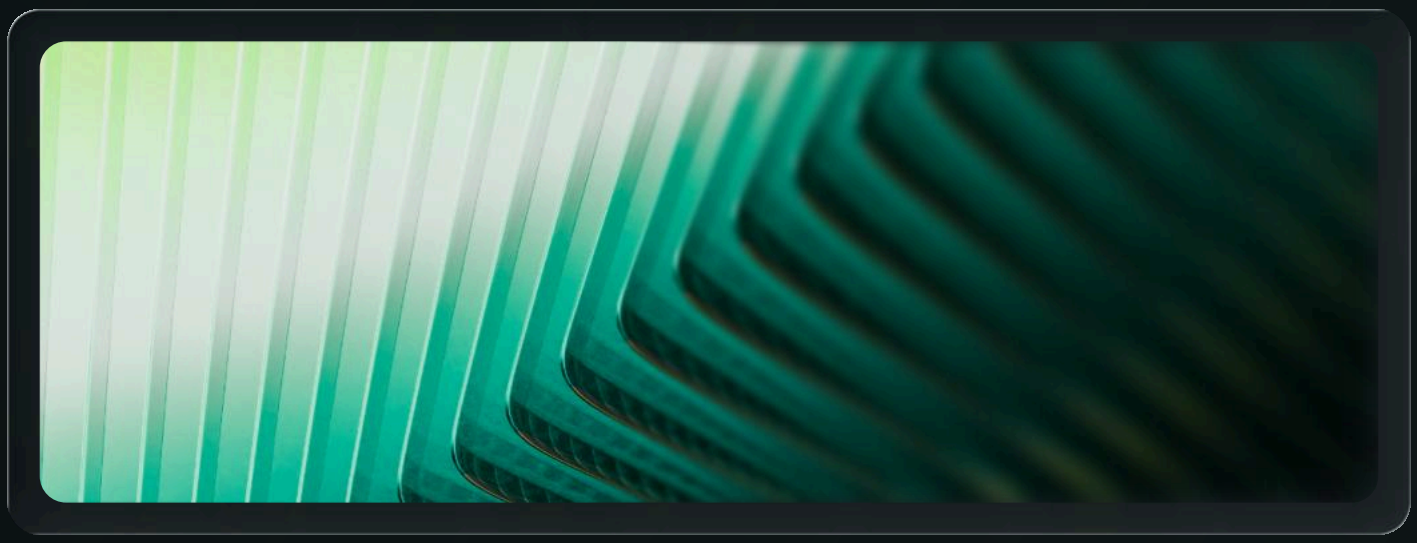
RENEW

Case Study



Targeted Long-Read Sequencing for Translational Oncology

Multi-Omic Variant and Methylation Profiling in Colon Cancer Using dTMS and Agilent SureSelect

Summary

Previous characterization work has demonstrated that Renew Biotechnologies (Renew) Direct Targeted Methylation Sequencing (dTMS) platform enables accurate detection of single-nucleotide variants (SNVs), short tandem repeats (STRs), haplotypes, and regional DNA methylation within a unified targeted long-read workflow. Here, we apply this hybrid capture-based approach to HCT116 colorectal carcinoma cells using the Agilent SureSelect Colon Cancer panel, demonstrating high-accuracy SNV detection and methylation profiling across targeted loci. These results highlight the potential of dTMS for integrated genomic and epigenetic profiling, supporting scalable multi-omic assay development within translational oncology.

Introduction

Genomic and epigenomic alterations that drive cancer development and progression frequently occur in structurally complex regions that are difficult to resolve with conventional short-read sequencing[1]. And while DNA methylation is increasingly recognized as a critical layer of tumor biology and a potential source of actionable biomarkers, conventional methylation assays often lack sensitivity, genomic context, or integration with CpG-level resolution.

Long-read nanopore sequencing addresses these limitations by enabling direct interrogation of both sequence and native methylation while preserving broader genomic context to resolve structurally complex features[2]. To scalably leverage this capability, Renew developed Direct Targeted Methylation Sequencing (dTMS), a targeted long-read platform designed for multi-omic interrogation of genomic variation and native methylation. Prior characterization in Genome in a Bottle reference samples demonstrated that dTMS enables accurate detection of SNVs, STRs, and regional DNA methylation within a unified workflow.

