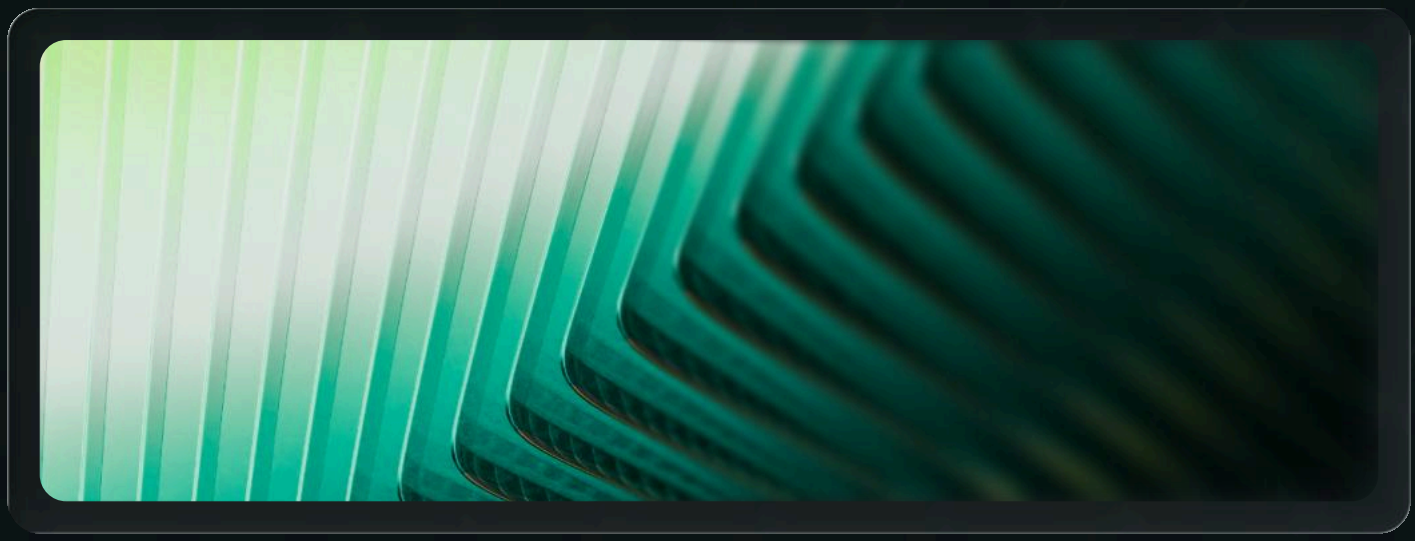
RENEW

Case Study



Scalable Oncology Insights with dWMS

5X Methylation Profiling and Structural Variant Detection

Summary

This case study highlights the utility of 5x direct Whole Methylome Sequencing (dWMS) for multi-omic biomarker discovery in oncology. By downsampling 30x ONT datasets from ovarian, lung, and lymphoma tumor samples, we demonstrate that low-pass dWMS enables cost-effective, high-confidence detection of genome-wide CpG methylation and large structural variants (SVs). These findings position dWMS as a powerful platform for translational research and clinical assay development.

Study Objectives

To evaluate whether 5x dWMS provides sufficient resolution to detect genome-wide methylation and large structural variants in cancer tissue, using repurposed oncology datasets—supporting applications in early biomarker discovery, translational assay development, and low-input tumor profiling.

Methods

Samples

Flash-frozen human ovarian, lung, and lymphoma tumor tissues (n = 1 each) were selected according to the client's original study protocol.

Sequencing Workflow

Genomic DNA (gDNA) was extracted and prepared using Renew Biotechnologies' (Renew) proprietary direct Whole Methylome Sequencing (dWMS) protocol. Native-read sequencing was performed on the Oxford Nanopore Technologies (ONT) PromethION platform, with reads aligned to the GRCh38 human reference genome using Minimap2.

Downsampling & Methylation Analysis

Each 30x dataset was downsampled to 15x, 10x, and 5x genomic coverage using a custom pysam (https://pysam.readthedocs.io/en/latest/faq.html)-based pipeline. Aligned BAMs were converted to WBL's proprietary .CH3 format, which compresses file size by ~95% while retaining base-resolution CpG detail.

Methylation analysis was conducted using ModSeqR, Renew's native-read R package for ONT methylation data. The tool supports CpG summarization by site, genomic region, or annotation window, and includes built-in modules for quality control, sliding window analysis, and differential methylation detection.

High-confidence CpG calls were defined as those with model-predicted probabilities ≥90%, and high-quality reads as those with base quality scores ≥10.

Renew CH3 File Format

Proprietary file format that compresses whole genome ONT methylation data by ~95%, enabling rapid downstream analysis with full-resolution CpG.

