



Human genomics with Oxford Nanopore

Introduction

The human genome holds vital information for understanding human variation and disease. However, with the first complete human genome sequence published in only 2022¹, a significant portion of this information has remained out of reach until very recently. Now, building on this milestone, researchers are harnessing ultra-rich Oxford Nanopore data to routinely sequence complete genomes at any scale, revealing the full landscape of human genomic variation.

From population-scale studies to the promise of personalised medicine, genomics provides crucial insights into human health, development, and both rare and complex diseases.

While legacy sequencing technologies have driven progress over recent decades, the use of short-read sequencing methods has left 'dark' areas of the genome — such as structural variants (SVs) and repetitive sequences — intractable to analysis, missing potential clinically relevant sequences². The need for multiple platforms and techniques to capture important multiomic information, across genomic variants and extending to epigenetic and transcriptomic analysis, can also be prohibitively costly and time consuming. Even the reference genomes underpinning this research often remain incomplete and fail to capture global genetic diversity, missing variants in underrepresented populations.

Oxford Nanopore sequencing is now addressing these critical gaps. With PCR-free sequencing and unrestricted read lengths, from short to ultra long, it is possible to capture and phase variants across the genome comprehensively from a single sequencing run (Figure 1). This spans from single-nucleotide variants (SNVs) to even large, complex SVs, including in regions that are challenging to resolve with short-read technology. Furthermore, PCR-free sequencing of native DNA captures epigenetic modifications alongside canonical sequences, without the need for bisulfite treatment or other special library preparation steps. Through flexible, real-time sequencing devices, this can be scaled from the identification of previously hidden variants in a single genome to research into complex, multivariate disease at the large cohort scale, with no need for sample batching.

Nanopore sequencing also makes possible the construction of highly complete whole-genome assemblies. Long nanopore reads enable access to the complete genome, including previously inaccessible regions, while ultra-long reads played a key role in the construction of the first truly complete human genome sequence¹. Now, they are sufficient to generate telomere-to-telomere (T2T) assemblies alone — without the need for complementary technologies — producing fully phased chromosomes, including centromeric sequences and other underexplored regions of the genome.

With accessible, scalable Oxford Nanopore sequencing technology, scientists are now addressing the crucial gaps in global representation across reference genomes, ushering in a new era of human genomics and pangenomics.

This guide introduces how to sequence human genomes with Oxford Nanopore, from the construction of new, highly complete reference assemblies to the comprehensive identification of variants.

While this guide focuses on the sequencing of human genomic DNA (gDNA), Oxford Nanopore sequencing also enables highly accurate characterisation of cDNA and native RNA, allowing you to unlock the deepest level of multiomic insights from a single technology.

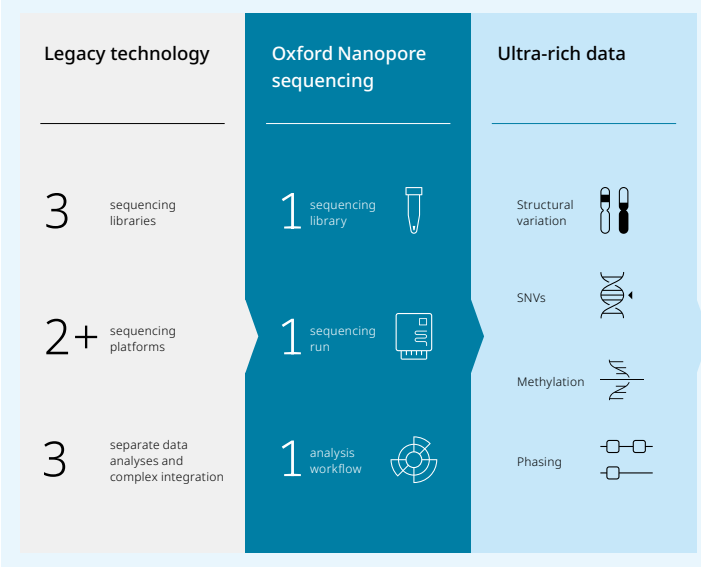
Find out more about multiomic Oxford Nanopore sequencing in the following getting started guides.

Investigating methylation in the human genome:
nanoporetech.com/methylation-guide

Bulk transcriptomics:
nanoporetech.com/bulk-transcriptomics-guide

Single-cell transcriptomics:
nanoporetech.com/single-cell-guide

Figure 1. Where legacy methods require multiple separate technologies, ultra-rich data from Oxford Nanopore reveals deep multiomic insights from a single platform.



