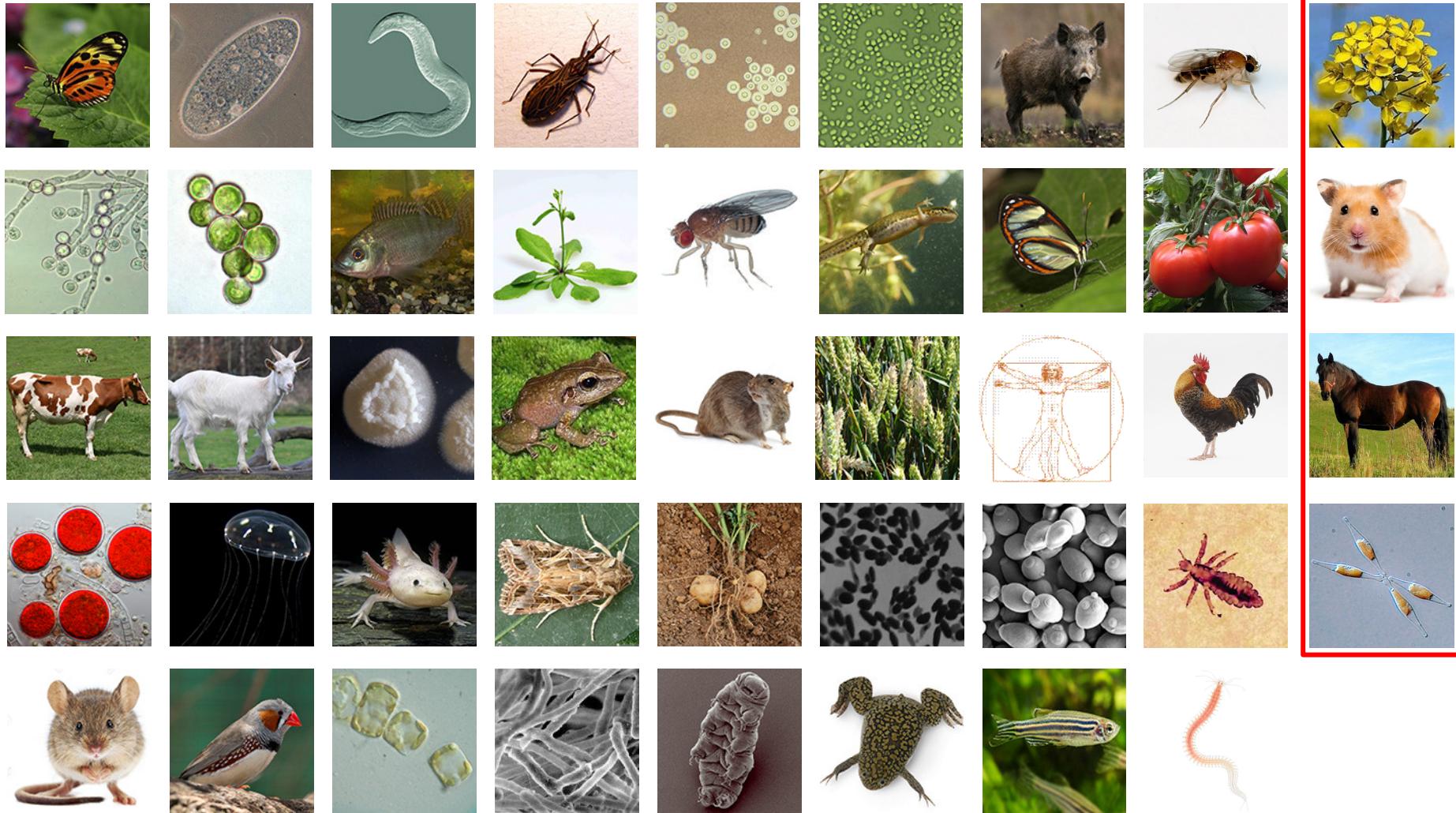
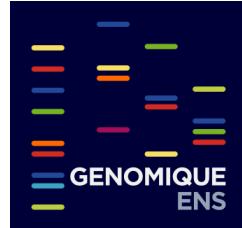
NextSeq 2000  
(*short reads*,  
Illumina, 2021)

MinION Mk1C, Mk1B &  
Promethion P2 solo  
(*long reads*, Oxford  
Nanopore Technologies,  
2022)

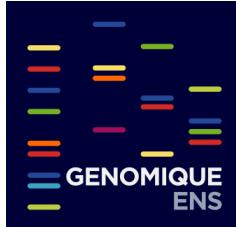


**Chromium X/iX**  
(10x Genomics, 2023)

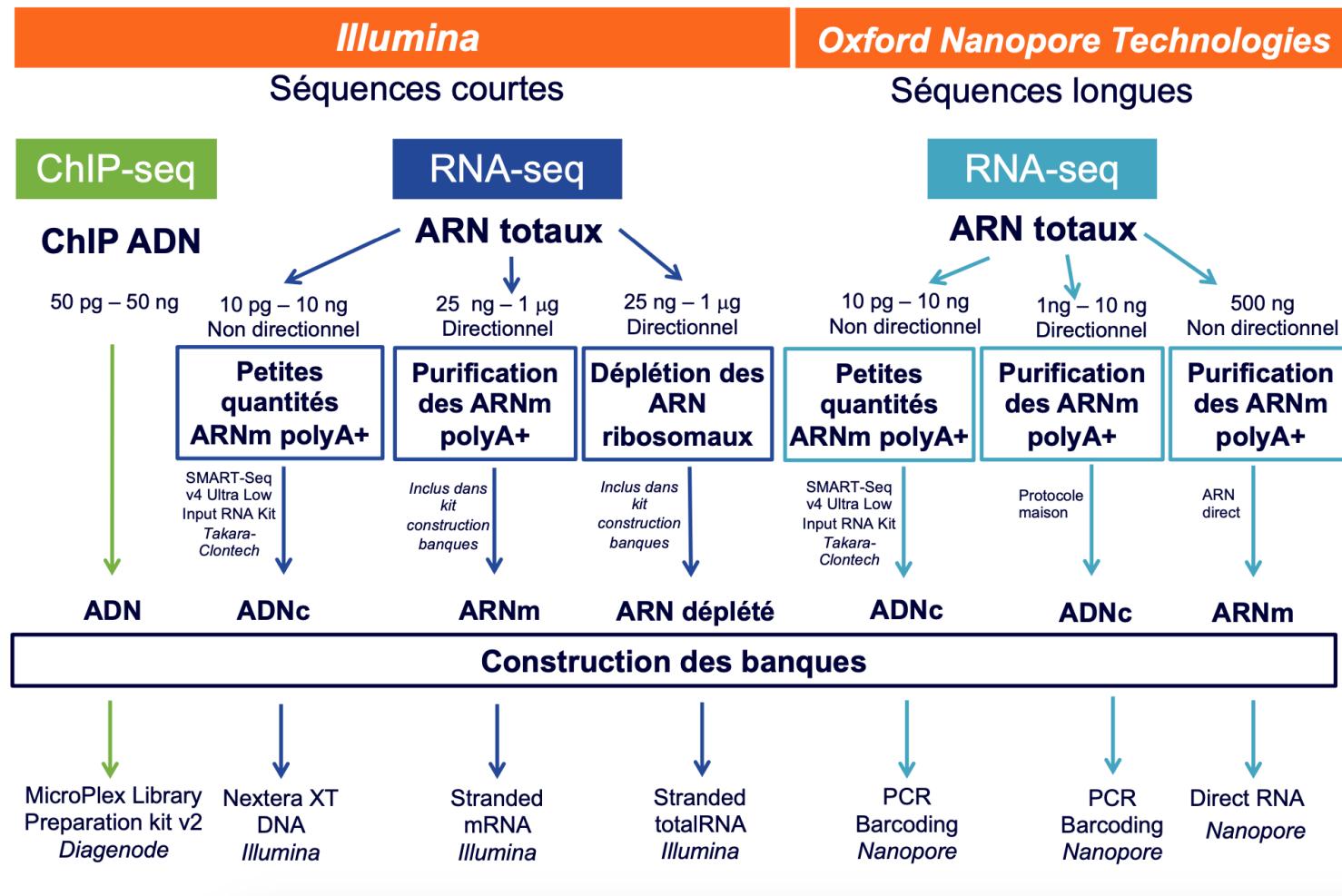
# Chez les organismes eucaryotes



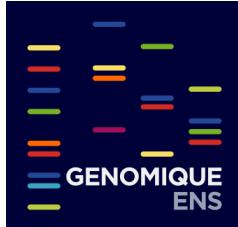
# Spécialistes en génomique fonctionnelle



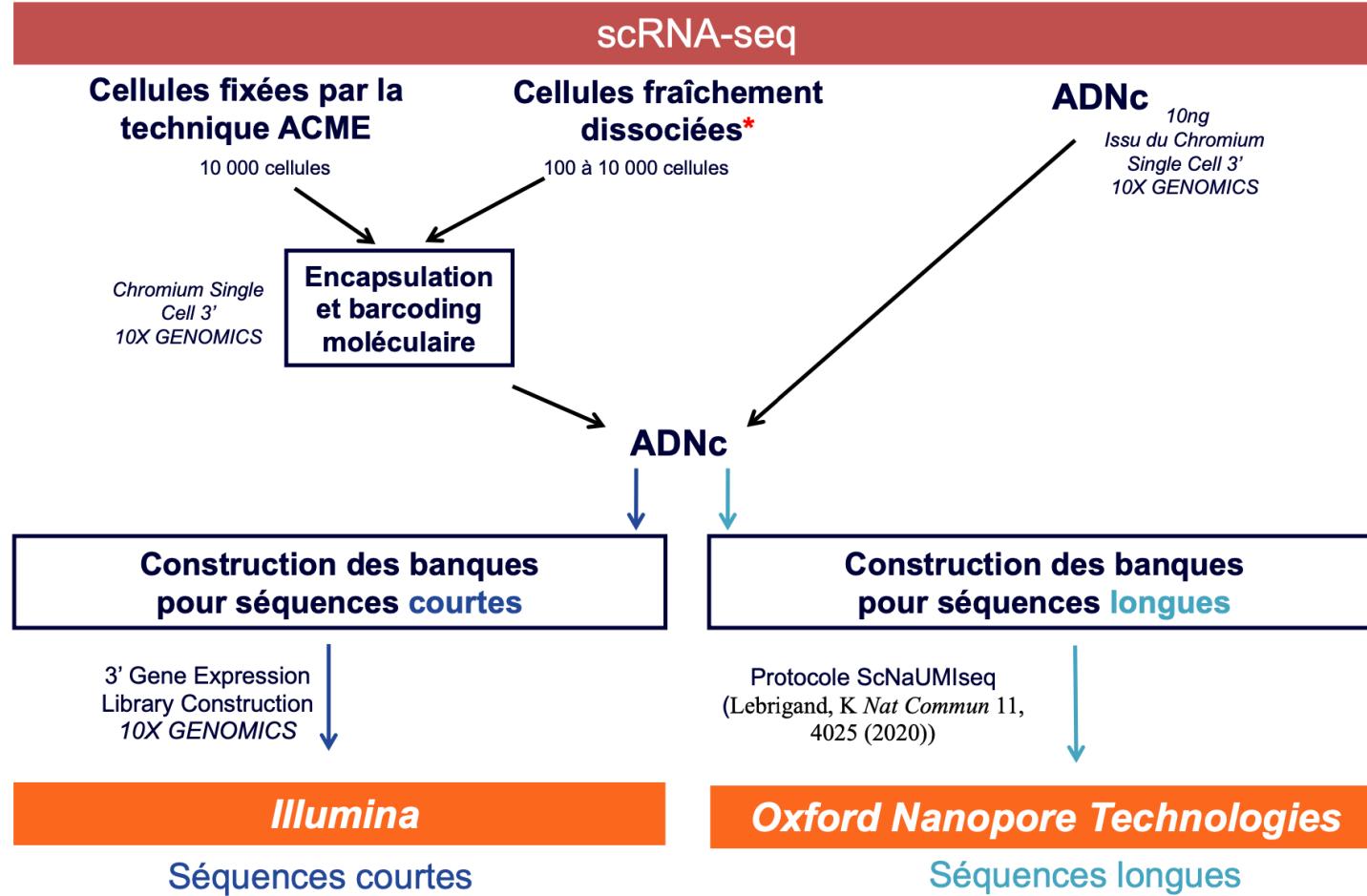
## Protocoles bulk de la plateforme GenomiqueENS



# Spécialistes en génomique fonctionnelle



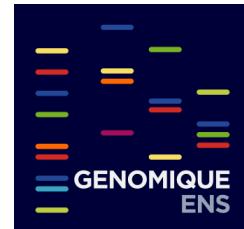
## Protocoles en Single Cell de la plateforme GenomiqueENS



Version 1 10/03/2023

\* Uniquement pour les utilisateurs de l'IBENS autonomes sur le 10X Chromium

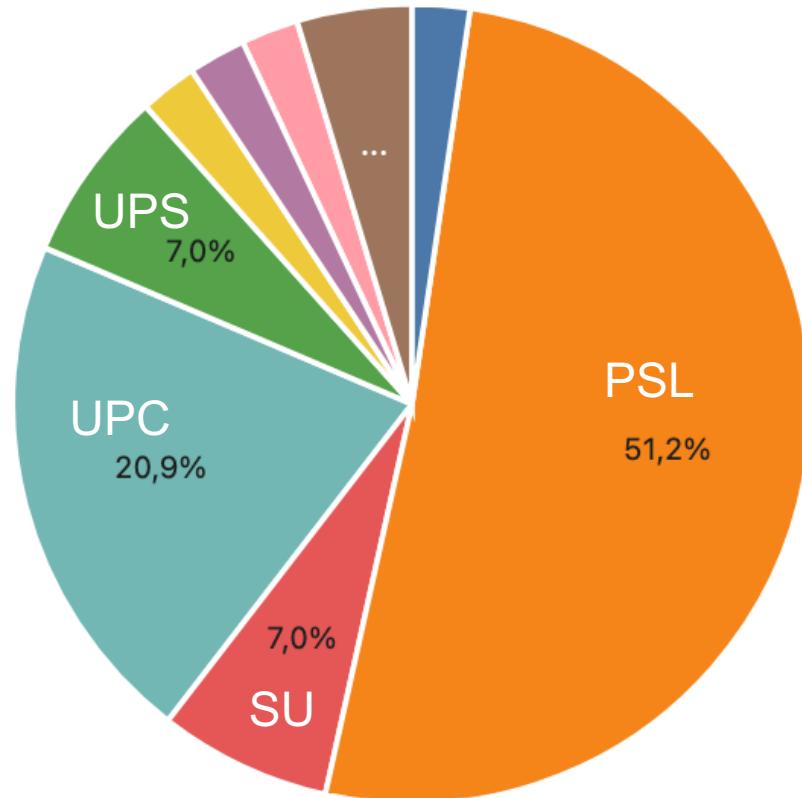
# Ouvert aux laboratoires extérieurs majoritairement en Ile de france



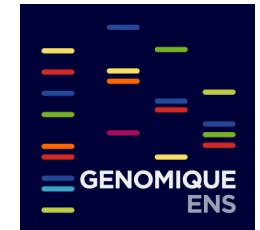
37 finished projects, 22 including analysis, 51,2 % PSL, 81 % Paris and 86 % ÎdF.

