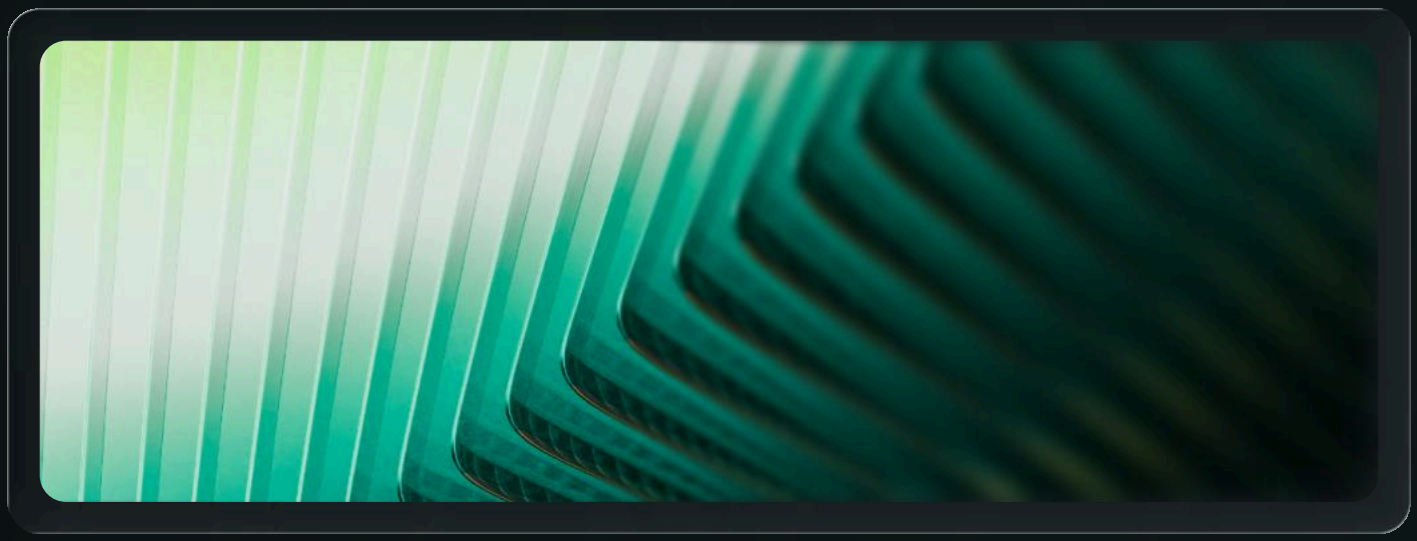
RENEW

Case Study



Native Methylation Detection With Renew Biotechnologies

Native Methylation Detection

Introduction

Unlocking the Plant and Animal Epigenome

DNA methylation is a fundamental epigenetic modification that governs gene expression, development, and adaptation across eukaryotes[1,2]. For plants and animals, understanding methylation patterns can provide key insights into phenotypic plasticity and diversity, environmental resilience, and evolutionary processes. However, accessing this wealth of information—especially in non-model organisms—remains hindered by technical challenges and outdated methodologies[3].

Traditional approaches, including bisulfite sequencing and MeDIP, often introduce biases, degrade DNA, or fail to fully capture methylation in complex genomic regions. These limitations leave major gaps in our understanding, especially for repetitive and polyploid genomes or species with unique methylation landscapes.

Renew Biotechnologies (Renew) bridges this gap with Direct Whole Methylome Sequencing (dWMS), an advanced platform built on Oxford Nanopore Technologies. dWMS directly detects native methylation without requiring bisulfite conversion or synthetic amplification, delivering high-resolution, whole-genome methylation insights across species. With dWMS, researchers can confidently decode the biological significance of methylation across diverse taxa.

The Problem

Challenges in Plant and Animal Epigenetics

Despite its transformative potential, epigenomic research faces enduring obstacles:

- Limited Methylation Maps: Many non-model organisms lack comprehensive methylation profiles, complicating efforts to study epigenetic mechanisms and their influence on gene regulation & adaptation.
- DNA Degradation and Bias: Bisulfite treatment damages DNA and introduces artifacts, while other methods fail to capture methylation patterns in repetitive and other structurally complex regions.
- Genome Complexity: The large, repetitive, and polyploid genomes of plants and animals complicate accurate methylation analysis using short-read sequencing approaches.
- Diversity Across Taxa: Existing methods often lack the flexibility to accommodate the wide-ranging epigenetic landscapes of different species, environments, and developmental stages.
- High Computational Barriers: Analyzing methylation data from large genomes often demands specialized bioinformatics expertise, limiting accessibility.