ModSeqR

- Renew's custom R package for native-read methylation analysis
- Summarizes CpGs by site, sliding window, or BED annotations
- Performs QC and regional differential methylation analysis
- Fully compatible with CH3 files generated from ONT dWMS

Structural Variant Detection

Large insertions, deletions, duplications, and inversions were identified and visualized at each coverage level using Samplot (https://github.com/ryanlayer/samplot) and custom scripts optimized for ONT native-read alignments.

Key Findings

Genome-Wide Methylation Coverage and Quality

High-coverage dWMS was performed on ovarian, lung, and lymphoma tumor samples (n = 1 each) using ONT PromethION with one sample per flow cell. All samples achieved >30x whole-genome coverage with alignment rates averaging 99.5%, confirming high-quality data suitable for methylation analysis and SV detection (Table 1). These datasets were subsequently downsampled to 15x, 10x, and 5x to evaluate dWMS performance at lower depths.

Given the consistency in sequencing quality and coverage across tumor types, data from the three samples were averaged to illustrate general dWMS performance trends across multiple cancer tissues at varying depths.

Cancer Type	Output	Coverage (x)	% Alignment	Avg Read Length (bp)
Ovarian	107.3	33.5	99.4	8353
Lung	138.4	43.2	99.6	8137
Lymphoma	111.8	37.1	99.5	5288

Table 1. Summary of sequencing performance metrics across tumor samples. dWMS was conducted on three human tumor types using ONT PromethION. Data were downsampled from 30x to assess methylation and SV detection at lower genomic coverage.

This study demonstrates the power of nanopore-based dWS to deliver scalable, high-resolution methylation data without bisulfite conversion[1,2] or PCR amplification[3]. Even at 5x genomic coverage, dWMS achieved 100% 1x CpG coverage across the genome (Figure 1) -far exceeding the reach of traditional Illumina EPIC arrays[4], which interrogate only ~ 3% of unique CpGs (~850,000 of ~28 million). In contrast, 5x dWMS provides native-read access to nearly all CpG sites, enabling base-resolution methylation analysis with full-molecule context.

CpG coverage depth scaled predictably with sequencing input: at 10x, ~90% of CpGs were covered at ≥5x, and at 15x, nearly 80% reached ≥10x coverage. Notably, even at 5x, dWMS captured more than 3x the number of CpGs covered at ≥10x depth than the EPIC array.

Across all coverage levels, the proportion of CpG sites classified as high-confidence (≥90% model probability) and high-quality (base quality ≥10) remained consistent, averaging 78.4% and 93.6%, respectively—highlighting the reproducibility and analytical integrity observed in this dataset using dWMS.

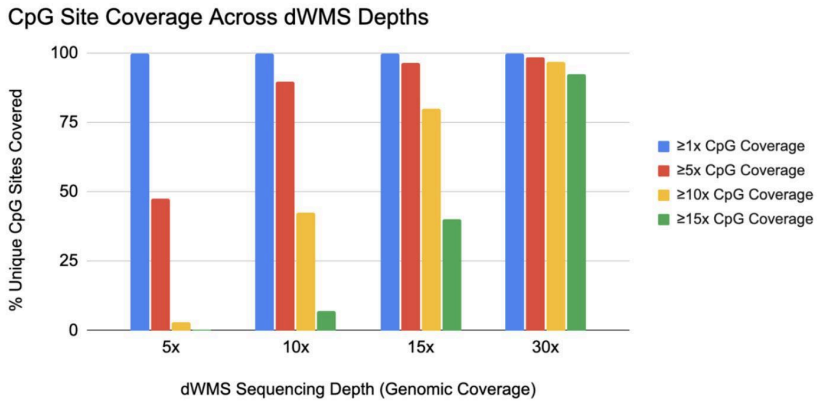


Figure 1. Genome-wide CpG site coverage across downsampled dWMS sequencing depths. Bar plots show the percentage of CpG sites covered at ≥1x, ≥5x, ≥10x, and ≥15x across 5x, 10x, 15x, and 30x genomic coverage using direct Whole Methylome Sequencing (dWMS). Data represent pooled results from three tumor types (ovarian, lung, lymphoma; n = 3). Even at 5x genomic coverage, 100% of unique CpGs were covered at least once, with increased depth achieved at progressively higher sequencing coverages. These findings demonstrate the scalability of dWMS for flexible methylation profiling—from broad discovery to high-confidence regional analysis.

