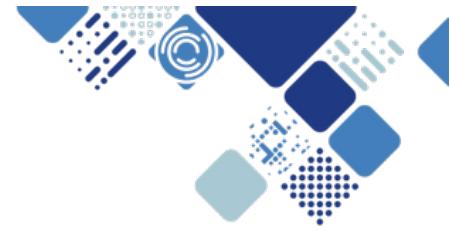


# Oxford Nanopore sequencing





# Oxford Nanopore sequencing

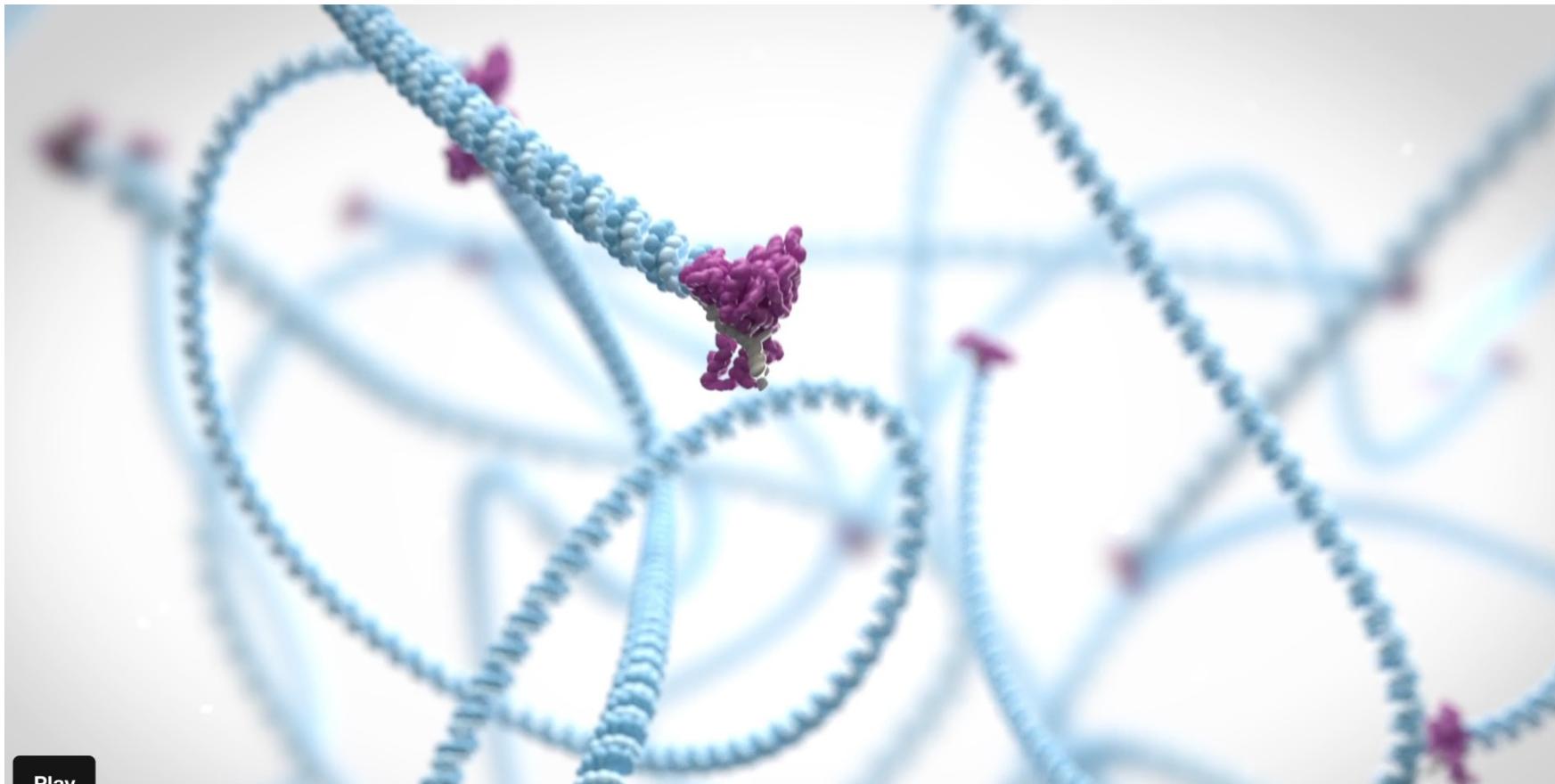
## Objective:

- To gain a greater depth of understanding of the principles of working with Long Reads and ONT sequencing
- To understand the ONT offering with regards to making an informed choice for your project

