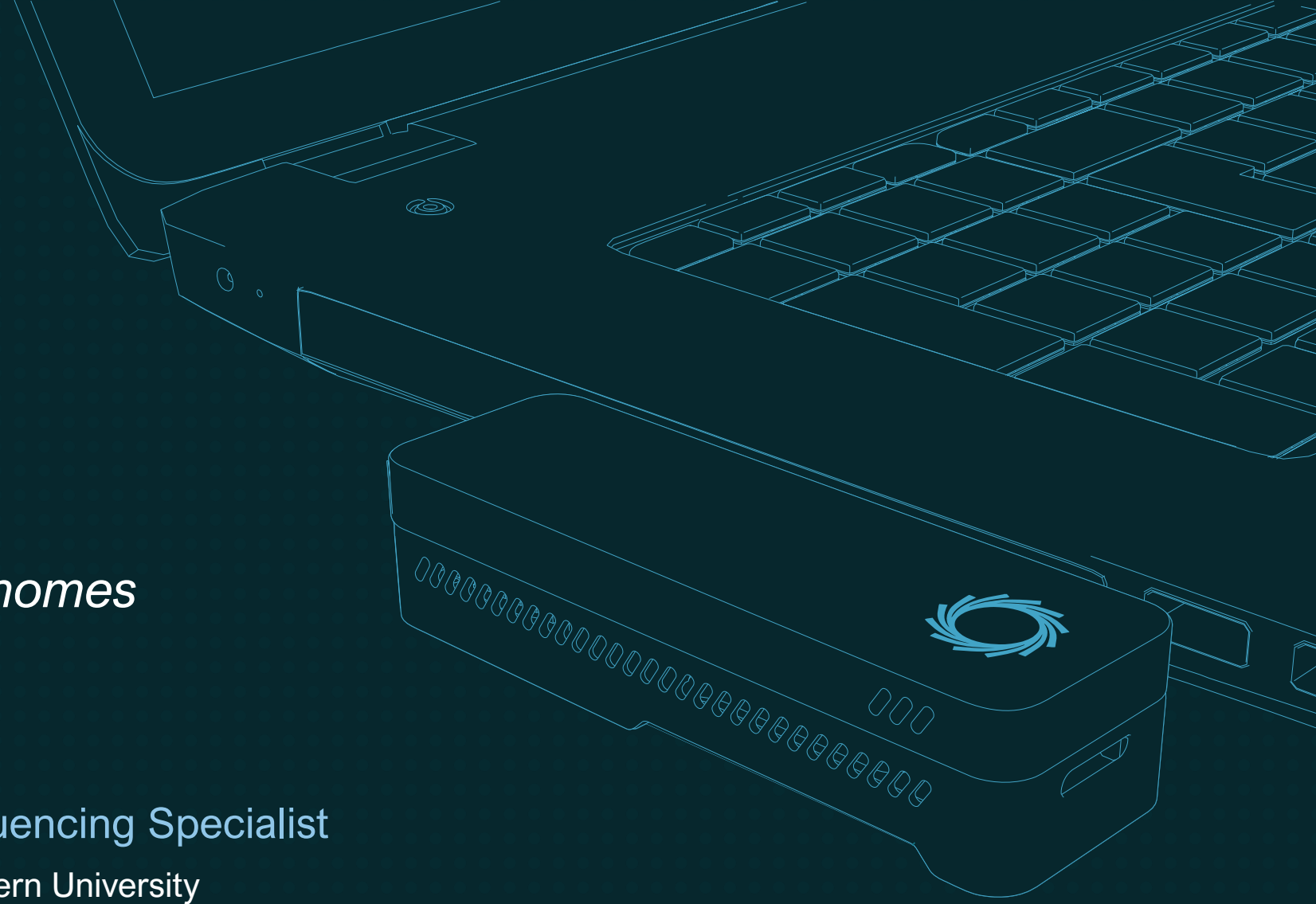
Oxford NANOPORE Technologies



Sequencing:

From plasmids to genomes

Roger Bialy, PhD – Regional Sequencing Specialist

London Regional Genomics Centre, Western University

March 1st, 2024

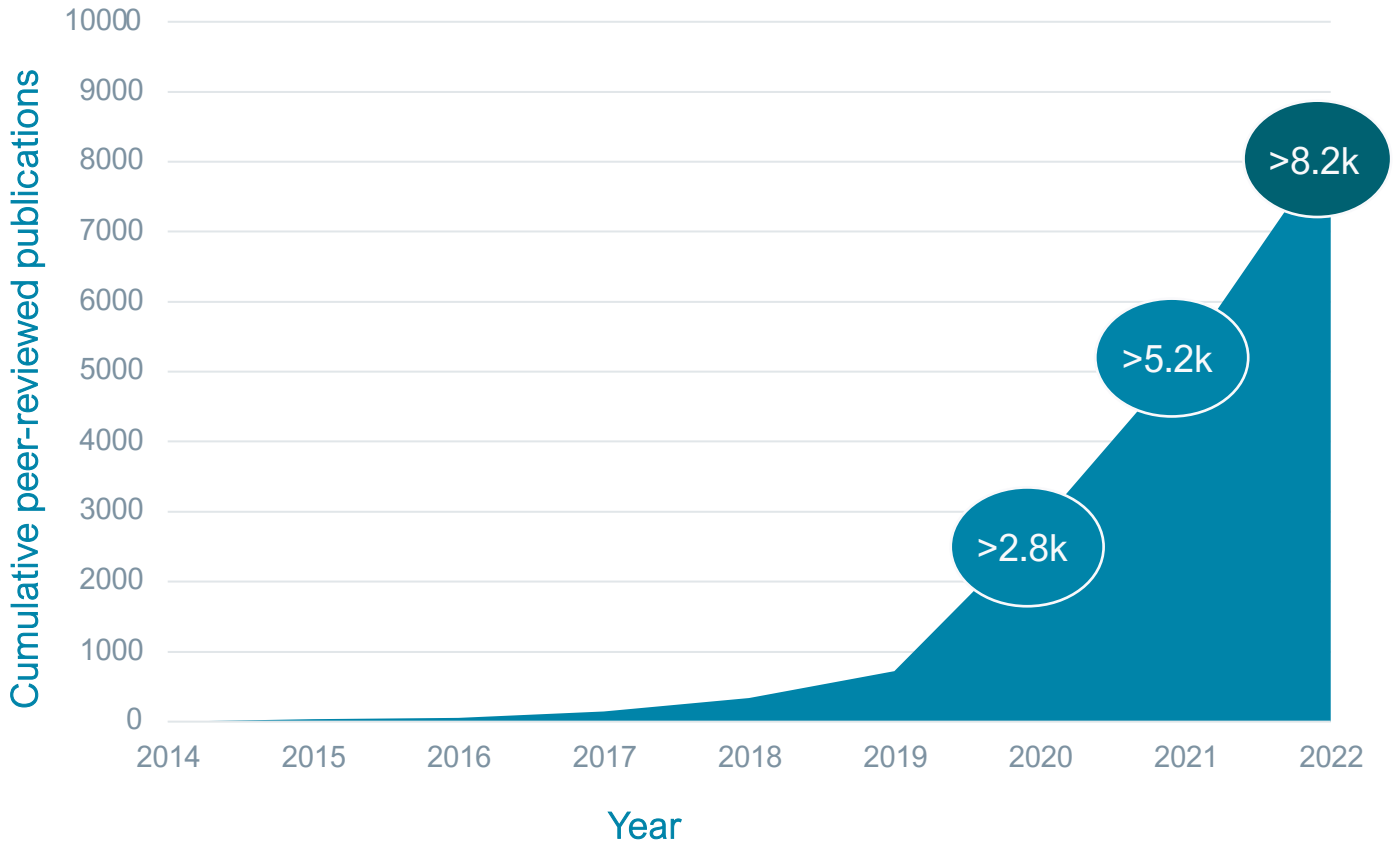
01

Introduction to Nanopore

Oxford NANOPORE Technologies

Scientific publications demonstrate broad uses of nanopore sequencing

In foundational research: human genetics, cancer, plant, animal, infectious disease, environmental genomics

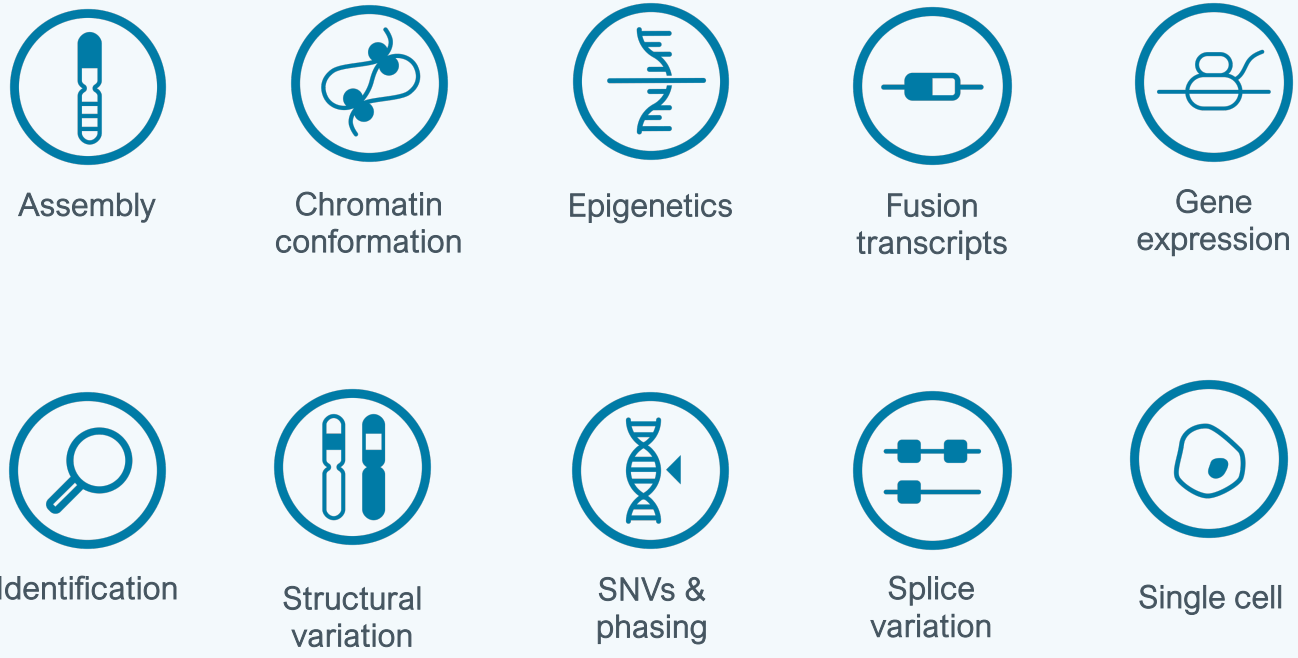


Oxford Nanopore's accessibility and broad utility is reflected in an increasingly large, international scientific evidence base

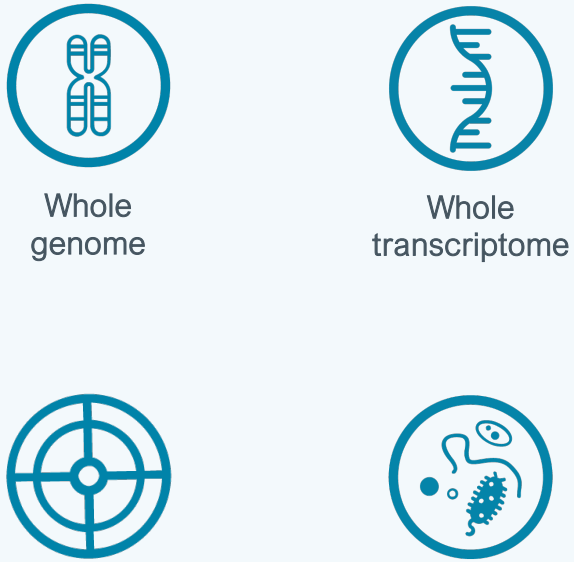
Delivering the widest range of biological analysis techniques

In the field, in research laboratories and in high throughput service environments