Here, we extend this approach to a targeted oncology setting using the Agilent SureSelect Colon Cancer panel, demonstrating broader compatibility with SureSelect hybrid-capture probe designs and a scalable strategy for integrated genomic and epigenetic profiling in translational oncology.

Study Objectives

This study evaluated whether targeted long-read sequencing enables integrated detection of genomic variation and regional methylation in colon cancer-relevant loci. Specifically, we aimed to:

1. **Detect sequence variation across targeted colon cancer genes**
2. **Characterize regional DNA methylation across captured loci**

Library Preparation & Sequencing

Genomic DNA was extracted and prepared using Renew's dTMS hybrid capture workflow with Agilent SureSelect Colon Cancer probes.

Library Prep	dTMS hybrid capture workflow
Probe Set	Agilent SureSelect Colon Cancer Panel (5282-0062)
Sequencing Platform	Oxford Nanopore PromethION
Samples	n = 4

Genomic DNA was extracted and prepared using Renew's dTMS hybrid capture workflow with Agilent SureSelect Colon Cancer probes.

Bioinformatics Processing

Sequencing data were processed using established long-read pipelines. Downstream analyses included SNV/indel detection and regional methylation profiling across targeted loci.

Reference Genome	GRCh38
Alignment	minimap2
Variant Calling	wf-human-variation, Clair3
Methylation Detection	modkit

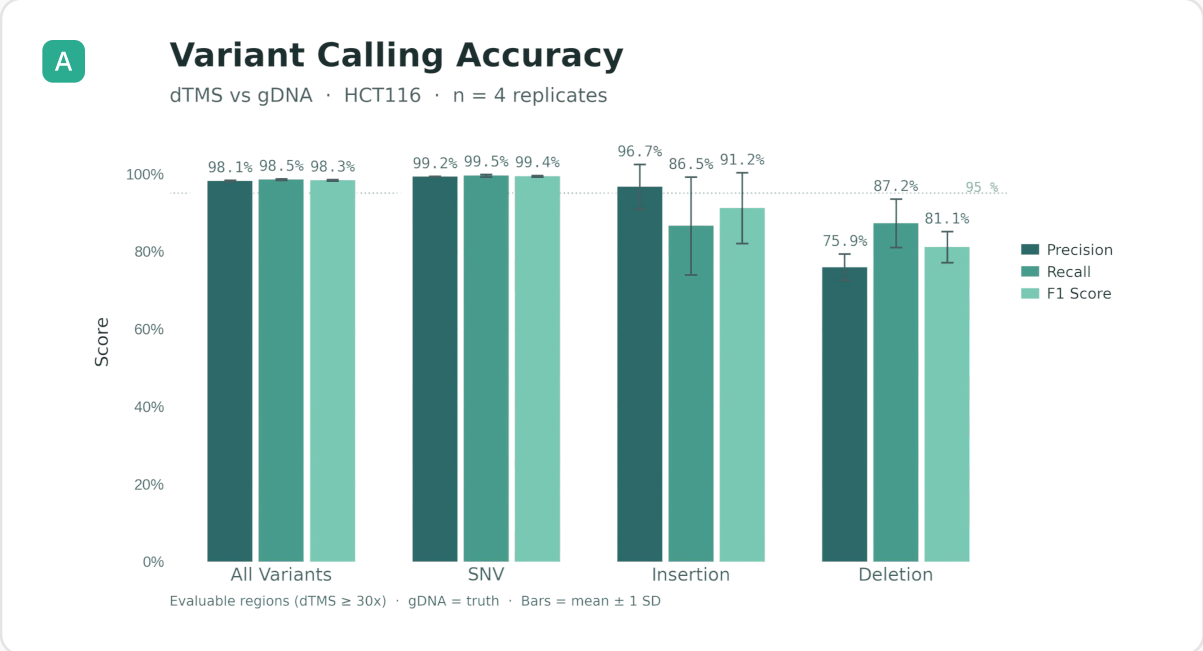
Key Findings

High-Accuracy Variant Detection Across Targeted Regions

Targeted long-read sequencing achieved a mean on-target coverage of 66x (up to 300x across replicates) and 1,464-fold enrichment, enabling high-confidence variant detection across colon cancer loci and suggesting strong target specificity.