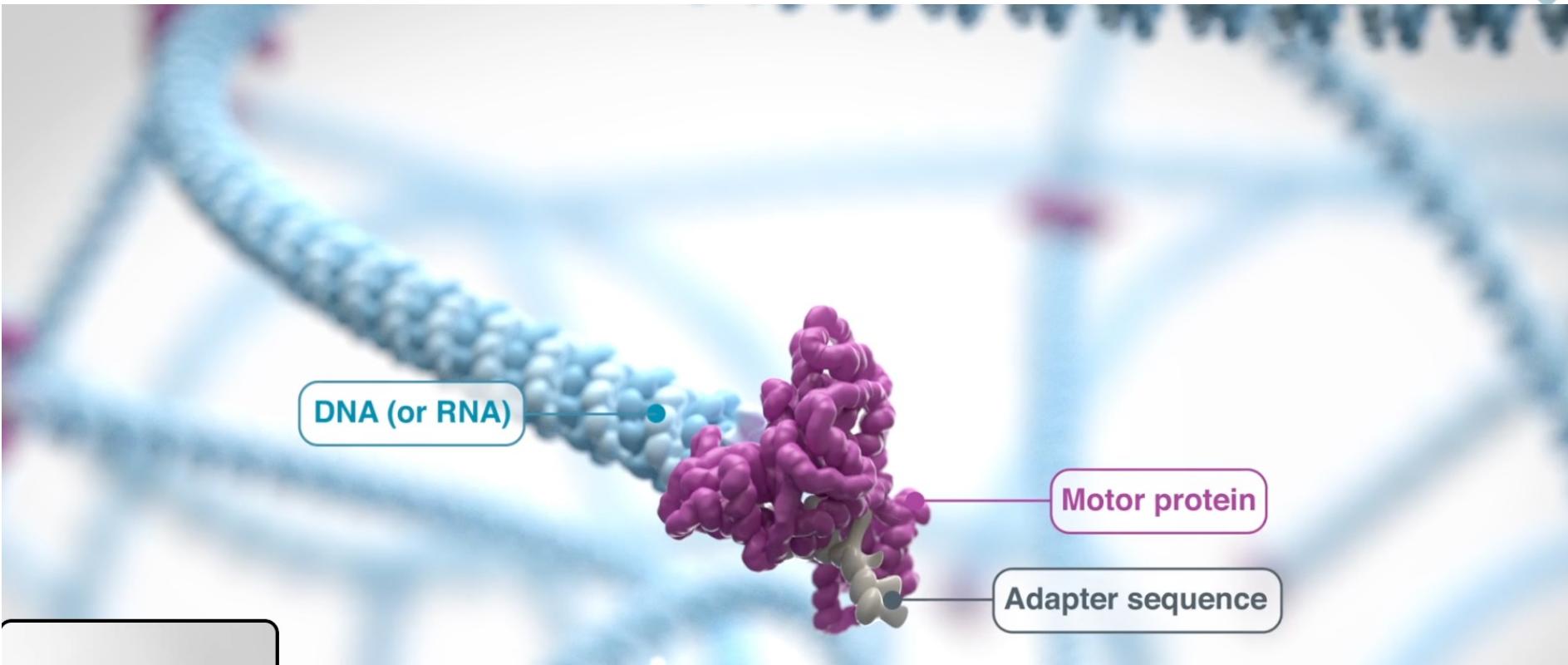


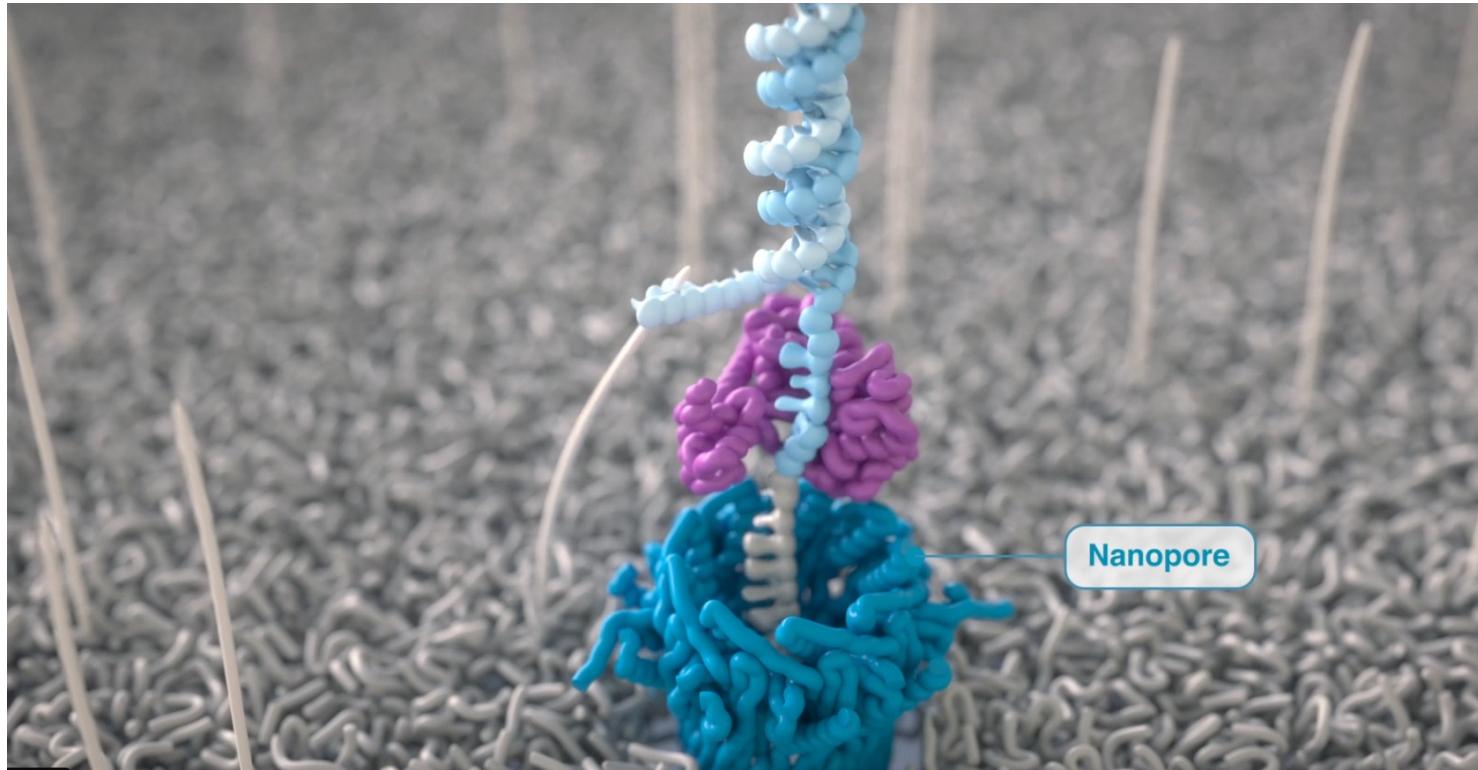


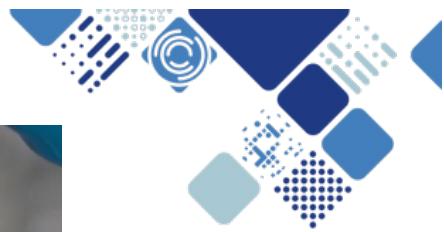