Investigations



Techniques



Find out more: nanoporetech.com/applications

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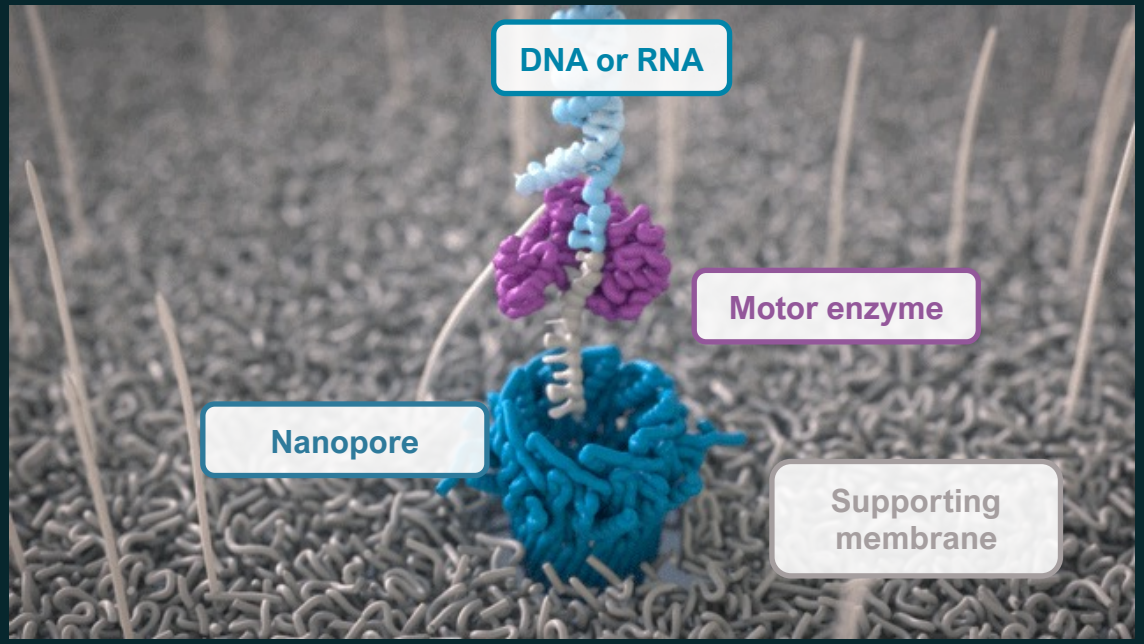
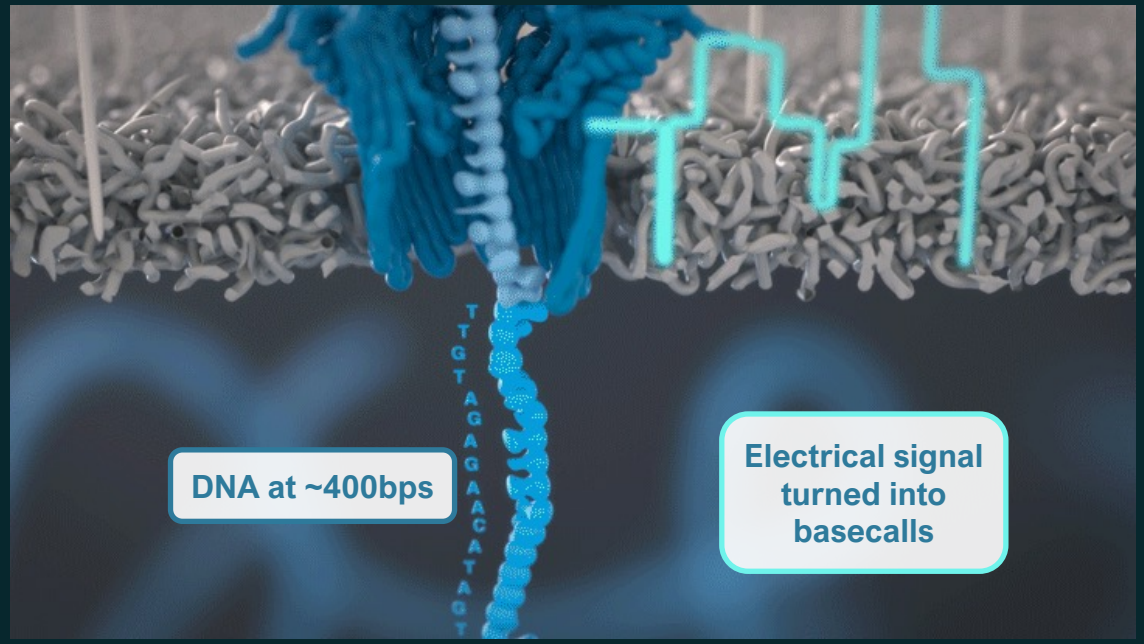
Nanopore sequencing: how it works

An adapted DNA strand is passed through a nanopore, an electrical signal is interpreted into sequence data





- Direct sequencing of DNA
- PCR free, no amplification bias
- Modified base detection
- Read length-agnostic
- Real-time analysis

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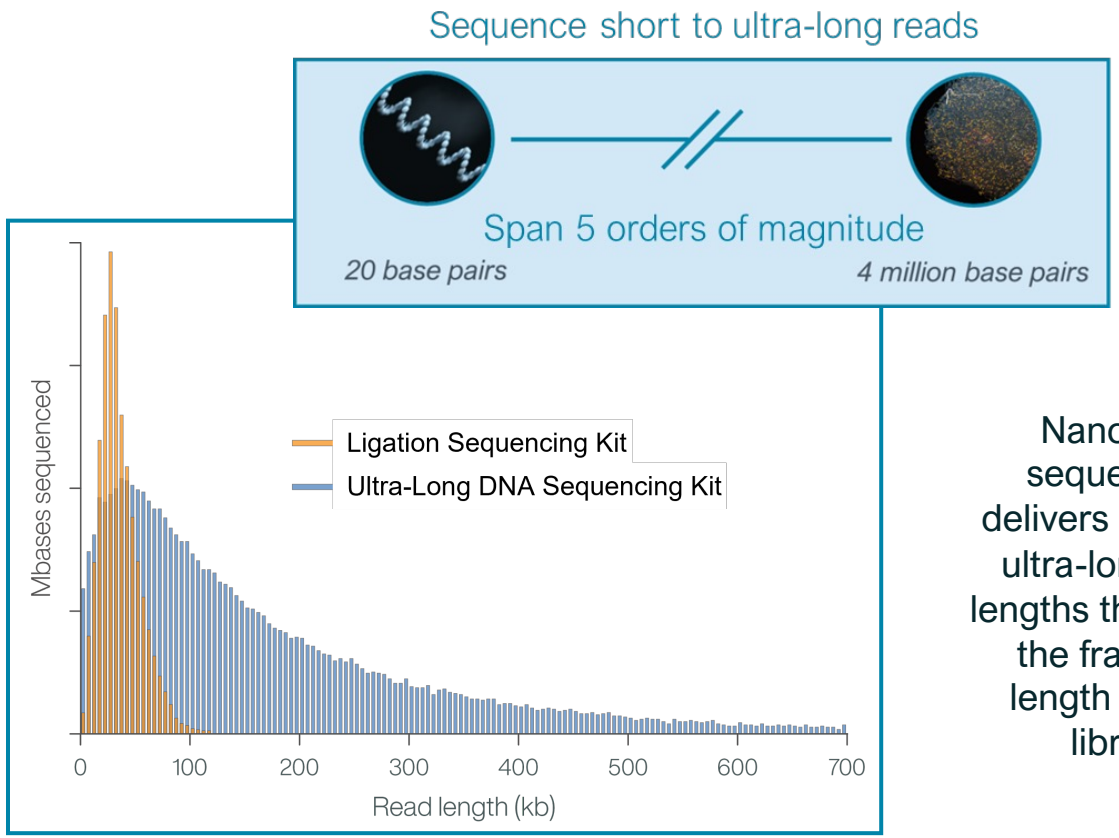
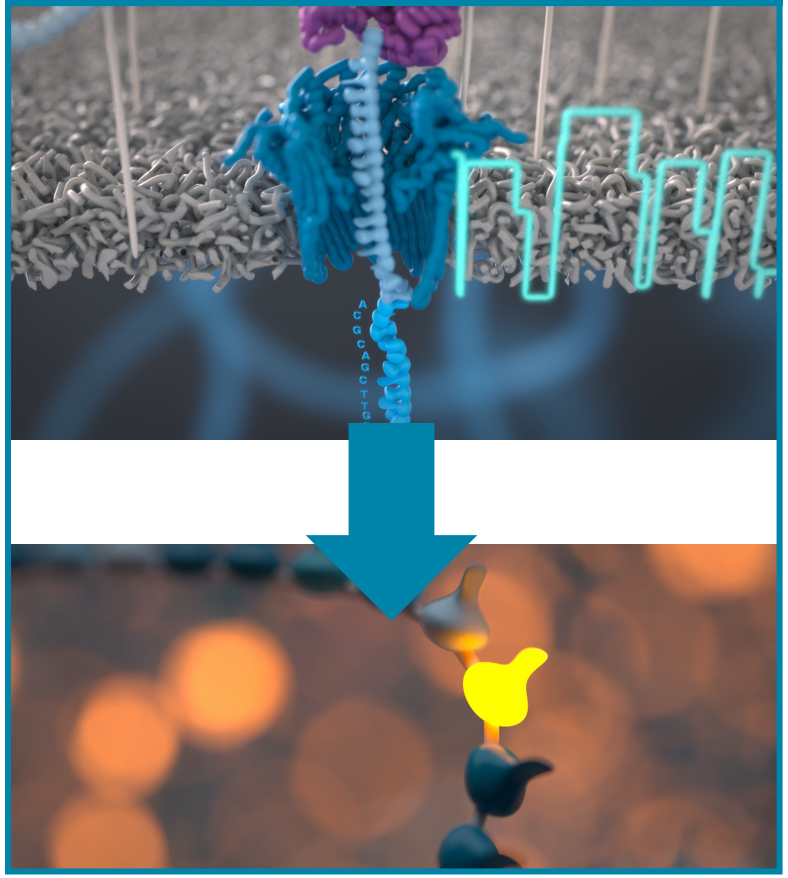
The benefits of PCR-free sequencing

Unrestricted read lengths



Nanopore sequencing delivers long and ultra-long read lengths that match the fragment length in your library

Methylation included



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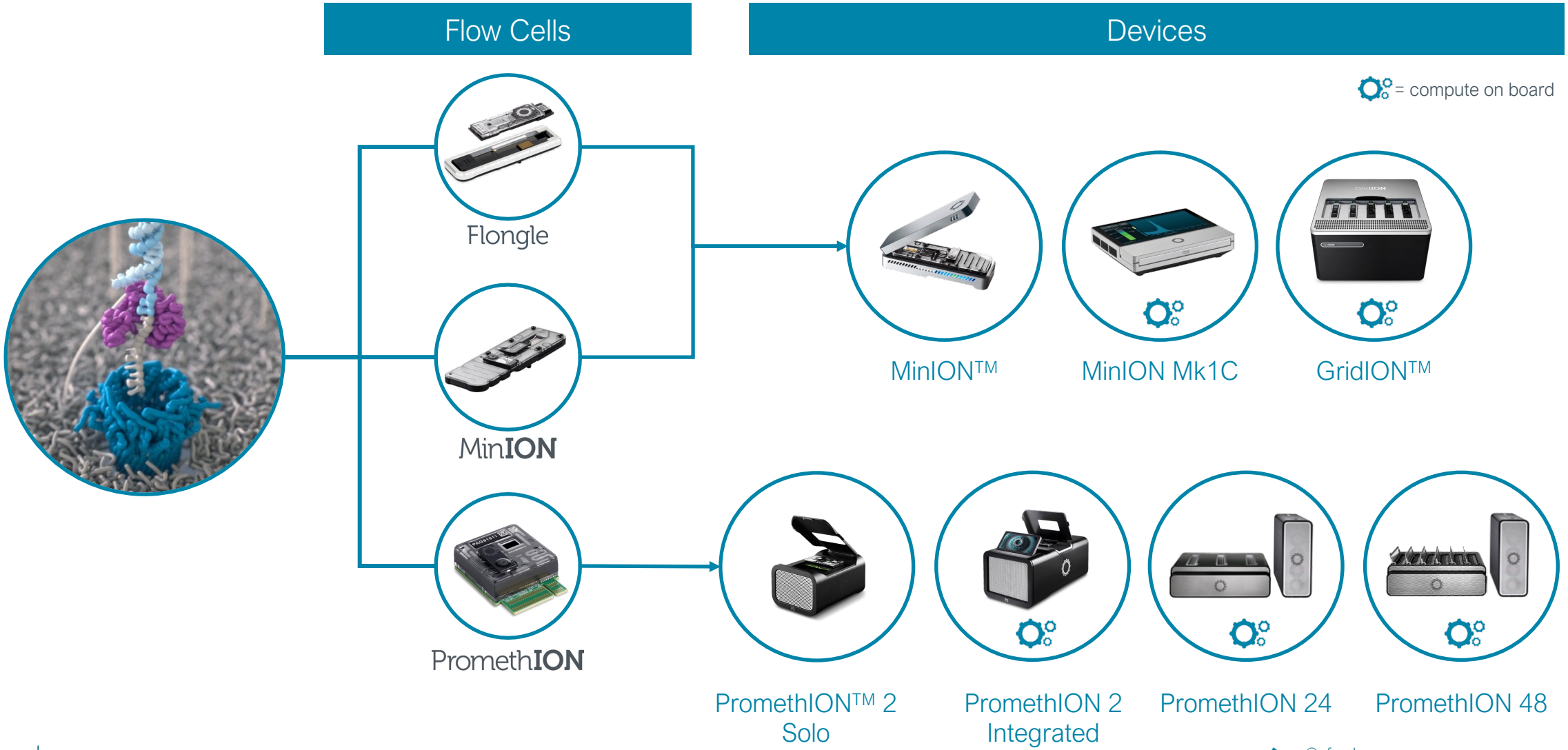
One core technology at any scale