1. Nurk, S., Koren, S., Rhie, A., Rautiainen, M. et al. The complete sequence of a human genome. *Science* 376(6588):44–53 (2022). DOI: <https://doi.org/10.1126/science.abj6987>
2. Ebbert, M.T.W. and Jensen, T.D. et al. Systematic analysis of dark and camouflaged genes reveals disease-relevant genes hiding in plain sight. *Genome Biol.* 20(1):97 (2019). DOI: <https://doi.org/10.1186/s13059-019-1707-2>

Demonstrating performance

- Cheng, H. and Qu, H. et al. Efficient near telomere-to-telomere assembly of nanopore simplex reads. *bioRxiv* 648685 (2025). DOI: <https://doi.org/10.1101/2025.04.14.648685>
- Oxford Nanopore Technologies. Poster. Available at: <https://nanoporetech.com/resource-centre/automated-single-platform-telomere-to-telomere-de-novo-genome-assembly-for-human-plant-and-animal-genomes> [Accessed 21 August 2025]
- GitHub. Dorado. Available at: <https://github.com/nanoporetech/dorado> [Accessed 21 August 2025]
- Rautiainen, M. et al. Telomere-to-telomere assembly of diploid chromosomes with Verkko. *Nat. Biotechnol.* 41(10):1474–1482 (2023). DOI: <https://doi.org/10.1038/s41587-023-01662-6>
- GitHub. wf-human-variation. Available at: <https://github.com/epi2me-labs/wf-human-variation> [Accessed 21 August 2025]

*Sequencing data resampled to match the read lengths and depths expected from a sample prepared using the [Human variation sequencing from 30 kb extracted cell line samples](#) protocol. See [page 5](#) for more information.

Assembling the human genome: from chromosomes to T2T

It is now possible to generate high-quality *de novo* human genome assemblies with a single sequencing platform.

In a publication from Cheng *et al.*, the team utilised their rapid assembly algorithm hifiasm (ONT) to generate near-T2T human genome assemblies from Oxford Nanopore-only data³. The assemblies generated using nanopore data with read length N50s of ~30 kb and >40x depth of coverage showed considerably higher contiguity than those generated with PacBio HiFi reads at similar depths of coverage³. With data from one to two PromethION™ Flow Cells, the near-T2T assemblies featured partially phased, complete diploid chromosomes.

Using ultra-long nanopore reads, assemblies can be pushed further to T2T, reference-quality genomes. The Applications Team at Oxford Nanopore Technologies combined ultra-long reads (read length N50 ~110 kb) with Pore-C data — a chromatin conformation capture technique — to produce the most complete and contiguous automated human genome assembly to date⁴. The assembly, generated using the correction tool Dorado correct⁵ and assembly algorithm Verkko⁶, featured fully haplotype-phased chromosomes and resolved centromeric regions ([Figure 2](#)).

For more information about assembling Oxford Nanopore data, see [page 9](#).

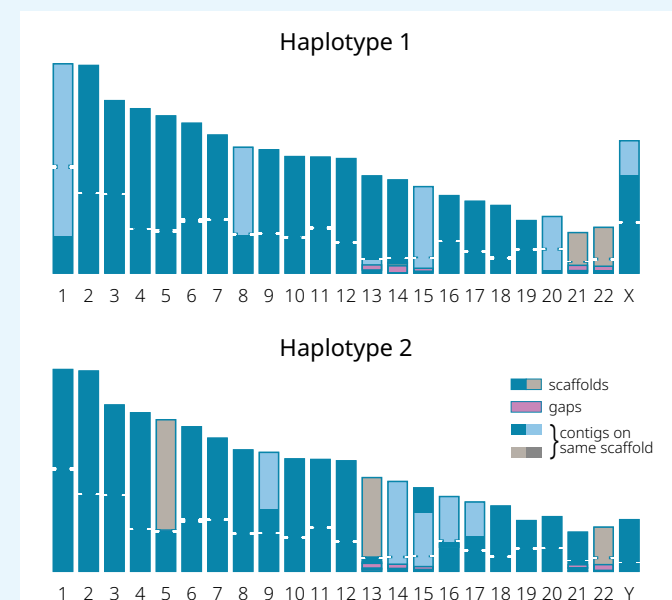
View the T2T assembly poster:
nanoporetech.com/poster-T2T-assembly

Uncovering variants and methylation across the genome

Combining scalable sequencing of native DNA with intuitive data analysis, Oxford Nanopore provides end-to-end workflows for the thorough identification of variants and epigenetic modifications in a single sequencing run. The technology delivers both higher overall depth of coverage than an equivalent workflow from an alternative long-read sequencing method (30x vs 20x) and more heterogeneous read lengths, resulting in approximately three times more individual reads.

