Oxford NANOPORE Technologies

WORKFLOW: HUMAN GENOME ASSEMBLY

Assembling the human genome using long nanopore sequencing reads

To gain a comprehensive insight into human genetic variation, and its potential impact on disease risk, it is important to obtain fully characterised, complete genomes. However, the presence of large structural variants (SVs) and repeat sequences have posed a significant challenge to assembling the human genome to completion.

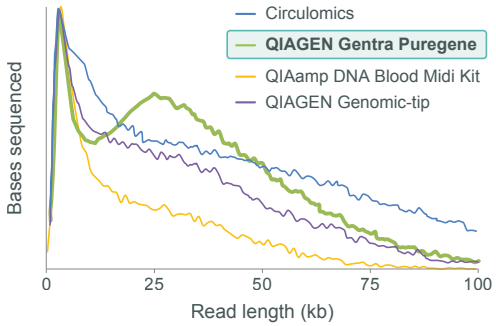
Unlike short-read sequencing, nanopore sequencing produces long and ultra-long reads, which enhance the resolution of SVs and repeats. With the high-output PromethION™ device, sequencing and assembling highly contiguous human genomes is now possible, with unprecedented efficiency.

Here we present a simple workflow for human genome assembly from a blood sample, using the PromethION sequencing platform.

EXTRACTION: obtaining high molecular weight DNA

Find more extraction protocol recommendations for your sample type, plus guidance on DNA storage and contaminants: **community.nanoporetech.com/extraction_methods**

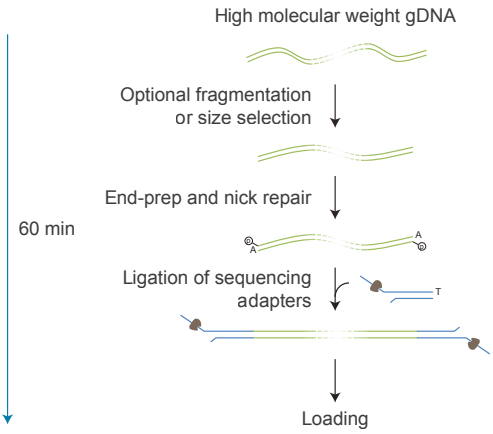
Selecting a suitable extraction method for obtaining high molecular weight DNA greatly depends on sample type. For DNA extraction from whole blood, we recommend the **QIAGEN Gentra Puregene Blood Kit**, which we have found maximises the production of long sequencing reads.



LIBRARY PREPARATION: selecting a kit

Find out more about size selection methods for long-read sequencing: **community.nanoporetech.com/extraction_methods**

Enriching for long and ultra-long (≥50 kb) gDNA fragments is important for performing genome assembly, to maximise the overlap of sequencing reads in analysis. We recommend size selection and light shearing of the extracted gDNA, which we have found to improve read length N50 — we suggest aiming for a read N50 of 25–35 kb. Internal testing has yielded good results using the **Circulomics Short Read Eliminator Kit** for size selection, to select for all fragments >10 kb, and the **Diagenode Megaruptor 3** for shearing.

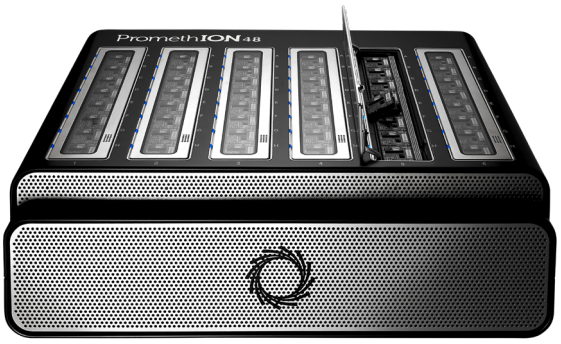


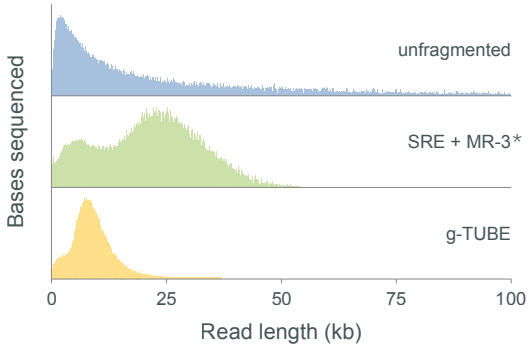
To prepare gDNA for sequencing, we recommend the **Ligation Sequencing Kit (SQK-LSK110)**, providing the greatest throughput and control over read lengths.

Find out more about library prep, including rapid and multiplexing options: **nanoporetech.com/products/kits**

SEQUENCING: generating high yields of long reads with PromethION

Find out more about PromethION:
nanoporetech.com/products/promethion





For assembly, we recommend sequencing a human genome to a minimum depth of 30x of 25–35 kb reads; this can be achieved by sequencing a single genomic sample on one PromethION Flow Cell for 72 hours, after following the extraction and library preparation protocols above. However, sequencing to a depth of 60x is advisable to obtain the best assembly metrics. We also recommend basecalling in high accuracy mode. Throughput can be maximised by performing a nuclease flush and loading fresh library every 24 hours.

The on-demand PromethION sequencing platform has the capacity to run up to 24 (P24) or 48 (P48) flow cells at any time, providing ultimate flexibility and adaptability to your sequencing requirements.

*Circulomics Short Read Eliminator Kit + Diagenode Megaruptor 3

Find out more about nanopore sequencing service providers: nanoporetech.com/services/providers

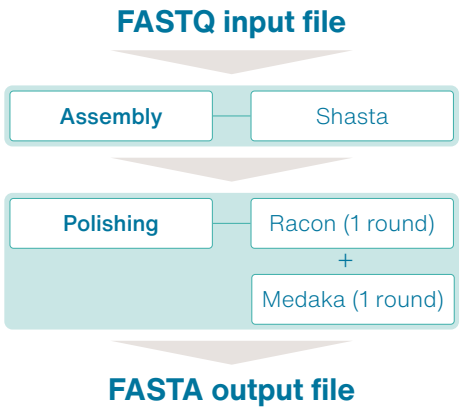
ANALYSIS: selecting an assembly tool

Find out more about data analysis solutions:
nanoporetech.com/analyse

To assemble a human genome, we suggest using the third-party tool **Shasta**, an assembler for rapid and accurate genome assembly from long sequencing reads[1]. Internally we have found that this tool has the shortest runtime and produces the greatest contig N50. We also recommend one round each of polishing with **Racon**[2] and **Medaka**[3]. All of these analysis tools can be found on GitHub.

Assembly can be performed using the on-board PromethION compute (requiring around 1 day to assemble a single human genome, including polishing); an Amazon Cloud instance (Amazon Web Services (AWS)) can also be used.

We alternatively recommend the combination of Flye (assembly) and Medaka (polishing) for high quality genome assembly; this requires AWS and has a longer runtime.



Find out more at: **nanoporetech.com/assembly**

References:
1. Shafin, K. *et al.* Nanopore sequencing and the Shasta toolkit enable efficient *de novo* assembly of eleven human genomes. *Nat Biotechnol.* 38: 1044-1053 (2020).
2. Vaser, R. *et al.* Fast and accurate *de novo* genome assembly from long uncorrected reads. *Genome Res.* 27(5): 737-746 (2017).
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