02

Technology Update

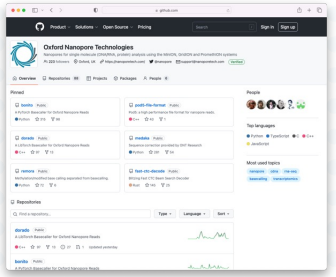
Oxford NANOPORE Technologies

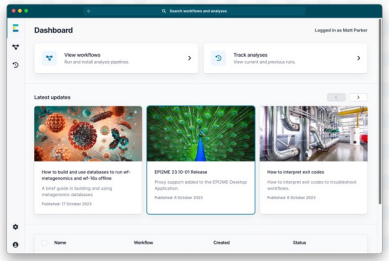
Secondary data analysis

EPI2ME

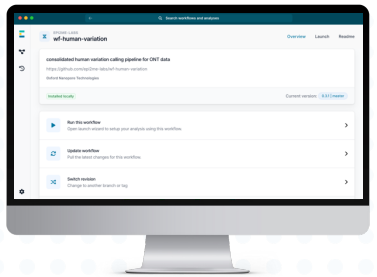
Available now

“Open-source” analysis pipelines become packaged for ease of use

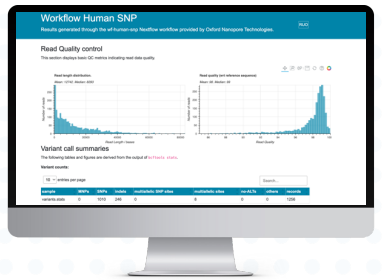




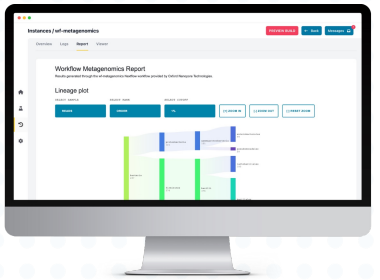
	GitHub	EPI2ME™ solutions
Location	User defined (laptop, cluster or cloud)	**Local, distributed or in the cloud**
Timing	Post-run	**Post-run** or real time
Configurability	User defined	**Pre-configured**
Reporting	User built	**Detailed output**, shareable reports
Operating systems	User defined	Windows, Mac, Linux
Expertise needed	●●●	●○○



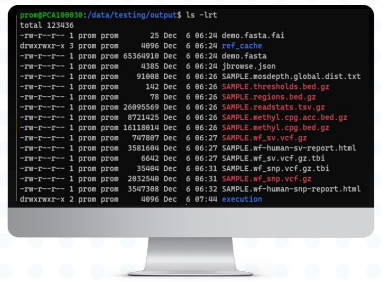
Intuitive interface
Pre-configured workflows



Sharable reports
Standard output files



Run locally or in the cloud
Post-run or real-time



Command-line access
Easy integration

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End-to-end workflows for SynBio and Amplicons

Delivering more complete, information rich answers

Plasmid

- **Entire plasmid** sequencing to verify mutations free status
- Resolves repetitive regions
- **No primers required**

- Sample to answer from 5 h
- Rapid Barcoding Kit based enabling 96 samples per run
- Affordable to set up, highly competitive to run
- EPI2ME *wf-clone-validation* workflow available

AAV

- **Entire recombinant adeno-associated virus** (rAAV) vectors sequencing, spanning **ITR-ITR**
- Identification of transgene & promoters of interest, truncated rAAV genomes or contamination

- Sample to answer from 6 h
- Native Barcoding Kit based enabling 96 samples per run
- Affordable to set up, highly competitive to run
- EPI2ME *wf-aav-qc* workflow available

Amplicons

- *de novo* amplicon consensus sequence generation (500 – 5,000 bp long)
- **Resolves repetitive regions**
- **No primers required**

- Sample to answer from 3 h
- Rapid Barcoding Kit based enabling 96 samples per run
- Affordable to set up, highly competitive to run
- EPI2ME *wf-amplicon* workflow available

End-to-end workflows for small genomes

Delivering more complete, information rich answers

SARS-CoV-2, Flu RSV *coming soon*

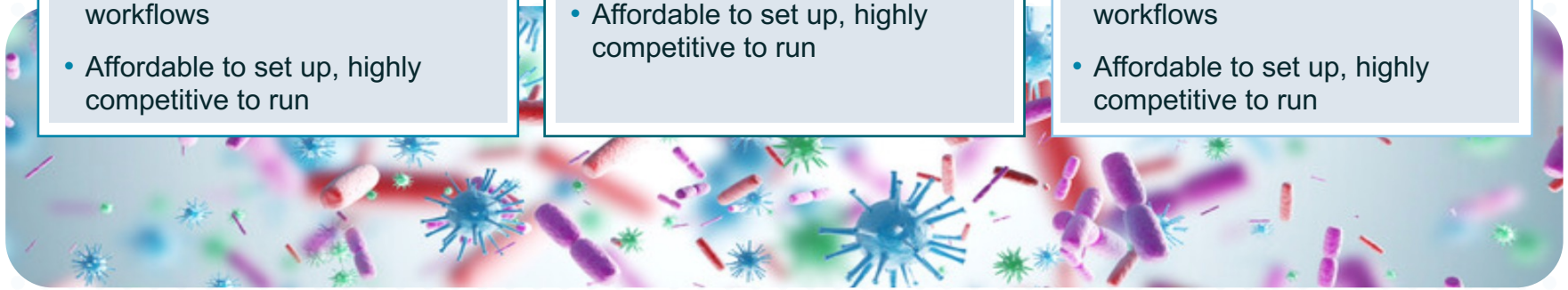
- **Full sample-to-answer workflow**
- PCR tiling approaches to sequence whole genome
- Tracking outbreaks, variants of concern
- EPI2ME *wf-artic*, *wf-flu* workflows available
- Up to 96 samples per run
- Rapid sample-to-answer workflows
- Affordable to set up, highly competitive to run

Bacterial assembly

- Isolate or metagenomic assembly workflows
- Alignment to reference or assembly
- Consensus annotation + resistance genes
- EPI2ME *wf-bacterial-genomes* workflow available
- Rapid sample-to-answer workflows
- Affordable to set up, highly competitive to run

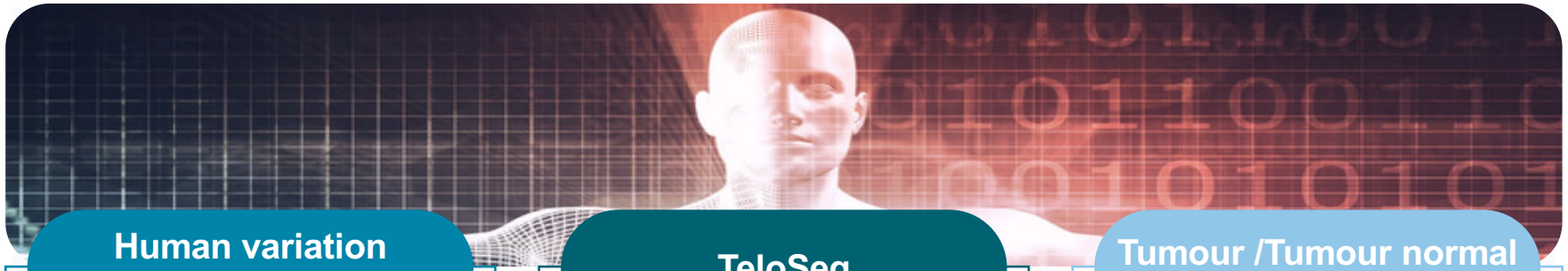
Metagenomics

- Amplicon-targeted or shotgun metagenomic samples sequencing and basecalling
- **Real-time classification**
- **Improved classification with long read context**
- EPI2ME *wf-metagenomics* workflow available
- Up to 96 samples per run
- Rapid sample-to-answer workflows
- Affordable to set up, highly competitive to run



End-to-end workflows for human applications

Delivering more complete, information rich answers



Human variation workflow

- **All variation captured inc. SNV, SV, CNVs, repeat expansions and methylation**
- Protocols for 10kb or 30kb read N50s for various sample input
- Extraction from cells or blood supported. Saliva, swabs *in development*
- Integration to interpretation providers
- EPI2ME *wf-human-variation* workflow

TeloSeq

- Sequencing from double-stranded telomeric ends of chromosomes, through the sub-telomeric regions
- >100-fold enrichment of telomeric regions
- Enabled telomere length measurement and chromosome arm resolution
- Full lab workflow with assisted informatics in GitHub
- EPI2ME workflow *in development*

Tumour /Tumour normal

Coming soon

- Paired tumor-normal WGS for deeper understanding of genomic and epigenomic variability in cancer
- Improved tumor type classification and identification of key mutations driving cancer progression
- All methylation markers retained alongside SNVs, SVs, CNVs
- EPI2ME workflow *wf-somatic-variation* available
- EPI2ME workflow tumour/tumour normal *in development*

Available now

High accuracy throughout all investigation types

Accuracy measurement adjusted to the specific experiment

Investigations accuracy

Simplex	Duplex	Assembly (Human)	Assembly (Bacterial)	SNP detection (F1 score human)	SV detection (F1 score human)	Methylation detection
99.6%	99.92%	99.998%	99.999%	SNV (WG): 99.8%[X] Indel (CDS): 99%[X]	96.2%[X]	99.5%[X] (5mC)
Q24[X] (raw read accuracy)	Q31[X] (single molecule accuracy)	Q47[Y] (consensus accuracy)	Q50[1] (consensus accuracy)	(variant calling)	(variant calling)	(raw read accuracy)

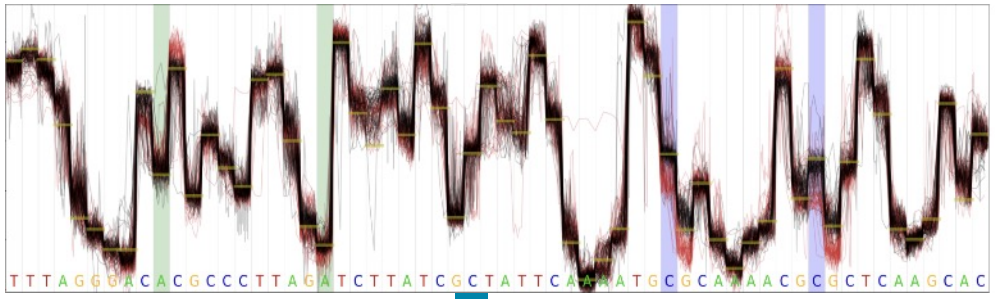
[1] Zymo mock community, R10.3 (LC 2021)
[X] Kit 14 / R10.4.1 / 400 bp
[Y] Accuracy at 60X depth, HG002, R10.3 (LC 2021)

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

DNA modifications: what's available now?