## Regroupement

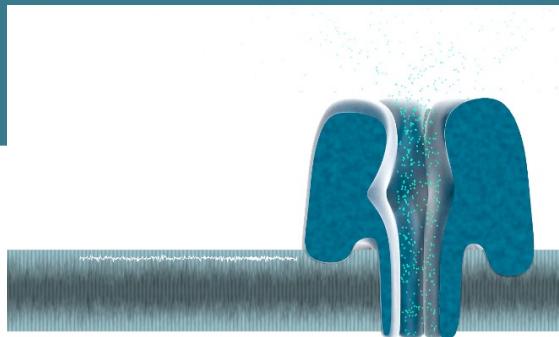
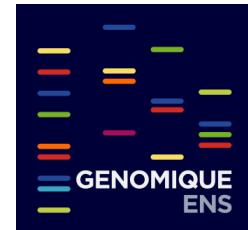
- INRAE
- Paris Sciences et Lettres
- Sorbonne Université
- Université Paris Cité
- Université Paris-Saclay
- Université de Bourgogne...
- Université de Brest
- Université de Caen Nor...
- Université de Lorraine



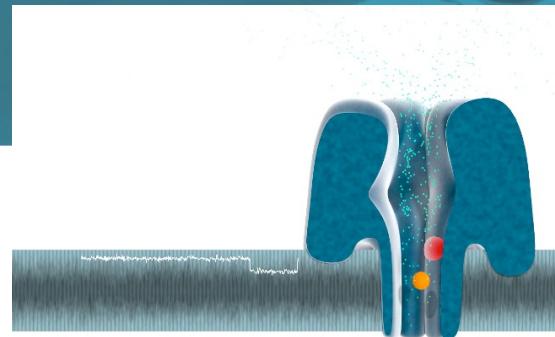
# Tour de table



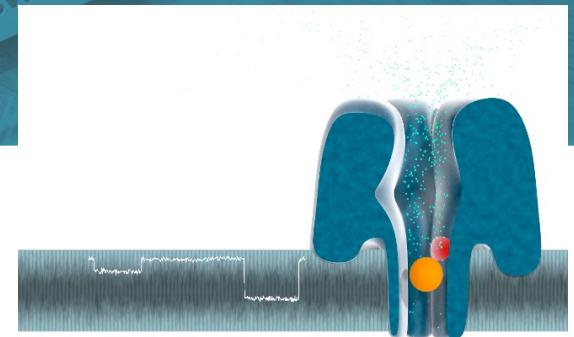
# Comment fonctionne les pores



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



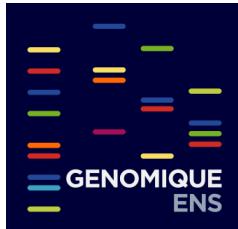
An ionic current is passed through the nanopore by setting a voltage across this membrane.



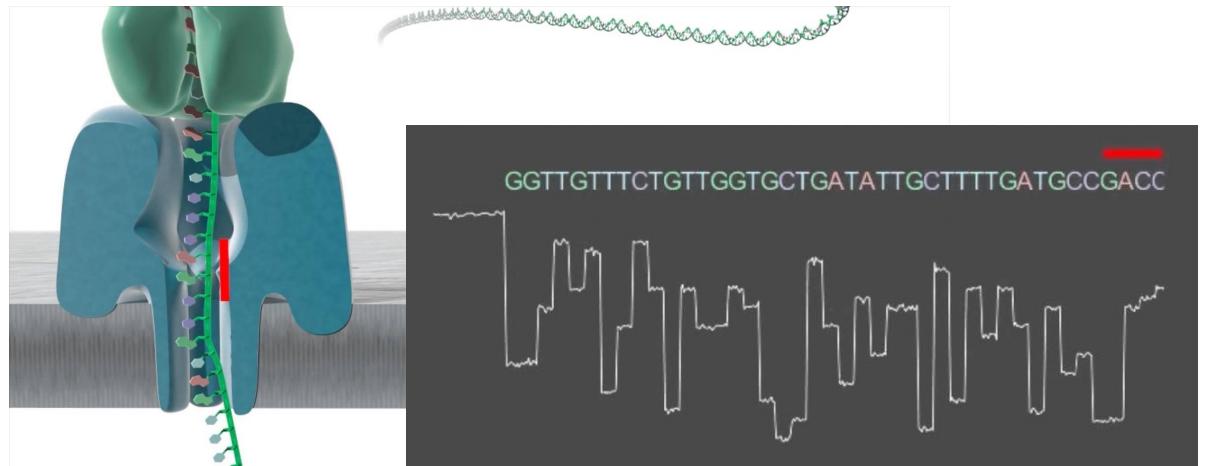
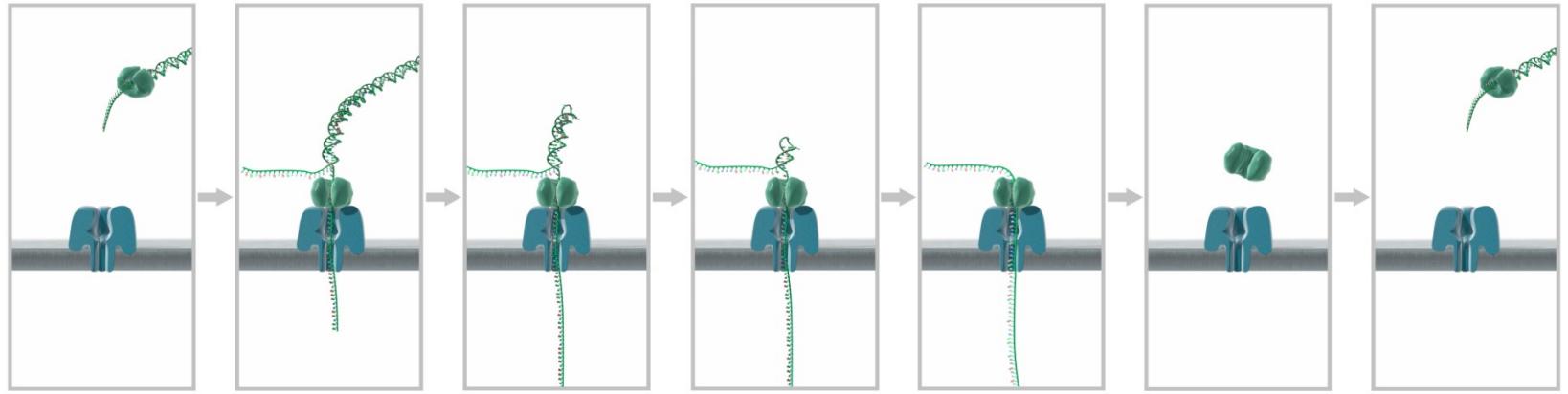
If an analyte passes through the pore or near it, this event creates a characteristic disruption in current.

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

# Séquencer avec des pores

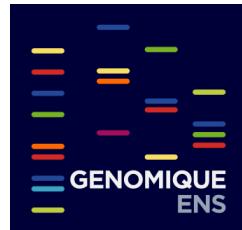


A strand of DNA is passed through a nanopore helped with a **motor protein** (helicase).

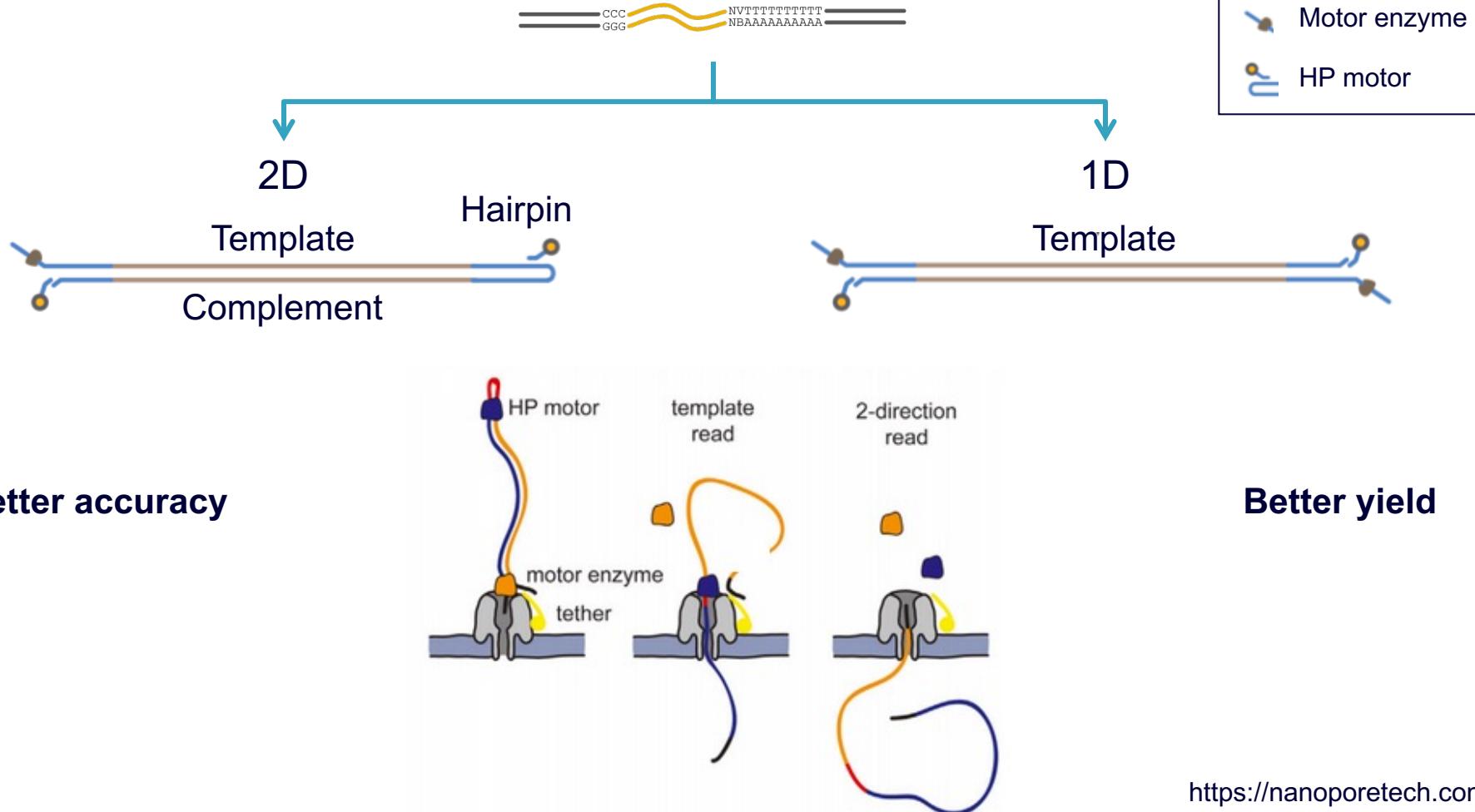


<https://nanoporetech.com/>

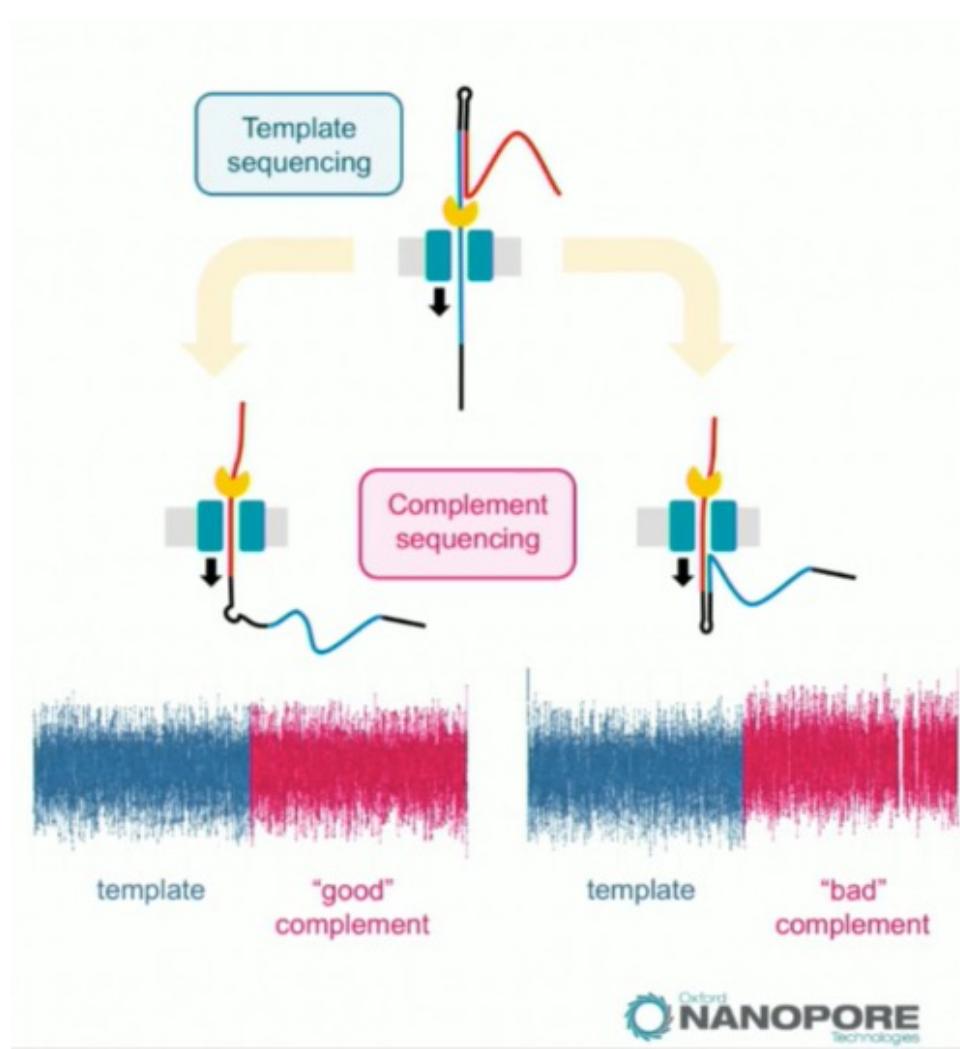
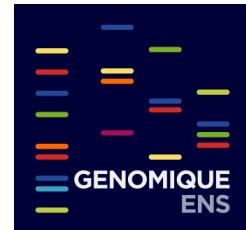
# cDNA library construction



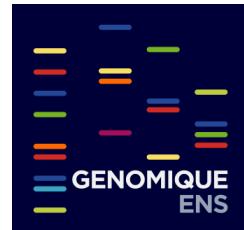
Full length cDNA from total RNA  
Poly A selection, non directional