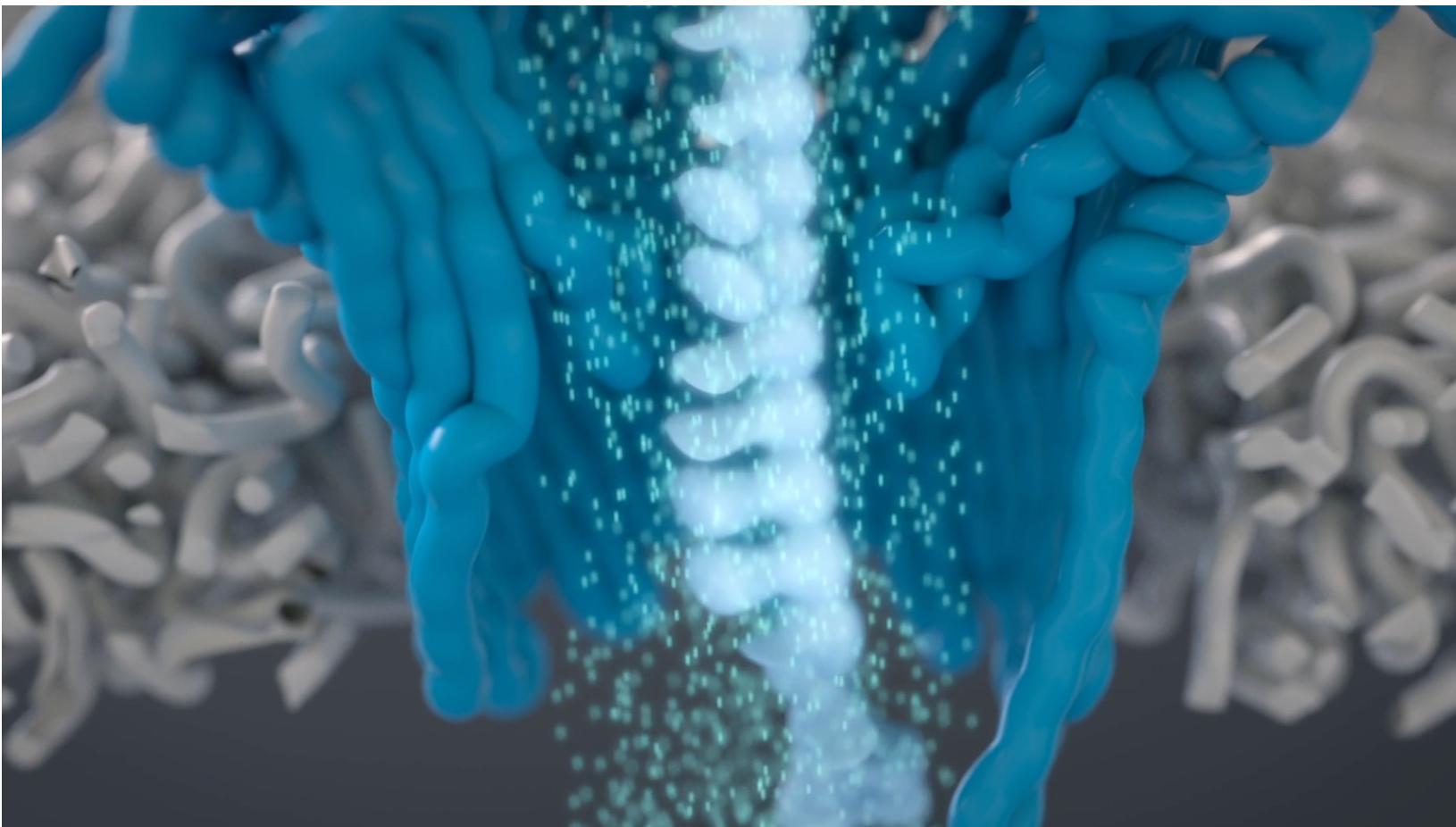
2048 membrane wells,  
each containing a nanopore