Comparison to matched whole-genome nanopore reference data demonstrated high accuracy, with 99.9% concordance for SNVs (Figure 1A). While INDEL detection demonstrated reduced performance, we observed that several deletions occurred within homopolymer regions, which are inherently challenging to resolve across sequencing platforms. Additionally, deletions were underrepresented within the targeted panel, amplifying their impact on overall precision, recall, and F1 performance.
A representative IGV visualization of a homopolymer-associated deletion is shown in Figure 1B with dTMS (top) and nanopore WGS (bottom), demonstrating similar patterns that suggest these challenges arise
from sequencing long mononucleotide stretches rather than
dTMS-specific limitations.

Representative examples of well-characterized pathogenic SNVs in KRAS and PIK3CA oncogenes were accurately captured with dTMS (Figures 1C–D) via concordance with whole-genome nanopore reference data and supporting reliable variant detection within clinically relevant loci.



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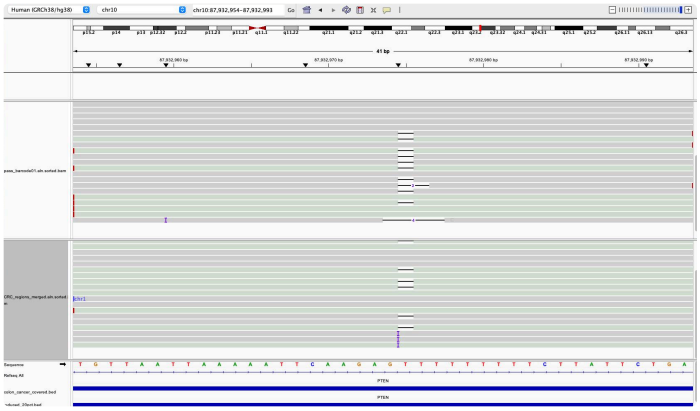

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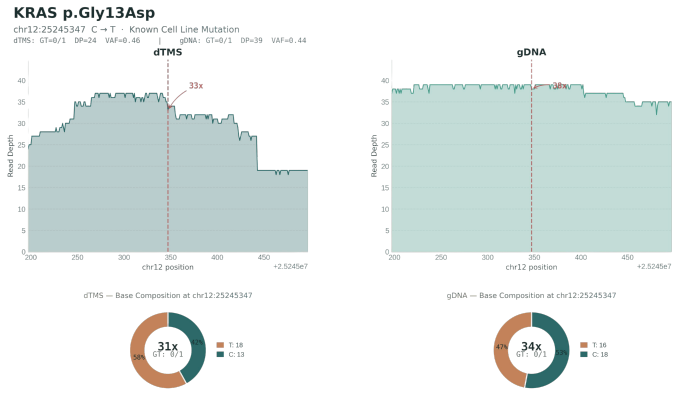

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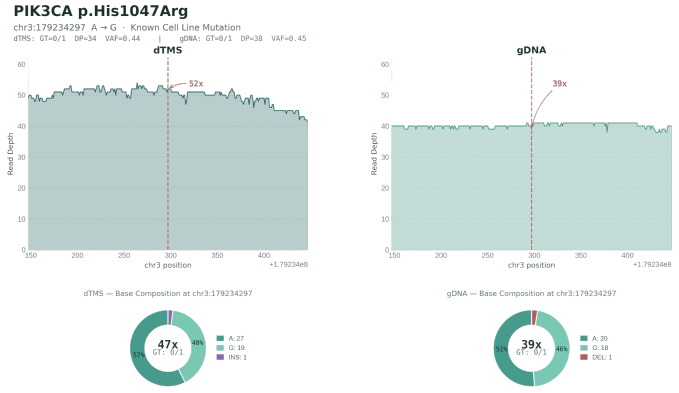

Figure 1. dTMS with Agilent SureSelect enables high-accuracy variant detection across targeted colon cancer loci. gDNA from HCT116 cells (n = 4) was sequenced using dTMS with the Agilent SureSelect Colon Cancer panel on the ONT PromethION platform. Variant calls generated using wf-human-variation and Clair3 were compared to matched nanopore WGS truth data. (A) Variant detection performance metrics. (B) Representative IGV view of a homopolymer-associated deletion, shown for both dTMS (top) and WGS (bottom). (C–D) Representative SNVs in well-characterized oncogenes (KRAS and PIK3CA), demonstrating concordance.

Regional Methylation Characterization

Methylation profiling across target regions demonstrated broad CpG coverage and enabled detection of region-level methylation patterns relevant to tumor biology. Accuracy was evaluated relative to HCT116 whole-genome nanopore sequencing reference data, yielding a mean correlation (R2 = 0.88) and mean absolute error (MAE) of 7.6% (Figure 2A). Per-sample regional accuracy is shown in Figure 2B.

