Non-Human Multiplexed Whole Genome Sequencing

Oxford Nanopore Technologies Services

Summary

Renew Biotechnologies (Renew), a certified Oxford Nanopore Technologies (ONT) service provider, offers Multiplexed Whole Genome Sequencing (mWGS) for high-throughput, cost-effective genomic analysis. Leveraging ONT's native long-read sequencing platform, this service enables the accurate resolution of complex genomic regions, detection of structural variants, and comprehensive methylation analysis-without the need for bisulfite conversion or amplification biases. By multiplexing up to 4 samples per flow cell, Renew's mWGS is optimized for researchers looking to reduce per-sample costs while maintaining robust sequencing performance. Ideal for larger-scale whole-genome analyses, biomarker discovery, and clinical genomics, Renew's mWGS service is tailored for applications requiring both sequence and methylation data. Key benefits include:

Key Features & Benefits

- **Long-Read Sequencing:** Resolves complex genomic regions, including repetitive sequences and structural variants, with long to ultra-long reads.
- **High-Accuracy Native Epigenetic Analysis:** Directly sequences native DNA, preserving methylation signatures without bisulfite conversion or PCR amplification biases, delivering Q20+ (99%) accuracy.
- **Cost-Efficient, High-Throughput Multiplexing:** Processes up to four genomes per flow cell, allowing for simultaneous sequencing of multiple samples with lower coverage, reducing bias and maximizing efficiency for large-scale projects.

Ideal Applications

- Population Genomics and Epigenomics
- Epigenomic Studies
- Genome and Epigenome-Wide AssociationStudies
- Large-Scale Biomarker Discovery
- Clinical Diagnostics (e.g., Prenatal Testing, Rare Disease Screening)

Protocol Overview

ONT Kit: Native Barcoding Kit (SQK-NBD114.96) | Platform: PromethION

Workflow

1. **Genomic DNA (gDNA) Extraction:** Clients prepare high molecular weight gDNA from their samples following their project's specific extraction protocol.
2. **Sample Receipt:** Extracted gDNA is received at Renew and quality checked to ensure that sample requirements are met (1 µg of gDNA, see QC standards below).
3. **Library Preparation (Figure 1):** gDNA is fragmented to the desired size profile (optional). Double-stranded gDNA ends are repaired, end-prepped, and dA-tailed. Unique dT-tailed barcode adapters are ligated on the dA-tailed template and barcoded samples are pooled together. Sequencing adapters are ligated onto the ends.
4. **Loading onto Flow Cell:** The prepared cDNA library is loaded onto the PromethION flow cell for sequencing.
5. **Real-Time Sequencing & Analysis:** Long-read, real-time sequencing occurs with direct data streaming and analysis.
6. **Results Generation:** Raw nanopore reads are basecalled and uploaded as BAM files in Renew's client portal. Clients receive notifications when sequencing is complete and when results are accessible in the portal.

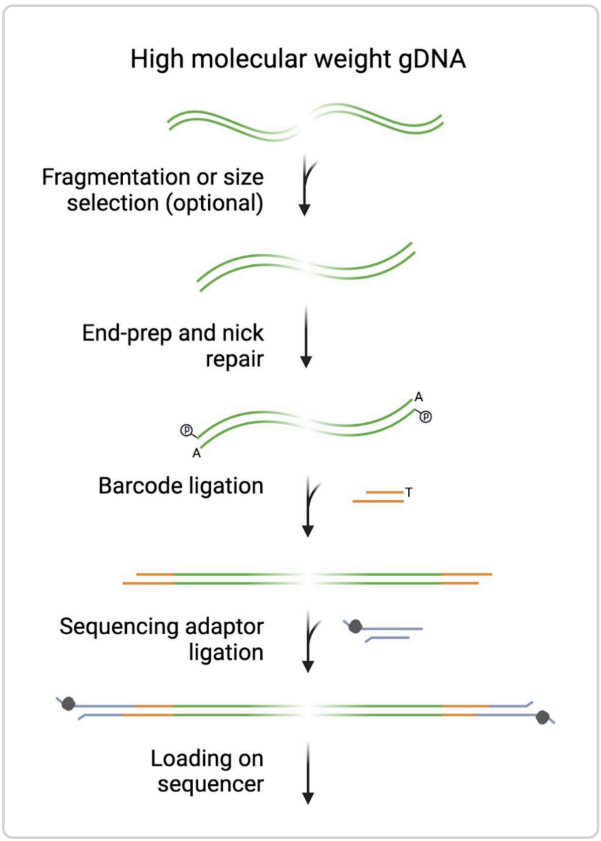

Figure 1. ONT Native Barcode Library Preparation Workflow for Multiplexed Whole Genome Sequencing. This schematic illustrates the key steps involved in preparing gNA samples for Multiplexed Whole Genome Sequencing using Oxford Nanopore Technologies' (SQK-NBD114.96) kit. The process includes DNA end repair and dA-tailing, barcode ligation, followed by sequencing adapter ligation to the prepared double-stranded DNA molecules. The library is loaded onto the flow cell for real-time, long-read sequencing on the PromethION platform. This method is optimized for high accuracy (Q20+) with long to ultra-long reads, suitable for various applications. This figure was adapted from ONT and created with BioRender.com.

