

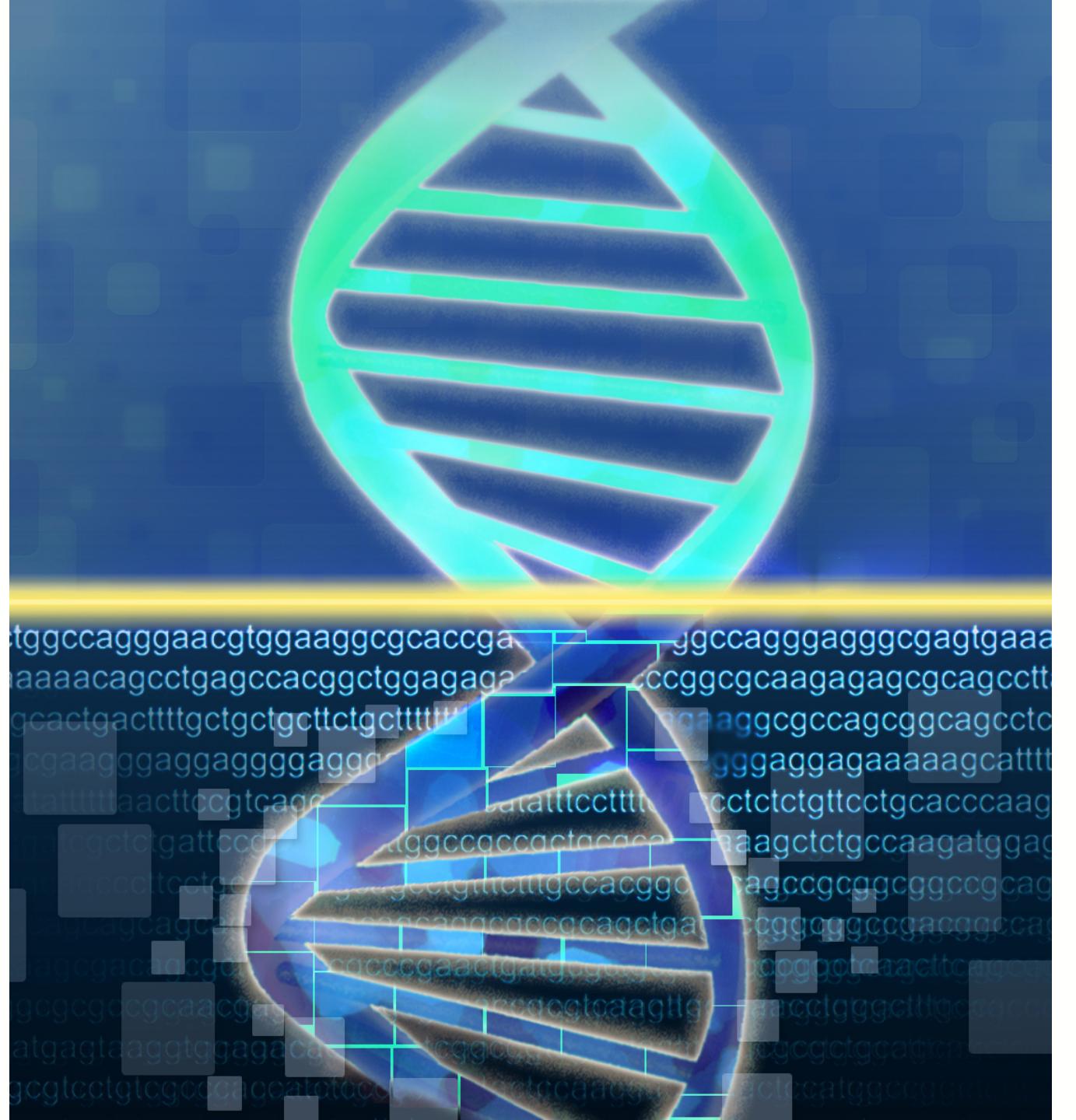


Introduction to Nanopore Sequencing

Kirstyn Brunker

RAGE workshop, NCDC, Abuja

12-16^h Feb 2024



Next generation sequencing



- Massively parallel sequencing technologies
- Simultaneously sequence millions of DNA fragments

Nanopore sequencing

A unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments



Disruptive technology

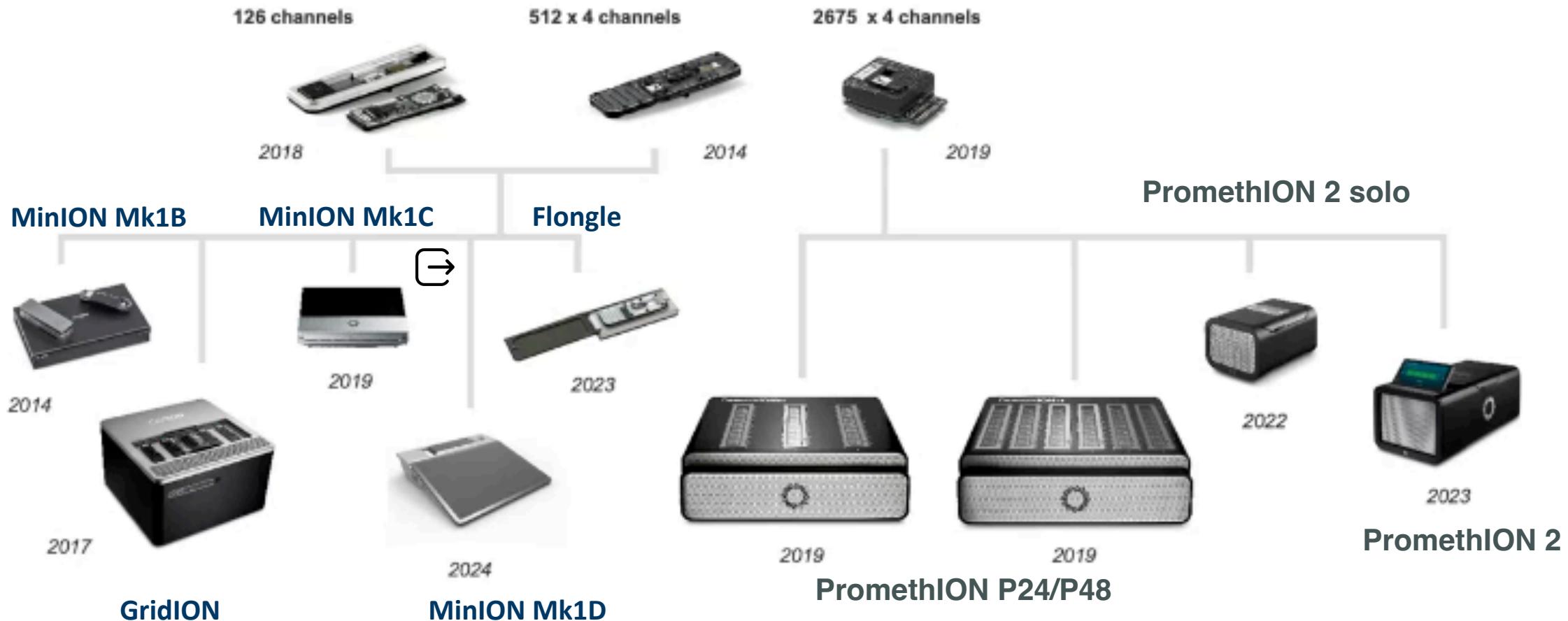
Enabling the analysis of **anything**,
by **anyone**, **anywhere**



