Accelerating clinical research with Oxford Nanopore large cohort sequencing

The analysis of large cohorts of clinical research samples is critical to advancing our understanding of disease mechanisms and identifying novel biomarkers. Furthermore, to address the lack of representation of global diversity in clinical research, whole-genome sequencing of research samples across underrepresented populations is crucial. Though genome-wide association studies (GWAS) have revealed numerous disease-associated loci of interest, the use of genotyping arrays does not provide base-level resolution, identifying only potential loci, which require subsequent fine mapping. Meanwhile, the use of legacy short-read whole-genome sequencing has precluded the analysis of regions such as repetitive sequences and large structural variants (SVs) that are inaccessible to the technology[1], despite over 30% of disease-causing variants spanning more than a single base[2].

The ability to comprehensively capture both genomic and epigenomic variation across the genome is essential to advancing clinical research. With any-length, PCR-free Oxford Nanopore sequencing reads, single nucleotide variants (SNVs), SVs, copy number variants (CNVs), short tandem repeats (STRs), and methylation can be captured within a single sequencing run, including in previously intractable regions of the genome. Through scalable sequencing of up to 2,496 human genomes per year on a single PromethION™ 24 device, Oxford Nanopore technology enables you to sequence at the throughput you need in your own lab, for the complete picture of the human genome.

Here we present an end-to-end workflow for genome-wide analysis of genomic and epigenomic variants across a large cohort of human clinical research samples using the PromethION 24 device.

Extraction:
obtaining high molecular-weight DNA

View documentation, including protocols and best-practice guidance: **nanoporetech.com/documentation**

To ensure high outputs of long reads in Oxford Nanopore sequencing, it is important to select an extraction method that preserves high molecular-weight DNA. When starting from clinical research blood samples, we recommend extracting DNA using the **QIAGEN Puregene Blood Kit**. To produce a fragment length of around 30 kb, we then recommend performing size selection with the Oxford Nanopore **Short Fragment Eliminator Kit**, followed by light shearing using a **Diagenode Megaruptor**. Our end-to-end protocol also guides you through how to quality check samples before proceeding, for optimal sequencing results.

Extraction and library prep protocols are easily automatable on various liquid handlers, ensuring a streamlined workflow and minimal hands-on time for high-throughput projects.

Find out more about options and support for automation: **nanoporetech.com/automation**

Library preparation:
preparing samples for sequencing at scale

Learn more about Oxford Nanopore library preparation methods: **nanoporetech.com/prepare**

To prepare your samples for sequencing, we recommend the **Ligation Sequencing Kit**. Providing a simple, fast, and scalable workflow, this PCR-free method is optimised for output and preserves long DNA fragments for sequencing — including epigenetic modifications, which can be directly detected alongside genomic variants without special library prep steps.

Manual prep of 24 samples by a single researcher, including quality control and normalisation, takes ~3 hours, while automated options require as little as ~2.5 hours and just 10 minutes of hands-on time.

Sequencing:
generating high coverage of thousands of genomes per year

Discover high-throughput sequencing on PromethION 24: nanoporetech.com/promethion

For genome-wide analysis of genomic and epigenomic variants, we recommend sequencing samples to ~30x depth of coverage. This can be achieved by sequencing each sample on one **PromethION Flow Cell** for 72 hours. To maximise output, use the **Flow Cell Wash Kit** to wash and reload the flow cell every 22 hours. You will have sufficient sequencing library from the previous step to cover this.

For the scale and flexibility to meet the needs of large cohort sequencing, the **PromethION 24** is ideal, enabling sequencing on 1–24 independent, high-output PromethION Flow Cells. Utilising the powerful compute of the device, basecalling keeps up with real-time data streaming even at full capacity.

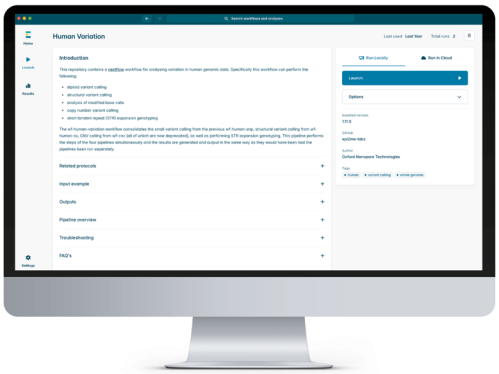
Using this workflow, a single PromethION 24 allows the sequencing of 48 human genomes to ~30x depth of coverage per week — a total of 2,496 per year — while the small footprint of the device makes it simple to scale up further for any throughput you need.

Analysis:
comprehensive variant calling in real time — from a single workflow

Find out more about data analysis with EPI2ME: nanoporetech.com/epi2me

Data analysis begins with real-time basecalling and methylation calling on the PromethION 24, utilising the high-performance models integrated into **MinKNOW**™, the software onboard the sequencer. We recommend basecalling in high accuracy (HAC) mode. For efficient data storage in large cohort projects, we recommend that you retain only FASTQ or BAM output files.

For comprehensive characterisation of genomic and epigenomic variants across the human genome, use the all-in-one **EPI2ME**™ human variation analysis workflow, **wf-human-variation**. This workflow enables analysis of SNVs, SVs, CNVs, and STRs, together with the calling of 5mC and 5hmC methylation in CpG contexts, and performs phasing. The workflow is implemented via an intuitive, point-and-click user interface or through the command line.



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

References:

1. Ebbert, M.T.W. et al. *Genome Biol.* 20(1):97 (2019). DOI: https://doi.org/10.1186/s13059-019-1707-2
2. Eichler, E.E. *N. Engl. J. Med*. 381(1):64–74 (2019). DOI: https://doi.org/10.1056/nejmra1809315

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