# Comprehensive human genomic variant and methylation analysis with long Oxford Nanopore reads

Capturing disease-associated genomic variants and epigenetic modifications across the entire genome is vital for effective clinical research. However, the use of legacy short-read technologies restricts variant detection to regions that can be amplified, missing potential variants of interest. Furthermore, methylation must be indirectly detected via lengthy methods such as bisulfite conversion, giving incomplete results.

In contrast, long, native Oxford Nanopore reads enable comprehensive, direct detection of single nucleotide variants (SNVs), structural variants (SVs), short tandem repeat (STR) expansions, and methylation across the human genome — in a single sequencing run. From streamlined library prep to high-output sequencing on PromethION™ and simple data analysis, this scalable, end-to-end workflow ensures that previously hidden, potentially pathogenic variants are identified, accelerating your clinical research. Further downstream data interpretation can be facilitated through the use of integrated tertiary analysis tools from industry-leading providers.

**Here we present a streamlined workflow for whole-genome human variant and methylation calling from a blood research sample, using native DNA sequencing on PromethION 24.**

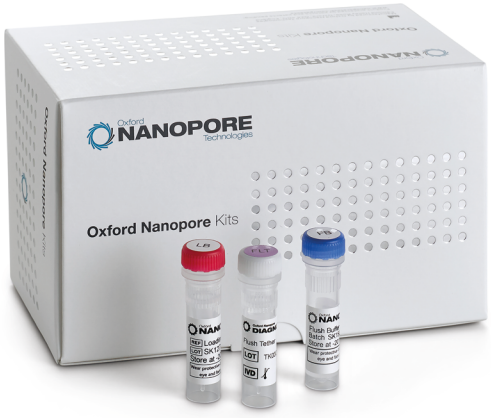
## Extraction and library prep:
preparing high molecular-weight DNA for sequencing

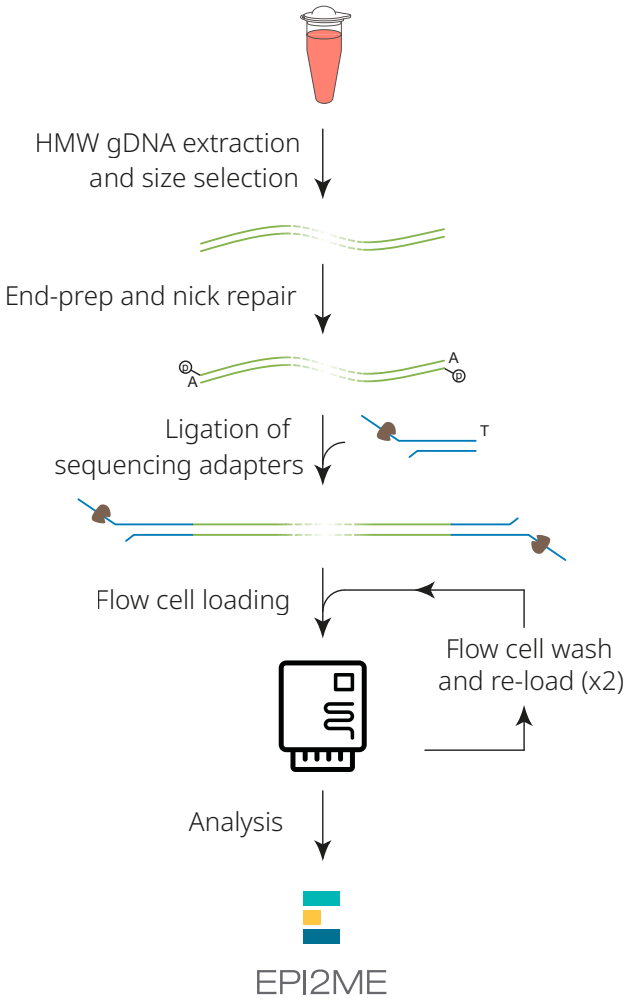
View extraction protocol recommendations, including for other research sample types such as buccal swabs and saliva: nanoporetech.com/extraction-methods

Extraction of high-quality, high molecular-weight DNA is critical to ensure high outputs of long reads in nanopore sequencing. We recommend extracting DNA from blood clinical research samples with the **QIAGEN Puregene Blood Kit**, then size-selecting for fragments >25 kb using the Oxford Nanopore **Short Fragment Eliminator Kit**.

While fragmentation is not a requirement for nanopore sequencing, light shearing can enhance sequencing output. For this, we recommend using the **Diagenode Megaruptor 3**, which produces a range of fragment lengths centred around ~30 kb.

Next, DNA samples are prepared for sequencing using the Oxford Nanopore **Ligation Sequencing Kit**. Optimised for high sequencing output, this PCR-free kit ensures native epigenetic modifications are retained, enabling their direct detection without the need for special library prep steps. Our end-to-end protocol also provides guidance on how to quality check your sample at each step.



## Sequencing:
generating high depth of coverage with PromethION 24

Learn more about PromethION devices:
nanoporetech.com/promethion

To call genomic and epigenetic variants across the human genome, we recommend sequencing your sample to ~30x depth of coverage; this can be achieved by sequencing on one **PromethION Flow Cell**. To maximise output, wash and reload the flow cell with fresh library twice using the Oxford Nanopore **Flow Cell Wash Kit**. You will have sufficient prepared library from the previous step for this process.

For high-throughput whole-genome sequencing and real-time basecalling, we recommend the **PromethION 24**, delivering sequencing on up to 24 independent PromethION Flow Cells and powerful onboard compute. For lower throughput needs, the **PromethION 2** devices provide PromethION-scale sequencing for any lab, with a compact design and sequencing on up to two flow cells.

## Analysis:
capturing variants and methylation from a single dataset

Discover streamlined data analysis with EPI2ME:
nanoporetech.com/epi2me

Data analysis begins with real-time basecalling and methylation calling using **MinKNOW™**, the software that controls Oxford Nanopore sequencing devices. We recommend using the high accuracy (HAC) basecalling mode.

Following basecalling, the EPI2ME™ **wf-human-variation** workflow provides all-in-one calling of SNVs, SVs, copy number variants, STR expansions, and methylation — covering both 5-methylcytosine and 5-hydroxymethylcytosine. The workflow also enables phasing of these variants. The **EPI2ME** platform provides data analysis solutions for all levels of experience, and can be run on a laptop, computer, cluster, or in the cloud. The workflow outputs a series of intuitive reports, VCF files listing variants, a BEDmethyl file containing methylation information, and QC metrics.

The results from the wf-human-variation workflow can be further explored by viewing in a track-based genome browser such as **IGV** and can be assessed for known pathogenicity through tertiary analysis software.

Oxford Nanopore is working with leading tertiary analysis partners to provide integrated analysis solutions to support the research of rare and undiagnosed diseases; we intend to expand our portfolio of end-to-end solutions in the future.

To support clinical research, the EPI2ME wf-human-variation workflow is being integrated with variant prioritisation and interpretation software solutions, with the long-term vision to transform clinical applications.

Learn more about nanopore-compatible data analysis solutions: nanoporetech.com/compatible-products-analysis

# Find out more at: nanoporetech.com/human-genomics

View the end-to-end protocol: nanoporetech.com/human-variation-sequencing-protocol

Oxford Nanopore Technologies

oxford-nanopore-technologies
@nanopore
@nanoporetech.com
**www.nanoporetech.com**

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