Single molecule, native modifications

Modifications are a built-in feature of nanopore signals



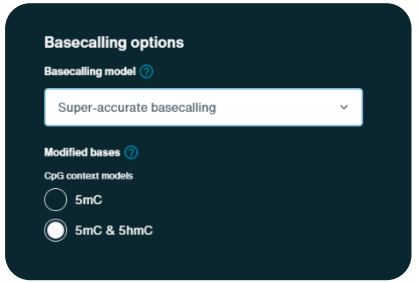
"CpG"

Basecaller optimised to find modifications in specific regions of interest

"All context"

Basecaller trained to find modifications no matter where they occur

Fully integrated – available now



MinKNOW

- Available live or post-run
- 5mC – "CpG" contexts
- 5mC and 5hmC – "CpG" contexts

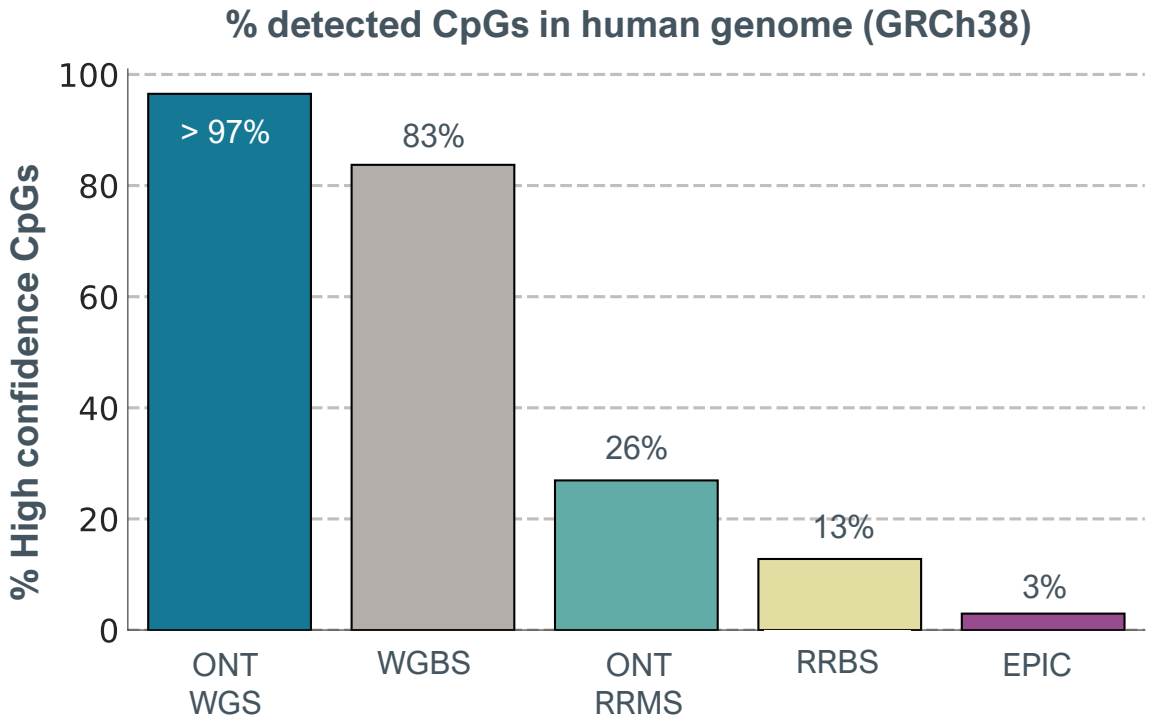
Open source offering in GitHub – available now

- All context 5mC
- All context 6mA
- Post-run via Dorado

```
dorado basecaller \
  dna_r10.4.1_e8.2_400bps_sup@v4.2.0 \
  pod5s/ \
  --modified-bases 5mC,6mA \
  > basecalls.bam
```

Oxford Nanopore provides superior methylation detection compared to other methods

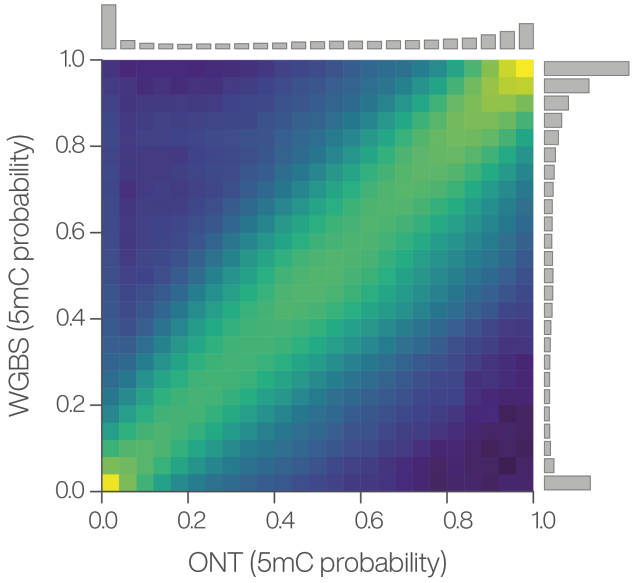
Nanopore sequencing detects significantly more CpGs vs EPIC arrays and bisulphite WGS, with high data correlation



- **EPIC arrays** cover only **~3%** of all CpGs in the human genome
- **Oxford Nanopore WGS** covers more than **97%**

Compared to bisulfite whole genome seq

- Higher number of CpGs called
- High correlation to whole genome bisulfite sequencing: 0.98
- Single-read, single-site accuracy ~99%



• CONF

03

Applications:
Microbial and Infectious Disease

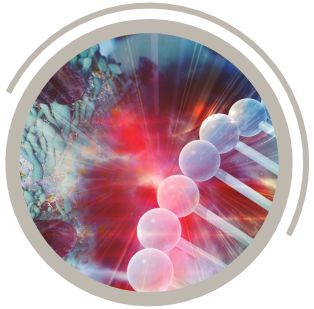
Oxford NANOPORE Technologies

Plasmid sequencing

Oxford NANOPORE Technologies

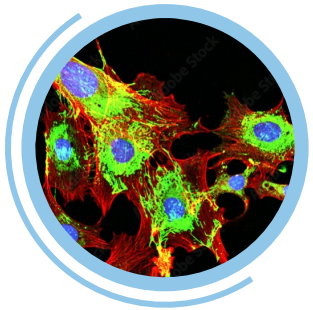
Plasmids are the backbone of molecular biology

Industrial applications

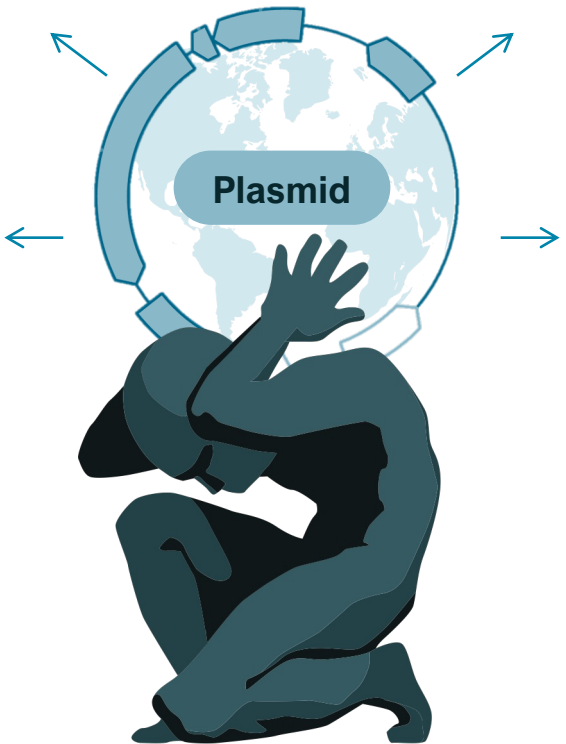


Gene therapy

Vaccine development



Basic research

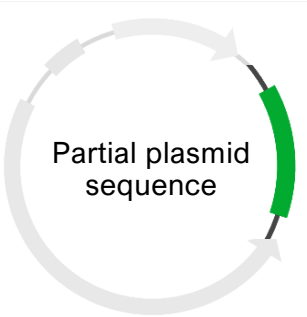


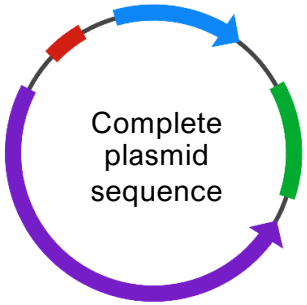
Genetic engineering

Oxford NANOPORE Technologies

Why implement a new plasmid sequencing workflow?

The power of complete plasmid sequencing, in the palm of your hand





Key Features	Sanger sequencing	Nanopore sequencing
Verify if plasmid insert and backbone are free of mutations	✗	✓
Avoid the need for primers and complicated primer walking	✗	✓
Resolve repetitive regions, dimers and deletions	✗	✓
Works across range of plasmid/BAC sizes and complexities	✗	✓
Simple end-to-end workflow with rapid turnaround time	✗	✓
Keep process in-house, ensuring data remains secure	✗	✓

Oxford NANOPORE Technologies

Case study: highly accurate, cost-effective nanopore-based plasmid sequencing

Generating complete maps of synthetic DNA constructs within 24 hours at a competitive cost

- Robust, full-length, accurate and contiguous plasmid assemblies
- Scales to large sample numbers at lower cost than Sanger sequencing
- Nanopore sequencing works across wide range of plasmid sizes and complexities, whereas short-read approaches struggle to resolve large repetitive regions

“The Sanger median per-base error rate was 0.4%, approximately a 10-fold higher rate than our Nanopore assembly approach”

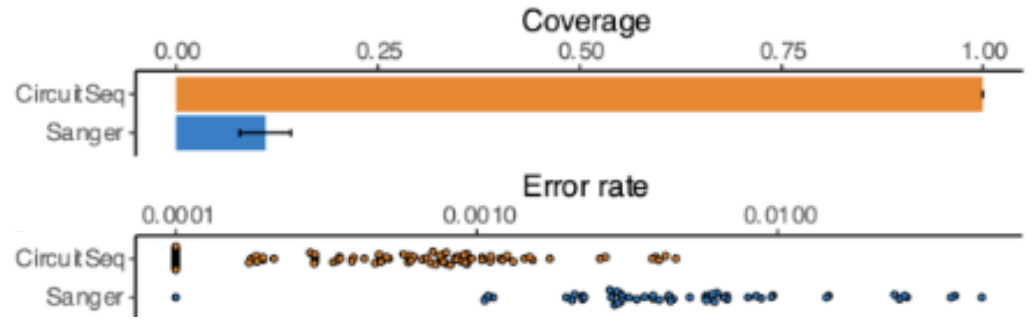
ACS Synthetic Biology

pubs.acs.org/synthbio

Letter

Multiplexed Assembly and Annotation of Synthetic Biology Constructs Using Long-Read Nanopore Sequencing

Francesco E. Emiliani,* Ian Hsu, and Aaron McKenna*



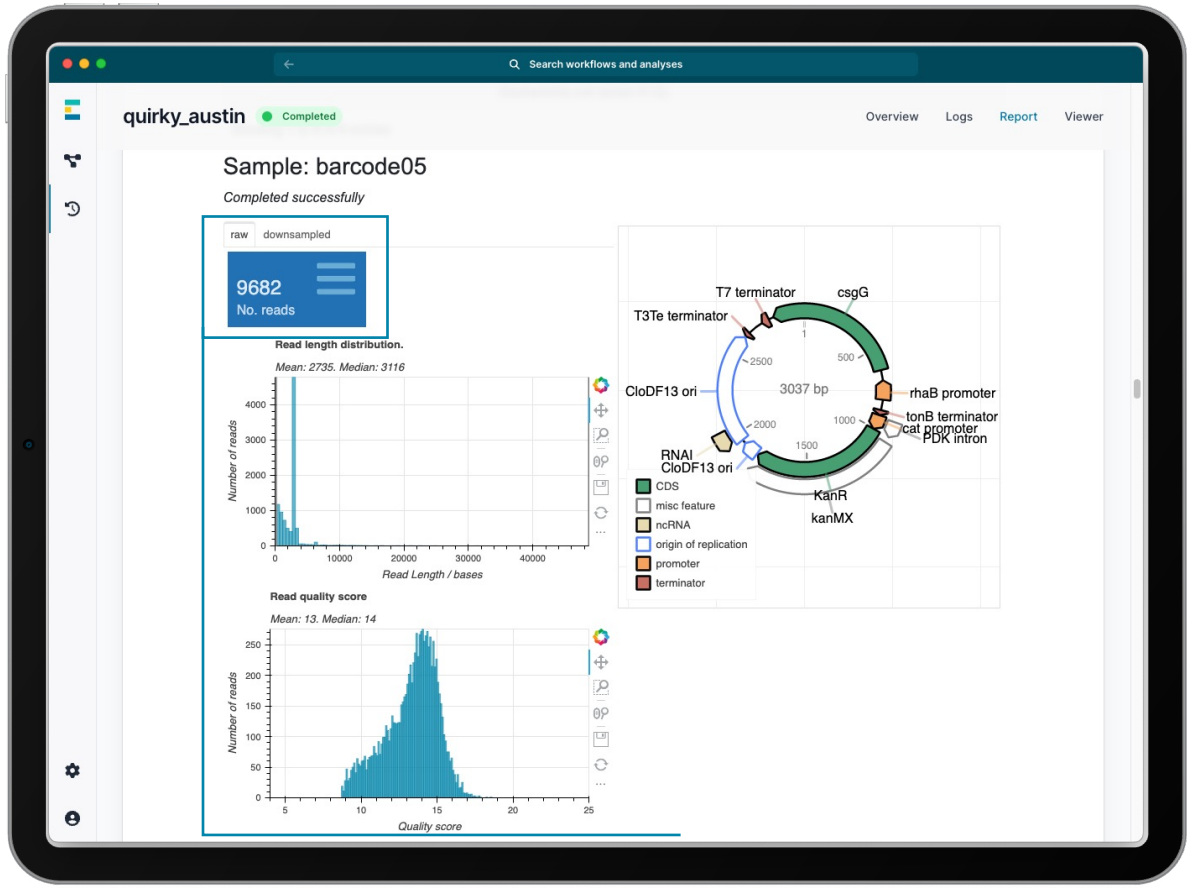
From Fig.1. Top: Proportion of the plasmid covered by nanopore-based assemblies (orange) and Sanger sequencing (blue). Bottom: the median per-base error rate is lower for nanopore-based assemblies (orange) compared to Sanger (blue).