To benchmark variant calling performance, the Applications Team sequenced the well-characterised Genome in a Bottle (GIAB) HG002 cell line on one PromethION Flow Cell and basecalled the data using the high accuracy (HAC) model (version 5.0). They then resampled the reads to match the sequencing metrics expected from a human blood sample*. The resulting data was analysed using the human variation EPI2ME™ data analysis workflow, wf-human-variation⁷. The easy-to-use human variation workflow provides all-in-one phased calling of SNVs, SVs, copy number variants (CNVs), repeat

Figure 2. T2T assembly of the human genome using Oxford Nanopore-only data.



Demonstrating performance

expansions, and 5mC and 5hmC methylation in CpG contexts (see [page 10](#) for more information).

The data demonstrated highly accurate SNV and small insertion/deletion (indel) calling across the genome, capturing over 99.9% of pathogenic ClinVar⁸ indels when excluding long homopolymers ([Figure 3](#))⁹. SVs were called with high accuracy across the genome ([Figure 4](#)), with results largely independent of the size or class of the SVs; they were also identified within highly repetitive contexts. Detection of large CNVs, spanning over 100 kb, reached beyond the limitations of the current reference genome; these were accurately resolved using the tool Spectre¹⁰.

Benchmarking 5mC methylation calling performance against whole-genome bisulfite sequencing (WGBS) with short-read technology revealed high correlation (0.98) between the techniques ([Figure 5](#)). The Oxford Nanopore data covered over 98% of CpGs from the human reference genome hg38 with >10 observations. Going beyond WGBS, nanopore sequencing also enables the calling of 5hmC from the same dataset.

Oxford Nanopore provides open-access datasets for benchmarking sequencing performance. This includes data for T2T human genome assembly and sequencing of GIAB samples.

View the variant calling poster:
nanoporetech.com/human-variant-calling-poster

Download and explore Oxford Nanopore sequencing datasets:
epi2me.nanoporetech.com/dataindex

- Landrum, M.J. et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res.* 42:D980–5 (2014). DOI: <https://doi.org/10.1093/nar/gkt1113>
- Oxford Nanopore Technologies. Poster. Available at: <https://nanoporetech.com/resource-centre/end-to-end-workflow-for-haplotype-resolved-genetic-and-epigenetic-variant-calling-using-nanopore-sequencing> [Accessed 21 August 2025]
- GitHub. Spectre. Available at: <https://github.com/nanoporetech/ont-spectre> [Accessed 21 August 2025]

Figure 4. Comprehensive SV calling across the HG002 genome from 30x depth of coverage.

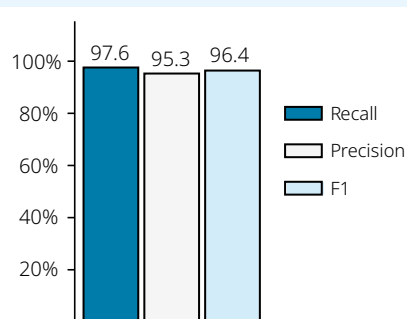


Figure 3. High-accuracy calling of SNVs and small indels in HG002 from 30x depth of coverage, excluding homopolymers ≥ 10 bp.