Nanopore Platform

Product family

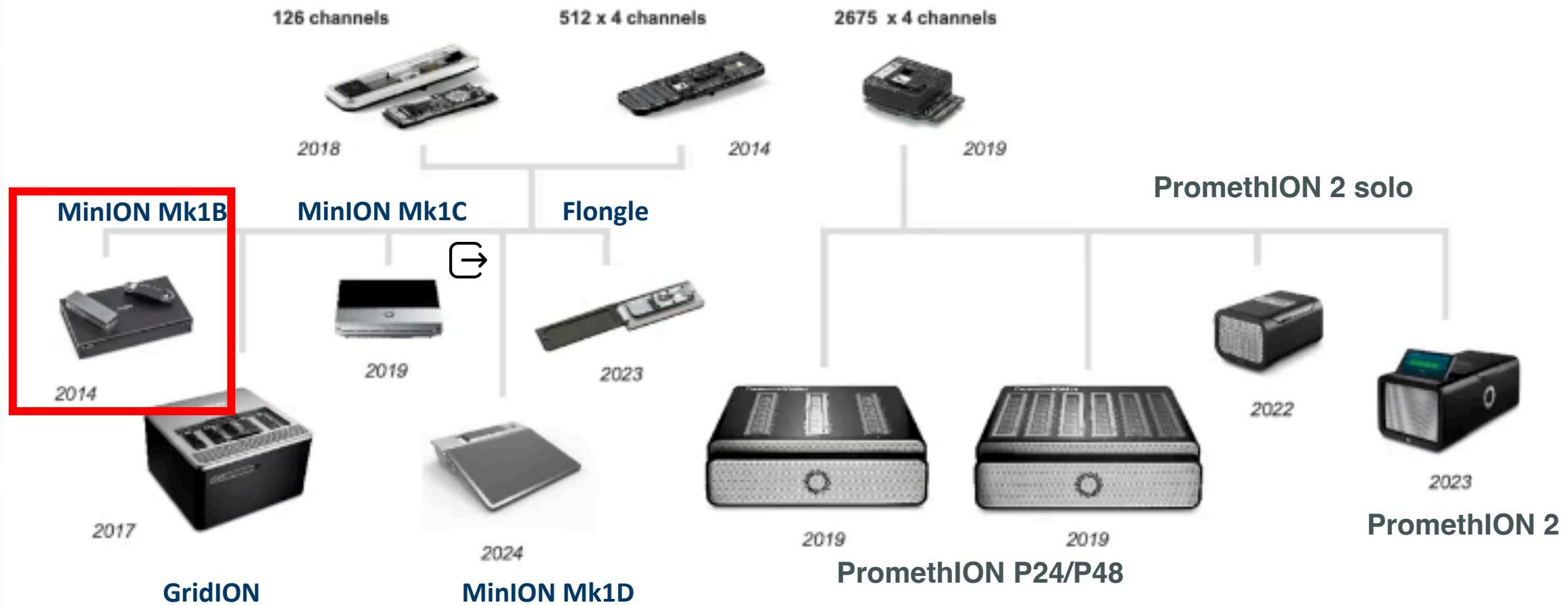
LC
LONDON
CALLING
2023



Nanopore Platform

Product family

LC
LONDON
CALLING
2023



How does it work?

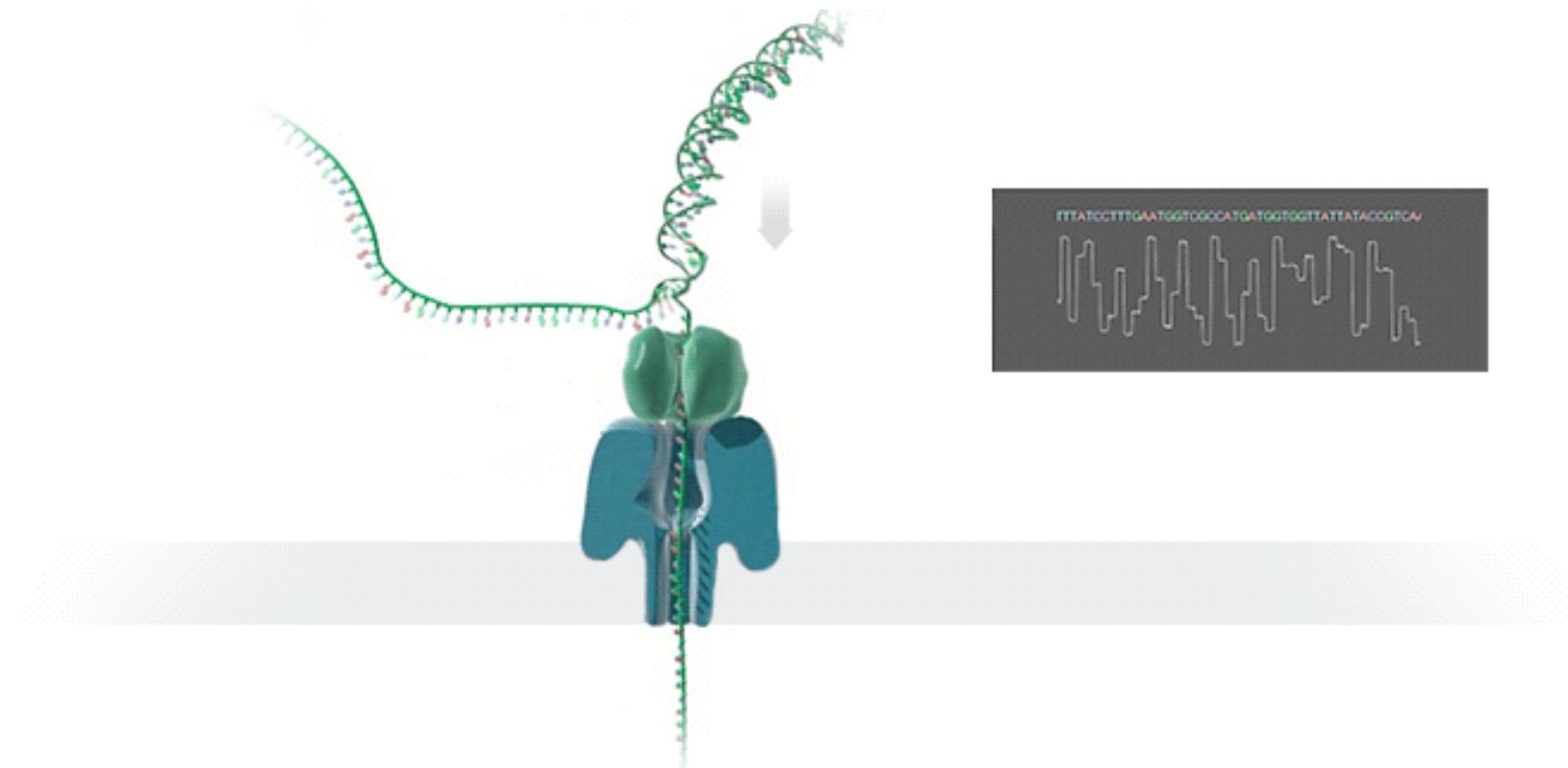


Image: Oxford Nanopore Tech

How does it work?