Emiliani, Francesco E., Ian Hsu, and Aaron McKenna. "Multiplexed Assembly and Annotation of Synthetic Biology Constructs Using Long-Read Nanopore Sequencing." ACS Synthetic Biology (2022).

Oxford NANOPORE Technologies

Example clone validation report in EPI2ME Labs

QC, reconstruction, and annotation of complete plasmids



Sample status

10 entries per page

Search...

Sample	pass/failed reason	Length
barcode01	Completed successfully	3097
barcode02	Completed successfully	3097
barcode03	Completed successfully	3097
barcode04	Completed successfully	7922
barcode05	Completed successfully	3037
barcode06	Completed successfully	10674
barcode07	Completed successfully	10674
barcode08	Completed successfully	10674
barcode09	Completed successfully	10674
barcode10	Completed successfully	6280

Insert sequences

This table shows which primers were found in the consensus sequence of each sample and where the inserts were found.

10 entries per page

Search...

Sample	start	end	primer	strand	Insert length
barcode01	2726	818	pRham	-	1189
barcode02	2737	829	pRham	-	1189
barcode03	991	2180	pRham	-	1189
barcode05	2603	695	pRham	-	1129
barcode06	4111	615	T7	-	7178
barcode07	5142	1646	T7	-	7178
barcode08	2352	9530	T7	+	7178
barcode09	5134	1638	T7	-	7178
barcode10	2345	5131	T7	+	2786

rhaB promoter	snapgene	100.0%	100.0%	promoter of the E. coli rhaBAD operon, conferring tight induction with L-rhamnose and repression with D-glucose in the presence of RhaR and RhaS (Giacalone et al., 2006)	699	818	119	-1
cat promoter	snapgene	100.0%	100.0%	promoter of the E. coli cat gene encoding chloramphenicol acetyltransferase	914	1005	91	1
KanR	snapgene	99.4%	100.0%	aminoglycoside phosphotransferase; aph(3')-Ia; confers resistance to kanamycin in bacteria or G418 (Geneticin®) in eukaryotes	1005	1821	816	1
CloDF13 ori	snapgene	100.0%	92.3%	Plasmids containing the CloDF13 (CDF) origin of replication can be propagated in E. coli cells that contain additional plasmids with compatible origins.	1949	2631	682	1
T7 terminator	snapgene	100.0%	100.0%	transcription terminator for bacteriophage T7 RNA polymerase	2764	2812	48	-1

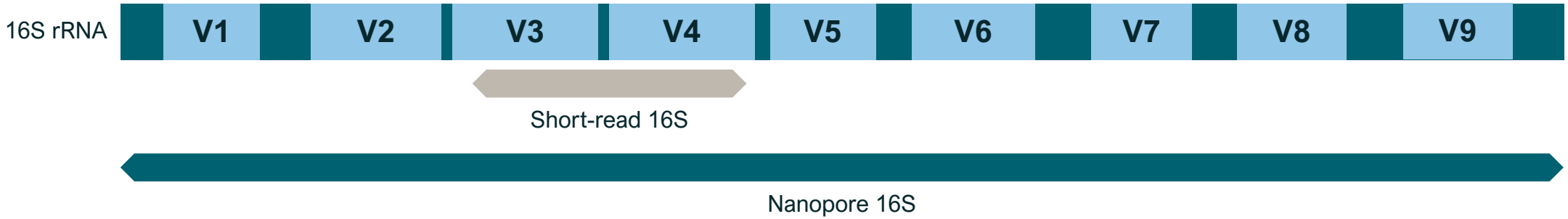
Oxford NANOPORE Technologies

Full-length 16S microbial identification

Oxford NANOPORE Technologies

Why implement nanopore sequencing for 16S analysis?

Full-length 16S rRNA sequencing improves taxonomic resolution of microbial communities



Key features	Sanger	Short-read	Oxford Nanopore
Read length	~500 bp	~150 – 300 bp	~1,500 bp
Resolves polymicrobial samples	✗	✓	✓
High taxonomic resolution[1]	✗	✗	✓
Rapid library preparation	✗	✗	✓
Real-time sequencing	✗	✗	✓

1. Zhang, et al. "The newest Oxford Nanopore R10. 4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling." Applied and Environ Microbio 89.10 (2023): e00605-23.

Oxford NANOPORE Technologies

Case study: Oxford Nanopore 16S sequencing with Q20+ chemistry

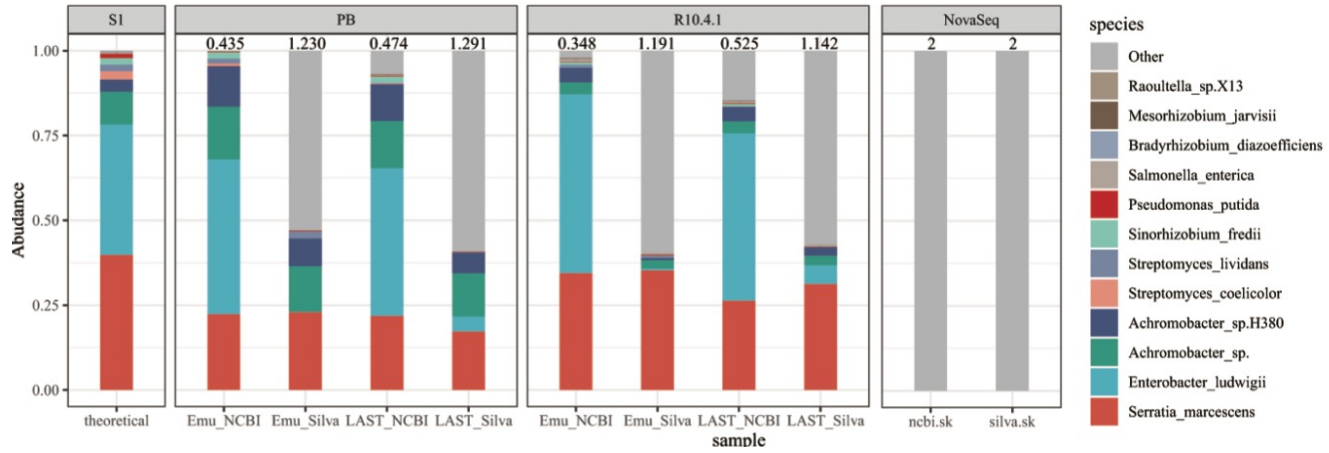
Accurate full-length 16S sequencing enables species-level profiling for environmental microbiota

- Authors assess the latest Q20+ chemistry for 16S experiments, generating raw read accuracy of ~99%
- Study highlighted that both Oxford Nanopore and an alternative long-read platform revealed similar microbial profiles, while short read sequencing had limited resolution for species-level identification

> *"Our findings show that the ONT R10.4.1 flowcell for full-length 16S* ***enabled species-level taxonomic identification for environmental samples****"*

The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling

AMERICAN SOCIETY FOR MICROBIOLOGY



From Fig. 2: Classification results on species levels with PacBio ('PB', left), Oxford Nanopore ('R10.4.1', middle), and short-reads ('NovaSeq', right) compared to abundance of sample ('S1'). 'Other' includes unclassified.

Zhang et al. "The newest Oxford Nanopore R10. 4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling." Applied and Environ Microbio 89.10 (2023): e00605-23.

Oxford NANOPORE Technologies

Bacterial isolate whole genome sequencing and antimicrobial resistance profiling

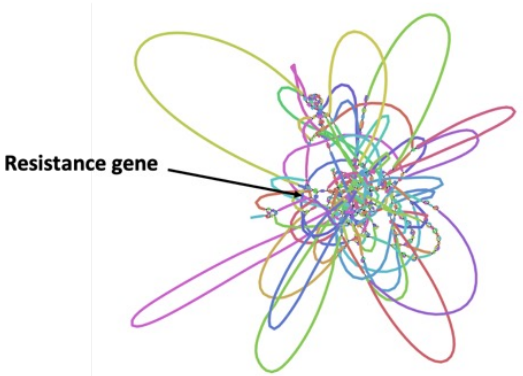
Oxford NANOPORE Technologies

Generating closed, high-quality bacterial genome and plasmid assemblies

- Most bacterial genome assemblies produced with short-read sequencing technologies are fragmented and incomplete[1]

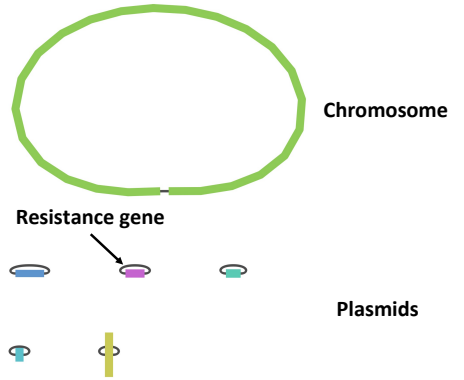
- Nanopore sequencing improves microbial genome assemblies, with the ability to:
  - Span repeat-rich sequences (characteristic of AMR genes) and structural variants
  - Determine which genes are on plasmids and which are on the chromosome[2]
  - Distinguish bacterial from plasmid transmission in healthcare settings to target interventions
  - Understand mechanisms and drivers of antimicrobial resistance for outbreak investigation

Short-read genome assembly



- ✗ Complete bacterial genome
- ✗ Resolve plasmids
- ✗ Locate AMR genes

Nanopore genome assembly



- ✓ Complete bacterial genome
- ✓ Resolve plasmids
- ✓ Locate AMR genes

1. Koren and Phillippy. "One chromosome, one contig: complete microbial genomes from long-read sequencing and assembly." Current opinion in microbiology 23 (2015): 110-120.
2. Figure from Dr. Kimberlee Musser, Wadsworth Center, NYSDOH, 'Improving bacterial disease public health testing with nanopore sequencing' presented at NCM 2023

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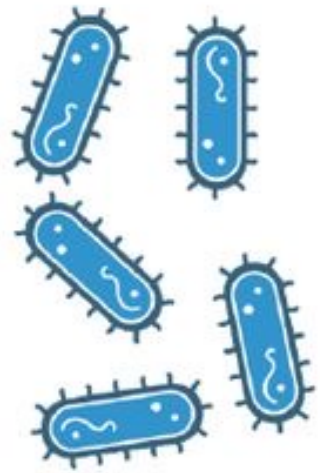
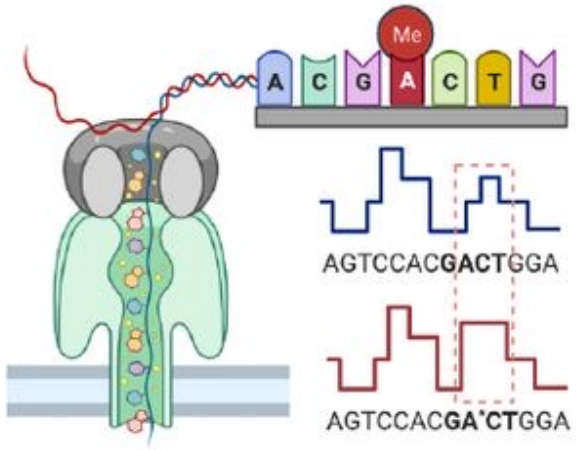
Updated basecalling models boost accuracy further in bacterial genomes

- Bacterial genomes contain different types of DNA methylation, such as 6mA, 4mC, 5mC
- New ONT basecallers have been trained on representative microbial samples, improving consensus accuracies further

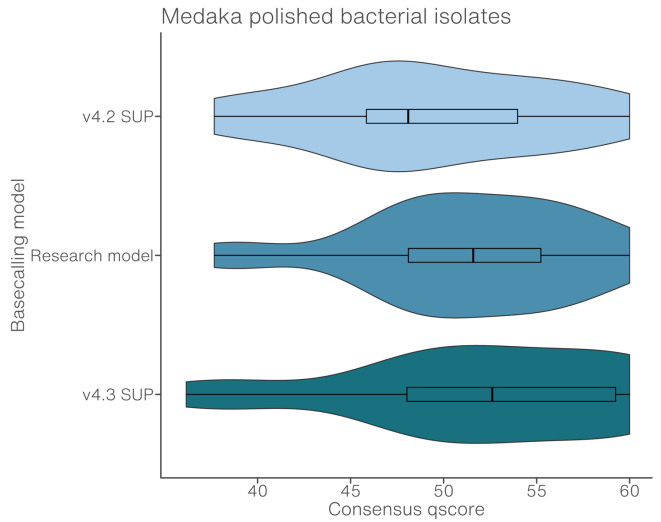
Bacterial genomic DNA contains modified bases