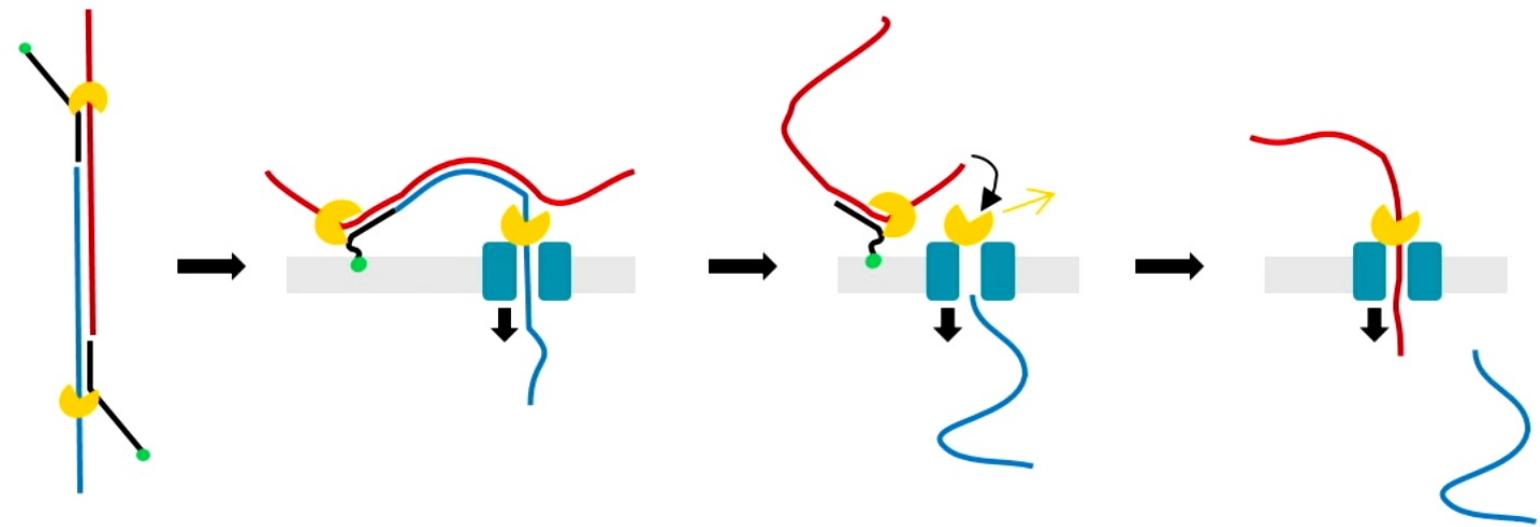
# Problème du 2D



# Evolution du 2D



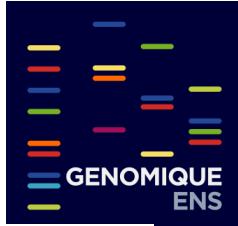
= duplex



© Copyright 2016 Oxford Nanopore Technologies

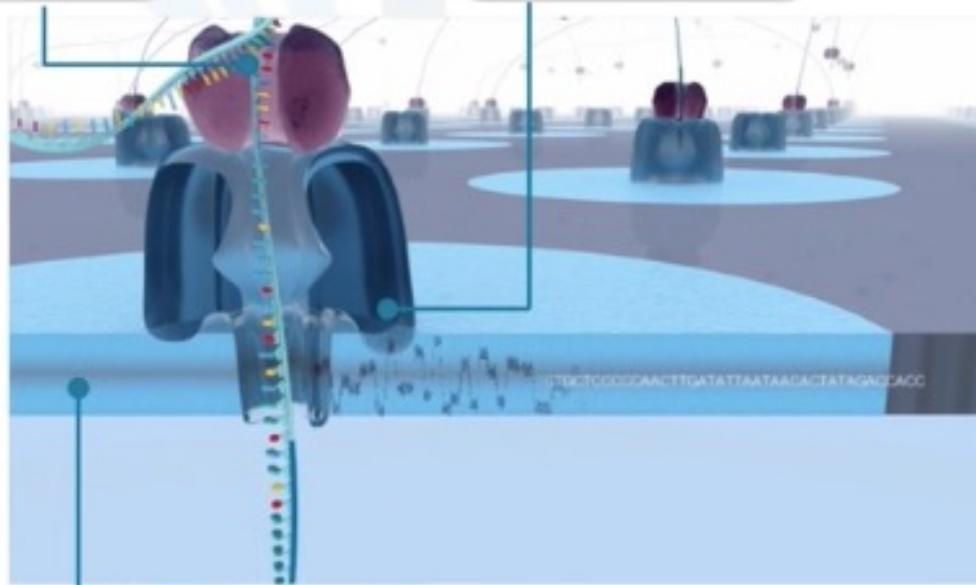
- = **Specific adapters**, that has a higher affinity to the pore, encourages the **complement strand** to immediately **follow the template strand**.
- = 60% whith the kit 14...

# Un pore



Motor  
(E6, E7, E8)

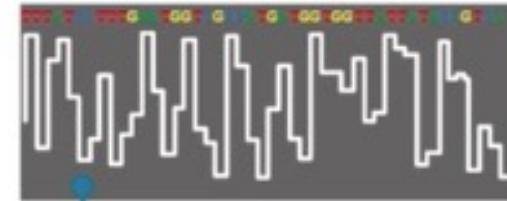
Nanopore reader  
(R7, R8, R9, R10 etc...)



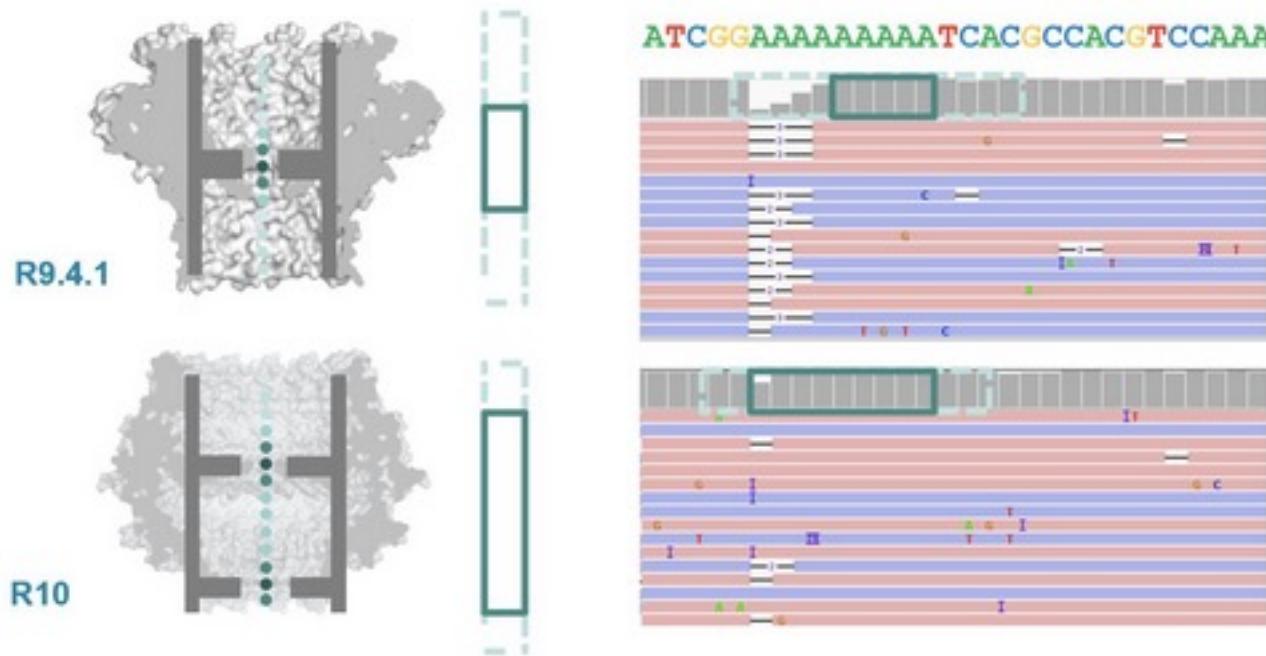
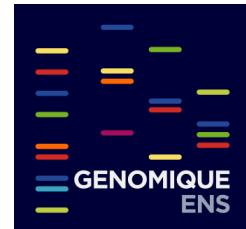
Membrane  
(M9, M10 etc...)

DNA passes through  
nanopore at 400+  
bases per second

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



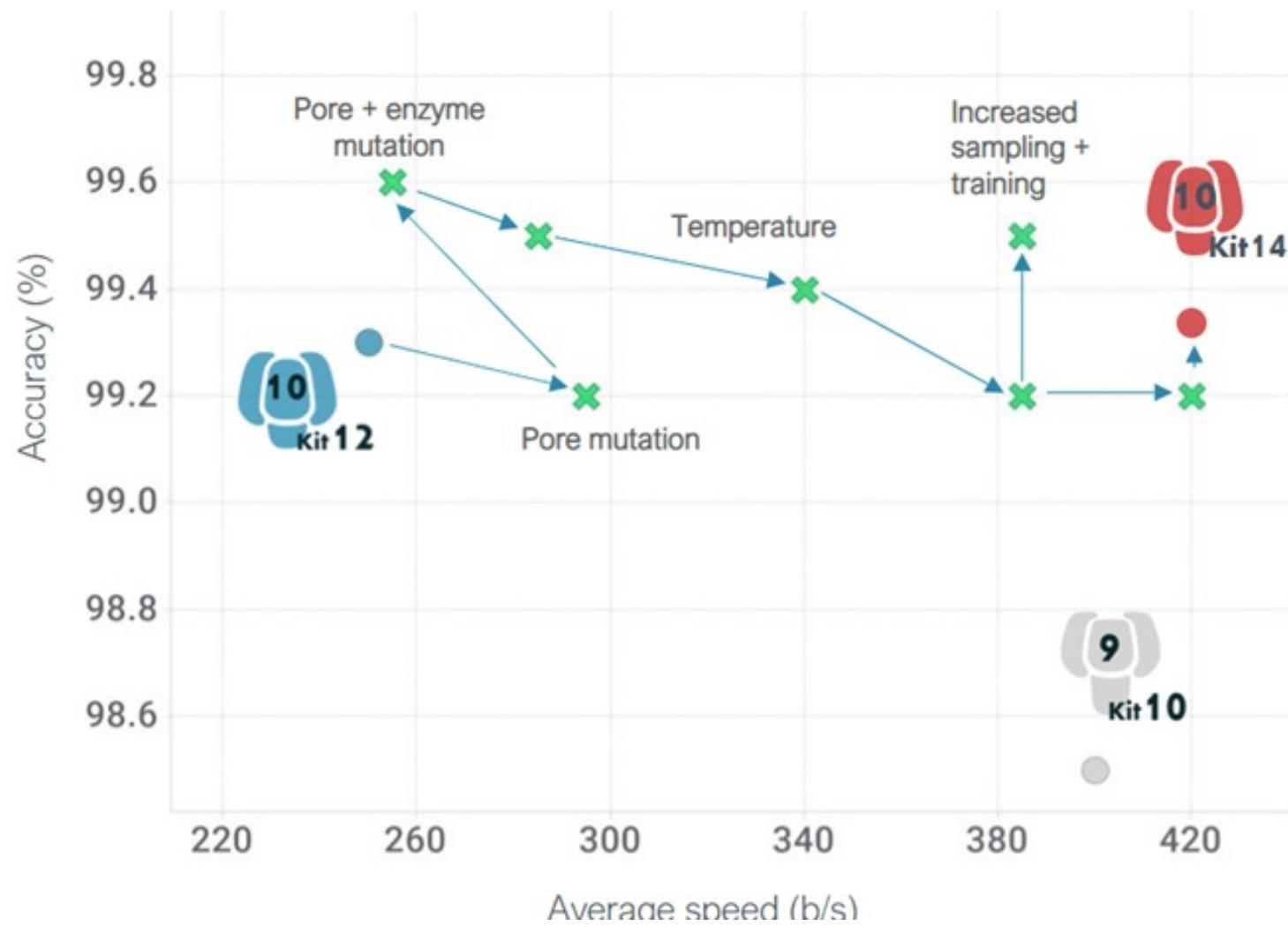
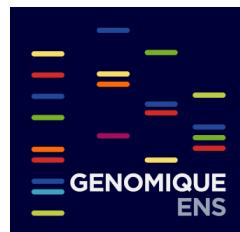
# Pore R10



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 doit s'arrêter en fin d'année 2023... 2024?

# Evolution constante

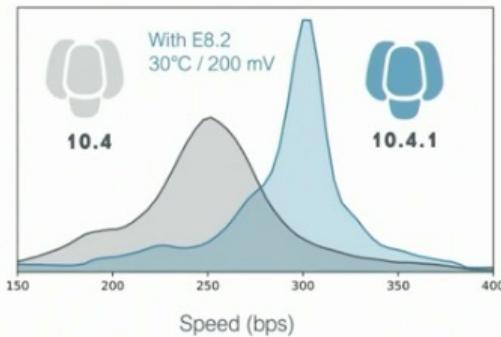


## Nanopore Accuracy

Chemistry update - motor and pore improvements

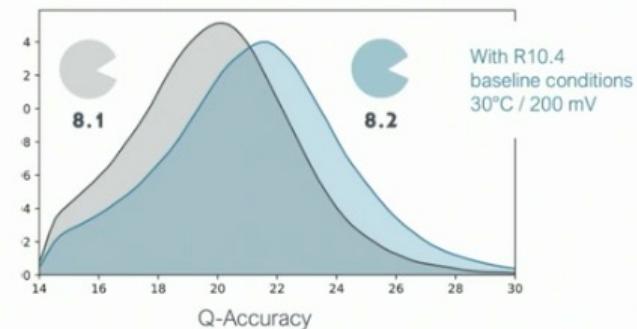
### New pore: R10.4.1

- 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



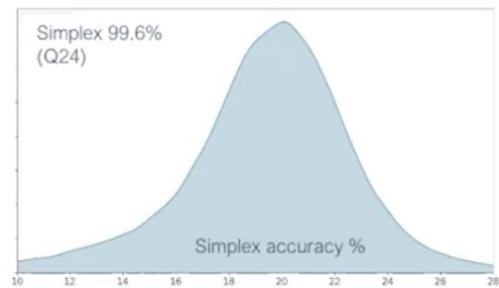
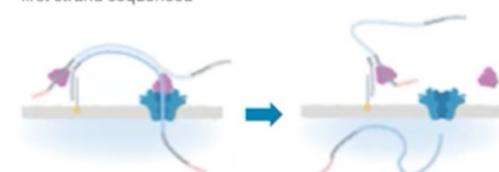
### New motor: E8.2

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



## Kit 14 — accuracy of 99% and higher throughout all investigations

One chemistry for highest accuracy and output

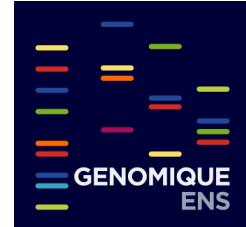
Combining the best chemistry features	Simplex: high accuracy & highest output	Duplex: Q30+ accuracy
<ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Simplex Q20+ accuracy</li> <li><input checked="" type="checkbox"/> Duplex ~Q30 accuracy</li> <li><input checked="" type="checkbox"/> High-quality homopolymer and indel calling</li> <li><input checked="" type="checkbox"/> High-capture adapters</li> <li><input checked="" type="checkbox"/> High output</li> </ul>	<p><b>Simplex: high accuracy &amp; highest output</b></p>  <p>Simplex 99.6% (Q24)</p> <p>Simplex accuracy %</p>	<p><b>Duplex: Q30+ accuracy</b></p> <p>Linear dsDNA molecule adapted on both ends and first strand sequenced</p> <p>Second strand captured and sequenced subsequently</p>  <p>Duplex 99.92% (Q31)</p> <p>Duplex accuracy (Q score)</p>



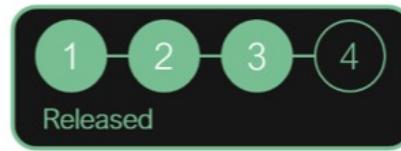
For further information about Q20+ chemistry please visit  
[nanoporetech.com/q20plus-chemistry](https://nanoporetech.com/q20plus-chemistry)



# Product phases



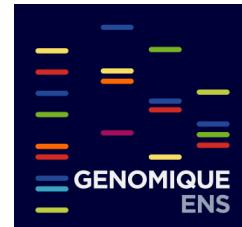
*Product phases and respective service warranty and change notification*



	Developer	Early Access	Released	Fully Released
	Trial concepts before product is specified	Get access to latest products	Use products that are fully available but <b>still evolving</b>	Use products that are <b>fully available with change notification enabled</b>

Availability:	By request only	Product live in main or private store	Product live in store	Product live in store
Lead Time:	-	Estimated lead time visible in EA store "Subject to availability"	Lead time visible in store "Subject to availability"	Lead time visible in store
Product warranty:	-	~ 1 months	1 – 3 months	3 months or more
Upgrade notification:	-	Subject to immediate change	1 - 3 months (project completion supported upon request)	3 - 6 months
Technical Support:	-	Community based technical support 9am – 5pm UK time	Your local technical support team working on your local hours	Your local technical support team working on your local hours

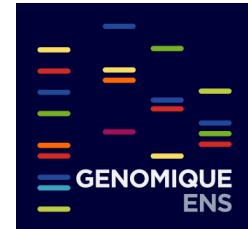
Visible in store	
<b>Ultra-Long DNA Sequencing Kit</b> SGR-ULX001	<b>Ligation Sequencing Kit</b> SGR-LSK010
Key features: Prep time: 810 minutes Input amount: Ultra-high molecular weight dsDNA from 5 million cells Read length: 100-150 kb Typical throughput: 2-3+ Gb in 8 hours, 8-16 Gb in 48 hours per flow cell on MiSeq/GenoME; 10-15+ Gb in 8 hours, 40+ Gb in 48 hours per flow cell on PromethION	Key features: Prep time: 80 minutes minutes Input amount: 1000 ng high molecular weight dsDNA 100+ ng DNA P performing fragmentation or PCR → fragment length: 2-3+ Gb in 8 hours, 8-16 Gb in 48 hours per flow cell on MiSeq/GenoME; 10-15+ Gb in 8 hours, 40+ Gb in 48 hours per flow cell on PromethION
\$1,200.00 <a href="#">Add to basket</a>	\$599.00 <a href="#">Add to basket</a>
<b>Cas9 Sequencing Kit</b> SGR-CRS010	<b>Ligation Sequencing Kit XL</b> SGR-LSK009-XL
Targeted sequencing of genomic regions using CRISPR-Cas9 <a href="#">Includes a Flow Cell Priming Kit</a>	A versatile sequencing kit optimized for throughput, long reads, and processing multiple samples simultaneously



In Q-Line products, software is frozen, and the product has been documented to a higher regulatory standard

-  Proven, locked-down technology
-  ISO 9001:2015 certified product manufacturing process
-  Full operating software and consumable version support and supply for at least 12 months post-purchase
-  Clearly defined, visible product update pathway and implementation support
-  Same pricing as standard Oxford Nanopore sequencing devices and consumables

# Flow Cells



Flongle up 2,8 to Gb



The Flongle Flow Cell can generate up to 2.8 Gb of data enabling direct, real-time DNA & cDNA sequencing on smaller, single-use flow cells.