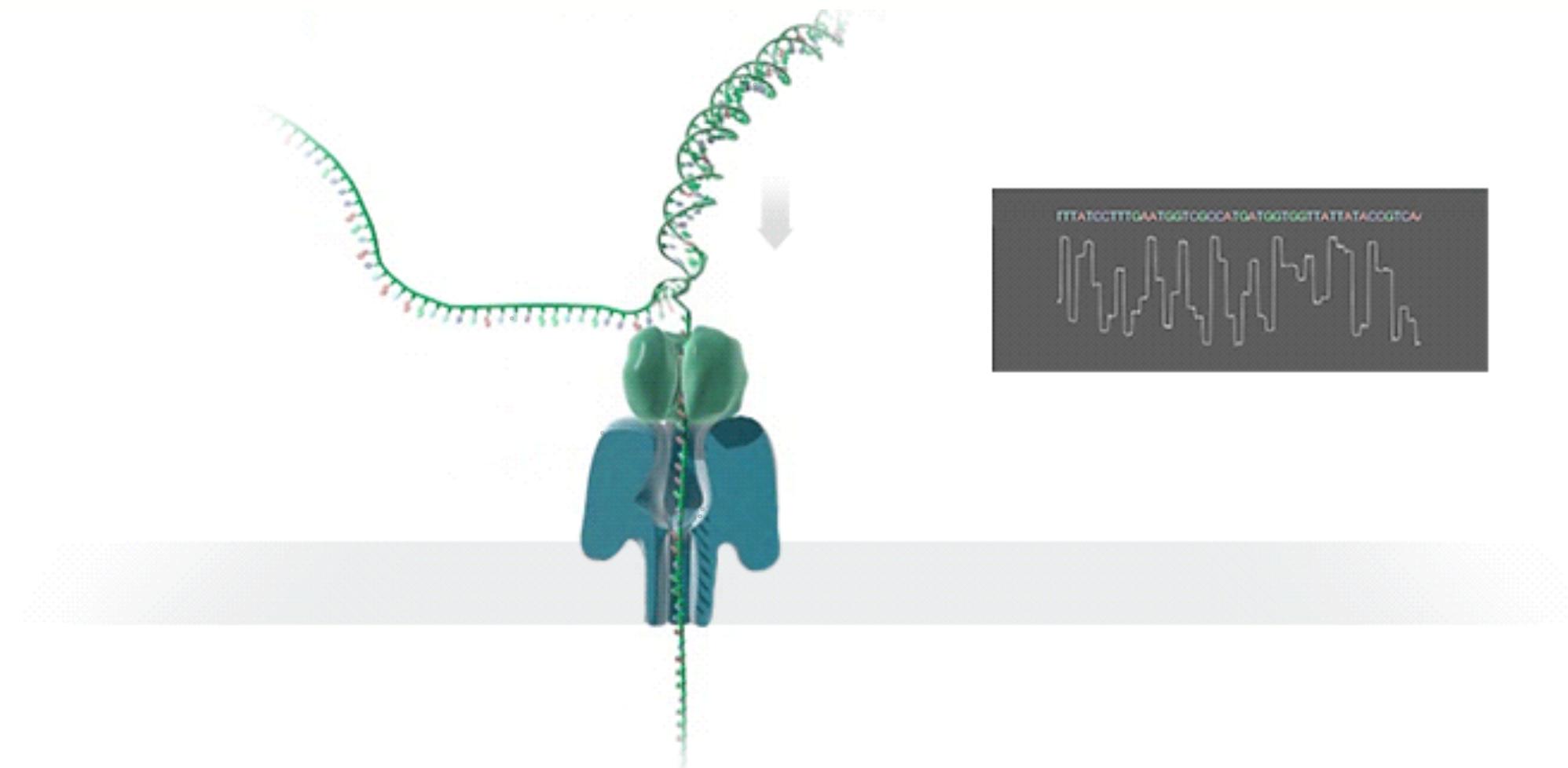
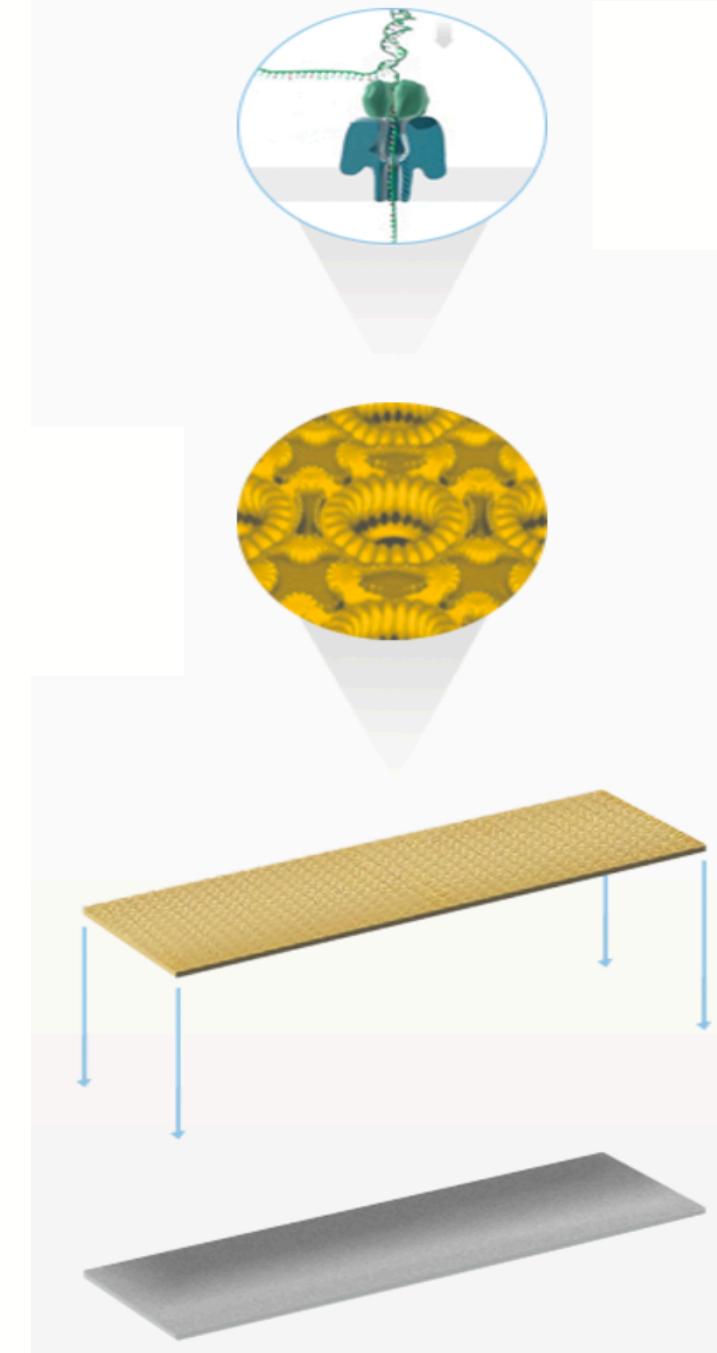