Nanopore sequencing distinguishes between canonical and modified bases[1]



New methylation-aware basecalling models produce higher consensus accuracy



1. Figure adapted from Crits-Christoph, et al. "MicrobeMod: A computational toolkit for identifying prokaryotic methylation and restriction-modification with nanopore sequencing." bioRxiv (2023)

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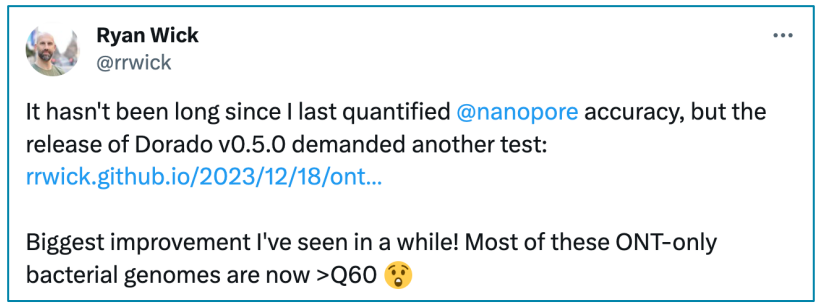
Community results: benchmarking nanopore-only bacterial genome accuracy

- Dr. Ryan Wick, Bioinformatician at the Doherty Institute, benchmarked bacterial genome accuracy using recently updated basecalling models
- Nanopore-only genome assemblies were compared to reference genomes
- Findings indicate that latest basecalling model yields large improvement over previous models in bacterial genome consensus accuracy

> *"Biggest improvement I've seen in a while! Most of these ONT-only bacterial genomes are now >Q60"*

View the full blog post at: https://rrwick.github.io/2023/12/18/ont-only-accuracy-update.html



Genome	Q score
Campylobacter jejuni	Q55.5
Campylobacter lari	Q49.2
Escherichia coli	Q67.2
Listeria ivanovii	Q57.7
Listeria monocytogenes	Q∞
Listeria welshimeri	Q64.5
Salmonella enterica	Q62.0
Vibrio cholerae	Q63.2
Vibrio parahaemolyticus	Q64.1
Average	**Q59.3**

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“Pathogen-agnostic” metagenomics

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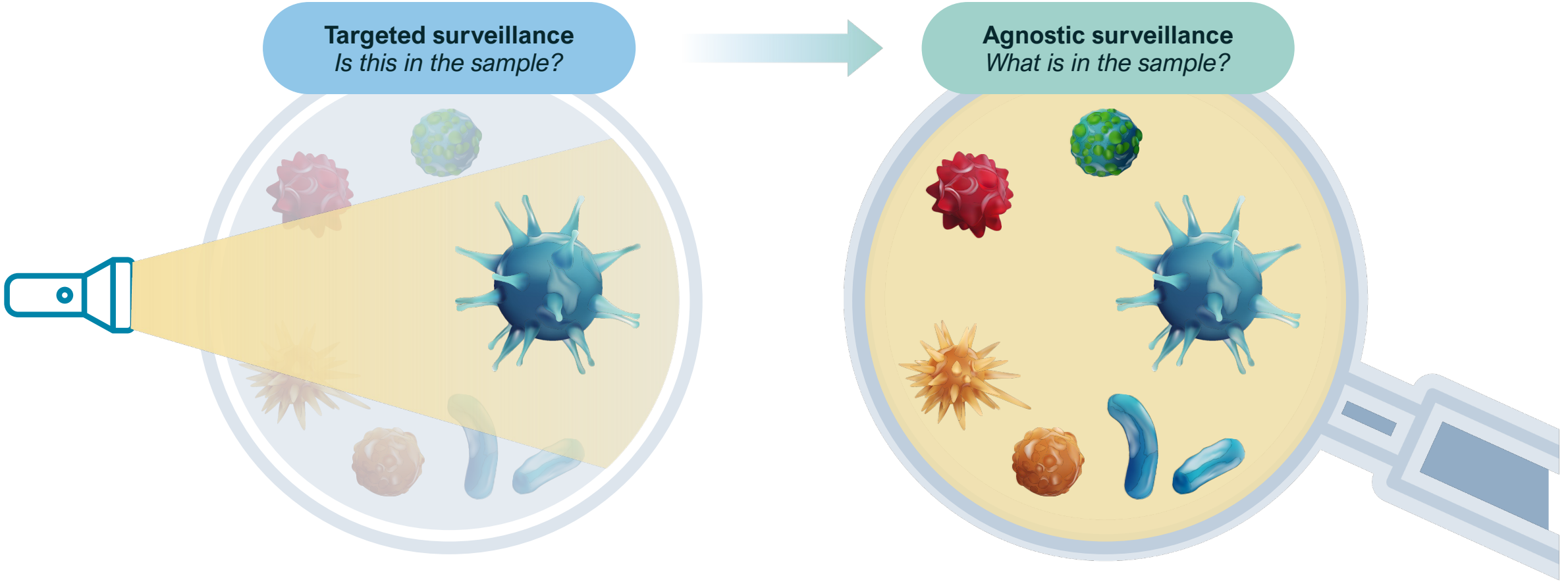
“Pathogen-agnostic” metagenomics

Nanopore sequencing is paving the way for rapid, unbiased pathogen surveillance methods

Today, pathogen surveillance strategies typically rely on retrospective investigations targeting known or suspected pathogens

Going forward, the field aims to move towards more agnostic sequencing methods for broad, real-time pathogen monitoring



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Metagenomics

The analysis of all genetic material present within a sample enables unbiased microbial characterisation

Standard approaches for identifying microbes, such as PCR, typically rely on targeting a limited number of specific organisms

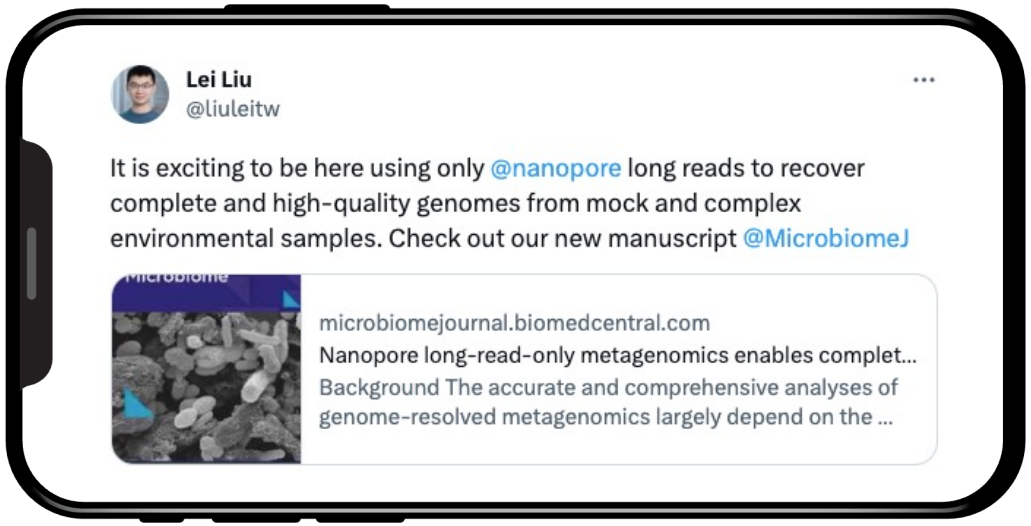
However, metagenomic approaches sequence all genetic material in a sample which can contain many different microbes, enabling users to:

- Detect known and unknown viruses, bacteria and fungi in a ‘hypothesis-free’ manner
- Detect novel variants of known pathogens that escape routine detection methods
- Extract virulence and resistance information in the same assay
- Deliver rapid results in situations where an urgent public health response may be required

New infections with significant public health impact continue to emerge, necessitating fast and comprehensive approaches for surveillance to detect rare, novel or emerging pathogens

Case study: nanopore-only metagenomics for complete, high-quality MAGs

- Lie et al. (2022) reconstructed reference-quality genomes from complex metagenomes using nanopore-only long reads
- In complex activated sludge (AS) samples, **275 MAGs were reconstructed** with median completeness of 90%
- Median N50 of MAGs was 735 Kb, which was **44 – 86X improvement compared to short-read-based MAGs**
- **Median contig number in each MAG was 9**, indicating long reads greatly closed gaps, obtained near-complete genomes



	Short-reads	Nanopore
Contigs N50 (Kb)	3.4	**103.9**
No. of HQ-MAGs	8	**94**
Median contig count per MAG	456	**9**
Median N50/MAG (Kb)	9	**735**

Liu et al. "Nanopore long-read-only metagenomics enables complete and high-quality genome reconstruction from mock and complex metagenomes." Microbiome 10.1 (2022): 209.

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04

Applications:
Whole Genome Sequencing

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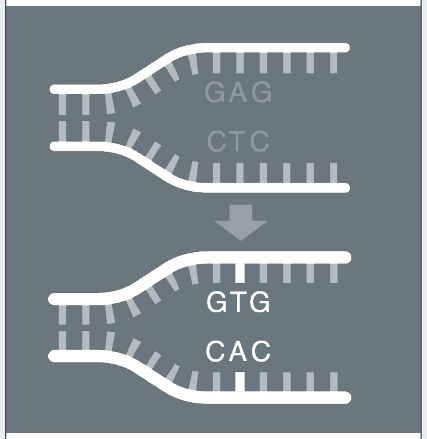
More comprehensive genomic insights

Traditional short-read SBS is limited to SNVs and indels

Nanopore sequencing provides this same information……with more added features, including any-length fragments, native DNA/RNA

01

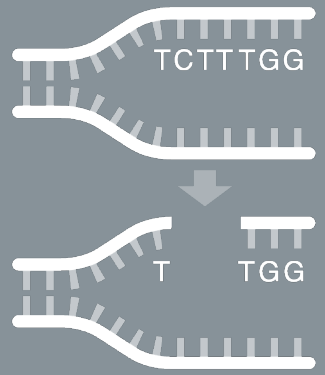
Single nucleotide variants



e.g. sickle cell disease

02

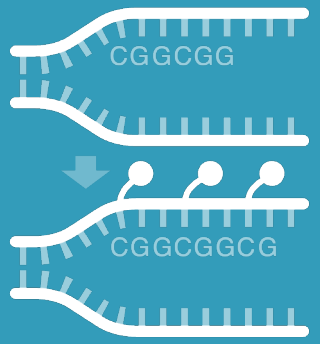
Insertions / Deletions



e.g. cystic fibrosis

03

Methylation in real time



e.g. fragile x syndrome

DNA methylation patterns are globally disrupted in cancer

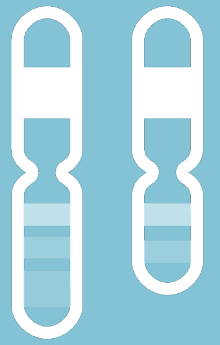
04

Structural variation

e.g. Alzheimer's, Parkinson's, Prader-Willi syndrome

05

Complex copy number variation



e.g. autism, schizophrenia, ADHD

Nanopore technology was used to assemble the largest animal genome to date, 30% larger than the previous assembly record of axolotl salamander

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Genome size

43 Gb

=

14 human genomes (3.2 Gb)

~90%

repeat content

vs

~50%

repeat content

"To overcome the challenges to sequence and assemble the even larger genomes of lungfish we used long and ultra-long read Nanopore technology"

Meyer et al. Nature. DOI: https://doi.org/10.1038/s41586-021-03198-8 (2021)

Ultra-long nanopore reads central to completing the human genome

Complete genome assembly of human haploid cell line CHM13

- Repeats (e.g. ribosomal DNA units, centromeric satellites, segmental duplications) comprise majority of gaps in GRCh38 reference
- Repetitive regions unresolvable with other technologies
- Ultra-long nanopore reads required for genome completion
  - E.g. 450 kb single read, spanning ribosomal DNA array on chr15
- Implications for genetic diseases

'Compared with GRCh38, T2T-CHM13 is a more complete, accurate, and representative reference for both short- and long-read variant calling across human samples of all ancestries'

Nurk *et al.*, 2022

Nurk *et al*., Science. https://doi.org/10.1126/science.abj6987 (2022)

Nanopore sequencing of human genomes provides a comprehensive view of haplotype-resolved variation and methylation