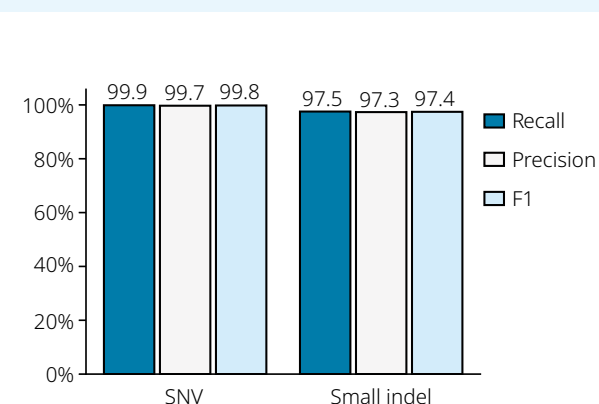
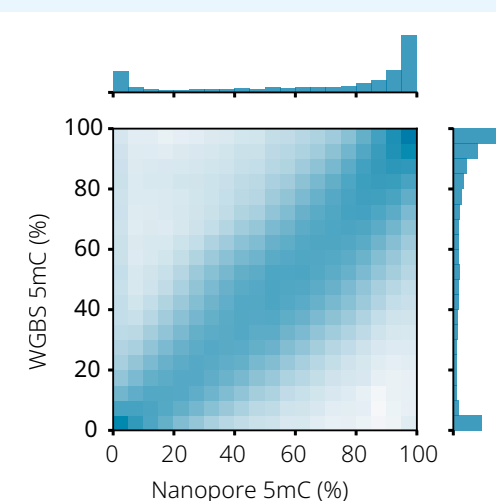
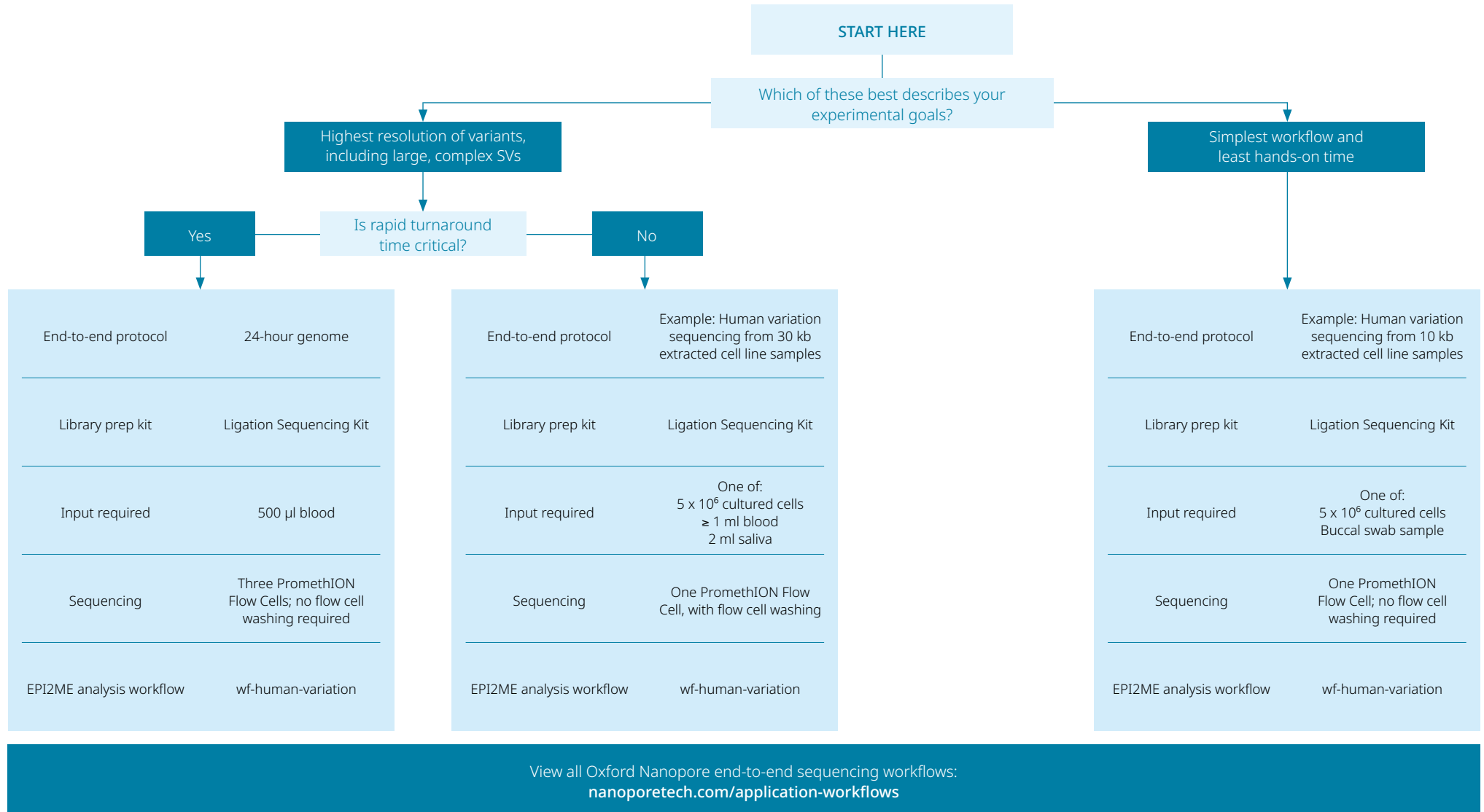


Figure 5. High correlation in 5mC calling between Oxford Nanopore native DNA sequencing and WGBS via short-read sequencing technology.