Minlon up to 50Gb



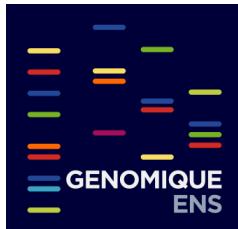
The MinION Flow Cell can generate up to 50 Gb of data for sequencing DNA, cDNA or native RNA in real-time.

Promethlon up to 290 Gb



The PromethION Flow Cell can generate up to 290 Gb for sequencing DNA, cDNA or native RNA in real-time.

# Instruments disponibles



3 types de FlowCells

126  
channels



512  
channels



3,000  
channels



Fongle

MinION

PromethION

Compatible with  
Flongle



MinION Mk1B



MinION Mk1C



GridION



PromethION 2 Solo/Integrated



PromethION 24/48

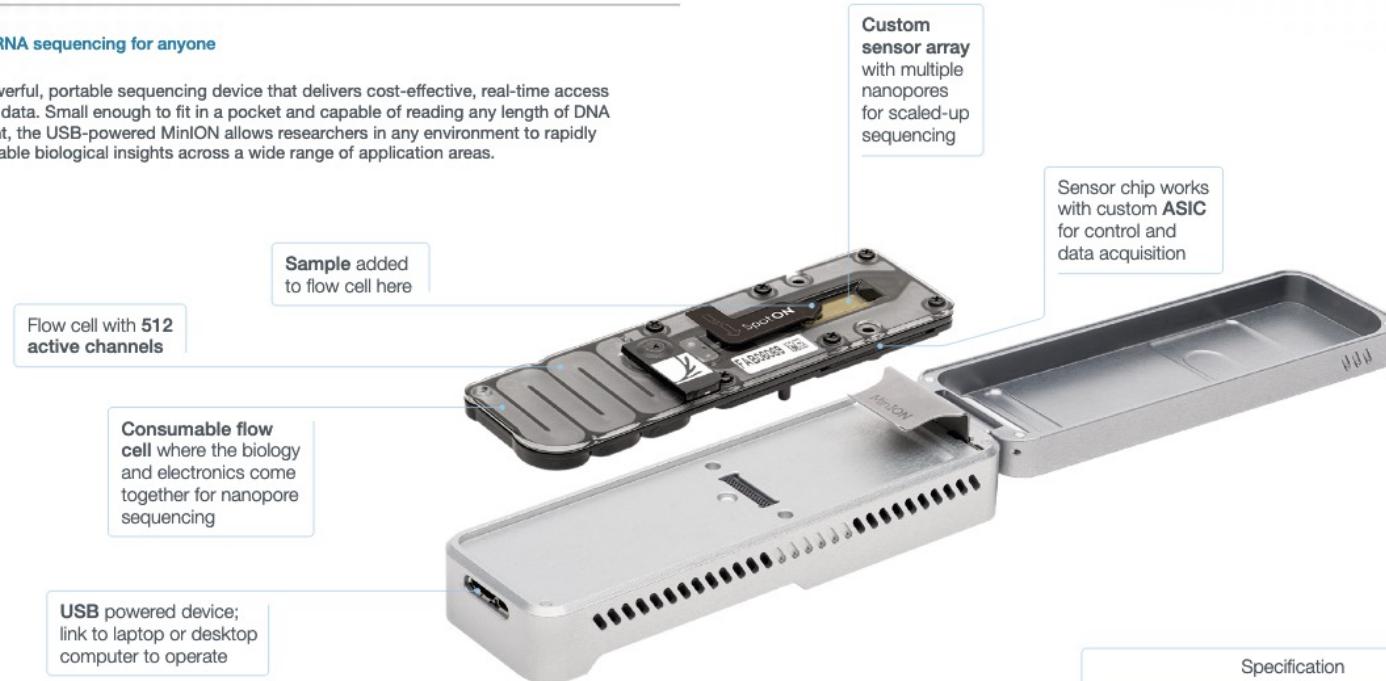


[nanoporetech.com/sequence](http://nanoporetech.com/sequence)

## 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](http://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

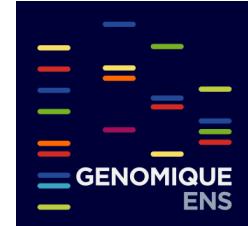
Weight  
420 g

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



Order now [store.nanoporetech.com/devices](http://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

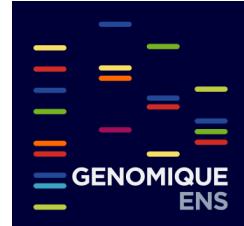
Weight  
11 kg

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



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

# PromethION 2



## PromethION 2 Solo and PromethION 2 Integrated

### Low-cost access to high-output PromethION sequencing

Offering the flexibility of two independent, high-output PromethION Flow Cells, the compact PromethION 2 devices deliver the benefits of high-coverage nanopore sequencing to users with lower sample processing requirements. Get fully integrated sequencing and analysis with PromethION 2 Integrated or expand your GridION/existing compute infrastructure with PromethION 2 Solo.

Two high-output flow cells can be operated individually or together for flexible, on-demand sequencing

Connect to GridION or existing compute infrastructure



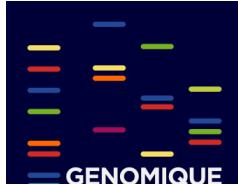
Service provider certification is available for PromethION devices



Specification		
<b>Weight</b> 1.5 kg	<b>Size</b> W 110 mm   H 87 mm   D 152 mm	<b>Compatible with</b> PromethION Flow Cells

Specification		
<b>Weight</b> 10.6 kg	<b>Size</b> W 180 mm   H 225 mm   D 430 mm	<b>Compatible with</b> PromethION Flow Cells

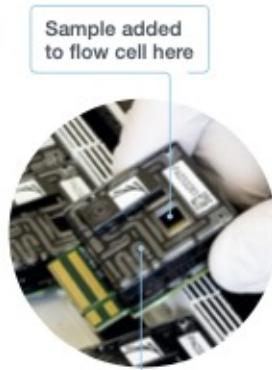
# PromethION



## 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

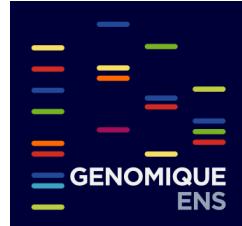
PromethION 48 can deliver over 7 Tb of data in a single run

### 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

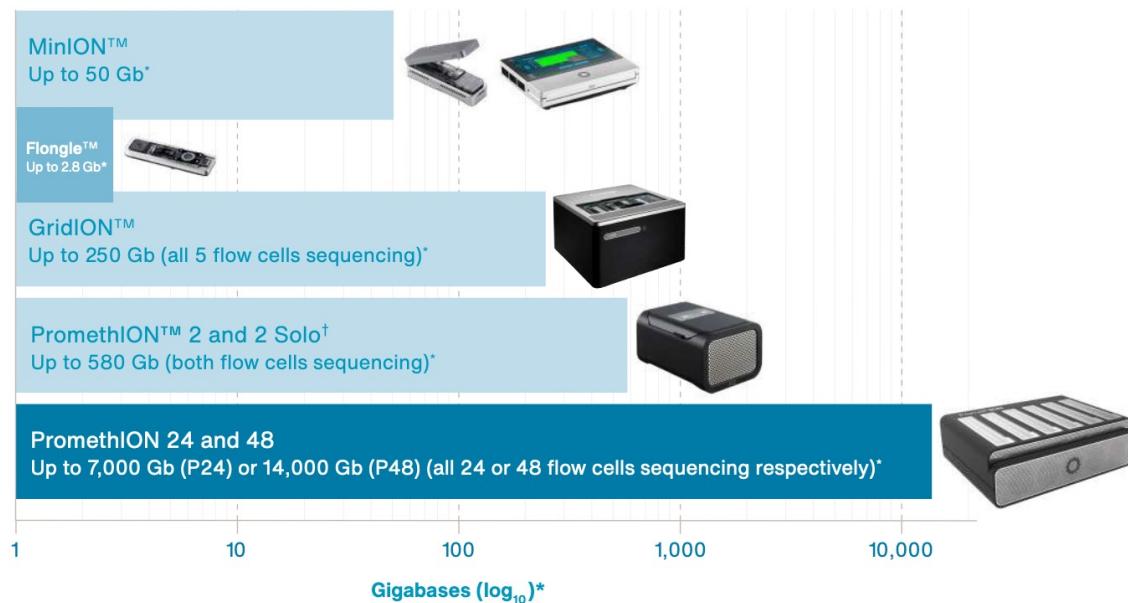
Order now [store.nanoporetech.com/devices](http://store.nanoporetech.com/devices)

# Instruments



Flexible, high-capacity benchtop sequencing offering any read length you need, in real time

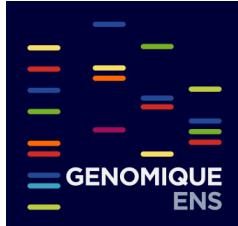
Offering the flexibility of 24 (PromethION 24) or 48 (PromethION 48) independently controllable, high-output flow cells and leveraging state-of-the-art algorithms and GPU technology, PromethION provides single or multiple users with on-demand access to terabases of sequencing data — ideal for large- and production-scale sequencing projects.



\* Theoretical max output (TMO). Assumes system is run for 72 hours (or 16 hours for Flongle) at 420 bases / second.  
Actual output varies according to library type, run conditions, etc. TMO noted may not be available for all applications or all chemistries.

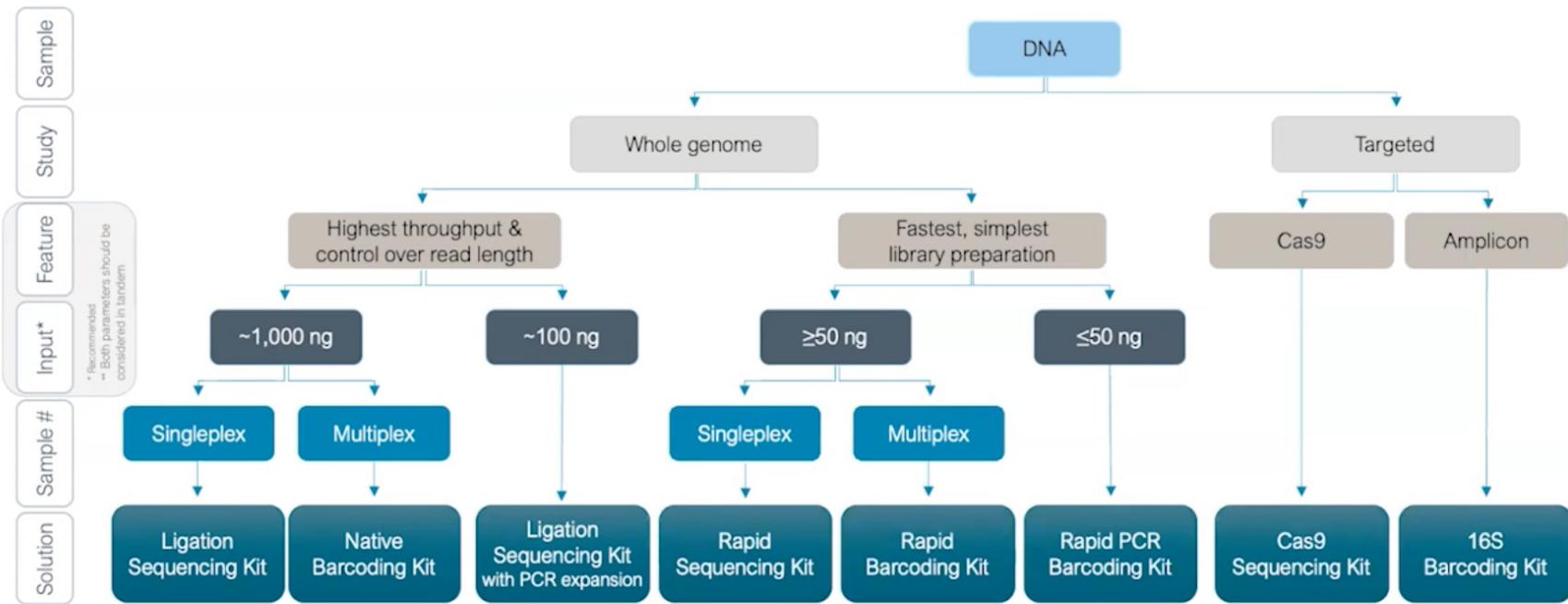
† PromethION P2 and P2 Solo devices are currently available for preorder, with Early Access devices expected to ship in 2022.

# Librairies ADN



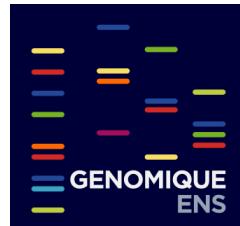
## Which DNA library prep kit do I choose?