Efficient and scalable wet-lab and computational protocol for 4,000 samples

NIH National Institute on Aging

- Short-read sequencing methods struggle to detect structural variants (SVs) in repetitive regions
- Here, Kolmogorov *et al.* share an **end-to-end**, nanopore-based protocol which could be *'a genuine alternative to short-reads for large-scale… projects'*, with **lower cost** and **greater throughput** than alternative long-read platforms

'Using a single PromethION Flow Cell, we can detect SNPs with F1-score better than…short-read sequencing'

Kolmogorov *et al.*, 2023

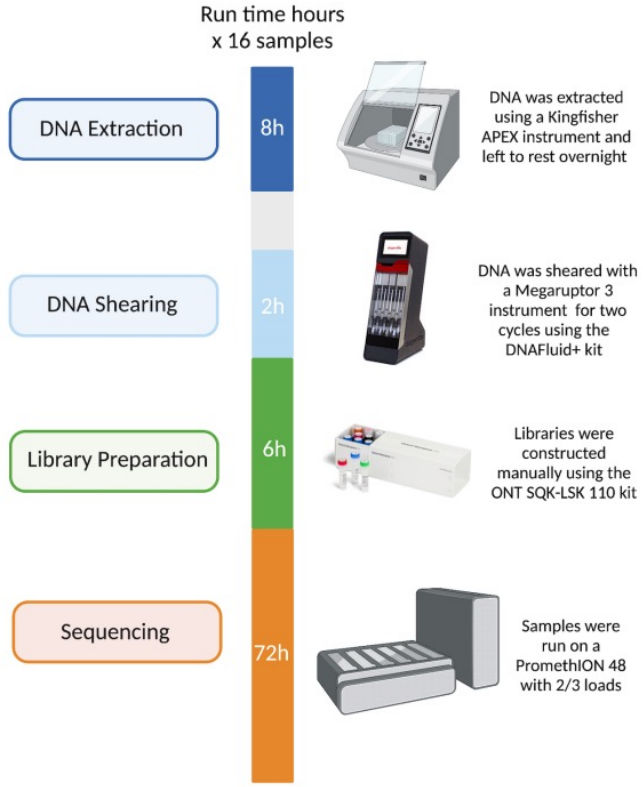

Figure 1. Single flow cell Oxford Nanopore Technologies (ONT) sequencing protocol. Adapted from: https://doi.org/10.1101/2023.01.12.523790

Kolmogorov et al.
bioRxiv
https://doi.org/10.1101/2023.01.12.523790
(2023)

05

Applications:
Adaptive Sampling

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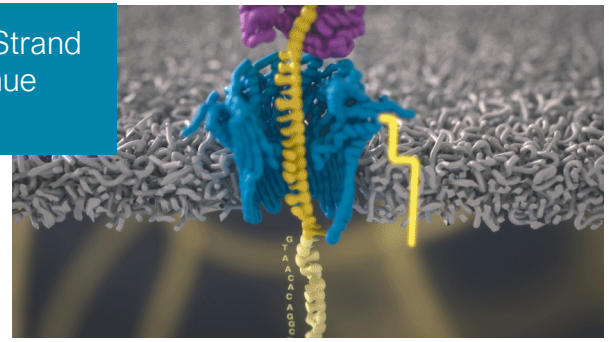
Adaptive sampling: targeted sequencing included

No additional sample prep needed

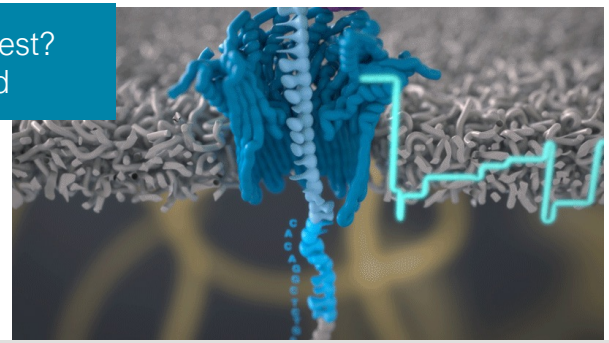
- **Software-controlled** enrichment/depletion of targets
- **Real-time**, any target number
- Enrich for **large regions** of interest or remove contamination
- You only need
  - Reference file (FASTA format)
  - Optional: coordinates for enrichment/depletion (bed format)

More at nanoporetech.com/resource-centre/adaptive-sampling-oxford-nanopore

Region of interest? Strand allowed to continue sequencing



Not region of interest? Strand rejected



Adaptive sampling: accept or reject strands based on their sequence

Case study: AS host depletion for pathogen and antimicrobial resistance detection

Nanopore Adaptive Sampling (AS) host depletion workflow provides 100% sensitivity, with 4.5-hour turnaround

- Traditional metagenomic sequencing approaches face the problem of high host:microbial DNA ratios, lowering the resolution of detection
- Here, adaptive sampling-mediated host depletion in metagenomic samples provided:
  - 8-fold enrichment in microbial sequencing yield
  - Real-time bacterial species identification and antimicrobial resistance (AMR) gene prediction
  - 100% specificity & sensitivity for pathogen detection
  - Turnaround time just 4.5 h

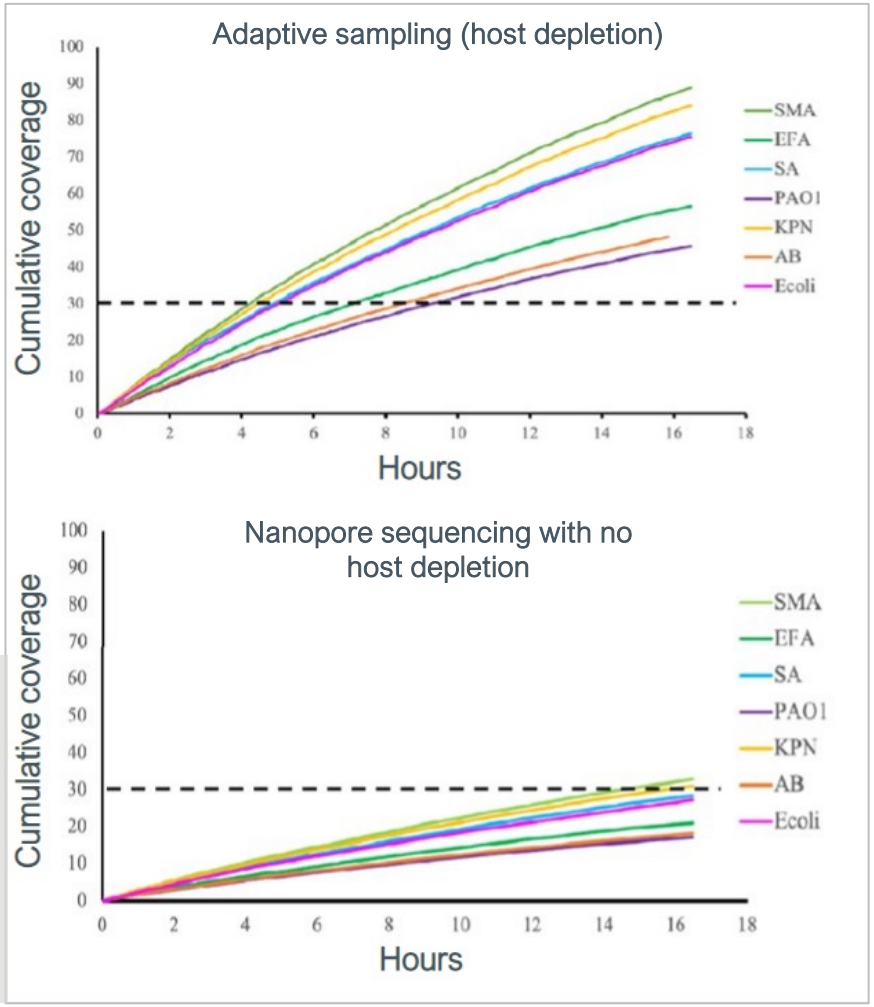


Figure: Cumulative depth of coverage over time for 7 microorganisms: *S. marcescens* (SMA), *E. faecalis* (EFA), *S. aureus* (SA), *P. aeruginosa* (PAO1), *K. pneumoniae* (KPN), *A. baumannii* (AB), and *E. coli*, with adaptive sampling and nanopore sequencing without adaptive-sampling-mediated depletion. Sufficient depth (i.e., >30× coverage) achieved after just 4.5 h of sequencing with adaptive sampling.

Cheng et al.
bioRxiv.
https://doi.org/10.1101/2022.07.03.22277093
(2022)

06

Applications:
Direct RNA Sequencing

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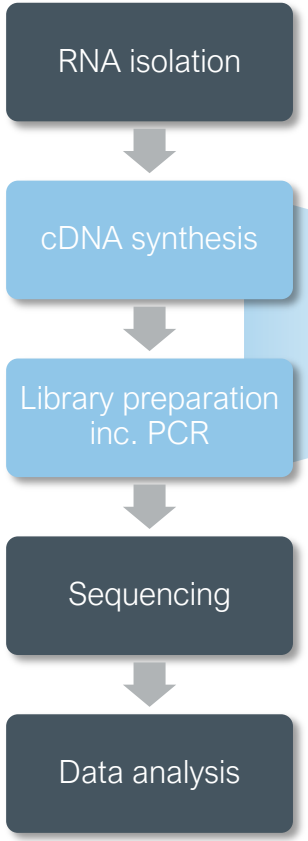
cDNA synthesis-based RNA sequencing cannot reveal all

Customers often highlight common challenges…



Loss of base modification data and poly(A) tail insights. Incomplete isoform mapping.

PCR bias: inaccurate expression quantification and downstream analyses

Cost- and time-consuming (batching required)

No real-time results

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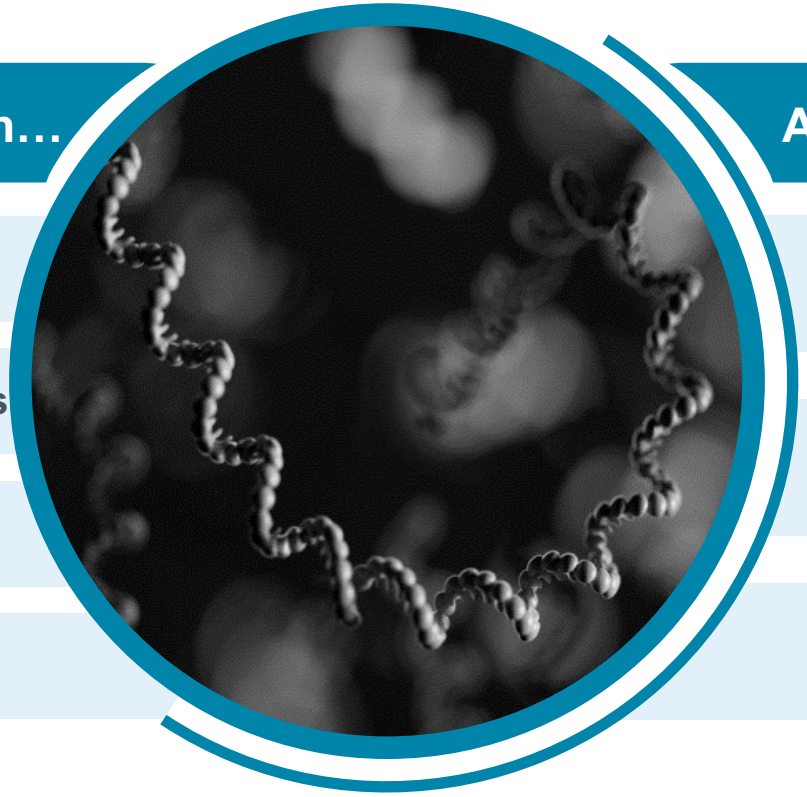
Direct, cDNA-free RNA sequencing reveals more biology

Uncover previously unseen biological insights

Reveal missing biology through…

- **RNA base modification** detection
- Highly accurate 3’ **poly(A) tail insights**
- Complete **isoform mapping**
- **Transcript quantitation**



Anyone, anywhere - Why wait?

