Rapid identification of pathogenic variants and methylation with whole-genome Oxford Nanopore sequencing

Up to 30% of children with a rare disease die before their fifth birthday[1]. With most rare conditions having a genetic cause, the timely identification of pathogenic variants is vital. Whole-genome sequencing (WGS) is central to this task, revealing information beyond what is possible with exome sequencing. However, the fixed run times and sample batching requirements of legacy short-read technology extend turnaround times or result in high costs when runs must be dedicated to single samples. The technique leaves behind challenging regions such as structural variants (SVs) and repeat expansions and cannot capture epigenetic modifications without additional sequencing runs. Furthermore, the lack of phasing information hinders the identification of compound heterozygosity.

Oxford Nanopore WGS enables faster-than-ever sample-to-answer turnaround times. Through streamlined sample preparation, real-time, on-demand sequencing, and a simple data analysis workflow, it is possible to go from sample to variant information in 24 hours. Crucially, PCR-free nanopore sequencing produces unrestricted read lengths and preserves epigenetic modifications. This enables thorough calling and phasing of small and large variants across the genome — from single nucleotide variants (SNVs) to complex SVs and repeat expansions — as well as capturing methylation information from a single dataset. The sample-to-answer workflow delivers comprehensive results in a rapid time frame, with no need for compromise.

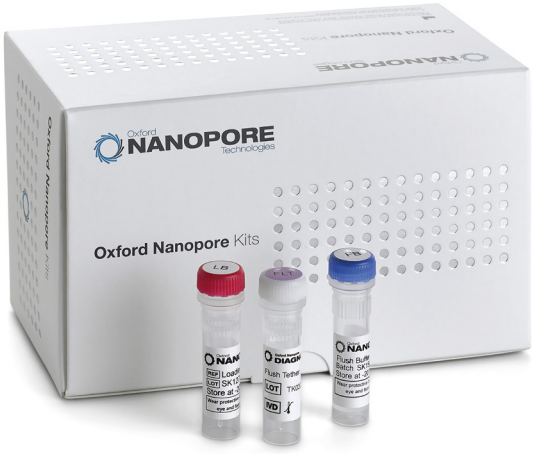
Here we present an end-to-end workflow for rapid whole-genome human variant and methylation calling from a blood research sample using high-output DNA sequencing on PromethION™ 24.

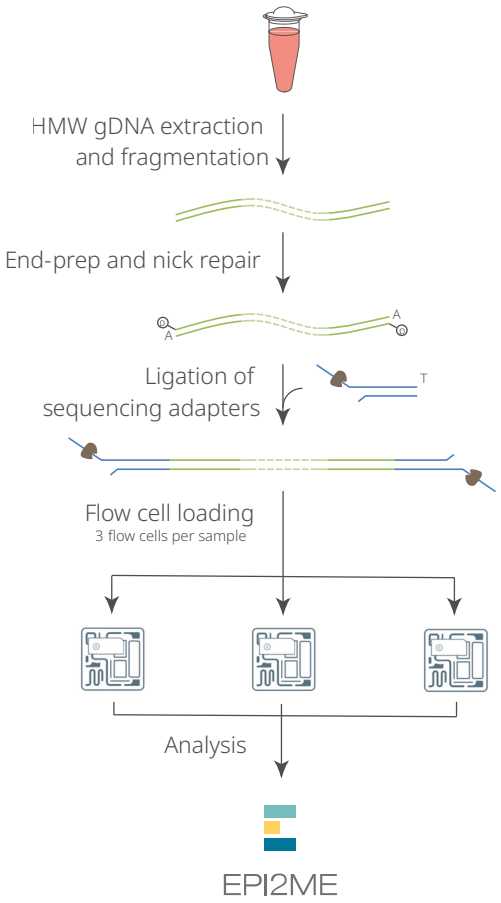
Extraction and library prep:
preparing samples for high-output sequencing

Learn more about extraction, sample handling, and QC: **nanoporetech.com/documentation/prepare**

First, extract high-molecular-weight DNA from your blood research samples using the **QIAGEN Puregene Blood Kit**. Next, use a **Diagenode Megaruptor 3** to perform light shearing of the extracted DNA, optimising for both long reads and high outputs in sequencing. The protocol also provides guidance on how to quality check your sample at each stage.

To prepare your extracted genomic DNA for nanopore sequencing, use the **Ligation Sequencing Kit**. This PCR-free approach preserves long fragments of native DNA for sequencing, including intact epigenetic modifications. From sample collection to sequencing-ready library, this process takes ~4–6 hours.





Find out more about Oxford Nanopore library prep: **nanoporetech.com/prepare**

Sequencing:
rapidly generating high data outputs on PromethION 24

Find out more about PromethION 24:
nanoporetech.com/promethion-24

For high outputs in a rapid time frame, sequence each of your research samples on three **PromethION Flow Cells** simultaneously; you will have sufficient library from the previous step for all three runs. Using this method, it is possible to generate ≥30x depth of coverage of the human genome, with a read length N50 of ~30 kb, from just 13–16 hours of sequencing.

The **PromethION 24** device combines sequencing on up to 24 independent flow cells with powerful onboard compute. Even at capacity, the device keeps up with real-time basecalling of canonical and modified bases using the high accuracy (HAC) model, as well as reference genome alignment, further reducing turnaround times. Using **MinKNOW™**, the software that controls Oxford Nanopore devices, you can monitor the experiment in real time and stop sequencing when you have generated sufficient data.

Analysis:
capturing variants and methylation from a single dataset

Discover compatible tertiary analysis solutions:
nanoporetech.com/compatible-products-programme

The **EPI2ME™** data analysis platform from Oxford Nanopore provides bioinformatics workflows for all levels of experience. The EPI2ME human variation workflow, **wf-human-variation**, enables all-in-one whole-genome calling of SNVs, insertions/deletions (indels), SVs, short tandem repeat expansions, and copy number variants, plus phasing, alongside analysis of 5mC and 5hmC methylation in CpG contexts.

You can access wf-human-variation through an intuitive point-and-click interface or via the command line. As an input, combine the three BAM files of basecalled, aligned data produced by MinKNOW into one file per sample. From this data, EPI2ME will output interactive reports, VCF files of variants, a BEDmethyl file of methylation data, and QC metrics; you can also view the data using your preferred genome browser.

The files produced by EPI2ME are compatible with tertiary analysis partner solutions for variant prioritisation and interpretation. Oxford Nanopore is working with leading tertiary analysis partners to provide integrated analysis solutions in order to support the research of rare and undiagnosed diseases; we intend to expand our portfolio of end-to-end solutions in the future with the long-term vision to transform clinical applications.

Learn more about the human variation EPI2ME workflow: nanoporetech.com/EPI2ME-human-variation

View the end-to-end protocol: nanoporetech.com/24-hour-genome

References:

1. The Lancet Global Health. The landscape for rare diseases in 2024. *The Lancet Global Health.* 12(3):e341 (2024). DOI: https://doi.org/10.1016/S2214-109X(24)00056-1

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