Let our tools help you choose the best solution



The **Protocol Builder** provides a range of end-to-end workflows to help select the best library prep solution  
[community.nanoporetech.com/knowledge/protocol\\_builder](https://community.nanoporetech.com/knowledge/protocol_builder)

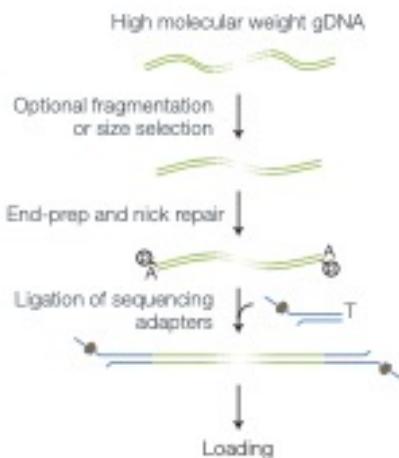
# Préparation des librairies ADN



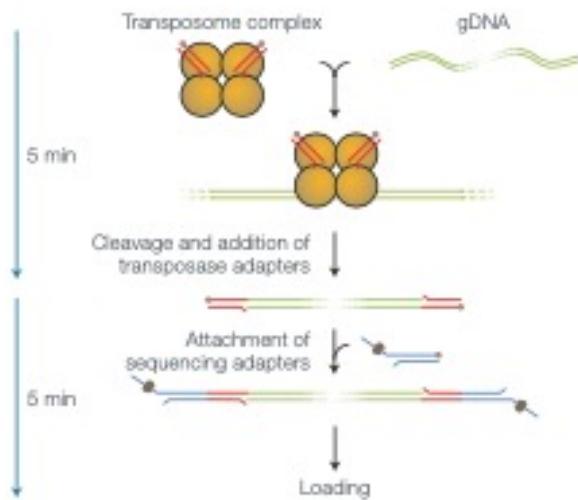
For maximum throughput

For minimal preparation time

## Ligation Sequencing Kit



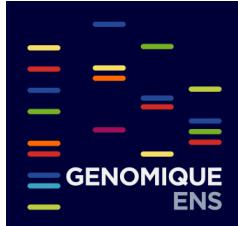
## Rapid Sequencing Kit with transposase



- 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

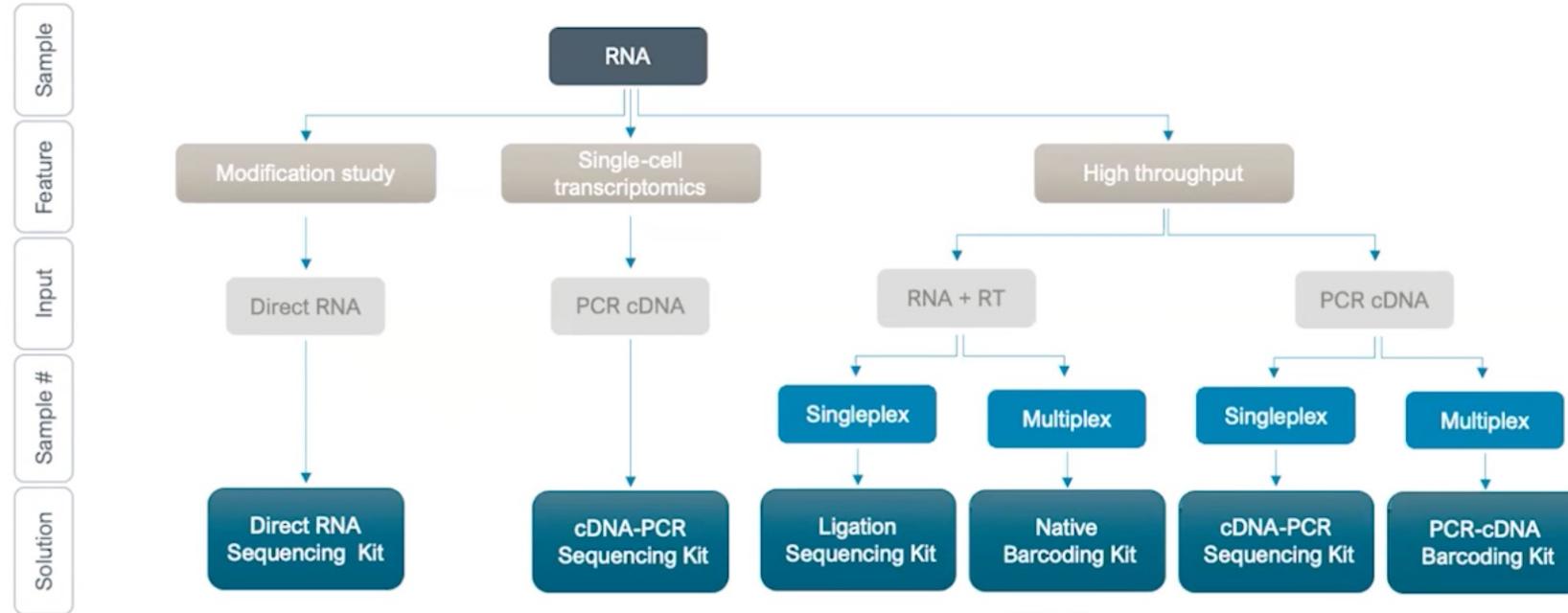
- The transposase simultaneously cleaves template molecules and attaches tags to the cleaved ends
- Rapid sequencing adapters are added to the tagged ends
- Fragment lengths are a result of the random cleavage

# Librairies ARN



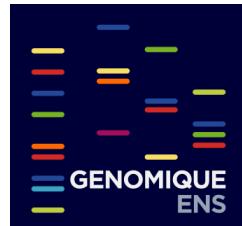
## Which RNA library prep kit do I choose?

Let our tools help you choose the best solution



The [Protocol Builder](#) provides a range of end-to-end workflows to help select the best library prep solution  
[community.nanoporetech.com/knowledge/protocol\\_builder](https://community.nanoporetech.com/knowledge/protocol_builder)

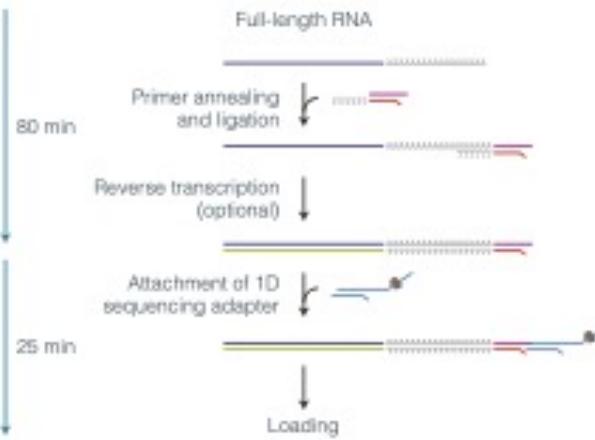
# Préparation des librairies ARN



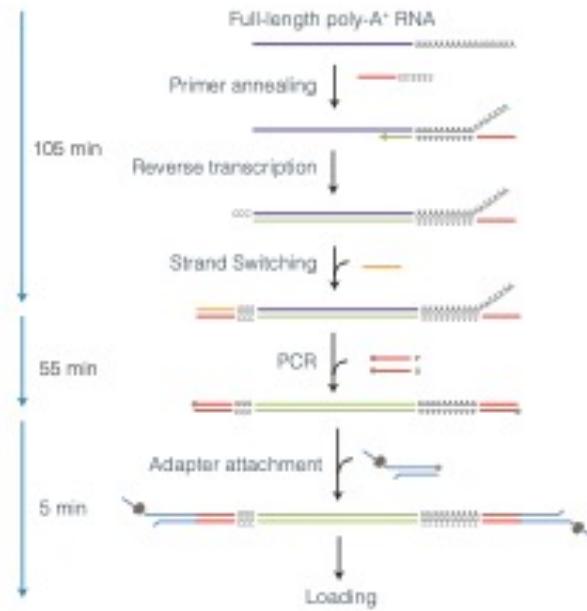
For sequencing the RNA molecule directly

For full-length transcript analysis with high throughput

## Direct RNA Sequencing Kit



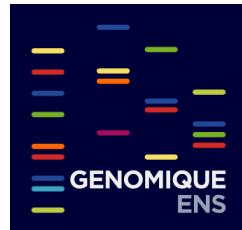
## PCR-cDNA Sequencing Kit



- 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

# Le nanopore sensor ou capteur

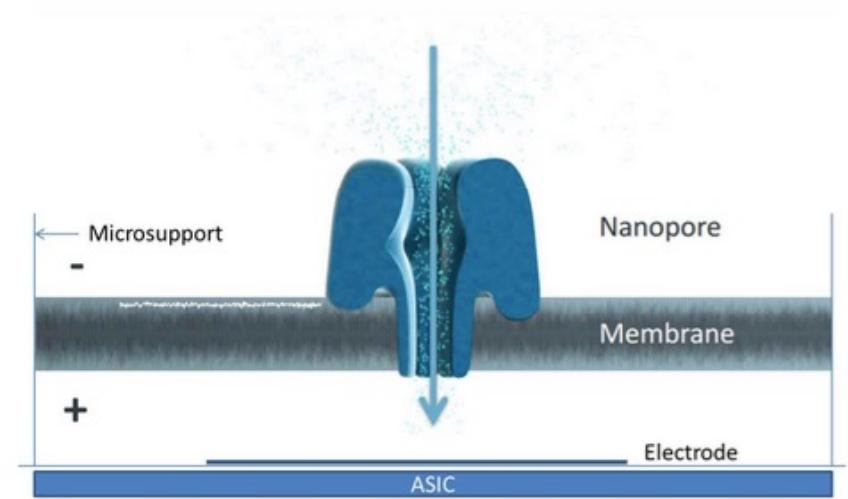


Composant du nanopore sensor:

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

(Application-Specific Integrated Circuit)

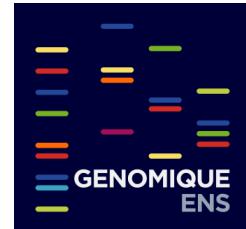
Chaque électrode est connecté à un canal sur le circuit intégré (ASIC: Application-Specific Integrated Circuit) qui contrôle et mesure le signal du pore



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

# Lien entre le nanopre sensor et le circuit ASIC

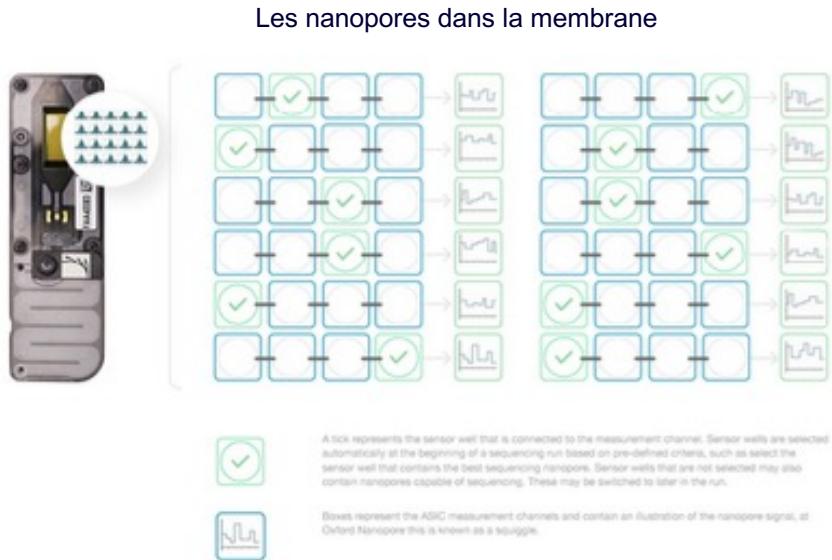
## (Application-Specific Integrated Circuit)



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

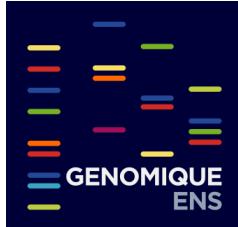
Un scan est fait au début de chaque expérience et l'ASIC choisi le meilleur nanopore sensor disponible



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

# Adaptative sampling

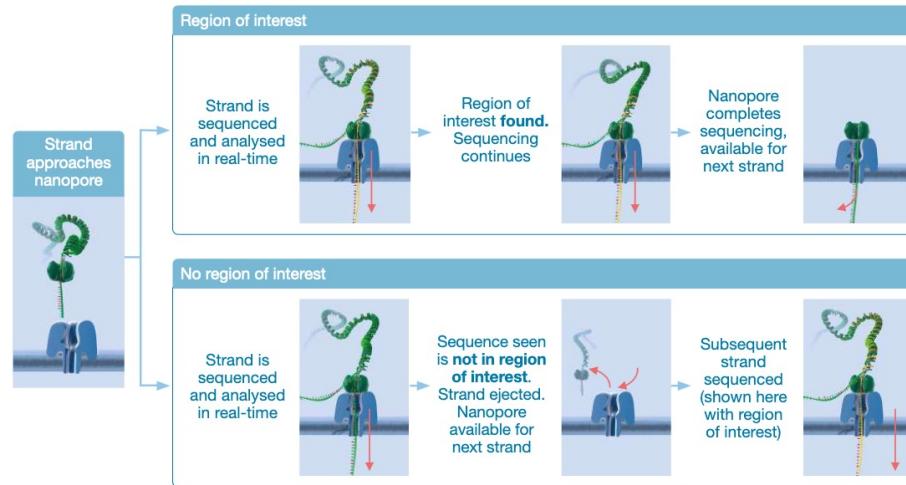


## A novel approach to targeted sequencing

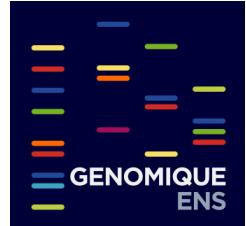
### Adaptive sampling

Adaptive sampling is a unique, on-device approach to targeted sequencing, which requires no upfront library enrichment steps. Using real-time basecalling, DNA fragments can be accepted or rejected for further sequencing based on their initial sequence composition. Furthermore, adaptive sequencing can be implemented in advance of, or even during, a run to increase coverage of specific targets.

- Target multiple regions of interest — without lengthy lab-based enrichment steps
- No limit on read length — expand targeted assays to include SNVs, SVs, and phasing
- Enrich long, native DNA molecules — retain base modifications
- Adjust enrichment in real time — enhance coverage of key regions or low-abundance species



More information [nanoporetech.com](https://nanoporetech.com)



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 a dark teal background. On the left, there is a sidebar with the following items:

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

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

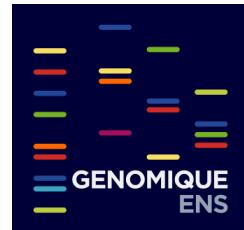
The central part of the screen is titled "Run options". It contains two input fields:

- Run length: 72 hours (with minus and plus buttons to adjust).
- Bias voltage: -180 mV (with minus and plus buttons to adjust).

Below these fields is a button labeled "Show Advanced User Options".

At the bottom of the screen, there are three buttons: "Connection Manager", "Back to Kit Selection", "Continue to Basecalling >", and "Skip to End >".

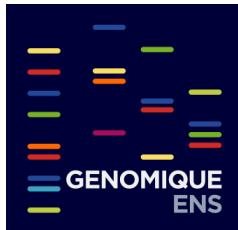
# Pore occupancy



- 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.



# Duty time plots



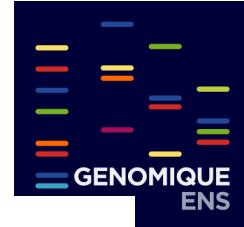
The duty time plot summarises the channel states over time.

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.