Image: Oxford Nanopore Tech



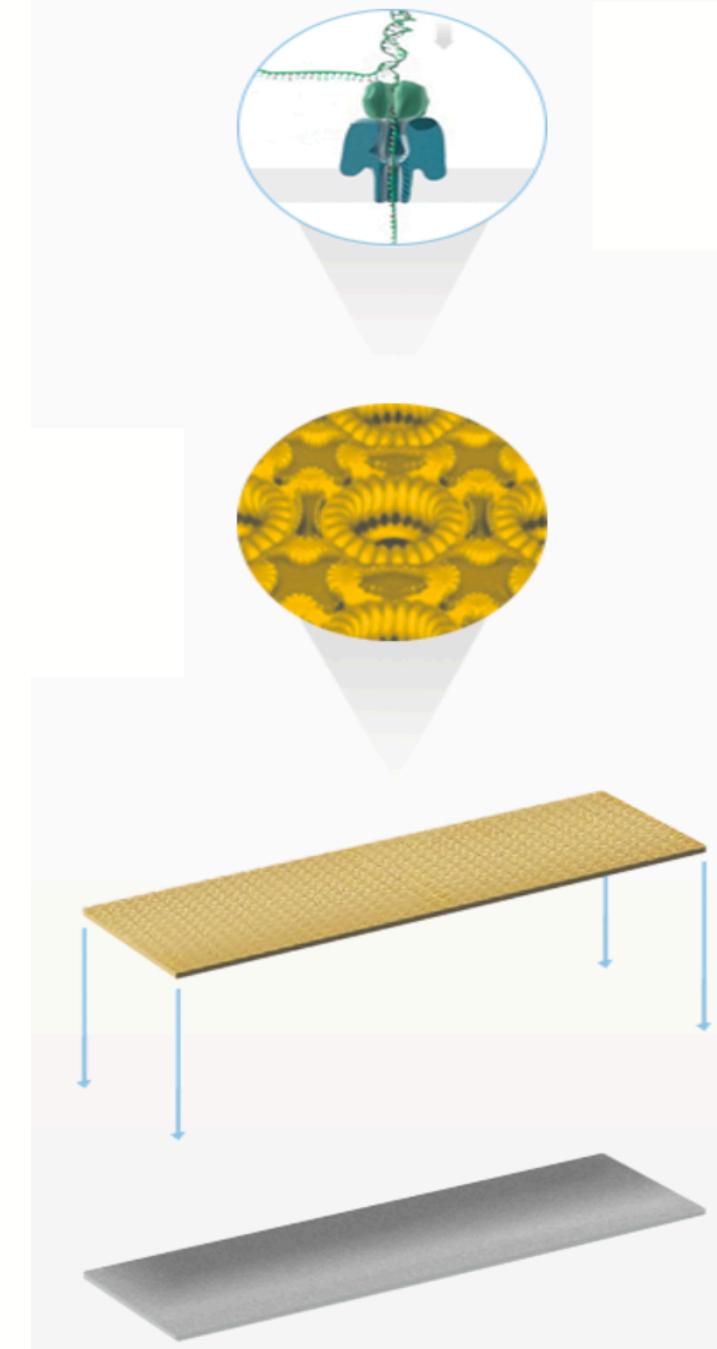
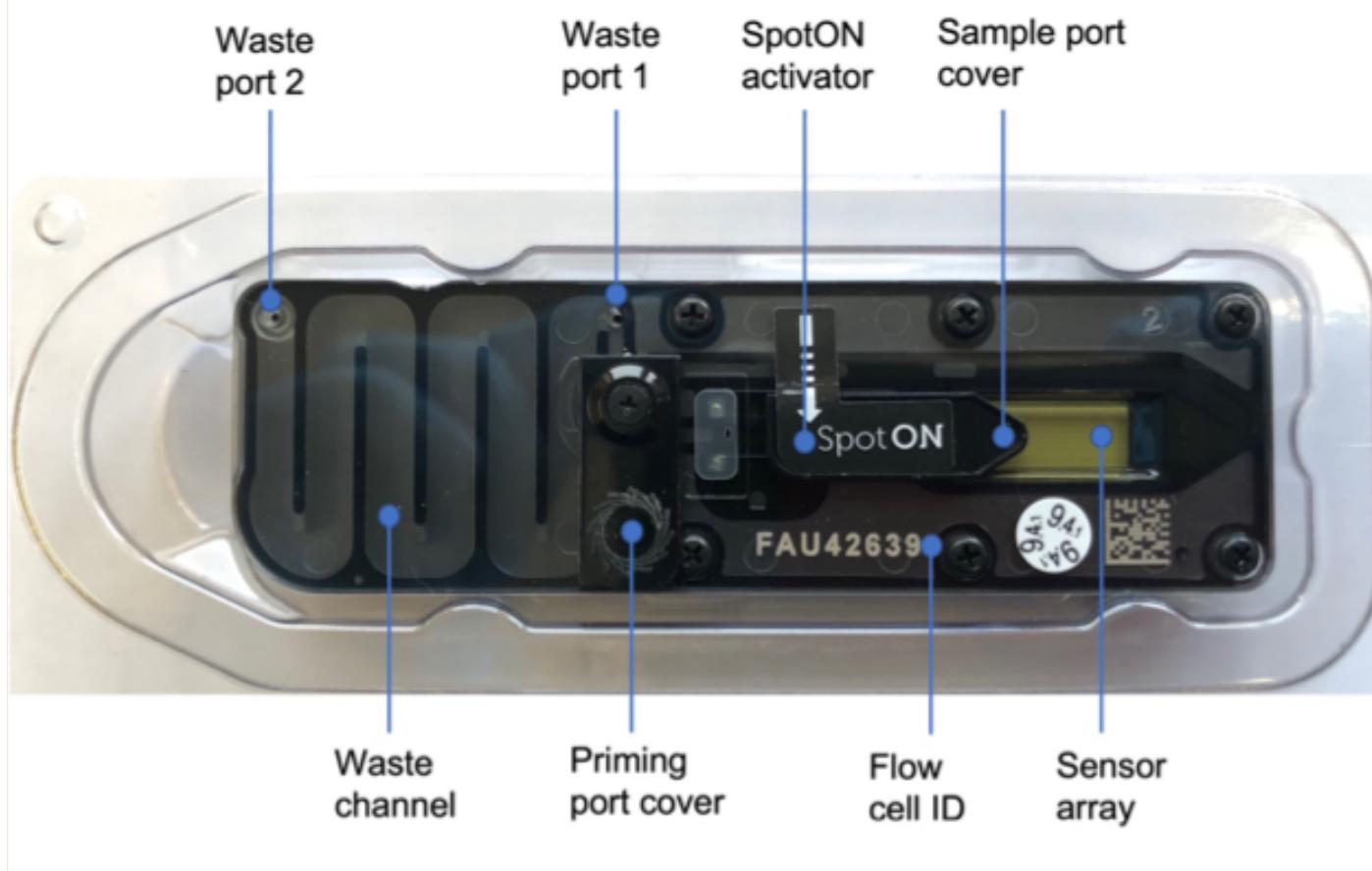
University
of Glasgow

Flowcell: a closer look





Flowcell: a closer look



Sequencing library preparation

Quality control

Fragmentation

End repair

Size selection

Amplification

Index/barcoding

Adaptor ligation

Quantification

Nanopore library preparation

- Determined by
 - Input type (RNA, cDNA, gDNA, amplicon, metagenomic...)
 - DNA concentration (from picograms to nanograms)
 - Fragment length
 - Index/barcoding
 - If it needs to be field friendly (i.e. no cold chain)
 - Speed- does it need to be a rapid prep?



How it is being used

Nanopore sequencing offers advantages in all areas of research...



