Accelerate your epigenetics research with Oxford Nanopore sequencing

Direct detection of DNA and RNA methylation

Legacy sequencing methods rely on short reads and laborious bisulfite or enzymatic conversion steps, which have inherent limitations, including the inability to phase modifications and incomplete conversion. Oxford Nanopore sequencing preserves these modifications and directly **captures and phases long-range epigenetic features, structural variants (SVs), single nucleotide variants (SNVs), and repeats** — all within a single dataset.

Uncover the complete methylome

With Oxford Nanopore sequencing:

- Capture 28 million CpG sites in the human genome
- Detect DNA (5mC, 5hmC, 6mA) and RNA (m^6A, m^5C, inosine) modifications at single-nucleotide resolution
- Phase genetic and epigenetic variants and identify differentially methylated regions (DMRs)

	Oxford Nanopore sequencing	Short-read sequencing (EM-seq or WGBS)	PacBio HiFi 5-base sequencing	Methylation arrays
SNVs	✓	✗	✓	✗
SVs	✓	✗	✓	✗
Genome-wide coverage	✓	Limited coverage in GC-rich regions	✓	✗
All context 5mC	✓	✓	Limited to CpG context	✗
Detect other base modifications	5hmC, 6mA, m^6A, m^5C, inosine	Requires further library prep	✗	✗

Regions with high-GC content can be challenging to sequence with EM-seq

Figure 1A demonstrates that Oxford Nanopore sequencing maintains consistent coverage of GC-rich regions compared with EM-seq (Figure 1B), which exhibits drop-outs that align with high-GC areas in Figure 1C. This demonstrates that enzymatic-based methods (e.g. EM-seq) struggle with converting GC-rich regions due to high repeat content, leading to uneven genome coverage. Furthermore, bases may also undergo more conversion events, leading to ambiguities in mapping and diminished coverage.

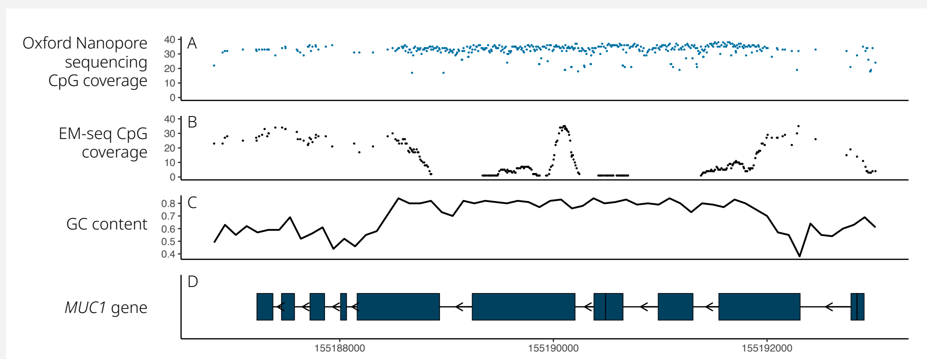


Figure 1. Comparison of CpG coverage across a high-GC region in the *MUC1* gene.
A) and B) Each dot indicates a CpG site sequenced by Oxford Nanopore technology or EM-seq, respectively.
C) The GC content curve quantifies the percentage of guanine and cytosine of the *MUC1* gene.
D) Gene promoters in *MUC1* gene.

Accurately capturing methylation

In humans, CpG methylation levels cluster near 0% (hypomethylated) or 100% (hypermethylated), creating a bimodal distribution. Oxford Nanopore sequencing, whole-genome bisulfite sequencing (WGBS), and EM-seq data capture this pattern, while raw PacBio data (PacBio_count) skews toward the centre, reflecting false positives and negatives. A methylation-based correction (PacBio_model) partially restores the bimodality, but it still differs from the true distribution seen with other methods.

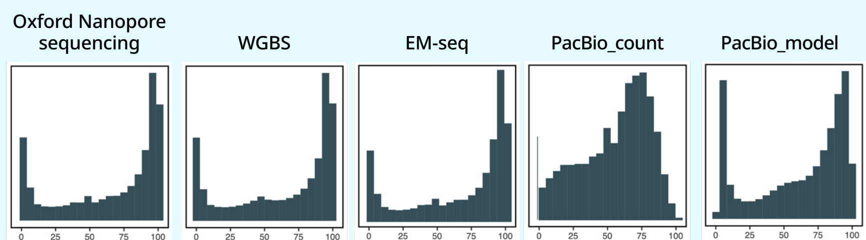
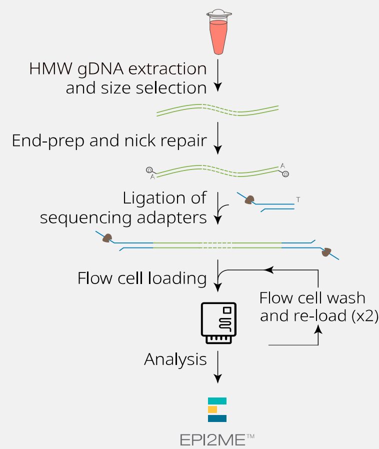


Figure 2. Comparison of methylation patterns in HG002.

The human variation end-to-end workflow

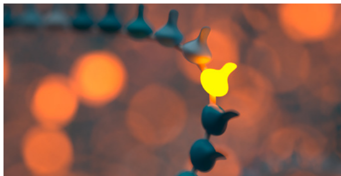
- SNV and indel detection
- SV (copy number variation, repeat expansions, large indels, inversions, and duplications) detection
- Genome-wide methylation (5mC, 5hmC, 6mA, 4mC*) detection
- Haplotype phasing
- Complete assembly

*Coming soon

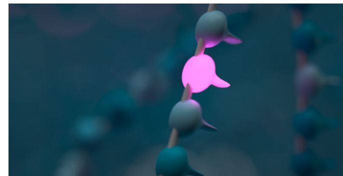


View the workflow overview

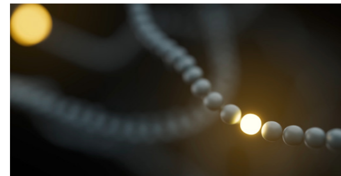
Highly accurate and comprehensive base modification analysis



5mC >99.7%



5hmC >96%



Plus, other modifications

View the benchmarking data

Solving the parent-of-origin effect in retinoblastoma

Retinoblastoma is a common paediatric eye cancer that has variable clinical outcomes depending on which parental allele of the *RB1* gene the mutation resides, known as the parent-of-origin effect[1,2]. A paternally inherited *RB1* variant is associated with a higher likelihood of developing bilateral tumours and secondary cancers in later life than a maternally inherited variant[3–5], highlighting the importance of determining the parent of origin to inform treatment plans.

The *RB1* gene has a DMR that varies between parental alleles, meaning it can be used to assign the parent of origin. Stacey *et al*. used Oxford Nanopore sequencing to target a region of the *RB1* gene containing the DMR and the pathogenic variant. The team captured both genetic and epigenetic information with long nanopore reads, enabling the variant to be analysed and phased with the DMR in a single sequencing run.

In this study, all variants — both inherited and *de novo* — were assigned a parent of origin without familial DNA samples. This research demonstrates that Oxford Nanopore sequencing can provide both epigenetic and genetic data in a single sequencing run, revealing a potentially important prognostic biomarker for this disease.

Find out more about methylation detection with Oxford Nanopore:
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