# Run RNA R9.4.1 chimie11



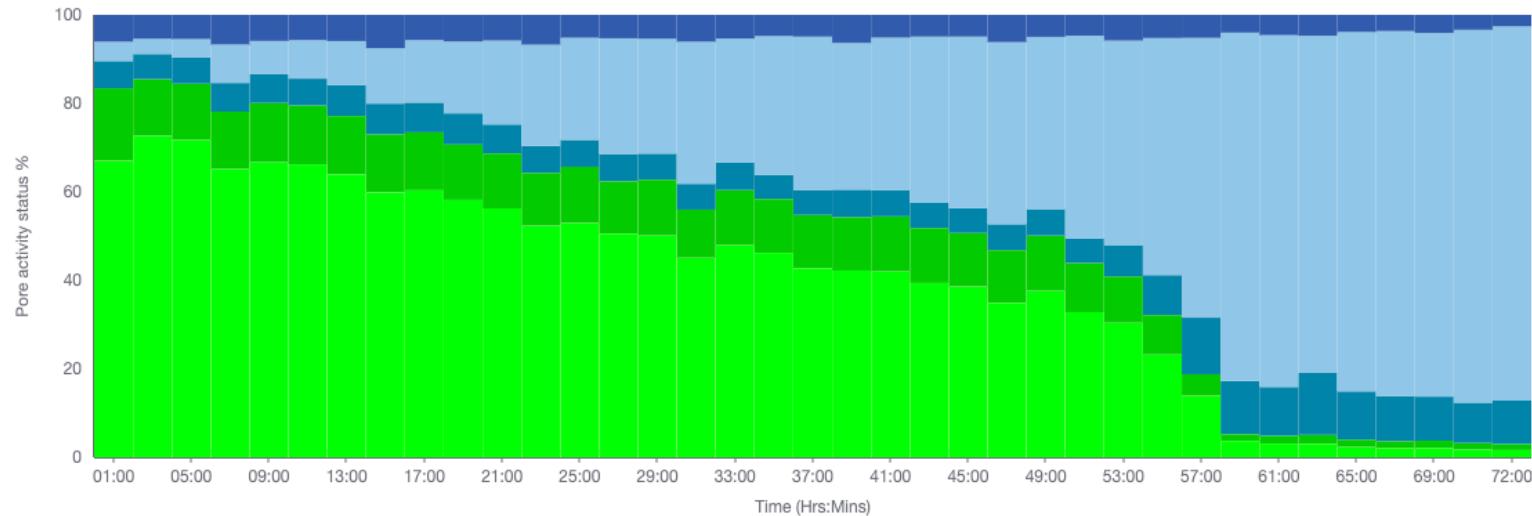
## ▲ PORE ACTIVITY

The Pore activity graph shows the performance of your sample as it is being sequenced during a run.

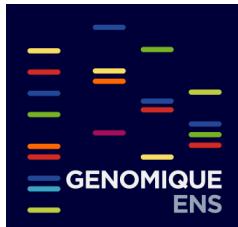
Show grouped

### Legend

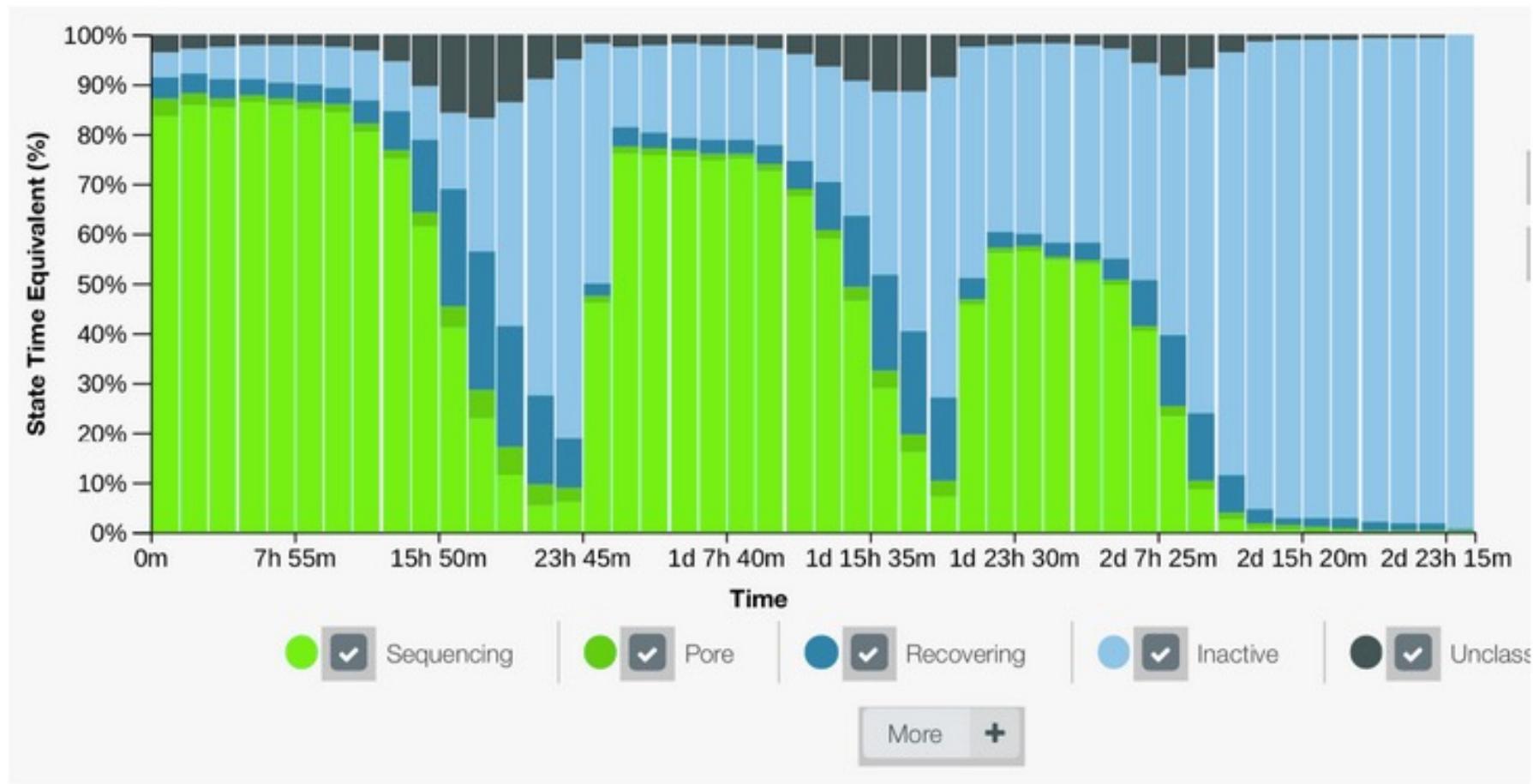
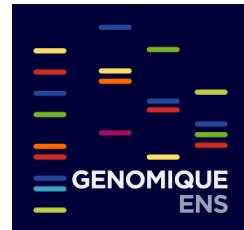
<span style="color: green;">●</span> Sequencing	<span style="color: green;">●</span> Pore available	<span style="color: darkblue;">●</span> Unavailable	<span style="color: lightblue;">●</span> Inactive	<span style="color: darkblue;">●</span> Unclassified
Pore currently sequencing	Pore available for sequencing	Pore currently unavailable for sequencing	Pore no longer suitable for further sequencing	Pore status unknown



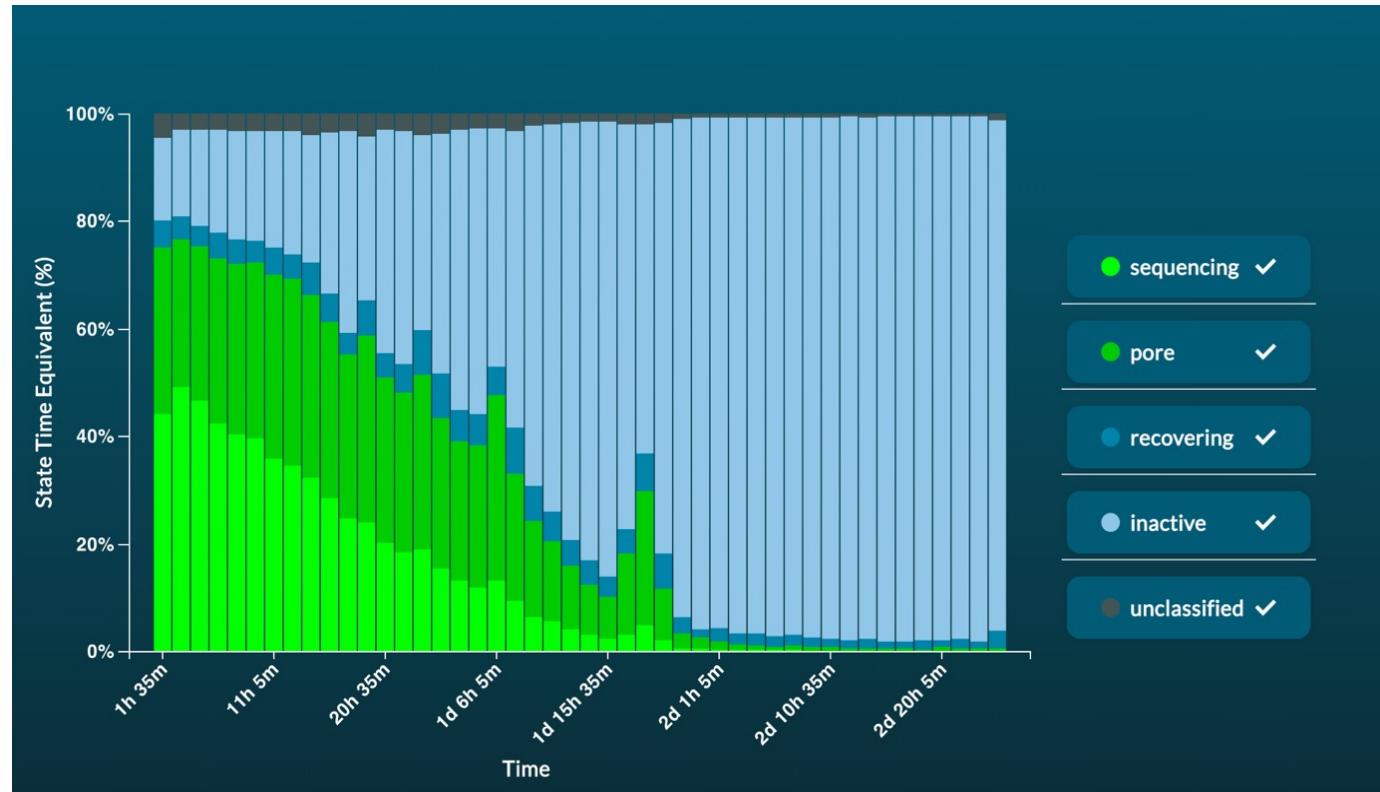
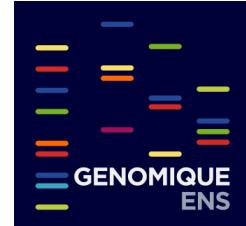
# Re dépôt



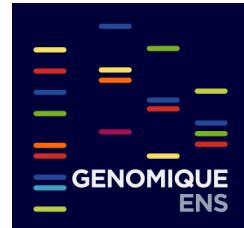
# Troubleshooting



# Troubleshooting



# Dépôt sur Flow Cell

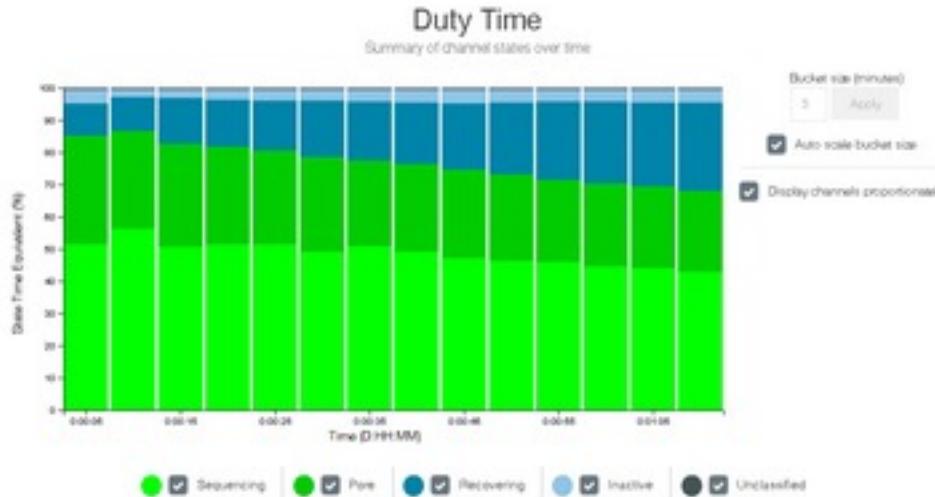


■ cDNA: d'après notre expérience

**20 à 40 fmol** sur du MinION- 4 à 10 M read

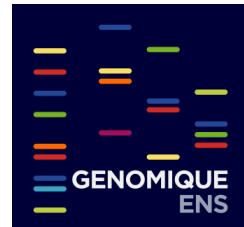
220 fmol sur du Promethion-150 M read- A tester avec moins