Microbiology



Environmental research



Microbiome



Basic genome research



Human genetics



Cancer research



Clinical research



Plant research



Transcriptome analysis

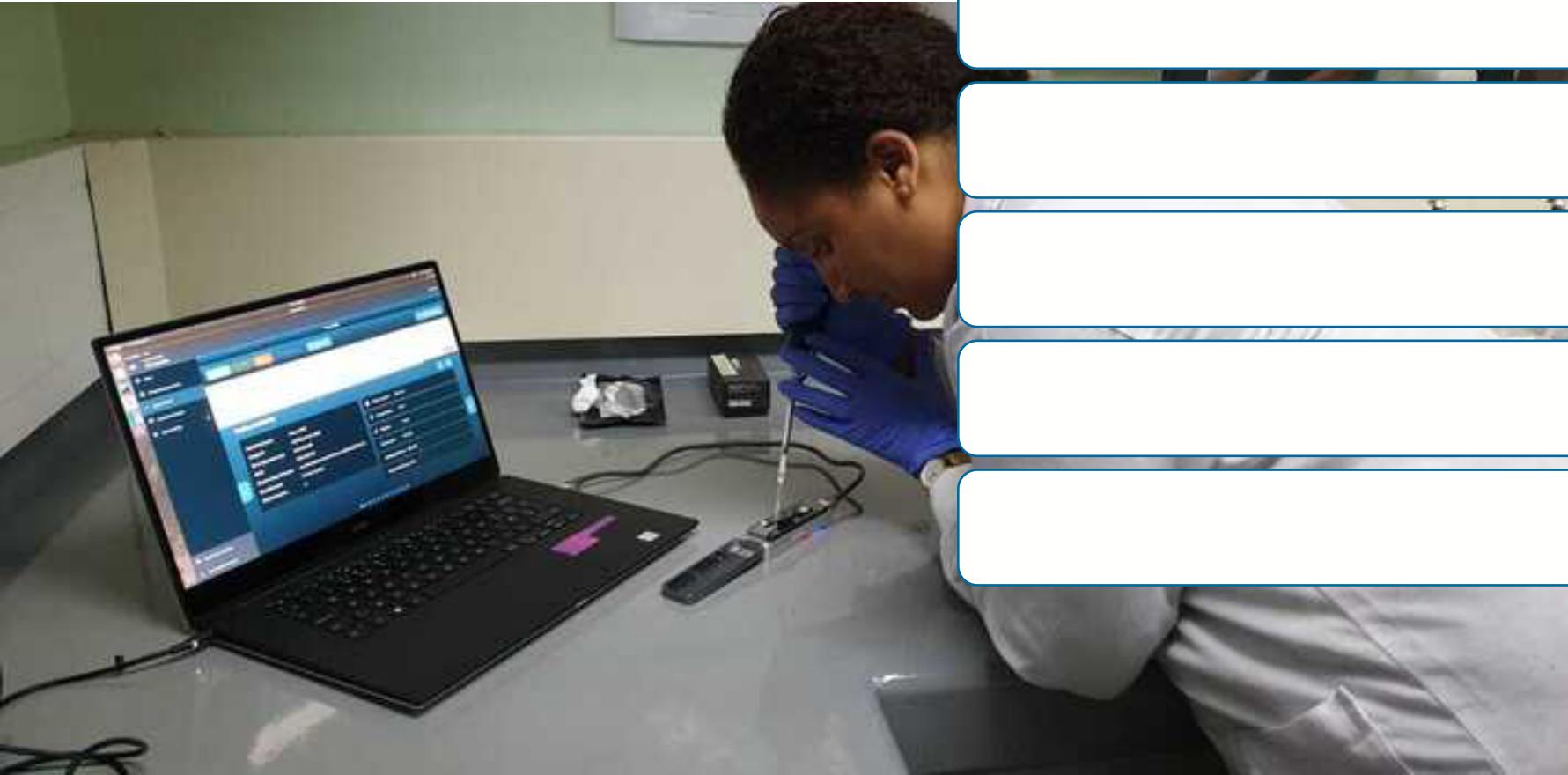


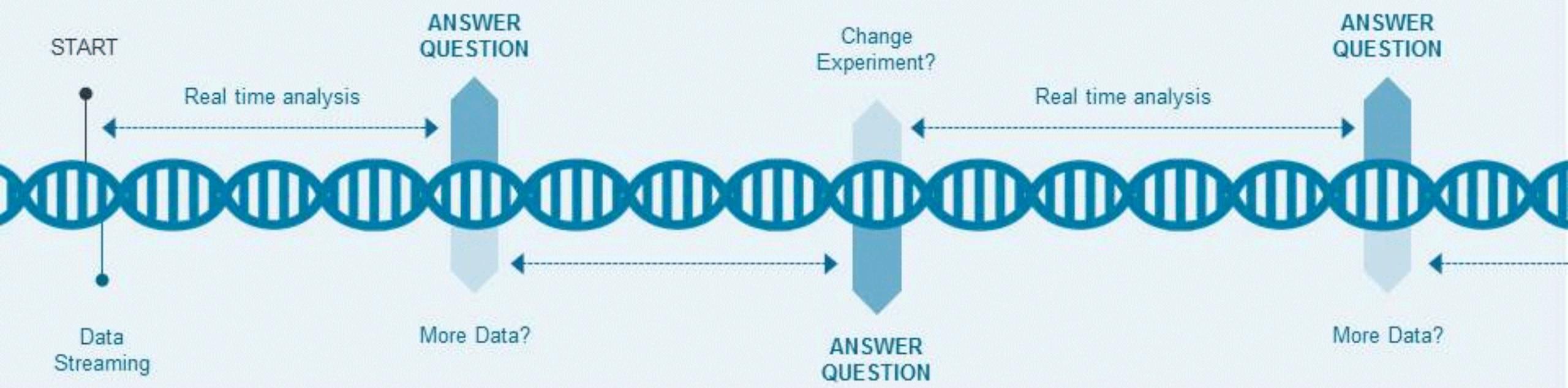
Population-scale genomics



Animal research

Advantages





SARS-CoV-2 Whole genome sequencing



7hr
RNA to answer

Of which ~1 hr
sequencing time

www.nature.com/nmeth/ January 2023 Vol. 20 No. 1

nature methods

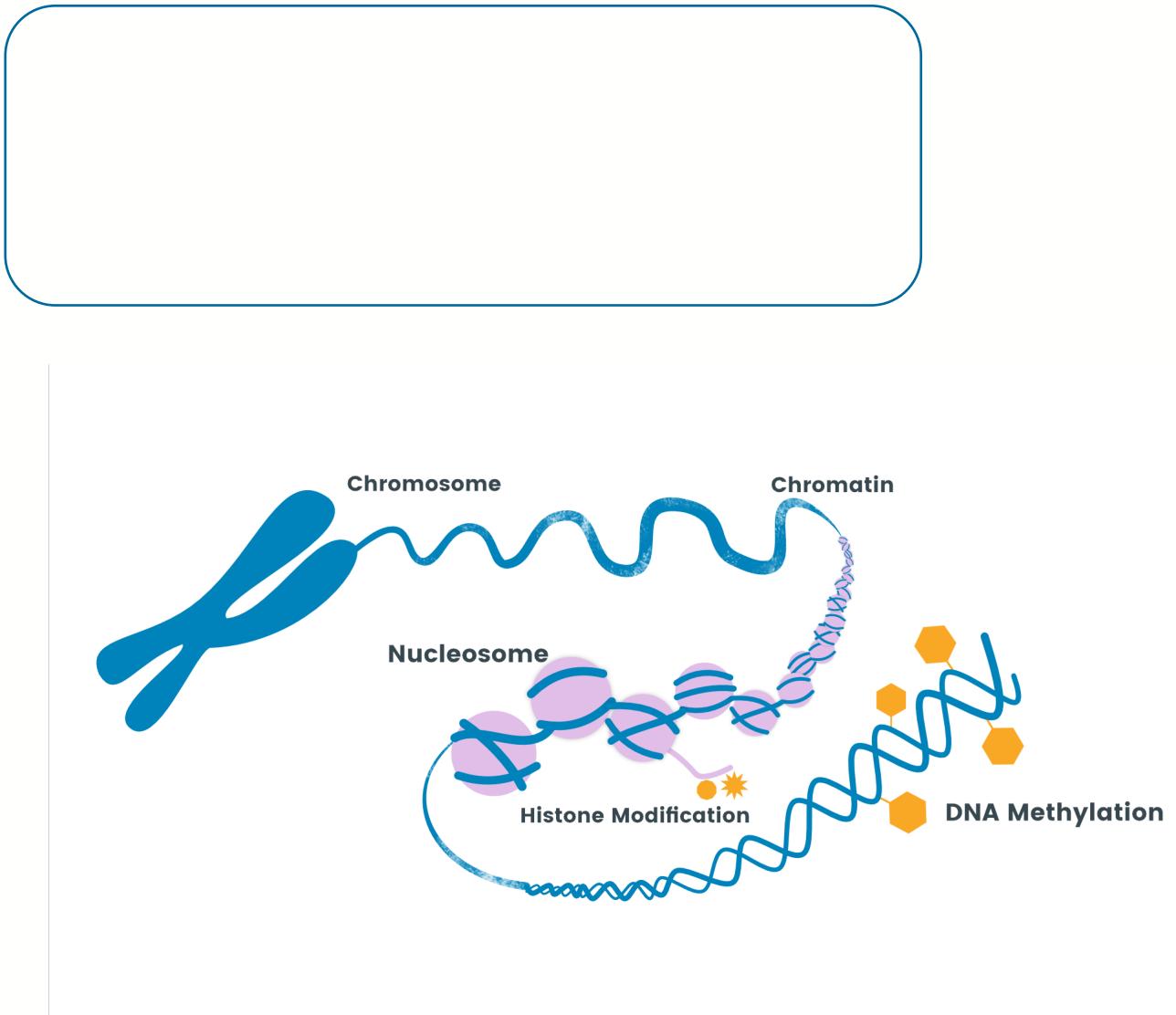
Method of the Year 2022:
Long-read sequencing



- Ultra-long reads (hundreds of kilobases)
- Ease of assembly and improved quality
- Span repetitive genomic regions
- Structural variant detection



- DNA and RNA methylation
 - Can measure long-range epigenetic patterns
 - Genome-wide methylation studies!