Sample Requirements

Parameter	Requirement
Sample Type	High molecular weight double-stranded gDNA
Minimum Input DNA	400 ng of gDNA per sample
Quality Control (QC)	260/280 ratio: 1.8 - 2.0 | 260/230 ratio: 1.9 - 2.5 High molecular weight DNA, confirmed by gel electrophoresis or Pulse Field Electrophoresis, with average fragment sizes ideally greater than 100 kb.

Throughput & Capacity

Parameter	Requirement
Read Lengths	Equal to fragment length. Thus read lengths will vary depending on the sample type and quality, and fragmentation protocol, which can be customized for your project's needs.
Samples per Flow Cell	4* (human genome)
Sequencing Yield per Sample	Approximate per-sample data yield scales with the number of samples per flow cell (approximately 100 Gb per flow cell)**.

Samples per Flow Cell	Approximate Data Yield Per Sample
1	100 Gb
2	50 Gb
3	33 Gb
4	25 Gb

*May vary depending on the size of the target organism's genome.

**May vary based on sample quality, quantity, genome size, sequencing conditions, and flow cell performance.

Data Output

File Format

Data is delivered via the Renew client portal, including sequencing files, methylation calls, variant outputs, and quality control reports. Optional services (available for an additional fee) include POD5 file delivery, alignment to client-supplied reference genomes, and custom bioinformatics analyses.

File Formats

File	Description
POD5	Raw sequencing data
BAM	Sequencing reads + base modifications

File	Description
CH3	Base modification output format
QC Report	Sequencing metrics and run statistics

QC Reports

For each batch of samples, we provide Quality Control Reports that include read length distribution, base quality, and yield, along with summary statistics such as total number of reads, average read length, and error rates.

Sequencing Coverage

Coverage: Scales with the number of samples per flow cell.

*May vary based on genome size, sample quality, and quantity.

Samples per Flow Cell	Approximate Data Yield Per Sample
1	30x
2	15x
3	10x
4	7.5x

Choosing the Right Approach

WGS vs. Multiplexed WGS: Key Differences

For researchers deciding between standard WGS and multiplexed WGS services, it's essential to consider the specific needs of your project. Each approach offers distinct advantages depending on whether you are seeking high-depth analysis for detection of rare variants or a more cost-efficient way to sequence multiple samples with lower coverage. Below, we outline the key differences to help you choose the most suitable service.

Feature	Multiplexed WGS	WGS
Sequencing Throughput	Enables sequencing of up to 4 samples per flow cell in a single run with lower coverage, making it ideal for larger-scale studies.	Optimized for single-sample analysis, focusing on deep sequencing of single genomes.
Sequencing Depth	Offers lower coverage per sample that scales with the number of samples per flow cell (e.g., 10x coverage for 3 samples per flow cell), ideal for population studies and early screening tests.	Provides deep coverage (approximately 30x) for whole-genome studies to detect SNPs, indels, structural variants, and rare or complex genomic features.
Epigenetic Modifications	Can also detect methylation (5mC and 5hmC). However, resolution may be lower due to reduced coverage per sample.	Detects native methylation patterns (5mC and 5hmC) without bisulfite conversion or amplification, ideal for epigenomic studies.
Cost Efficiency	More cost-effective, with reduced sequencing depth, ideal for analyzing larger cohorts or studying samples that need to be run in parallel.	Higher cost per sample as a result of deep sequencing requirements.
Processing Complexity	Additional sample barcoding and demultiplexing are required for handling multiple samples per flow cell.	Standard workflow for WGS with extensive data generation per sample.
Ideal For Researchers	Ideal for researchers who wish to multiplex samples, reduce per-sample costs, and maintain data quality for larger-scale genomic projects.	Ideal for researchers requiring comprehensive, high-resolution whole-genome analysis, including de novo genome assembly, rare variant discovery, and precision epigenetic studies.

Summary

- Multiplexed WGS is ideal for studies where multiple genomes need to be sequenced in parallel, offering cost efficiency at the expense of per-sample sequencing depth, making it perfect for population-level studies and higher-throughput projects.
- WGS is best suited for researchers who require deep, high-resolution sequencing of single genomes to capture rare variants and detailed epigenetic information.

Common Challenges and Solutions

Concern	Renew's Solution
Low DNA Input or Quality	Renew's Multiplexed Whole Genome Sequencing service is optimized for high sensitivity, handling inputs as low as 400 ng of gDNA with reliable results. For projects with lower input or quality, we offer custom sample preparation solutions.
Sequencing Efficiency	Our rigorous quality control protocols are designed to optimize sequencing yield. We employ refined QC processes to enhance performance and efficiency for every run, providing reliable results.
Sample Contaminants	To provide reliable and accurate data, stringent purification protocols are used to remove contaminants and prepare samples to high-quality standards.

Service Inclusions

Library Preparation: Full library prep services are included, utilizing the specified ONT kit.

Data Delivery: All sequencing data is provided as unaligned BAM files through Renew's secure cloud-based Laboratory Information Management System (LIMS), offering clients a clear and transparent view of the entire process.

Contact and Pricing

Contact us for more information or request a quote

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