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

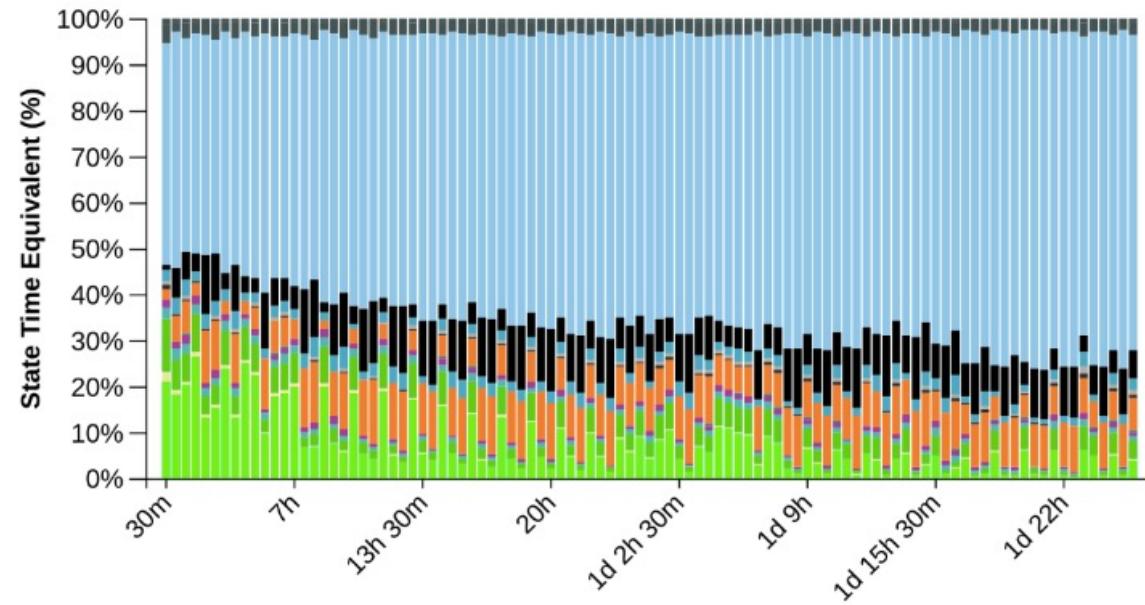


**Large proportion of recovering pores.**  
Contaminants or too much librairie

# Troubleshooting



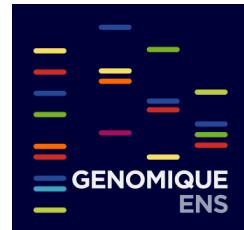
Duty time Categorised



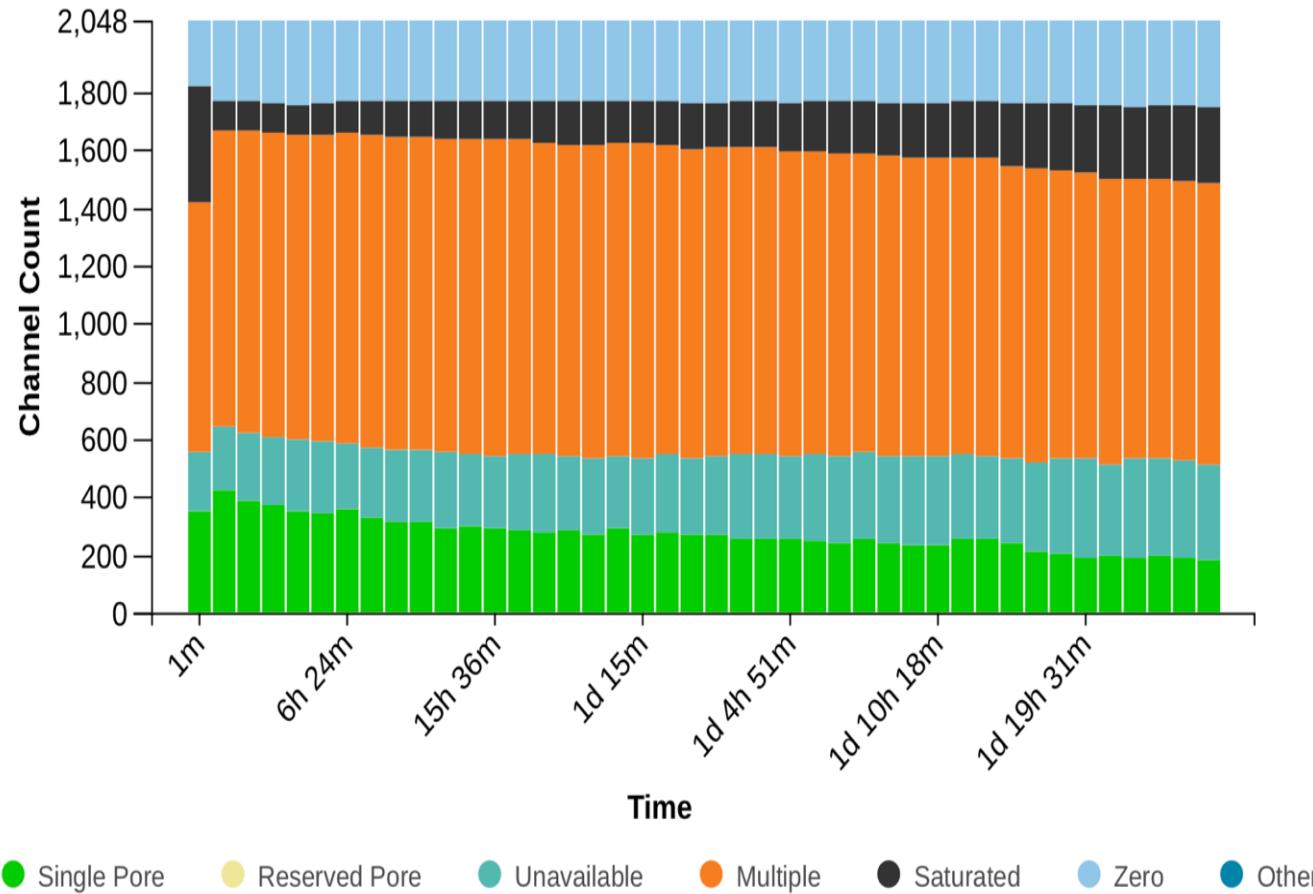
Time



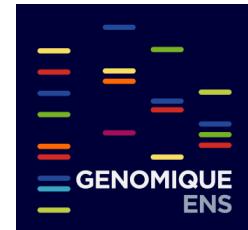
# Troubleshooting



## Mux Scan Categorised

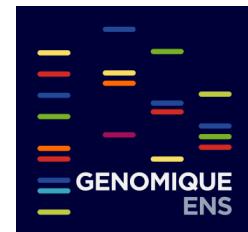


# Troubleshooting P2 solo v22.07.7



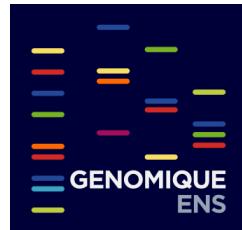
Using Linux I tried to roll back to v22.07.5 a no pores were available for the flowcells used with the patch. What did 22.07.7 do to these flowcells?

# Troubleshooting

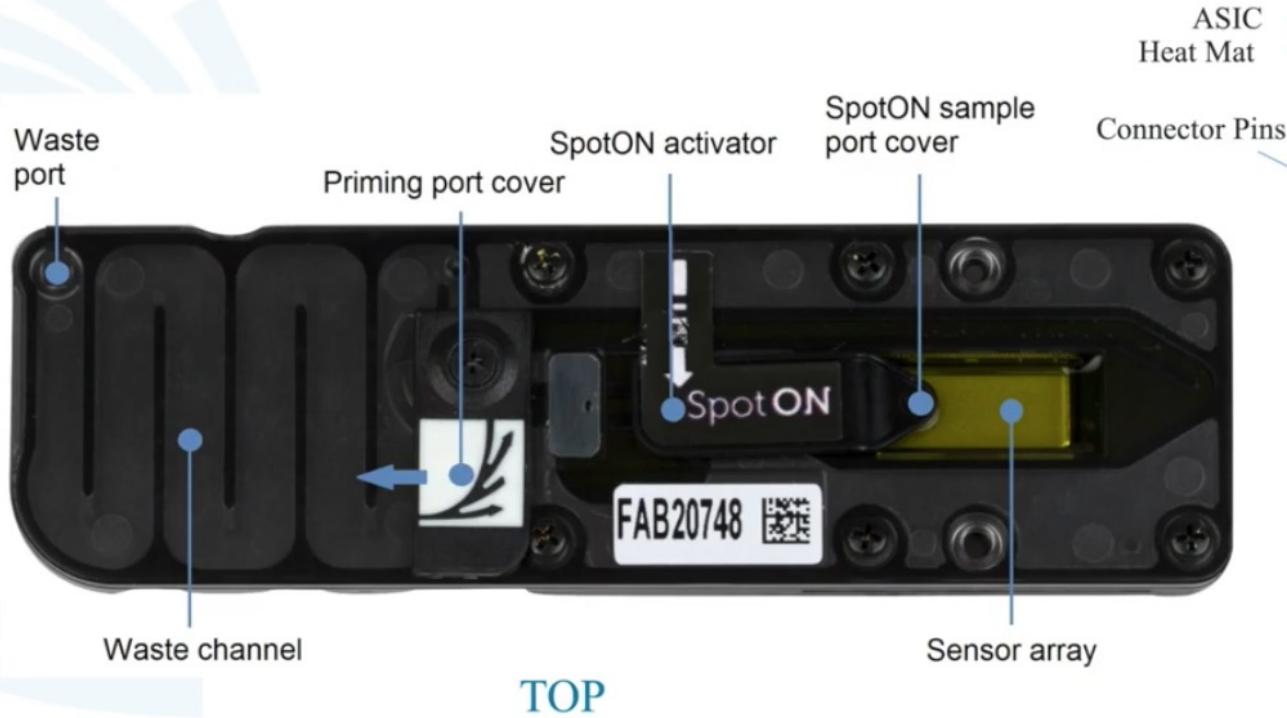


[https://community.nanoporetech.com/protocols/experiment-companion-minknow/v/mke\\_1013\\_v1\\_revbl\\_11apr2016/troubleshooting-your-run-from-the-duty-time-plots](https://community.nanoporetech.com/protocols/experiment-companion-minknow/v/mke_1013_v1_revbl_11apr2016/troubleshooting-your-run-from-the-duty-time-plots)

# Flow Cell du MinION



## MinION FLOW CELL COMPONENTS?

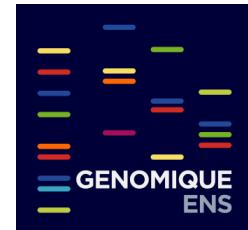


ASIC  
Heat Mat

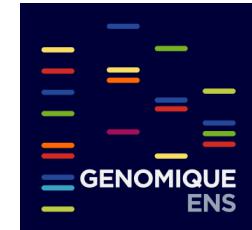
Connector Pins



# Flow Cell PromethION

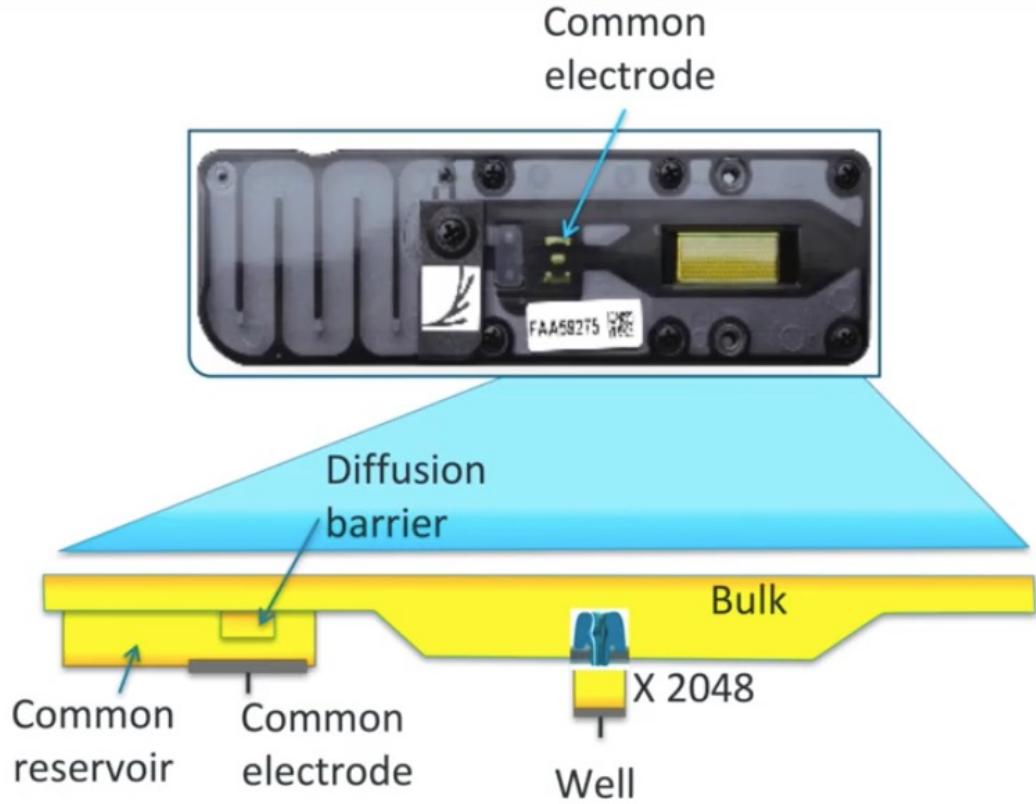


# Dépôt sur Flow Cell



## 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

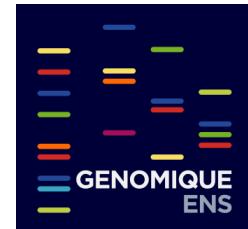


Y-adapter



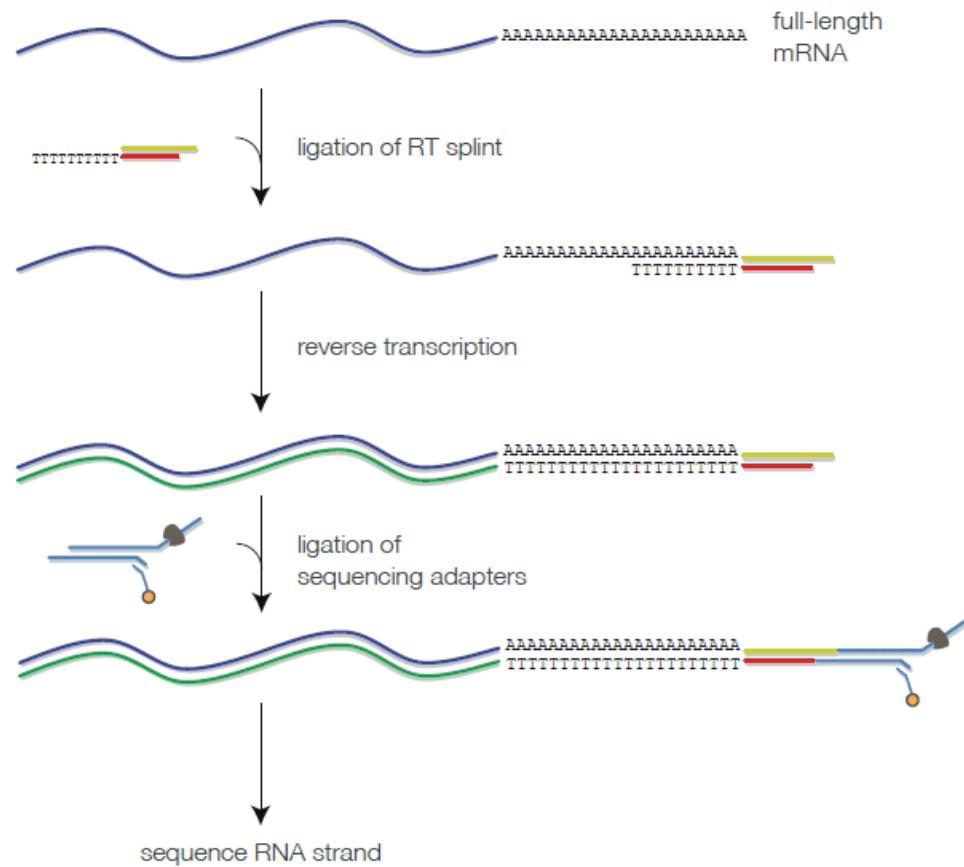
Library DNA

# Direct RNA sequencing

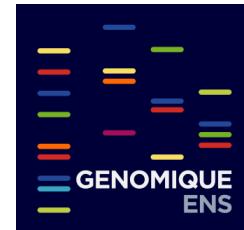


Native RNA strand is sequenced:

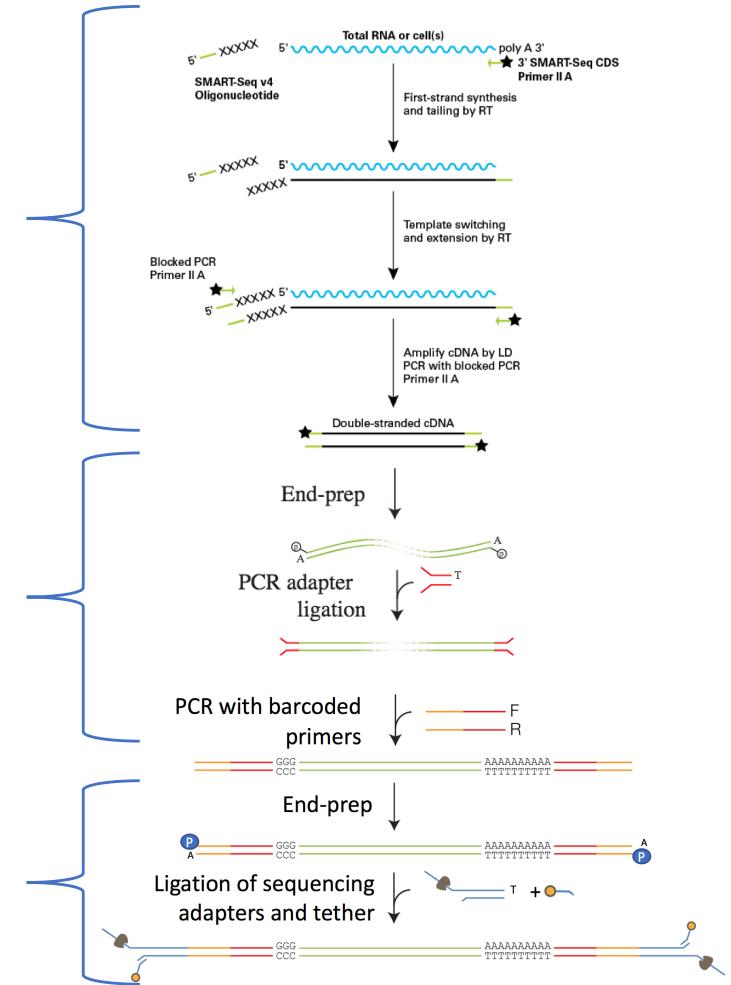
- No PCR biases;
- Modified bases detection (example methylation);
- 500 ng polyA+ RNA required.