Which approach do I choose for whole-genome human variant calling?

For information on targeted Oxford Nanopore sequencing, see [page 9](#).



View all Oxford Nanopore end-to-end sequencing workflows:
nanoporetech.com/application-workflows

From sample to answer

Preparation

How do I prepare samples for sequencing?

Oxford Nanopore provides verified extraction protocols for a wide range of sample types, preserving high molecular-weight DNA for sequencing. For example, for human variant detection, protocols are available for blood, cell line, buccal swab, and saliva research samples. We also include guidance on how to quality check your samples to ensure high-quality DNA is taken forward into the next step.

For library prep, multiple Oxford Nanopore sequencing kits are available to suit the needs of your experiment, including considerations such as read length, turnaround time, input amount, and multiplexing needs. For example, with the Ligation Sequencing Kit, long DNA fragments can be prepared for sequencing without PCR in ~90 minutes.

In our application workflows, recommended extraction and library prep methods are included as part of the end-to-end protocol.

View protocols for extraction and library prep, and end-to-end application workflows: nanoporetech.com/documentation

Learn more about Oxford Nanopore sequencing kits: nanoporetech.com/prepare

Application workflows provide sample-to-answer guidance

Application workflows

Sort by: Most relevant

Ultra-Long DNA Sequencing Kit V14 (SQK-ULK114)

Extraction of ultra-high molecular weight (uHMW) gDNA - Reliably generate and sequence ultra-long read length N50s (>50 kb) - High yield - Compatible with R10.4.1 flow cells For Research Use Only. This is an Early Access product. For more...

Protocol | Last updated 7/2/2025

[FLO-MIN114](#) [FLO-PRO114M](#) [Whole genome sequencing](#) [PromethION](#) [+6](#)

Telomere-to-telomere sequencing (T2T) from blood and cells using SQK-APK114, SQK-LSK114, and SQK-ULK114

For Research Use Only. This is a registration-based Early Access product.

Protocol | Last updated 5/9/2025

[FLO-PRO114M](#) [Whole genome sequencing](#) [PromethION](#) [SQK-ULK114](#) [+4](#)

Reduced representation methylation multiplex sequencing (RRMS) from cells using SQK-NBD114.24

For Research Use Only.

Protocol | Last updated 7/8/2025

[FLO-PRO114M](#) [Whole genome sequencing](#) [PromethION](#) [SQK-NBD114.24](#) [+2](#)

Preparation

What read length do I need?

With Oxford Nanopore sequencing, read length is unrestricted, ranging from short to ultra long; the current record read length stands at over 4 Mb¹¹. Choosing a read length depends on your experimental goals.

For assembly, a read length N50 of >40 kb will deliver chromosome-scale assemblies³, spanning challenging regions of the genome that cannot be resolved with short-read sequencing. The use of ultra-long reads — spanning over 100 kb — delivers T2T assemblies.

For a simple, streamlined variant calling workflow, our protocol producing reads around 10 kb in length provides a hands-off method of generating high sequencing outputs. For best resolution of large and complex SVs and repetitive sequences, we recommend our protocols that generate reads of around 30 kb.

Learn more about any-length Oxford Nanopore reads: nanoporetech.com/read-lengths-blog



11. Oxford Nanopore Technologies. Ultra-Long DNA Sequencing Kit V14. <https://store.nanoporetech.com/uk/ultra-long-dna-sequencing-kit-v14.html> [Accessed 21 August 2025]

From sample to answer

Sequencing

What device should I choose?

The range of Oxford Nanopore devices ensures that sequencing can be tailored to your experimental goals.

For whole-genome sequencing projects, high-output PromethION Flow Cells, compatible with any sequencer in the PromethION range, are ideal. A single PromethION Flow Cell can produce 100–200 Gb native gDNA reads, representing ~30–60x depth of coverage of the human genome.

The PromethION 24 sequencer combines the capacity to sequence on up to 24 independent flow cells with powerful onboard compute, delivering high-throughput, on-demand sequencing. Basecalling and alignment can also be performed in real time on the device. For lower throughput needs, the small-footprint PromethION 2 range enables sequencing on up to two independent flow cells, for PromethION-scale sequencing in any lab. If you are using adaptive sampling to enrich regions of the human genome (see [page 9](#)), a PromethION Flow Cell can be used to generate the coverage you need across targets of interest.