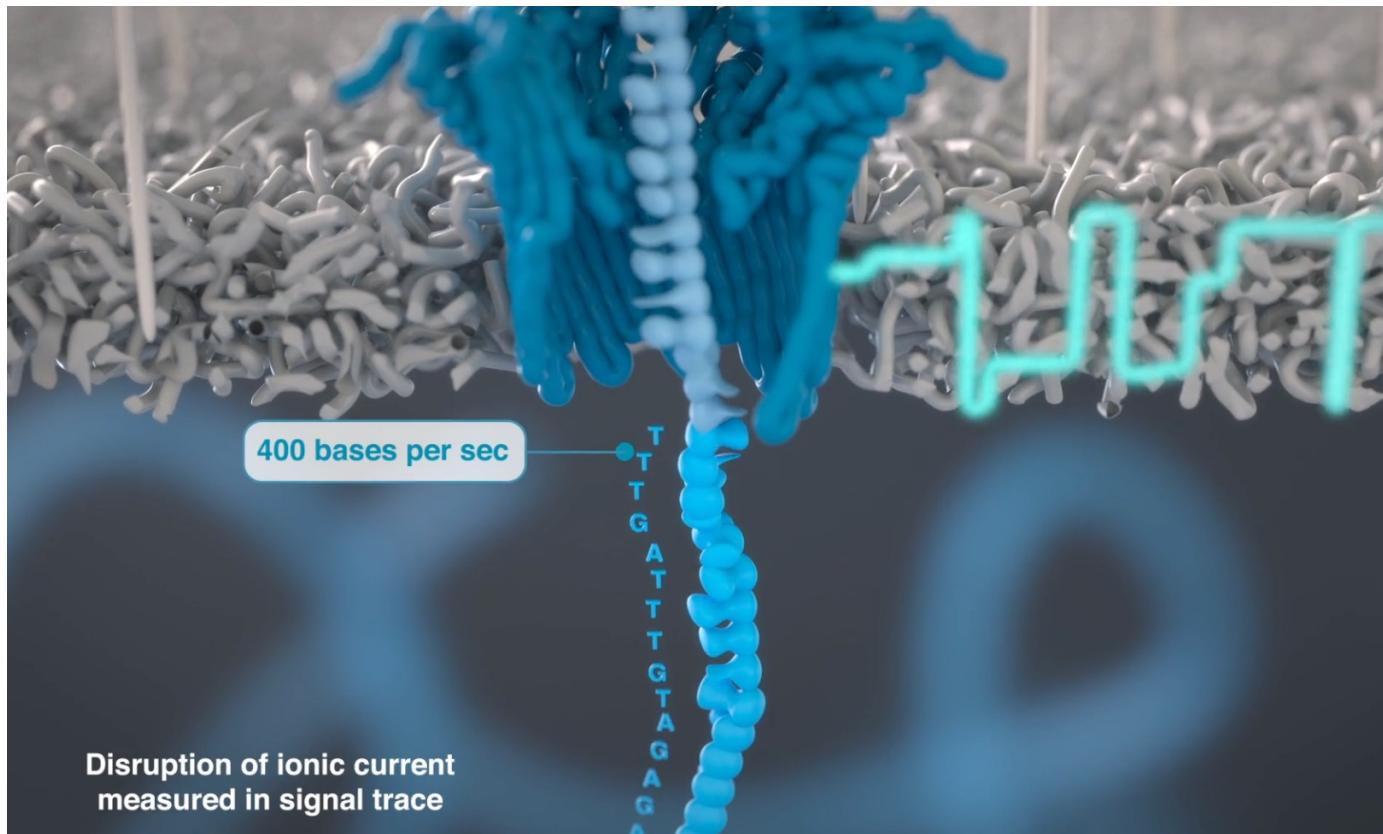


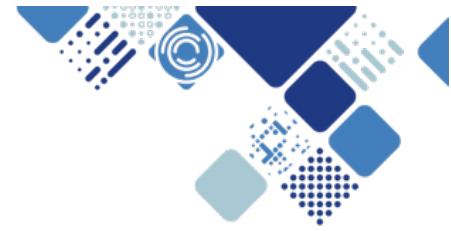
Play











# Oxford Nanopore sequencing

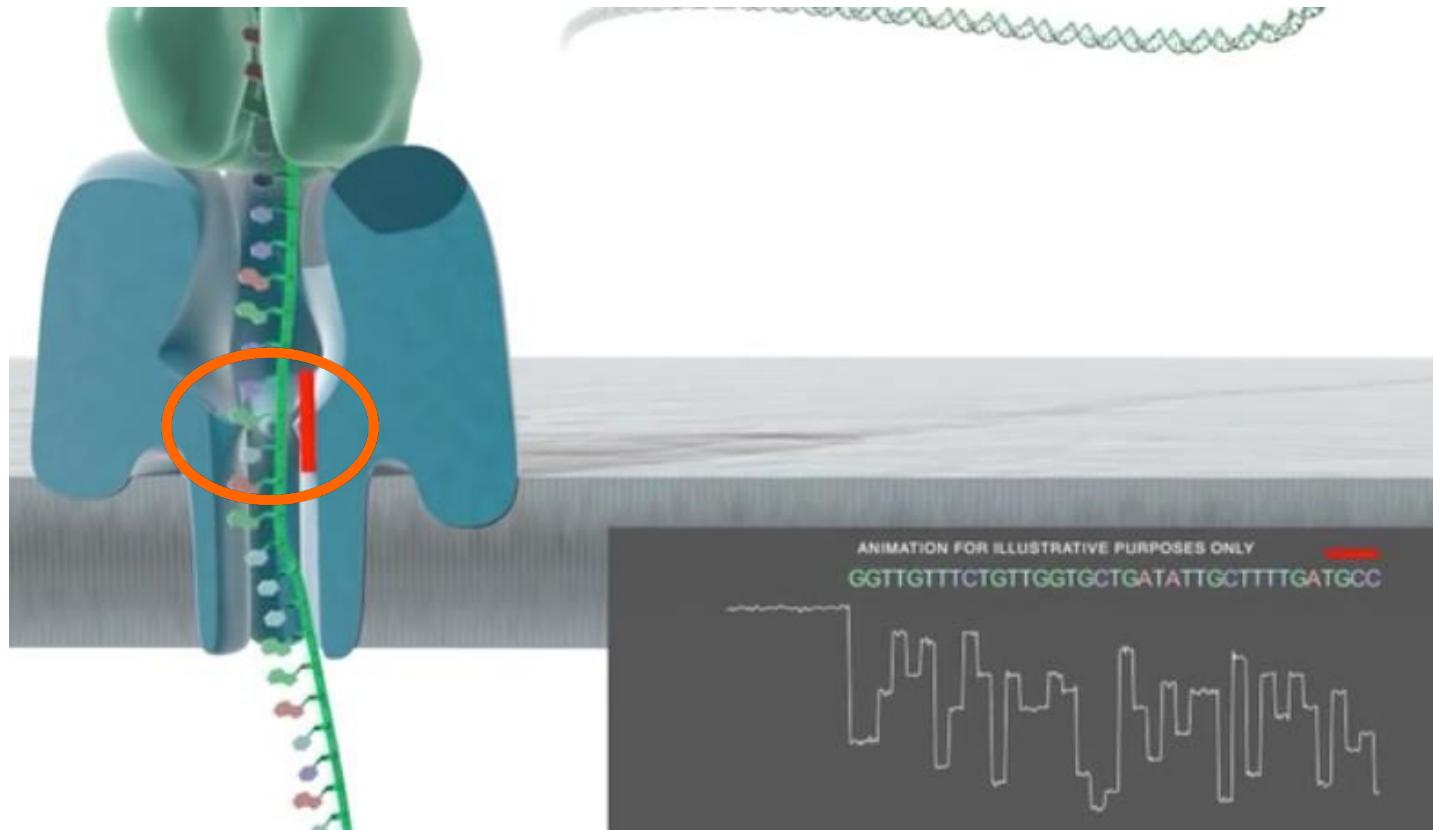


Image c/o Oxford Nanopore, <https://www.youtube.com/watch?v=3UHw22hBpAk>



## Q20+

- Combination of latest Nanopore(R10.4.1) and Library Construction kit (“Kit 14”) will give single strand accuracy of “99.3%” and duplex (where both strands of DNA are basecalled) of “~99.9%” Duplex = methylation information & high quality basecalls from single strand –no need for consensus (which can obscure rare variants)
- This is currently in early access testing and not fully commercially available Some applications, e.g. Direct RNA sequencing, may not be available as quickly as others

N.B. Illumina NovaSeqstats suggest >75% reads at Q30 at 2x250 or >90% reads at Q30 at 2x50bp

<https://emea.illumina.com/systems/sequencing-platforms/novaseq/specifications.html>

Q10 = 90% Q20 = 99% Q30 = 99.9%



# DNA Extraction Methods



Kit?

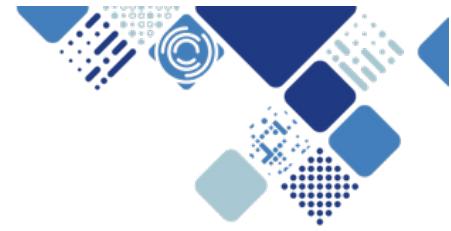
Phenol  
Chloroform?

Nucleus  
isolation?

Magnetic  
beads?

Bead  
beating?

You cannot get long reads from short fragments!



# DNA extraction methods

The four S's!

Safety

Species

Suitable (cost,equipment)

Scale up (or size)