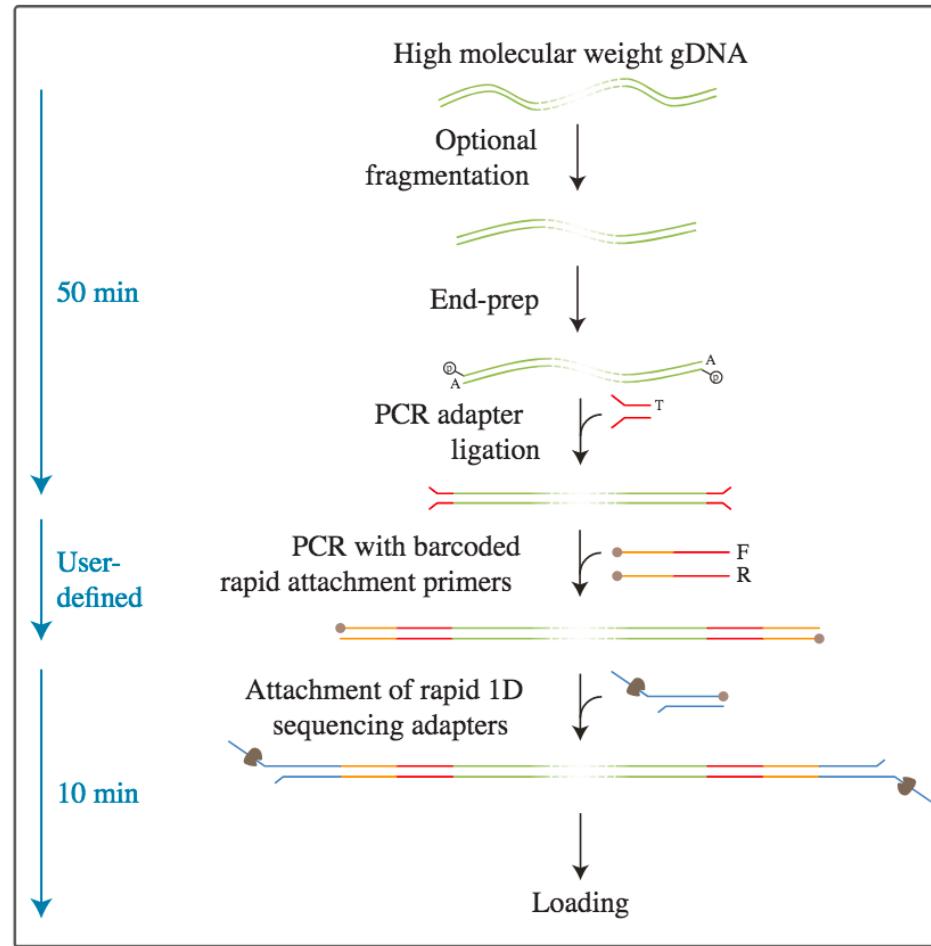
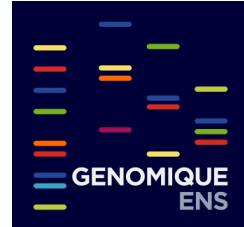
# SmartLengthMinION\_A2018



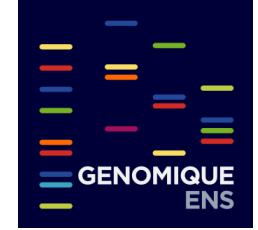
Kit	Etapes	Quantité Requise
SmartSeq v4 clontech	1er brin cDNA	10 ng d'ARN
	2 <sup>nd</sup> brin cDNA	
	PCR 9 cycles	Minimum 5 ng
PCR Barcoding kit - ONT	End prep	5 à 10 ng pour la PCR
	Ligation Barcode adapter (BCA)	
	PCR 18 cycles	2 à 3 µg post PCR
Ligation Sequencing kit 108 - ONT	Multiplexage	1 pmol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

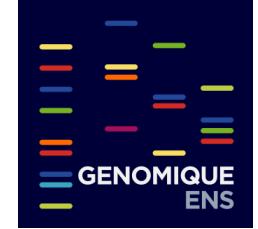


# SQK-PBK004 PCR Barcoding Kit

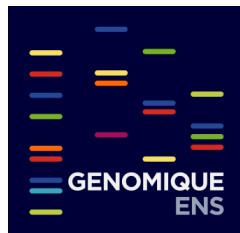








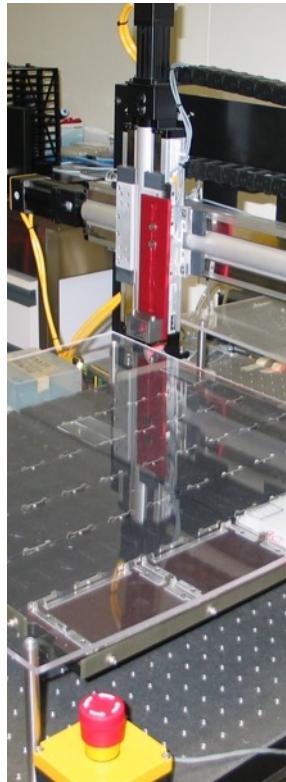
# 20 ans de génomique à l'ENS



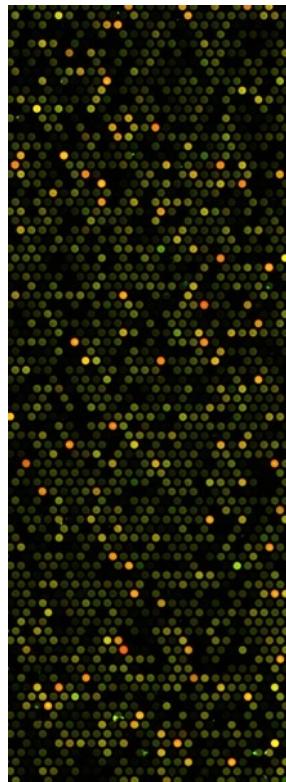
1996



1999



2005



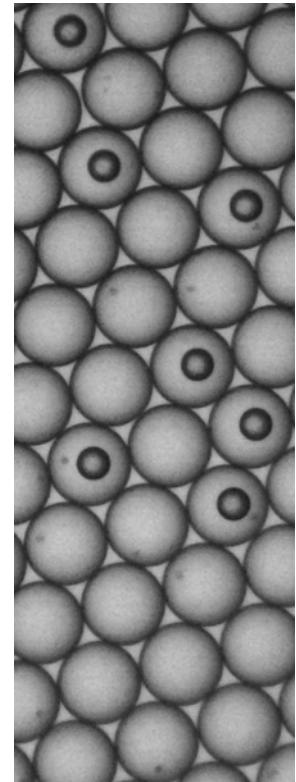
2010



2016



2017



Projet  
génome de  
la levure

Puces à  
ADN maison

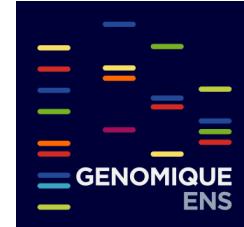
Puces  
transcriptome

Séquençage  
à haut débit

Séquençage  
nanopore

Cellules  
uniques

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



Morgane THOMAS-CHOLIER - 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%

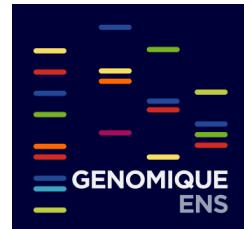


Catherine SENAMAUD-BEAUFORT  
AI Inserm 100%

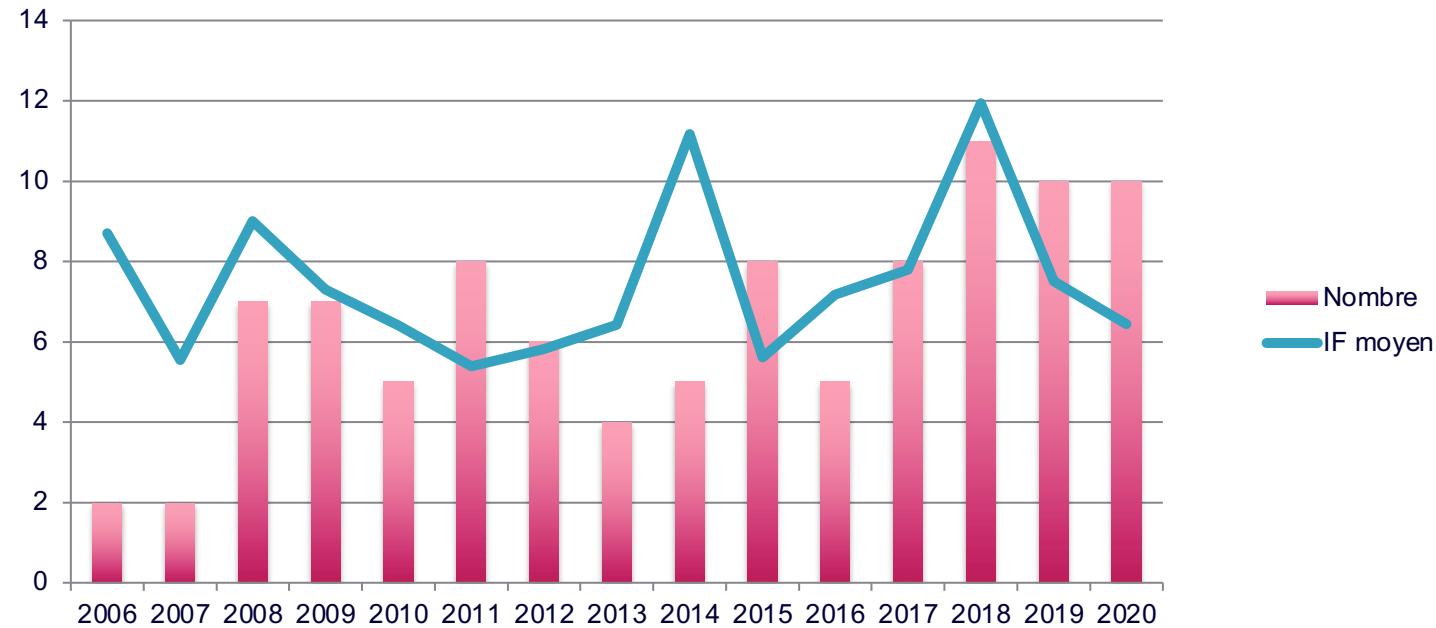


Expérimentation

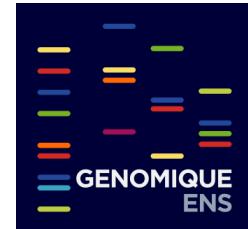
# Publications



- 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

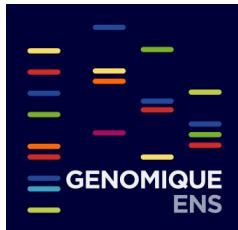


## Bioinformatique

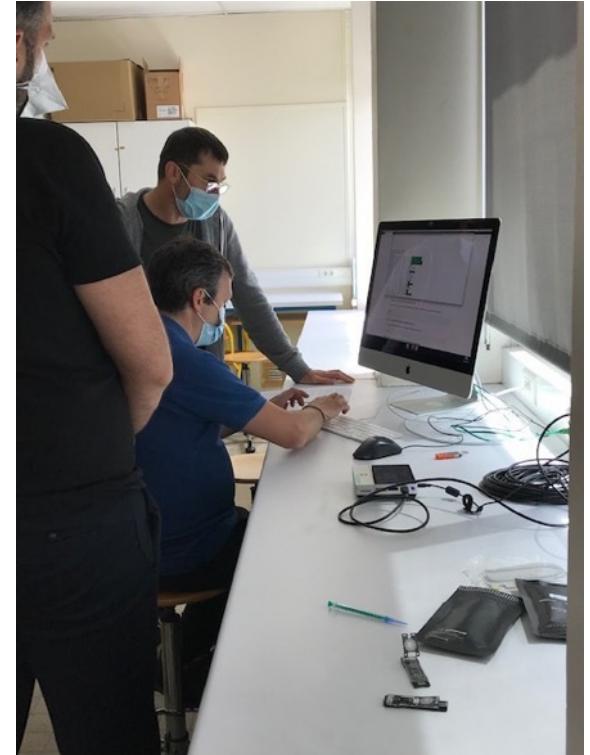
### ToulligQC 2

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

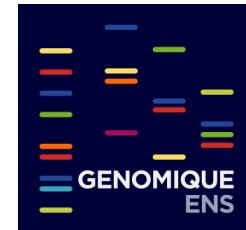
# Création d'une formation MinION



- 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

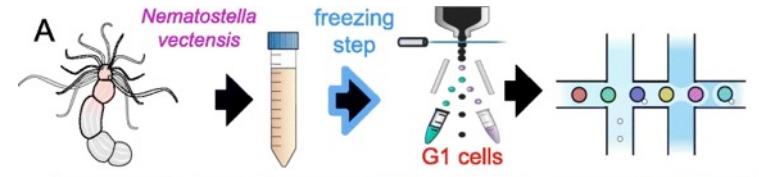
## Bioinformatique

### Réorientation des lectures Nanopores

- Intégré à notre pipeline RNA-seq Eoulsan

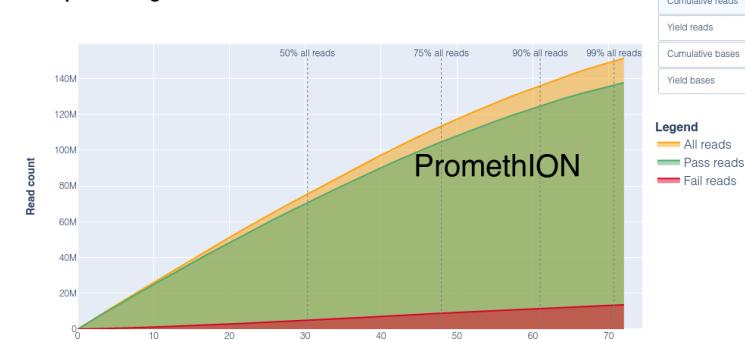
### Détection et quantification d'isoformes

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

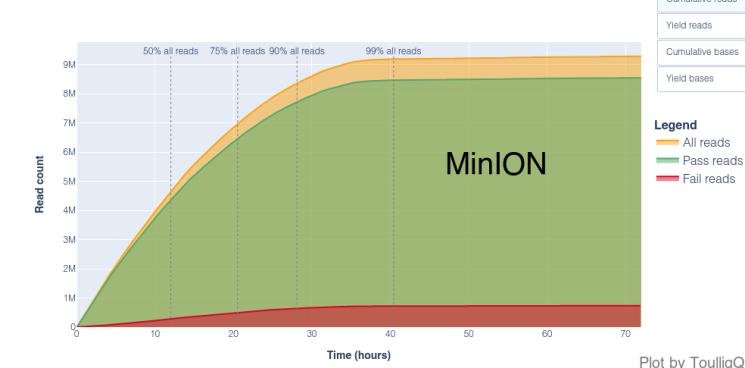


ACME dissociation + FACS + 10X Genomics

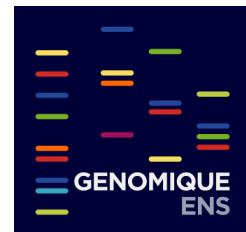
Yield plot through time ⓘ



Yield plot through time ⓘ



# Nouveaux tarifs RNA-seq Illumina



Nouveaux protocoles banques RNA-seq

+ flowcell haut débit P3 NextSeq 2000

+ QC et bioinfo incluse

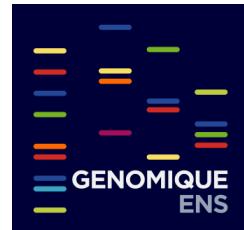
= **baisse de jusqu'à 50% du coût du RNA-seq par échantillon**

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