- **Richer insights:** Highly-accurate, native RNA data
- **Faster, real-time results** from near-sample without batching
- **Accessible and affordable**, with scalability for more use cases

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07

Single-cell RNA Sequencing

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Obtain full-length transcripts from single cells with nanopore technology

Compatible with the most widely used single-cell sample preparation approach

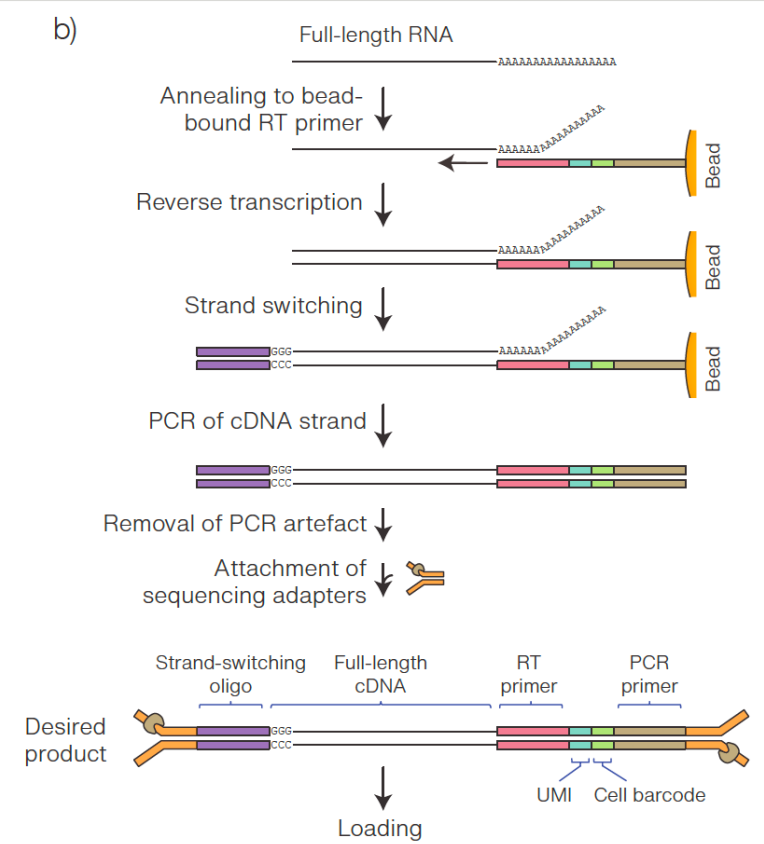


Figure: Diagram of 10x + Nanopore single-cell preparation protocol.
View poster: nanoporetech.com/resource-centre.

- Generate full-length cDNA from 10x Genomics libraries by skipping the fragmentation step required with short-read approaches
- Prepare full-length cDNA for nanopore sequencing
- Typical output:
  - ~100 M reads per PromethION Flow Cell
  - ~80 M cell & UMI assigned reads

Full-length isoforms can reveal isoform switching during cell development

Many isoform switch events cannot be identified using short-read approaches

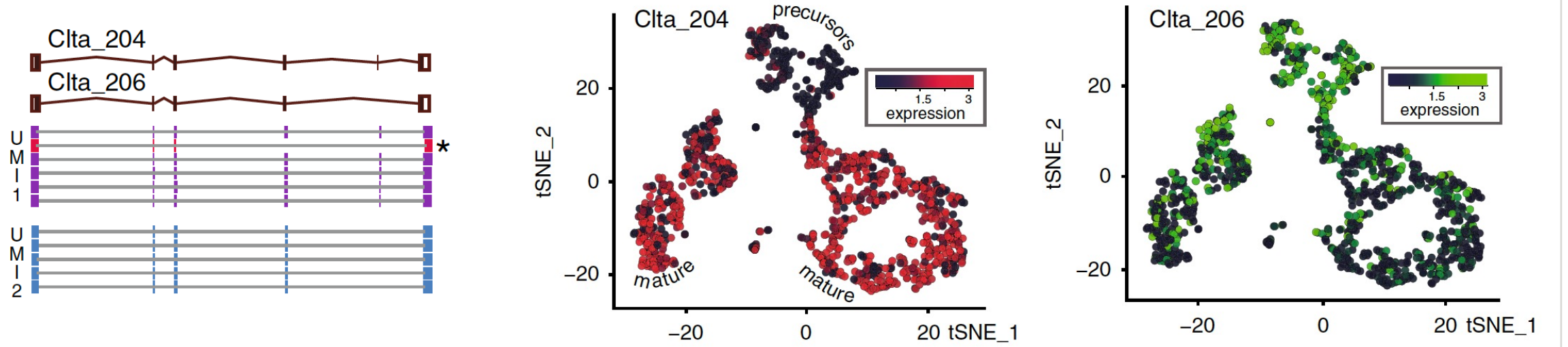


Figure: Differential isoform expression identified in mouse brain using single-cell nanopore data: full length isoforms obtained from nanopore data (left); Clta 204 is predominantly expressed in mature cells (middle); Clta 206 is predominantly expressed in radial glial precursor cells (right). Clta = Clathrin Light Chain A.

- 76 genes in mouse brain found to have differentially expressed isoforms between cell types
- Example: *Clathrin Light Chain A* (*CLTA)* isoforms 204 and 206 undergo isoform switch
- Finely tunes protein function for the intricacies of neuronal, and therefore brain, development

Lebrigand *et al*. (2020). Nature Communications. https://doi.org/10.1038/s41467-020-17800-6

Measure isoform expression in spatial context

- 10x Visium spatial libraries use the same barcodes as 10x single-cell libraries
- Demux of Visium libraries is supported by sockeye
- Long reads enable detection of isoforms, SNPs, fusions, etc from spatial libraries.
- In the published example (right) the mouse midbrain (red circle) predominantly expresses isoform Snap25-201 and not Snap25-202. These phenomenon are invisible to short reads in most cases

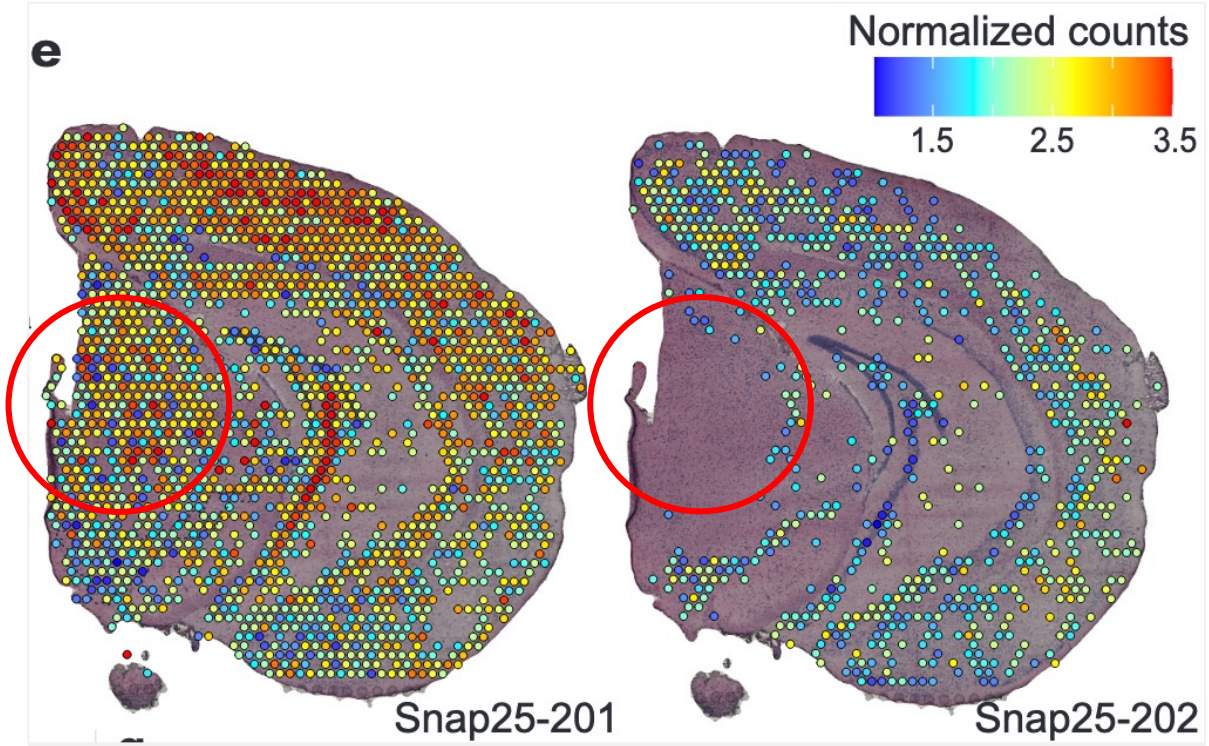


Figure adapted from Lebrigand *et al*. (2020). bioRxiv. https://doi.org/10.1101/2020.08.24.252296

Isoform-level single-cell nanopore sequencing in cancer research

Use nanopore data alone for simultaneous analysis of genotype and phenotype

'The discovery of cancer-specific genes using gene expression levels may only be revealing the tip of [the] iceberg of transcriptional diversities in cancer'

Shiau et al, 2023

- Nanopore-only pipeline:
  - scNanoGPS* analysis toolkit deconvolutes nanopore reads into single cells and single molecules, without short-read guidance
  - Simultaneous identification of cell-type-specific isoforms and gene expression profiles (phenotypes) and mutations (genotypes)

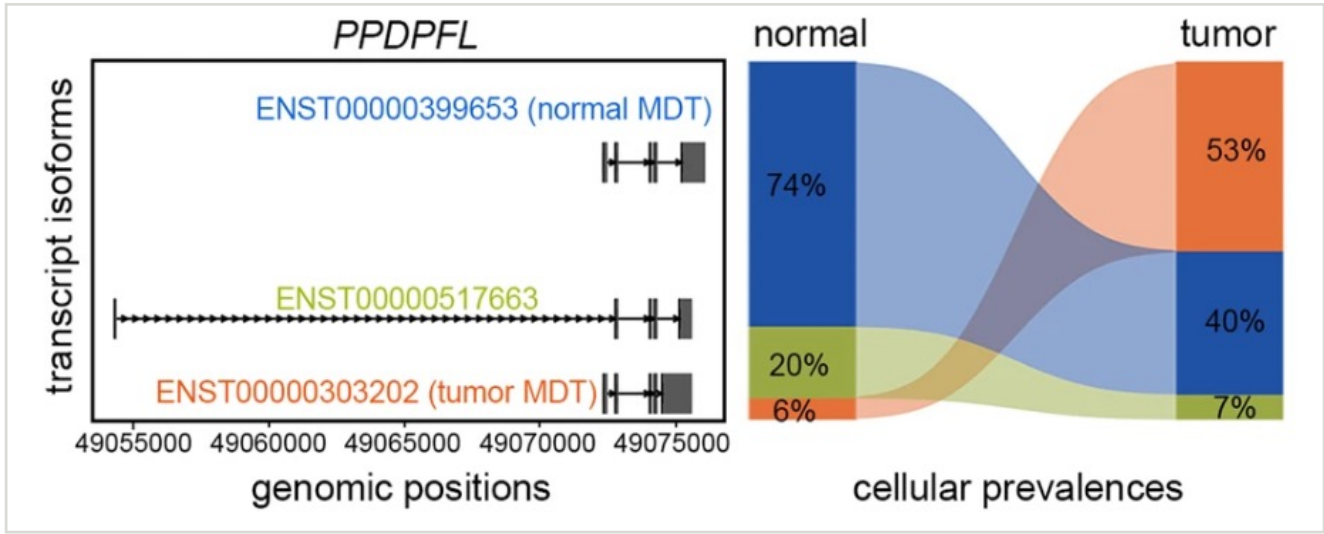


Figure: Isoforms of gene *PPDPFL* expressed in normal cells vs tumour single cells, revealing no significant differences at the level of gene expression, but significant differences in the underlying isoform breakdown (Adapted from: Shiau *et al*. 2023).

*single cell Nanopore sequencing analysis of Genotypes and Phenotypes Simultaneously

Shiau et al.
bioRxiv.
https://doi.org/10.1101/2023.01.24.525264
(2023)

Oxford Nanopore solution for 10x Genomics single-cell and spatial sequencing

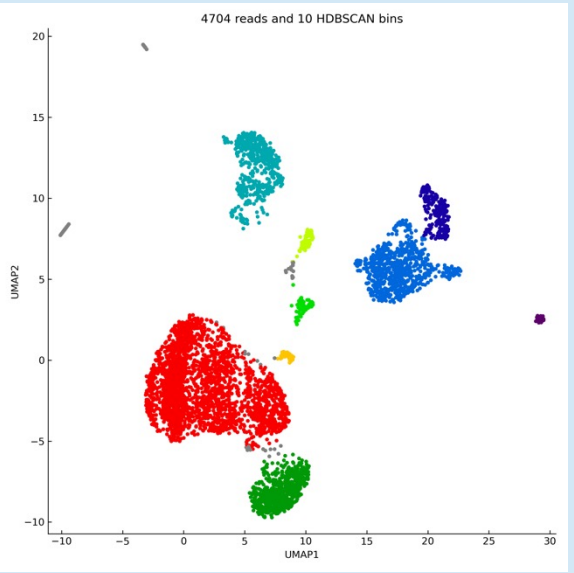
Sample and library preparation

Nanopore sequencing

Data analysis

EPI2ME

wf-single-cell



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Thank you