**What is the best  
method for your  
application?**



# Assessing your DNA fragments

Purity



Nanodrop

Length



Tapestation or FemtoPULSE  
or gel

Amount



Qubit



# Library construction

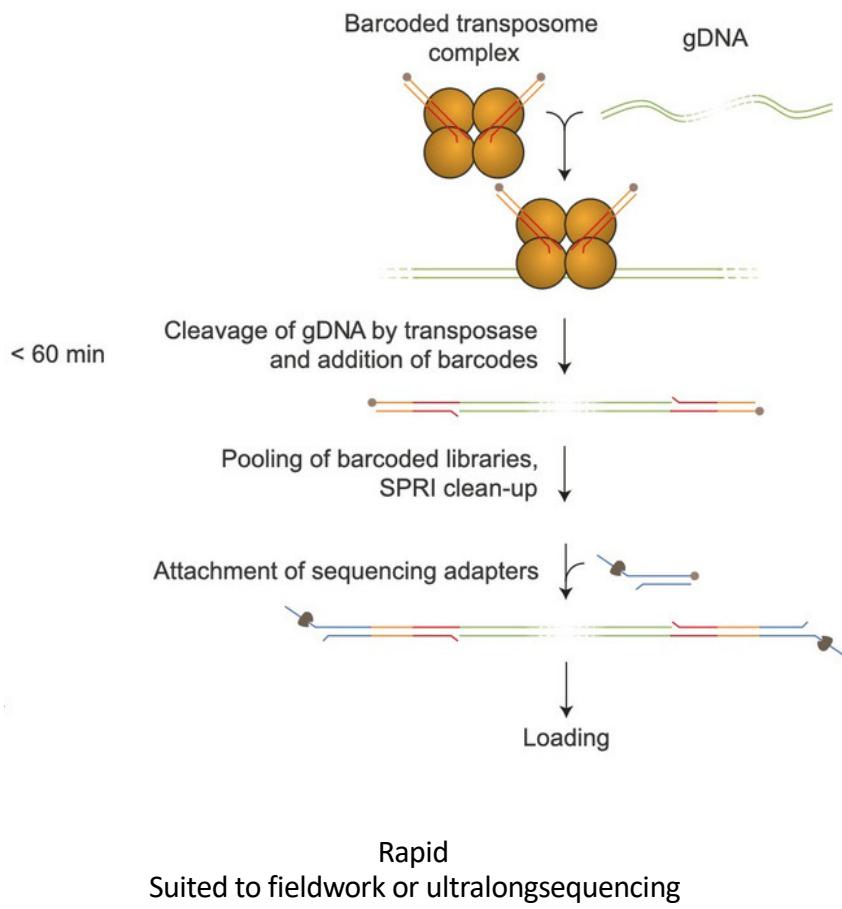
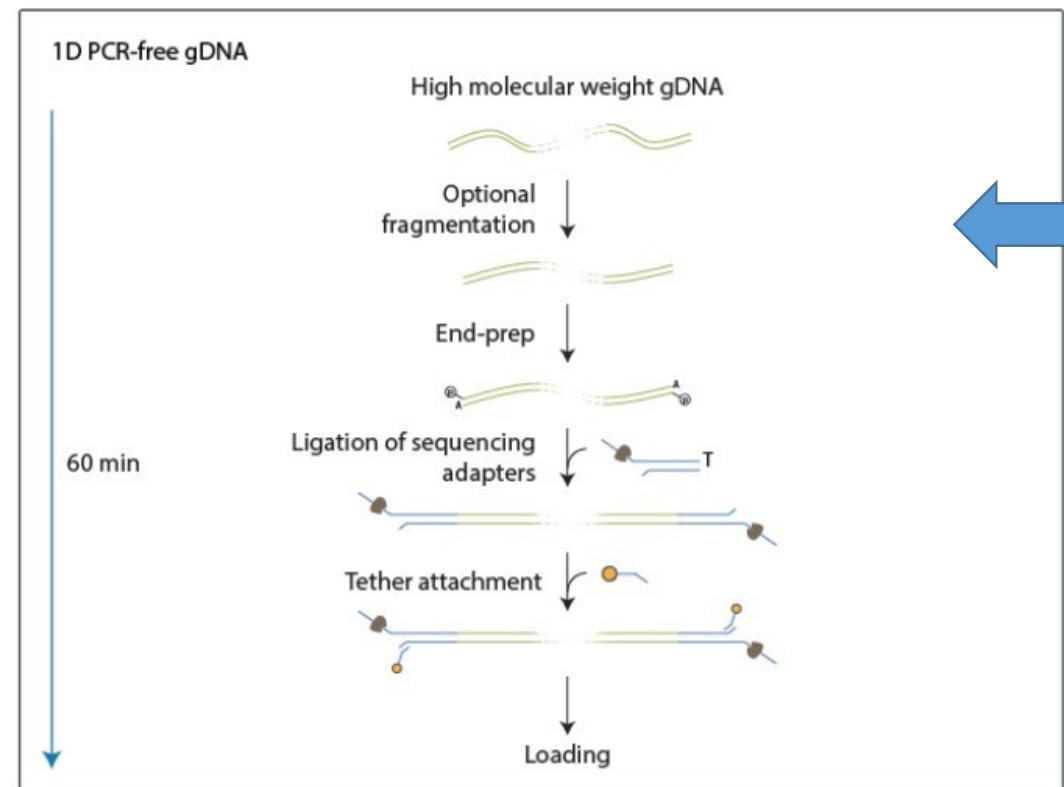
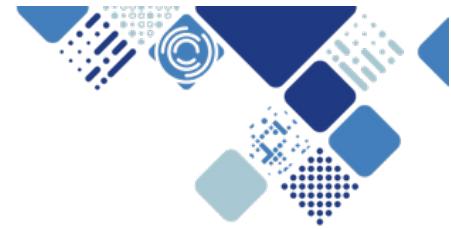


Image c/o Oxford Nanopore





## Optional Fragmentation

Not recommended for some applications, e.g. short fragments, PCR products

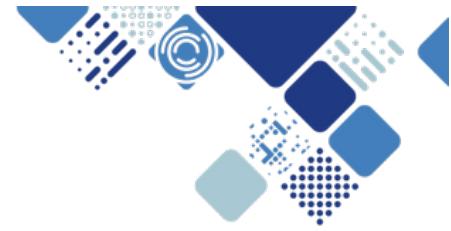
Can be useful when some very long fragments are present but high throughput is priority

Options include G-TUBE (historically recommended by ONT), needle shearing, Megaruptor



# Ligation vs Rapid

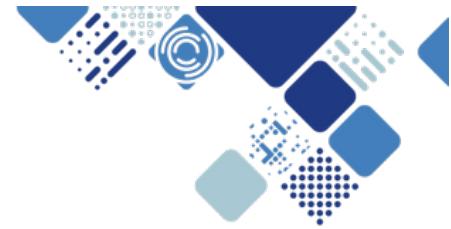
	Ligation	Rapid
Yield Time	Green	Red
Cost Data	Red	Green
quality	Green	Green
Robustness	Green	Red
	White	White



# Multiplexing

Make best use of a flow cell capacity by combining >1 sample together Each sample is “labelled” with a known DNA sequence Oxford Nanopore barcodes are longer than Illumina, at 24 bases This allows confidence despite the higher per-base error rate, and makes use of Oxford Nanopore’s long reads