For lower output needs, a MinION™ Flow Cell provides an average of 15–35 Gb of data, enabling low-pass sequencing of the human genome or high depth of coverage in targeted experiments, such as amplicon panels. MinION Flow Cells are compatible with both the portable MinION Mk1D, which can be plugged into a laptop for sequencing in any location, and the flexible GridION™, providing capacity for sequencing on up to five flow cells.

Discover the PromethION range:

nanoporetech.com/promethion

Find out more about Oxford Nanopore sequencing devices:

nanoporetech.com/sequence

PromethION 24



PromethION 2 Integrated



From sample to answer

Sequencing

How do I scale up sequencing?

For large cohort and population-scale projects, Oxford Nanopore sequencing can be scaled up to meet the throughput you need. Preparation of 24 samples for whole-genome sequencing using the Ligation Sequencing Kit takes ~3 hours and can be easily automated on various liquid handlers.

A single PromethION 24 device can sequence up to 2,496 genomes per year, while integrated compute ensures that basecalling keeps up with real-time data streaming even at full capacity.

Learn more about large cohort sequencing:
nanoporetech.com/large-cohorts-workflow



Sequencing

How do I perform rapid whole-genome sequencing?

For when rapid turnaround time is the priority, Oxford Nanopore has developed an end-to-end workflow enabling you to go from a blood research sample to whole-genome analysis in just 24 hours.

This method sequences one gDNA sample across three PromethION Flow Cells on a PromethION 24 to produce over 30x depth of coverage of the human genome from just 13–16 hours of sequencing time. This data is then combined and analysed using the EPI2ME workflow wf-human-variation, delivering rapid variant and methylation analysis. This data can then be optionally taken forward for further tertiary analysis.

Find out more about rapid whole-genome sequencing:
nanoporetech.com/rapid-wgs-workflow



From sample to answer

Sequencing

How do I perform targeted sequencing?

With nanopore sequencing, it is possible to target specific genes, panels, or larger regions of the human genome — with or without PCR.

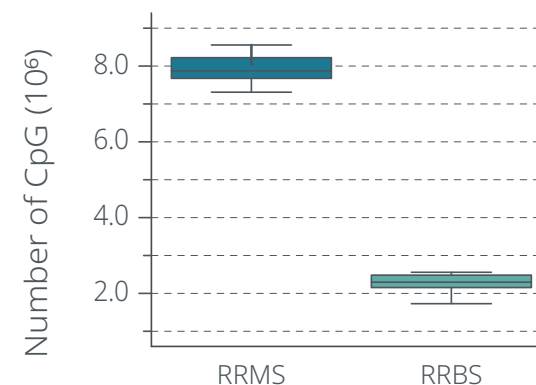
For regions that are amenable to amplification, samples that have been enriched with locus-specific primers can be prepared for sequencing using either the Ligation Sequencing Kit or, for preparation in just ten minutes, the Rapid Sequencing Kit; multiplexing options are also available.

For PCR-free targeted sequencing, adaptive sampling, a method unique to Oxford Nanopore, provides enrichment or depletion of regions of interest during real-time sequencing itself. With no need for any special library prep, the bioinformatics-based technique allows the targeting of regions that cannot be amplified, including large SVs, repeat expansions, and methylated regions, directly from native DNA. The method also underpins Reduced Representation Methylation Sequencing (RRMS), which captures methylation across 100% of CpGs in the human genome on a single MinION Flow Cell¹².

12. Oxford Nanopore Technologies. Poster. Available at: <https://nanoporetech.com/resource-centre/reduced-representation-methylation-sequencing-rrms-captures-100-cpg-islands-and> [Accessed 21 August 2025]

Find out more about targeted nanopore sequencing: nanoporetech.com/targeted-sequencing-oxford-nanopore

RRMS delivers methylation information across more high-confidence CpGs than short-read reduced representation bisulfite sequencing (RRBS)¹²



Data analysis

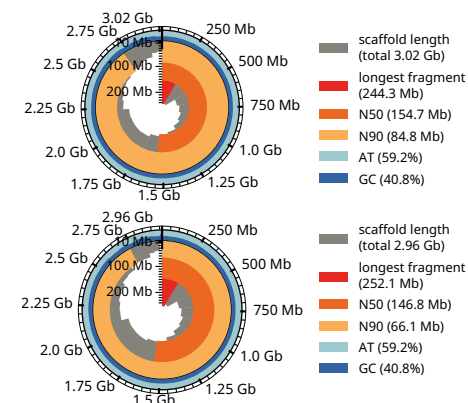
How can I perform whole-genome assembly?