Portability and accessibility

- Take the lab to the sample
- Research & diagnostics in remote locations
- Rapid, on-site sequencing

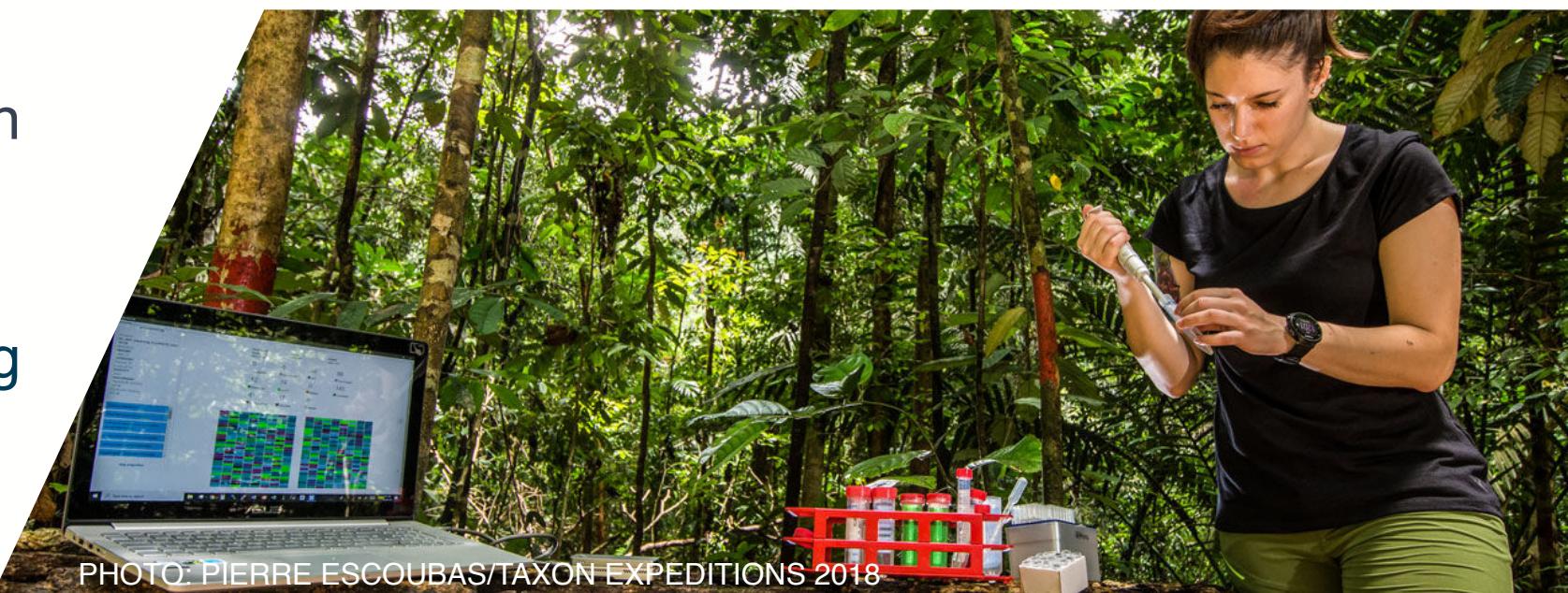
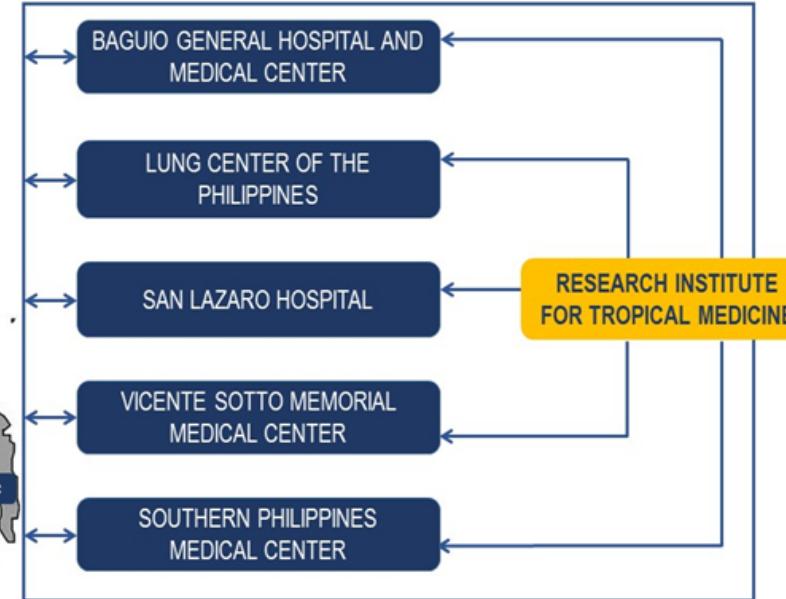


PHOTO: PIERRE ESCOUBAS/TAXON EXPEDITIONS 2018

COVID – an explosion of decentralized sequencing capacity



Genomic Epidemiology of
COVID in the Philippines
(GECO PH)

Disadvantages

Error rate

Insertion and deletion errors

AGCTTTTTTCGCA

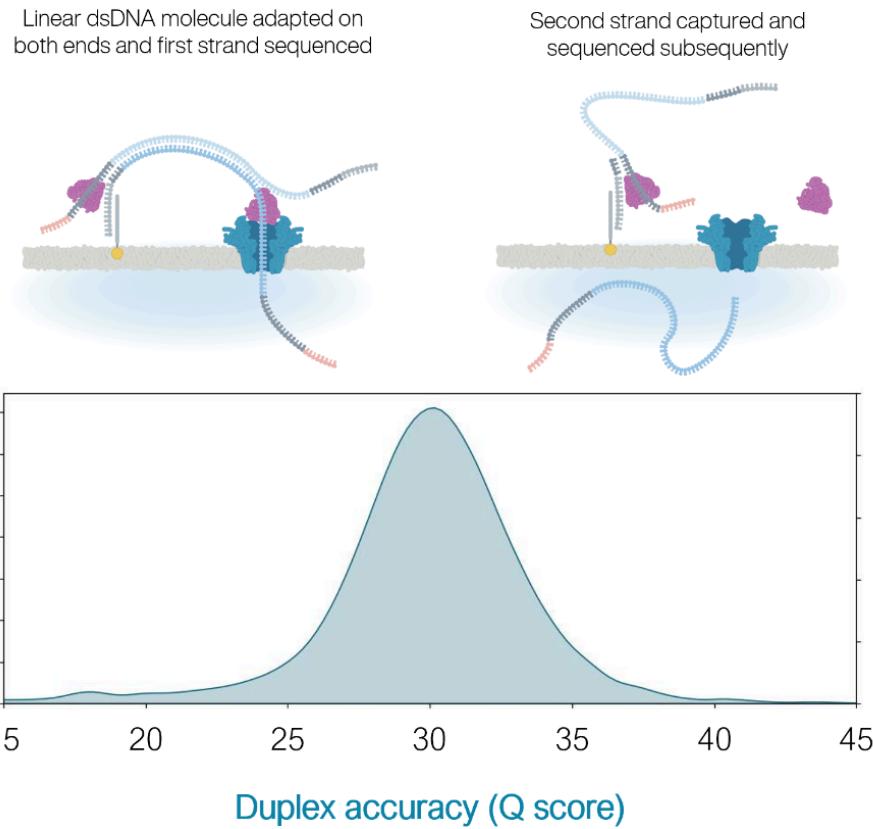
Homopolymers

Complex data analysis

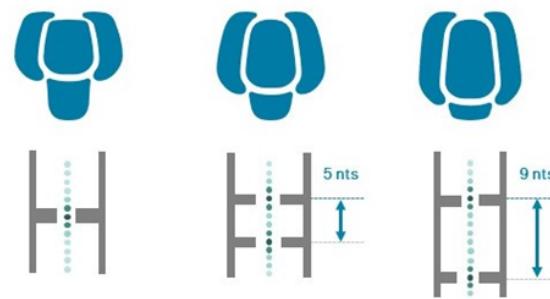
A	T	A	T	C	C	G	G	-	C	A	A
A	T	A	T	-	-	-	G	-	C	A	A
A	T	A	T	C	C	G	G	C	C	A	A

TCCTGAGTAATT


Latest kits/flowcells



- Kit V14 chemistry with R10.4.1 flowcells
- Higher accuracy and yield
- Capable of duplex reads



R9.4.1

R10.3

R10.4.1

Products unavailable or being phased out

Kit 11 chemistry
R9.4.1 flowcells



Nanopore Sequencing

Headline numbers – May 2023

	Raw read accuracy	99.5%, Q22
	Duplex accuracy	>99.9%, >Q30
	SNPs (F1 score, All/CDR ¹)	SNV: 99.9% Indel: 90.7% / 99.5%
	Assembly (Human, diploid)	Telomere to telomere ² Q42
	Assembly (Bacterial)	Circular ² >Q50
	SV detection (F1 score)	96%
	Methylation accuracy (5mC, raw read)	99.7%