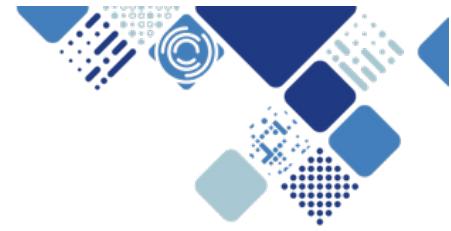
Component	Sequence
NB01	AAGAAAGTTGTCGGTGTCTTG
NB02	TCGATTCCGTTGTAGTCGTCTG
NB03	GAGTCTTGTGTCCCAGTTACCA
NB04	TTCGGATTCTATCGTGTTCCTA
NB05	CTTGTCCAGGGTTGTGTAACCTT
NB06	TTCTCGCAAAGGCAGAAAGTAGTC
NB07	GTGTTACCGTGGGAATGAATCCTT
NB08	TTCAGGGAACAAACCAAGTTACGT
NB09	AACTAGGCACAGCGAGTCTGGTT
NB10	AAGCGTTGAAACCTTGTCCCTCTC
NB11	GTTCATCTATCGGAGGGAAATGGA
NB12	CAGGTAGAAAGAACAGAATCGGA



# Basecalling

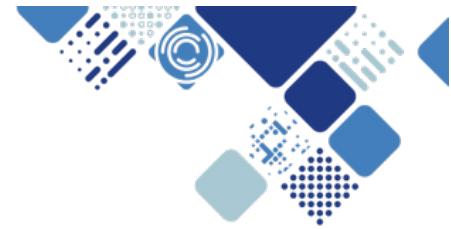
Current or recently announced basecallers now allow:

- Real time methylation information
- Demultiplexing of samples
- Accurate sequencing of short fragments (>20bp)



# Oxford Nanopore Platforms and Products





# Oxford Nanopore sequencing



MinION Up  
to 50Gb



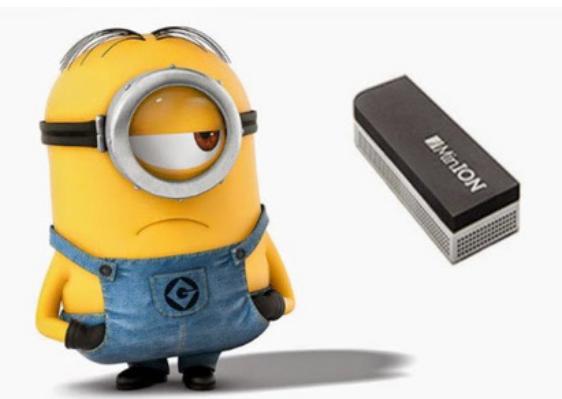
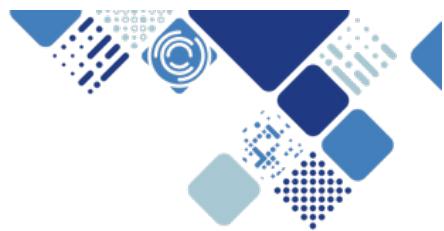
GridION Up  
to 250Gb



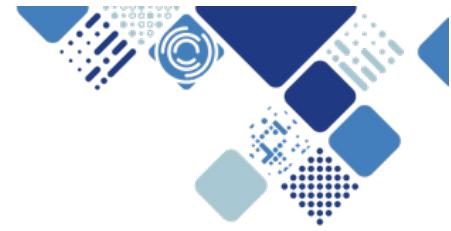
PromethION Up to  
220Gb per flow cell  
10.5Tb per instrument

Image c/o Oxford Nanopore





Images center and right c/o Oxford Nanopore



## Why is it so small?

Other machines need to have cameras and lasers to take pictures of the DNA

They need to have moving parts to add chemicals to synthesise the DNA

The MinION measures electrical signals from DNA that is already present

# MinION

Don't forget cost  
of computer!



## MinION Mk1D US\$4,950.00 Pack

[Configure package >](#)

1x MinION Mk1D Sequencing Device  
MIN-101D

1x Control Expansion Kit  
EXP-CTL001

1x Flow Cell Wash Kit  
EXP-WSH004

1x MinION Mk1D Standard Support  
STANDARD12M-MK1D

5x Flow Cell (R10.4.1)  
FLO-MIN114

- or -

5x Flow Cell (RNA)  
FLO-MIN004RA

1x Sequencing kits

[Configure package >](#)

## MinION Mk1D US\$2,999.00

[Configure package >](#)

1x MinION Mk1D Sequencing Device  
MIN-101D

1x MinION Mk1D Standard Support  
STANDARD12M-MK1D



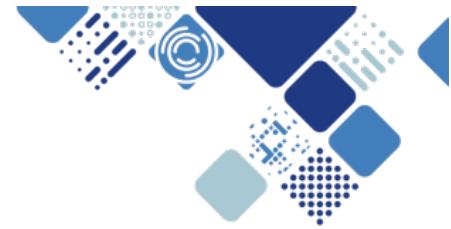
Prices c/o Oxford Nanoporestore, taken 27/03/2025



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

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





**Chemistry type:**

R10.4.1



**Pack size:**

Select ...



1 Flow cell **US\$800.00**  
US\$800.00 each

12 Flow cells **US\$8,100.00**  
US\$675.00 each

24 Flow cells **US\$14,400.00**  
US\$600.00 each



# GridION



Image c/o Oxford Nanopore

The screenshot shows the GridIONx software interface. At the top, there is a navigation bar with links for 'HOME', 'DOCUMENTATION', 'New Experiment', 'Running Experiments', 'Live Plots', 'Messages', and 'Flow Cells'. Below the navigation bar, the main area is titled 'NEW EXPERIMENT'. It features five icons representing different flow cell configurations. Below these icons is a dropdown menu labeled 'Pick a script...'. Further down is a text input field labeled 'Sample ID'. At the bottom right of the main area is a blue button labeled 'Start Experiment'.



# GridION



Image c/o Oxford Nanopore

GridION

US\$58,800.00

Configure package >

1x GridION Sequencing Device Mk1  
GRD-MK1

1x Assurance - GridION  
ASSURANCE

1x GridION Standard Support  
STANDARD12M-G

- or -

1x GridION Standard Support (2 year)  
STANDARD24M-G

- or -

1x GridION Standard Support (3 year)  
STANDARD36M-G

Configure package >



# PromethION



Image c/o Oxford Nanopore

48 flow cells

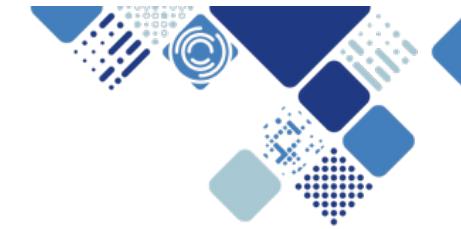
Each flowcell  
originally “50Gb”  
now maximum is  
290Gb

Up to 14Tb of  
sequence

Rapidly evolving



# PromethION



PromethION US\$431,500.00

24

[Configure package >](#)