Several analysis tools are available for the assembly of high-quality whole human genomes from Oxford Nanopore-only data, each offering benefits to suit different experimental goals.

The tool hifiasm (ONT)³ is a fast, effective assembler that does not require GPU, making it ideal for streamlined assembly at scale. Compatible with data produced from Ligation Sequencing Kit-prepared libraries, hifiasm (ONT) delivers chromosome-level assemblies from read length N50s of ~30 kb.

For T2T-quality assembly from ultra-long reads, combining read correction via Dorado correct⁵ with assembly via the tool Verkko⁶ produces highly complete results. With the assembler Verkko optimised for ultra-long reads, acrocentric assembly, and ribosomal RNA (rRNA) scaffolding, this technique provides T2T, haplotyped whole-chromosome contigs, including resolved centromeres. Verkko assembly also supports the integration of Pore-C data, enabling full chromosome phasing. Alternatively, hifiasm (ONT) can be utilised for a faster, GPU-free approach.

Assembly statistics for the GIAB reference genome HG002, assembled from ultra-long reads using Dorado correct and Verkko⁴



From sample to answer

Data analysis

How do I analyse genomic variants and methylation across the human genome?

Oxford Nanopore provides easy-to-use data analysis workflows for all levels of expertise through the bioinformatics platform EPI2ME. Featuring a wide range of preconfigured workflows, EPI2ME can be accessed through an intuitive point-and-click user interface or via the command line. The human variation EPI2ME workflow, wf-human-variation, provides comprehensive calling of genomic variants and methylation analysis from a single pipeline.

MinkNOW™ is the software that controls Oxford Nanopore sequencing devices and performs basecalling. This includes calling of both canonical and modified bases in a single run, with no need for extra library preparation steps such as bisulfite treatment; modification calling is simply selected in MinkNOW prior to starting a sequencing run. The software can also be used to perform alignment, or this can be done via EPI2ME. From an input BAM file, wf-human-variation calls SNVs, SVs, CNVs, short tandem repeats, and 5mC and 5hmC methylation in CpG contexts, and performs phasing. The workflow outputs intuitive reports and annotation files; the results can be visualised within EPI2ME or your chosen genome browser.

Long Oxford Nanopore reads can span large, complex variants, even in highly repetitive regions. In one example demonstrating the performance of wf-human-variation, the Applications Team detected an SV within a segmental duplication in a clinical research sample, resolving its precise breakpoints⁹.

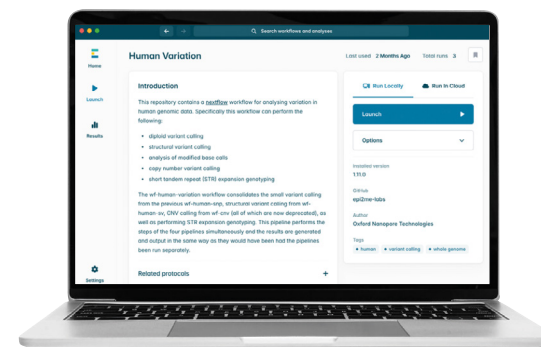
Discover streamlined data analysis with EPI2ME:

nanoporetech.com/EPI2ME

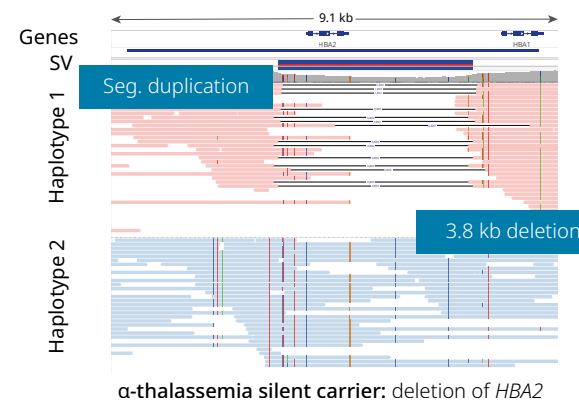
Find out more about wf-human-variation:

nanoporetech.com/EPI2ME-human-variation

The EPI2ME human variation workflow, wf-human-variation, provides all-in-one human genomic variant and methylation calling



Calling of an SV within a segmental duplication in a clinical research sample using wf-human-variation⁹



Case studies

Case study 1: expanding reference genomes to reflect global diversity — T2T assembly with Oxford Nanopore

High-quality reference genomes are the backbone of precision medicine. However, until recently, the reference genomes available have lacked representation from diverse populations, and due to the limitations of legacy sequencing approaches, many remain incomplete. Now, researchers are demonstrating how T2T assemblies, generated exclusively with Oxford Nanopore sequencing, could help close both of these gaps.