Headline accuracy figures Kit14 with 5kHz run conditions and SUP basecalling

1: RefSeq coding regions

2: Applications assembly poster (LC2023)

3: Future chemistry, 800bps (NCM2022)

4: Short fragment mode (LC2022)

Test accuracy, e.g. LamPORE:
Sensitivity 99.1%, Specificity 99.6%

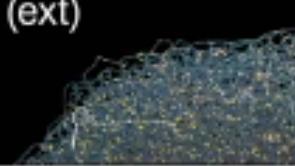
Instrument record (int):
10 Tbase output, 48 flow cells

Flow cell records:
350 Gbases (int)³, 245 Gbases (ext)

Short Fragments:
> 250 M reads at ~200 bases⁴

Read length records:
4.2 Mbases (int), 2.3 Mbases (ext)

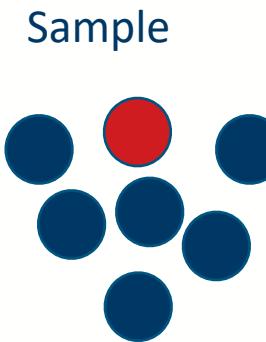
Longest Q40 read:
130 kbases



For further information about specific tools and methods used to generate these results please visit nanoporetech.com/accuracy.

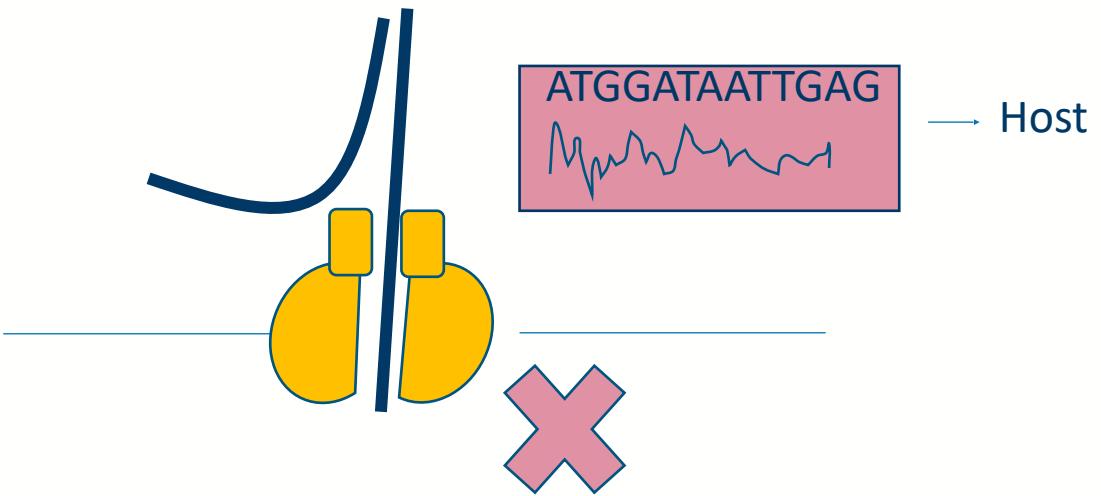
Adaptive sampling

- Ability to "select" what passes through the nanopore
- Avoid complex sample preparation
- Provide a reference sequence(s) and choose to **enrich** or **deplete** any matches



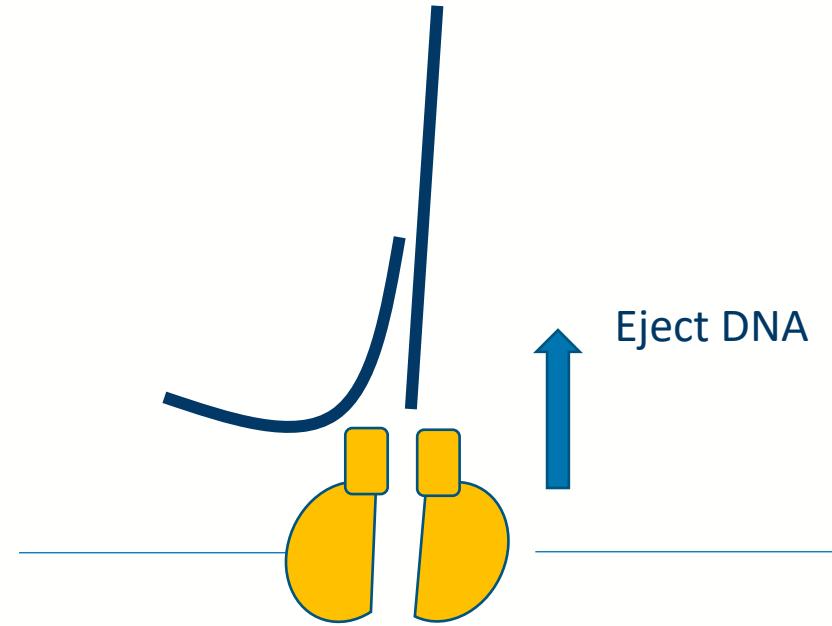
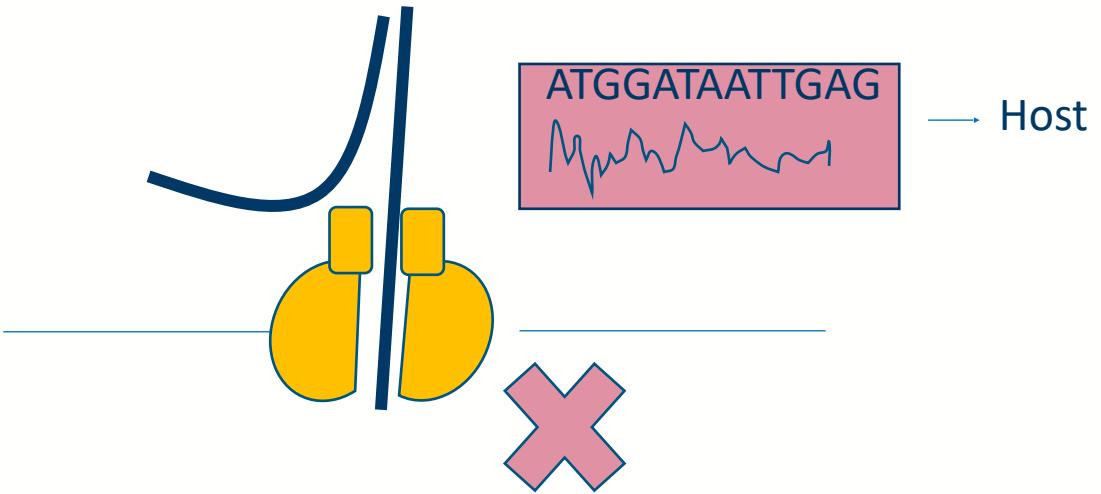
- Target
- Non-target