1x PromethION 24 Sequencing Unit  
PRO-SEQ024

1x PromethION Data Acquisition Unit  
PRO-PRCA100

1x PromethION Advanced Training  
SUPP008

1x P24 Standard Support  
STANDARD12M-P24A

- or -

1x P24 Standard Support (2 year)  
STANDARD24M-P24A

- or -

1x P24 Standard Support (3 year)  
STANDARD36M-P24A

[Configure package >](#)



Image c/o Oxford Nanopore





## P2

A “mini”  
PromethION

Also as “solo” model –  
plugs into GridION or  
computer



Image c/o Oxford Nanopore



**PromethION 2** US\$27,955.00  
**Solo Pack**

[Configure package >](#)

1x PromethION 2 Sequencing Unit Solo  
PRO-SEQ002

1x Control Expansion Kit  
EXP-CTL001

2x Flow Cell Wash Kit  
EXP-WSH004

4x PromethION Flow Cell Packs (R10.4.1)  
FLO-PRO114M

- or -

4x PromethION Flow Cell Packs (RNA)  
FLO-PRO004RA

1x P2 Solo Standard Support  
STANDARD12M-P2S

- or -

1x P2 Solo Standard Support (2 year)  
STANDARD24M-P2S

- or -

1x P2 Solo Standard Support (3 year)  
STANDARD36M-P2S

3x Sequencing kits

**PromethION 2** US\$89,000.00  
**Integrated**

[Configure package >](#)

1x PromethION 2 Integrated Sequencing Unit  
PRO-INT002

1x Assurance - P2I  
ASSURANCE

1x P2i Standard Support  
STANDARD12M-P2I

- or -

1x P2i Standard Support (2 year)  
STANDARD24M-P2I

- or -

1x P2i Standard Support (3 year)  
STANDARD36M-P2I

[Configure package >](#)

# Flongle

## < Flongle Advanced Pack

FLGMiniSP



The Flongle Advanced Pack enables users to get started with Flongle. This pack includes a Flongle adapter and Flongle Flow Cells (R10.4.1).

US\$4,815.00

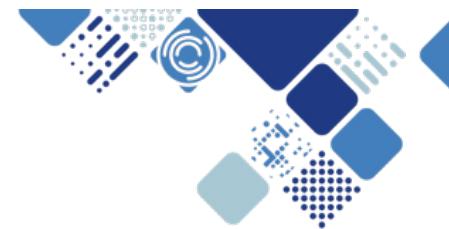
Flongle Advanced Pack

- 1 +

Add to basket

2 Early Access

48 Flongle Flow cells



## < Flongle Starter pack

FLGIntSP



The Flongle Starter Pack enables users to get started with Flongle. This pack includes a Flongle adapter and Flongle Flow Cells (R10.4.1).

US\$1,995.00

Flongle Starter pack

- 1 +

Add to basket

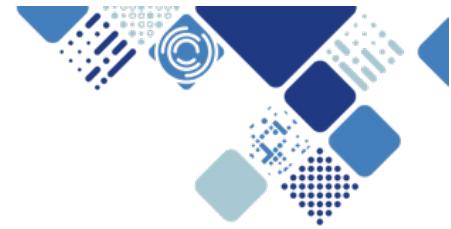
2 Early Access

Adapts MinION to take  
cheap, low throughput flow  
cells

Closed store BUT still  
available

Image c/o Oxford Nanopore





# Q Line

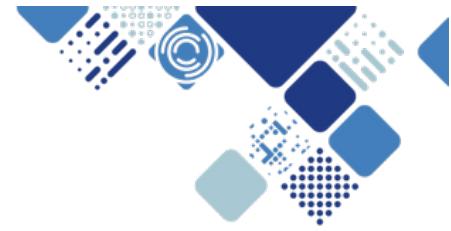
ISO 9001:2015 certified manufacturing process

12 months guaranteed supported software & consumables

Clearly mapped out upgrades

Same price as standard devices & consumables

Allows in house validation of assays for long term use



# Where has Oxford Nanopore been used?





*MUSE/Science Museum of Trento*

The MinION device can sequence small genomes, such as those of bacteria and viruses, displaying the results as they are generated.

<http://www.nature.com/news/pint-sized-dna-sequencer-impresses-first-users-1.17483>







Image c/o NASA, USA



Instead of shipping samples to fully-equipped laboratories for analysis, the MinION device can send sample data via a USB. This information can then be sequenced and analysed within 24 hours, instead of the weeks it usually takes. Photograph: Tommy Trenchard/EMLabs



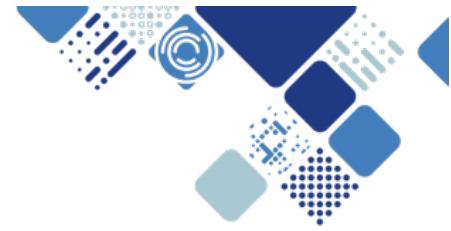
Image from The Guardian Online

<https://www.theguardian.com/science/2016/feb/03/from-eбола-to-zika-telephone-lab-gives-real-time-dna-data-on-outbreaks>



# SARS-CoV-2



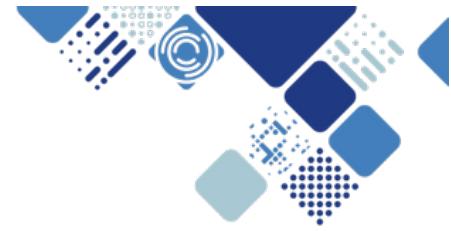


# Concluding thoughts



# Oxford Nanopore sequencing

Advantages	Disadvantages
Portable	Can require highly pure DNA
Rapid	Can require large amounts of input DNA
Low start up costs	Less supported analysis
Native DNA sequencing	
Long read	
Multiplexing options	
Fleet of sequencers	



## Floatation

Oxford Nanopore Technologies floated on the London Stock Exchange in 2021



**London**  
Stock Exchange

Currently valued at ~£979 million (\$1.26 billion –for comparison Illumina ~\$12.96 billion) down from about £2.2 billion (Illumina down from \$31 billion)

What does this mean for your science?

- perhaps greater visibility of how the company is performing
- otherwise, not much



## Additional technologies



Base4



[press.office@sanger.ac.uk](mailto:press.office@sanger.ac.uk)



- All scripts/software = open source
- Slides & links available on your course webpage
- Questions?