Prasad Sarashetti (Genome Institute of Singapore, Singapore), who leads T2T and pangenomics efforts for the Asian Reference Genome project, recently shared results from a study aimed at assembling complete, high-quality T2T genomes using only Oxford Nanopore sequencing data — without the need for a hybrid approach using multiple technologies¹³.

The team assembled three human genomes using both a hybrid method — PacBio HiFi reads combined with Oxford Nanopore ultra-long reads — and an Oxford Nanopore-only pipeline, which utilised Pore-C chromatin conformation capture for phasing. When evaluating the results, Prasad pointed out a striking trend: you can clearly see [Oxford Nanopore]-only approaches produce a lot more telomere-to-telomere contigs than the hybrid approach itself.

Although contiguity values (NG50s) were similar across most of the assemblies, the Oxford Nanopore-only assemblies consistently outperformed those generated using the hybrid approach in terms of the number of complete chromosome-length sequences; they also exhibited fewer structural errors. This resulted in one particularly impactful finding: an SV in a clinically relevant repetitive region that was absent from African and European reference genomes but appeared to be a fixed allele in South Asian populations.

The team's findings provide compelling evidence that Oxford Nanopore sequencing can stand alone in delivering complete, reference-quality, phased human genome assemblies. For scientists aiming to accurately reconstruct reference genomes — and to close longstanding diversity gaps in human genomics — these results offer a practical and powerful path forward.

Watch the talk (London Calling 2025):
nanoporetech.com/precision-genomics-showcase

Case study 2: Oxford Nanopore sequencing at the large cohort scale — new insights from the NIHR BioResource

In mechanisms of disease research, the variants contributing to the risk of developing complex conditions can be challenging to identify. With legacy short-read sequencing technologies offering an incomplete picture, researchers at the NIHR BioResource (United Kingdom) — a national platform for health research — turned to Oxford Nanopore sequencing, in the first project of its kind to use the technology on this large a scale for research into multiple diseases¹⁴. In total, the study encompasses nanopore sequencing of 22,000 whole human genomes.

At the London Calling 2025 conference, Dr Nathalie Kingston and Dr Kathy Stirrups described how they harnessed Oxford Nanopore sequencing for three flagship translational research studies. Performing high-throughput sequencing of up to 384 genomes per week on PromethION devices and data analysis with EPI2ME, they aimed to gain a better understanding of the genetic and epigenetic landscape of multiple diseases. Although the full datasets are still being analysed, early findings have already validated their approach.

In one project, they sequenced research samples from participants in the UK Eating Disorders Genetics Initiative with severe phenotypes. The team have already identified repeat expansions that align with these phenotypes, which may represent a breakthrough that could eventually point to new therapeutic targets. In another

project, sequencing research samples from participants in the Genes and Cognition cohort, they obtained haplotype-level methylation information that could shed light on heritability of dementia. The team also studied clinical research samples from a rare disease cohort. Here, the method identified known variants, including *de novo* SNVs, SVs, and compound heterozygosity, and also provided haplotyped methylation data from the same sequencing run. Furthermore, the long reads enabled phasing without the need for parental sample sequencing.

Together, these projects highlight the ultra-rich data revealed by Oxford Nanopore sequencing, revealing insights that matter for the future of precision health.

Watch the talk (London Calling 2025):
nanoporetech.com/NIHR-LC25

13. Sarashetti, P., De La Vega, F., Kang, Y., Bediako, Y. Precision genomics requires a diverse reference. Presentation. Available at: <https://nanoporetech.com/resource-centre/precision-genomics-requires-a-diverse-reference> (2025) [Accessed 21 August 2025]
14. Kingston, N. and Stirrups, K. Using long-read sequencing for translational health research. Presentation. Available at: <https://nanoporetech.com/es/resource-centre/using-long-read-sequencing-for-translational-health-research> (2025) [Accessed 21 August 2025]




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