Large Structural Variant Detection

To assess the performance of dWMS for structural variant (SV) detection we selected four large rearrangements spanning insertions, deletions, inversions, and duplications from the ovarian, lung, and lymphoma samples (Figure 2). Although not cancer-specific, these variants were chosen for their size and clarity to illustrate the resolution and consistency of SV detection across coverage levels. Each variant was confidently identified at 5x, 10x, 15x, and 30x, underscoring the power of native-read sequencing to detect complex rearrangements missed by short-read methods.

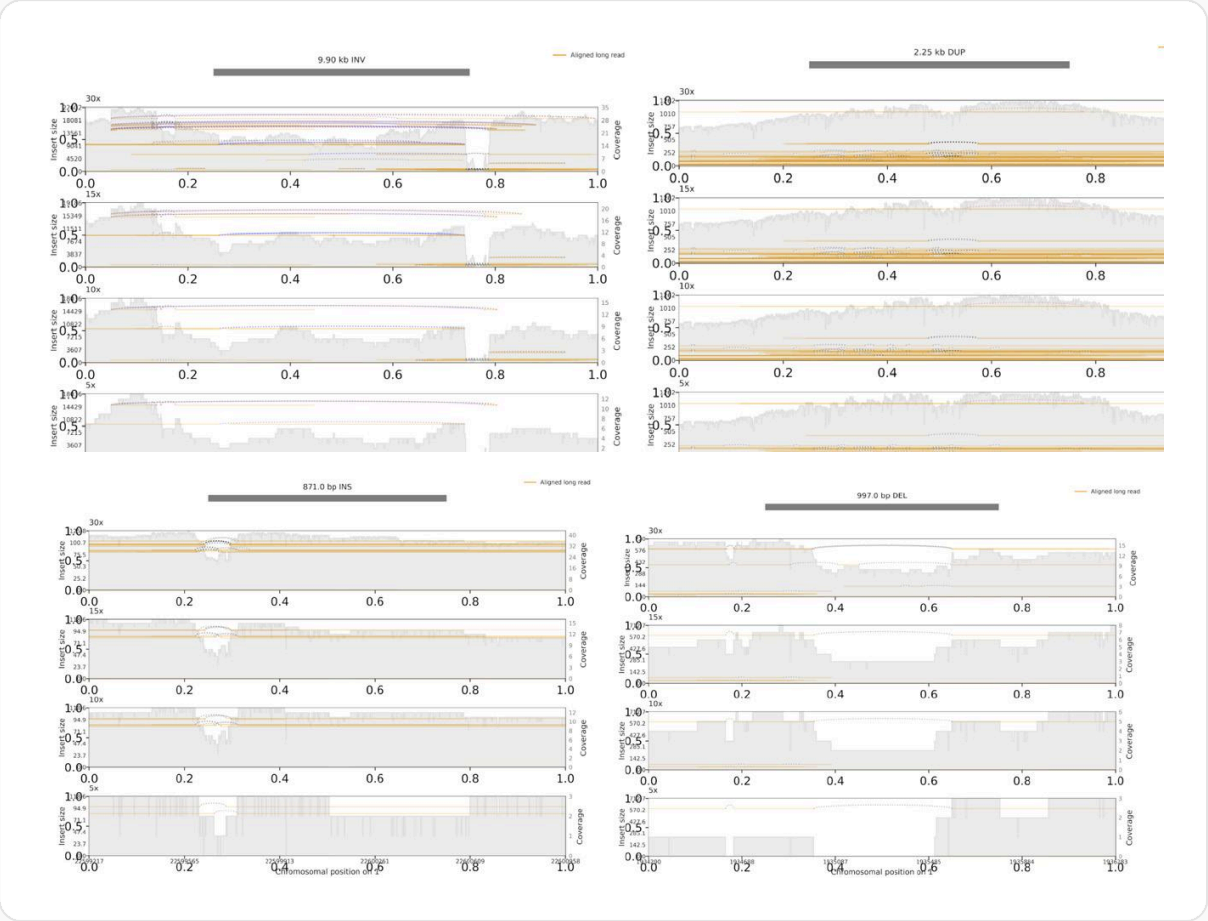


Figure 2. Representative structural variants detected across 5x–30x dWMS coverage. Variants include a 471 bp insertion (A, ovarian), 997 bp deletion (B, lymphoma), 9.9 kb inversion (C, lung), and 2.25 kb duplication (D, ovarian). All were confidently detected across depths, including 5X sequencing coverage, and visualized with Samplot, demonstrating the robustness of native-read SV detection at low-pass coverage.

Conclusions

Renew's 5x dWMS platform delivers high-resolution methylation and SV profiling from a single native-read assay. Even at reduced coverage, dWMS achieves broad CpG detection and resolves large genomic rearrangements, offering a cost-effective, scalable solution for biomarker discovery and assay development in oncology research.

Reference List

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4. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome biology. 2016;17:1-17.