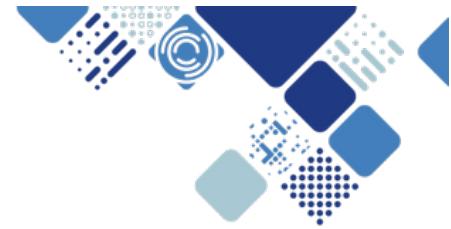
[kj6@sanger.ac.uk](mailto:kj6@sanger.ac.uk)



@kim\_judge\_



# Analysis session



# Objective

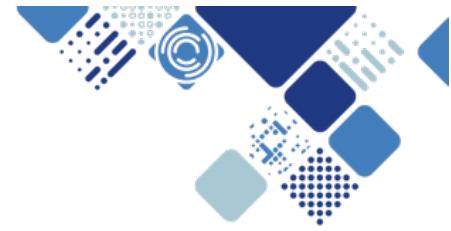
Check our data

Assemble bacterial data generated using the latest Oxford Nanopore Chemistry (as used this morning)

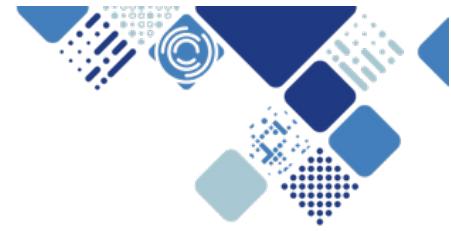
Check completeness of assembly

Investigate assembling other samples

Each genome can have many assemblies!



# Why?



**Let's find out  
reads and check  
them out**

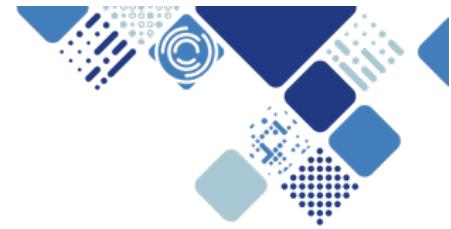
head  
tail  
ls -l

assembly-stats





# Depth of Coverage and Theoretical Depth of Coverage



# 1. Install minimap2 and miniasm

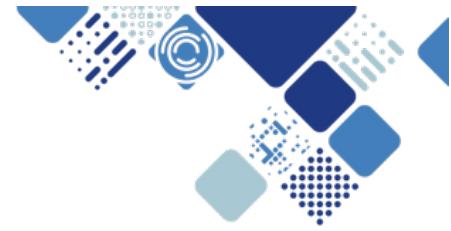
Search for the website

<https://github.com/lh3/miniasm>

Copy the commands into your terminal

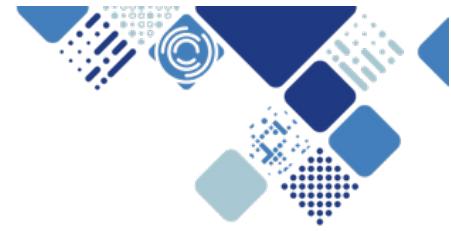
```
git clone https://github.com/lh3/minimap2 && (cd minimap2 && make)
```

```
git clone https://github.com/lh3/miniasm && (cd miniasm && make)
```



## 2. Run the overlap

```
minimap2/minimap2 -x ava-ont </path/to/sample1.fastq> </path/to/sample1.fastq> > <sample1.paf>
```



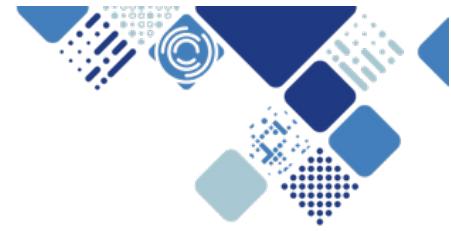
### 3. Run the assembly

```
miniasm/miniasm -f </path/to/sample1.fastq> <sample1.paf> > <sample1.gfa>
```

Minimap2 & miniasm will work with compressed (zipped) data files, so you can try running:

```
minimap2/minimap2 -x ava-ont </path/to/reads.fq> </path/to/reads.fq> | gzip -1 > <reads.paf.gz>
```

```
miniasm/miniasm -f <reads.fq> <reads.paf.gz> > <reads.gfa>
```



## 4. Assess the assembly

**Process the assembly to get fasta format**

```
awk '$1=="S" {print ">"$2"\n"$3}' sample1.gfa > sample1.fa
```

**Then Try:**

head  
wc-l

tail  
ls -l

assembly-stats



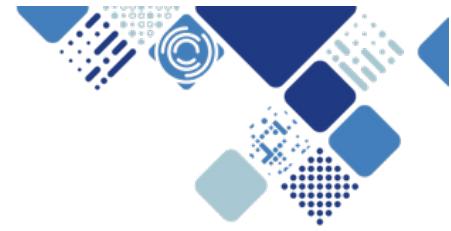
# Overview

```
minimap2/minimap2 -x ava-ont </path/to/sample1.fastq> </path/to/sample1.fastq> > <sample1.paf>
```

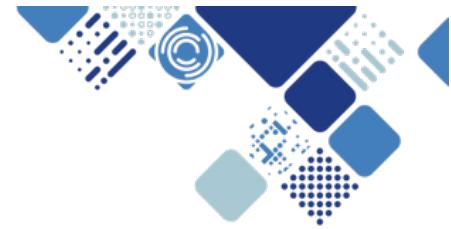
```
miniasm/miniasm-f </path/to/sample1.fastq> <sample1.paf> > <sample1.gfa>
```

```
awk '$1=="S" {print ">"$2"\n"$3}' <sample1.gfa> > <sample1.fa>
```

```
assembly-stats <sample1.fa>
```



## 5. Try on other samples



You could consider submitting part of your finished assembly to BLAST

Google NCBI BLAST

Click Nucleotide BLAST

Paste in the box

National Library of Medicine  
National Center for Biotechnology Information

Log in

BLAST® » blastn suite

Home Recent Results Saved Strategies Help

blast blastp blastx tblastn tblastx

Standard Nucleotide BLAST

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#) [Query subrange](#)

From  To

Or, upload file [Choose file](#) No file chosen [?](#)

Job Title   
Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Choose Search Set

Database  Standard databases (nr etc.)  rRNA/ITS databases  Genomic + transcript databases  Betacoronavirus  Experimental databases

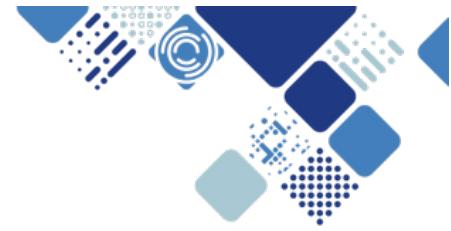
Core nucleotide database (core\_nt) [?](#)

Organism Optional Enter organism name or id—completions will be suggested   exclude [Add organism](#)  
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude Optional  Models (XM/XP)  Uncultured/environmental sample sequences

Limit to Optional  Sequences from type material

Entrez Query Optional  [YouTube](#) Create custom database  
Enter an Entrez query to limit search [?](#)



# Round up and final questions