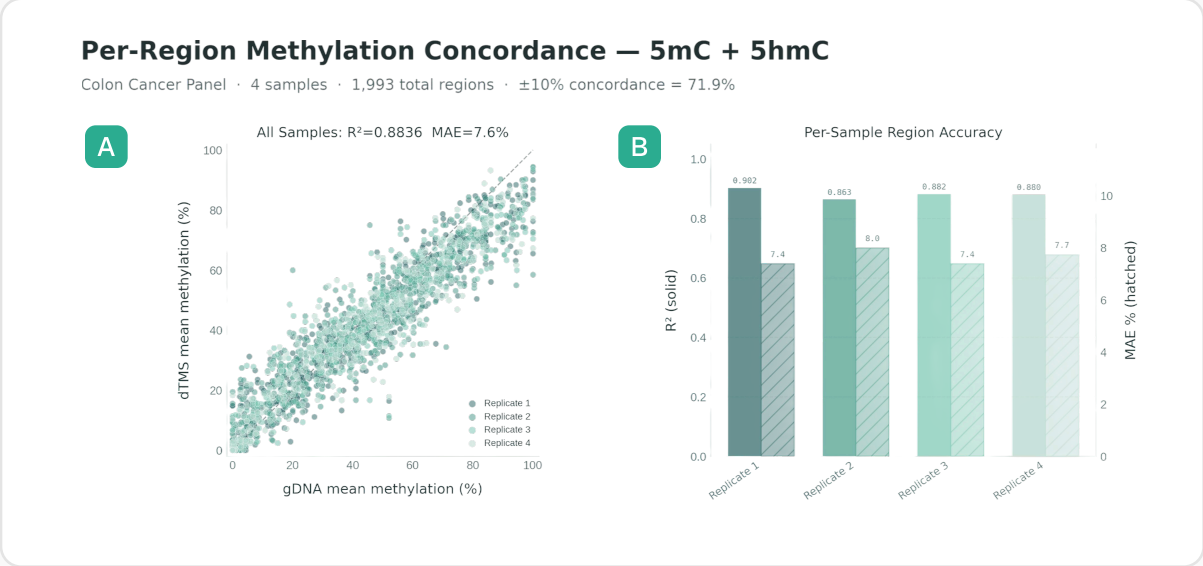

Figure 2. dTMS with Agilent SureSelect enables accurate regional methylation profiling across targeted colon cancer loci. gDNA from HCT116 cells (n = 4) was sequenced using dTMS with the Agilent SureSelect Colon Cancer panel on the ONT PromethION platform. Methylation calls generated using modkit were compared to matched WGS reference data, demonstrating high per-base and per-region concordance (A) and per-sample region accuracy (B).

Discussion

This study demonstrates that targeted long-read sequencing enables integrated detection of genomic variation and DNA methylation within cancer-relevant regions. By combining hybrid capture with native nanopore sequencing, dTMS preserves structural context while enabling epigenetic profiling within a single assay, addressing key limitations of conventional approaches.

Prior characterization studies with Genome in a Bottle samples established that dTMS enables accurate detection of SNVs, STRs, and regional DNA methylation within a unified long-read workflow. Here, we apply this approach to a targeted oncology setting using the Agilent SureSelect Colon Cancer panel, achieving high-accuracy variant detection and methylation profiling across relevant loci. While STRs were not evaluated in this study due to panel constraints, previous work supports their detection within the same framework.

dTMS has also been applied to the Agilent lung cancer panel, demonstrating compatibility across SureSelect panel designs and enabling scalable multi-omic profiling across oncology applications. Together, these findings support continued optimization of dTMS for targeted multi-omic assay development with strong potential for clinical translation in oncology.

Reference List

1. Cosenza MR, Rodriguez-Martin B, Korbel JO. Structural Variation in Cancer: Role, Prevalence, and Mechanisms. Annu Rev Genomics Hum Genet. 2022;23:123-52.
2. Fujimoto A, Wong JH, Yoshii Y, Akiyama S, Tanaka A, Yagi H, et al. Whole-genome sequencing with long reads reveals complex structure and origin of structural variation in human genetic variations and somatic mutations in cancer. Genome medicine. 2021;13(1):65.