Real-time basecalling and alignment



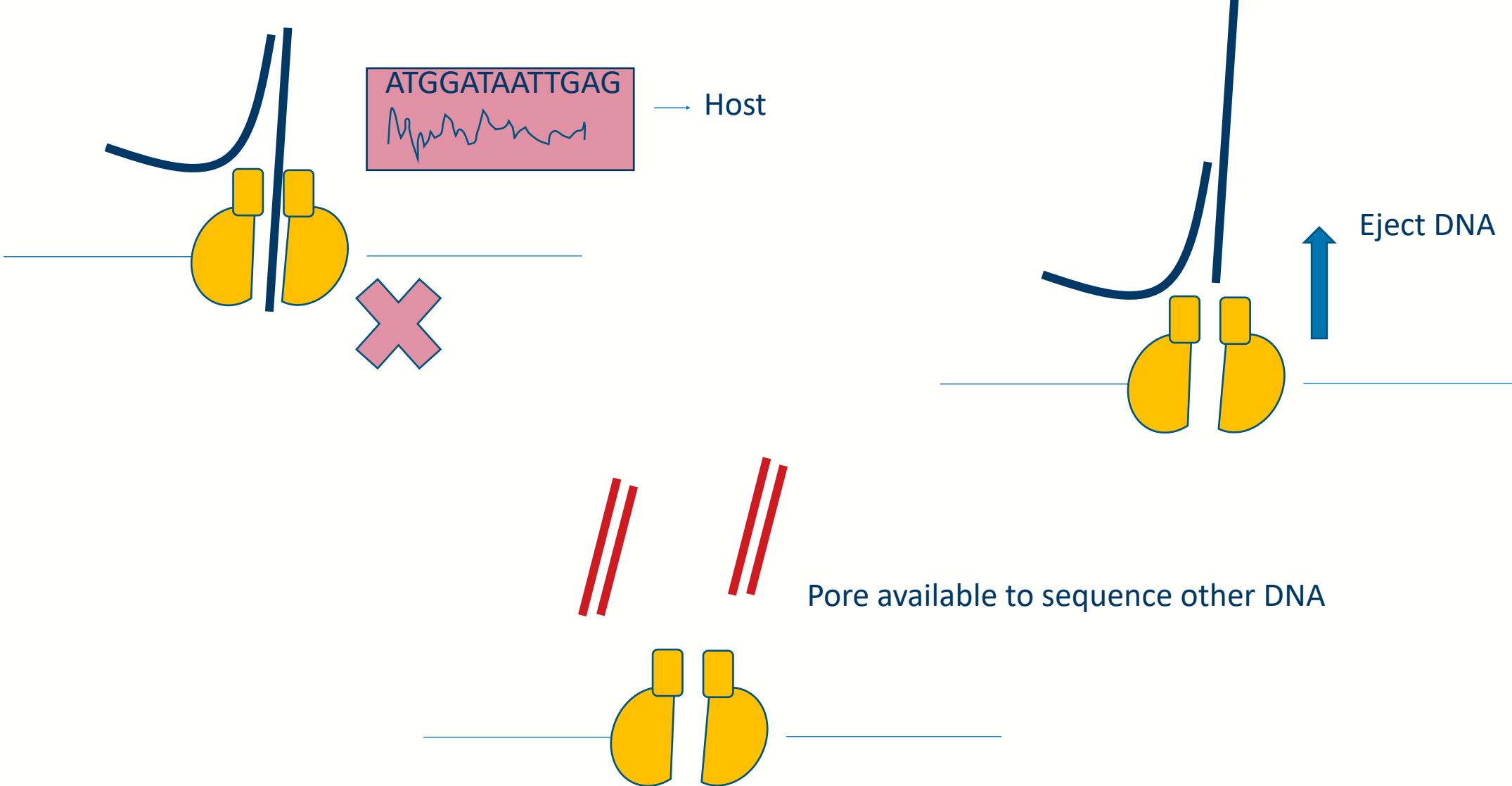
- Target
- Non-target

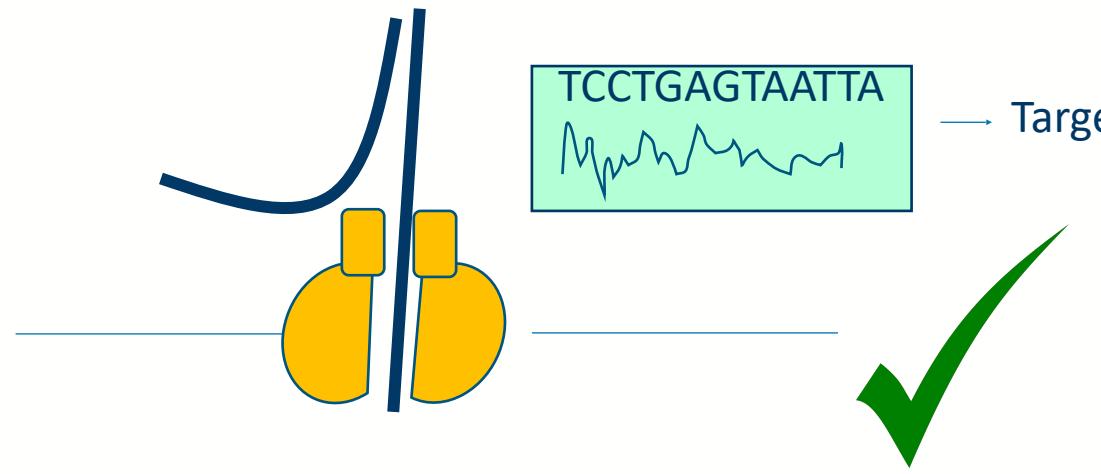
Real-time basecalling and alignment



- Target
- Non-target

Real-time basecalling and alignment





→ Target → Continue
sequencing

Higher
yields

Faster
experiment

Caveats

More effective
with longer reads

Faster pore
decline

Need a good
reference

Recovery protocol



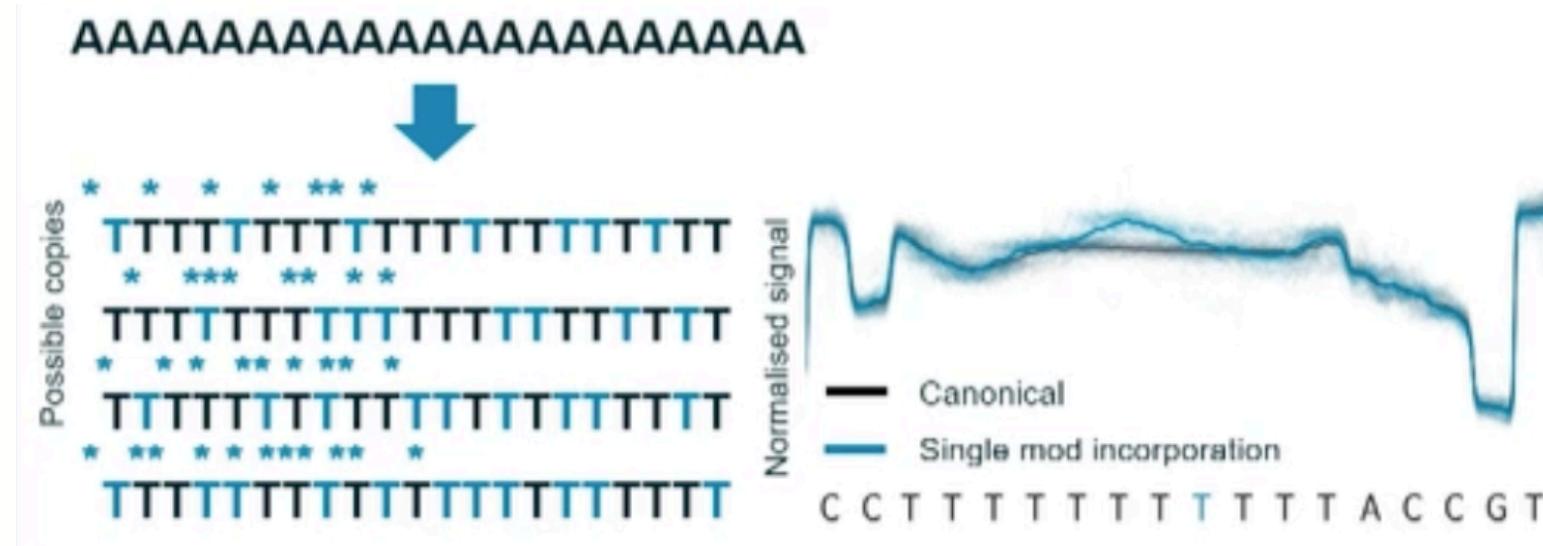
Recover a library from the flowcell and reload it on another flowcell!

Useful if:

- Want more data from the same library
- Library fails early in run
- If flowcell is blocked

Homopolymer accuracy fix

- Modifications to break up homopolymers (5B4)
 - Quality score improvements (92 to 99% at 90X coverage)
 - With polished assembly Q50



Fancy nanopore video!

- <https://nanoporetech.com/support/how-it-works#fullVideo&modal=fullVideo>

Let's get sequencing!

