Assessing variants across the human genome with interaction-free sequencing on PromethION

View the protocol

This overview introduces a protocol for sequencing and analysing variants across a whole human genome, using a PromethION™ to deliver high sequencing output without the need for interaction with the sequencing device once the sample is loaded. Ideal for those who are new to Oxford Nanopore sequencing or wish to minimise hands-on time. This end-to-end method generates sequencing data with a read length N50 of ~10 kb and does not require washing or reloading of fresh library during sequencing — enabling a simple and flexible workflow.

This protocol can be used to detect single nucleotide variants (SNVs), copy number variants (CNVs), short tandem repeats (STRs), and structural variants (SVs) across the genome. In addition, because Oxford Nanopore sequencing does not require amplification, native DNA modifications, such as methylation, can be detected with no additional library prep.

Samples: human cell lines or tissue samples

1 Extract genomic DNA

Extract genomic DNA from human cell samples using the Puregene Cell Kit (QIAGEN)

Quantify each sample three times using a Qubit fluorometer

2 Shear DNA samples

Shear DNA using a g-TUBE (Covaris) — for a fragment length of ~10 kb, centrifuge DNA in a g-TUBE at 4,300 x g for one minute at room temperature

Check the read length of each sample using the Femto Pulse System (Agilent)

3 Prepare libraries

To prepare DNA ends for the addition of sequencing adapters, use the NEBNext FFPE DNA Repair Mix and the NEBNext Ultra II End Prep Enzyme Mix from the NEBNext Companion Module v2 (NEB)

4 Ligate sequencing adapters

Attach the sequencing adapters from the Ligation Sequencing Kit V14 using Salt-T4 DNA Ligase (NEB), purify using 0.4x AMPure XP Beads (Beckman Coulter), washing twice with Long Fragment Buffer (LFB), and quantify 1 µl of library using a Qubit fluorometer

5 Sequence

Prepare 200–300 ng of a single library for loading by combining 32 µl of the library with 100 µl of Sequencing Buffer (SB) and 68 µl of Library Beads (LIB)

Load 200 µl of the prepared library on to a PromethION Flow Cell, set up the sequencing run on MinKNOW™ using the high accuracy (HAC) basecaller, and sequence on your chosen PromethION device

Washing or reloading of the flow cell is not required

Sequence to ~30–40x depth of coverage — this can be achieved by sequencing a single library for 72 hours on one PromethION Flow Cell

6 Analyse

Use the wf-human-variation workflow[1] from EPI2ME™ to analyse the BAM file produced by MinKNOW

The workflow provides all-in-one calling of SNVs, SVs, CNVs, STRs, and methylation — covering both 5mC and 5hmC — and enables phasing of these variants

The workflow outputs a series of intuitive reports, VCF files listing variants, a BEDMethyl file containing methylation information, and QC metrics

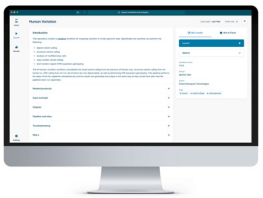
The results from the workflow can be further explored by viewing in a track-based genome browser such as IGV

For users preferring an easy-to-use graphical interface, this preconfigured workflow is free to access from the EPI2ME Desktop Application

For users with advanced bioinformatics experience, the workflow is simple to run in the command line

Both options can be run on local compute or in the cloud

Kits, devices, and software



Library preparation
Ligation Sequencing Kit V14

Sequencing
PromethION Flow Cells on PromethION devices

Analysis
EPI2ME wf-human-variation

View the end-to-end protocol:
nanoporetech.com/interaction-free-human-WGS-protocol

References:

1. GitHub. wf-human-variation. Available at: https://github.com/epi2me-labs/wf-human-variation [Accessed 28 August 2025]

Oxford Nanopore Technologies

www.nanoporetech.com

phone +44 (0)845 034 7900

email support@nanoporetech.com

oxford-nanopore-technologies

@nanopore

@nanoporetech.com

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