Renew's Epigenomic Solutions

Direct Whole Methylome Sequencing

This addresses these challenges by providing:

1. Native Methylation Detection: dWMS avoids harsh chemical treatments and PCR amplification, preserving DNA integrity to capture direct methylation signals without degradation or artifacts.
2. Comprehensive Long-Read Coverage: The method maps methylation patterns across entire genomes with long reads, resolving repetitive and structurally complex regions.
3. Adaptable Across Species: From model organisms to novel species, dWMS delivers reliable insights into methylation with flexible coverage.

ModSeqR Technology: Native Methylation Detection

To complement dWMS, Renew has developed ModSeqR, a user-friendly R package designed to simplify methylation data analysis for researchers:

Renew's ModSeqR software simplifies data analysis, empowering researchers to visualize and interpret methylation profiles without extensive bioinformatics expertise.

1. Accessible Tools: Streamline the interpretation of complex nanopore methylation data without requiring advanced bioinformatics expertise.
2. Actionable Insights: Perform methylation profiling, quality control, differential analysis, and visualization with ease.
3. Integration Ready: Combine epigenetic data with genome assembly and transcriptomics for a holistic understanding of biological processes.

Objective

To demonstrate the quality of dWMS for methylation analyses across diverse plant and animal genomes, regardless of species.

Methods

Genomic DNA was extracted from homogenized tissues using tissue-specific protocols. Mouse and zebrafish DNA were extracted with the Qiagen DNeasy Blood and Tissue Kit, while Arabidopsis and mung bean DNA were extracted with the Qiagen DNeasy Plant Pro Kit, and all samples were eluted in water. Libraries were prepared and sequenced on a single PromethION flow cell using Renew's dWMS protocol, with multiplexing adjusted based on genome size and study objectives.

Results

Thale Cress (Arabidopsis thaliana) 140 Mb Genome

As a model organism for plant genomics, A. thaliana's compact genome offers an ideal system for benchmarking methylation detection technologies. Using dWMS, four samples multiplexed per flow cell achieved average genomic coverage >220X and CpG coverage >99.9%. Expanding to a 16-plex configuration maintained high performance, with average coverage >48X and CpG coverage >99.4% (Table 1). These results underscore dWMS's scalability and precision in small-genome methylation mapping, setting a standard for plant epigenomic studies.

Metric	4-Plex Batch Avg	16-Plex Batch Avg
Genomic Coverage (Depth)	> 220X per sample	> 48X per sample
CpG Coverage	> 99.9% per sample	> 99.4% per sample

Table 1. A. Thaliana methylation metrics using dWMS.

Zebrafish (Danio rerio) 1.4 Gb Genome

Renowned for its rapid development, external fertilization, and ease of genetic manipulation, D. rerio has emerged as a cornerstone model for developmental biology, with over 70% genomic similarity to humans[4]. Using dWMS, genomic DNA was sequenced from embryos at 48 and 72 hours post-fertilization. At 48 hours, the platform achieved average >30X genomic coverage per sample, capturing over 91% of CpGs across the genome. At 72 hours, the platform maintained strong performance with average >34X coverage and >92% CpG capture (Table 2). These results demonstrate dWMS's ability to resolve methylation dynamics across developmental stages, offering valuable insights into vertebrate epigenetic regulation.

Metric	48 hr Batch Avg	72 hr Batch Avg
Genomic Coverage (Depth)	> 30.9X per sample	> 34.6X per sample
CpG Coverage	> 91.4% per sample	> 92% per sample

Table 2. D. Rerio methylation metrics at 48 (n = 2) and 72 (n = 2) hours post-fertilization using dWMS.

Mouse (Mus musculus) 2.5 Gb Genome

As a key mammalian model for biomedical research, M. musculus was used to evaluate dWMS on complex genomes. Whole brain DNA was extracted from CD1 mice. Data were averaged across controls, and two groups of mice that were exposed to morphine and two different concentrations of lysergic acid diethylamide (LSD). Sequencing achieved an average >3.7X genomic coverage and CpG coverage >72% (Table 3), approximately 22X the number of CpG sites (900k) typically covered by methylation arrays[5]. These results highlight dWMS's potential in resolving mammalian genomic complexity and studying epigenetic responses to drug exposure.

Metric	Batch Avg
Genomic Coverage (Depth)	> 3.7X
CpG Coverage	> 72%

Table 3. Average M. musculus methylation metrics for control, and morphine and LSD-treated whole mouse brain samples using dWMS (n = 9). Samples were multiplexed at 3 samples per PromethION flow cell.

Conclusion

Together, these case studies illustrate the transformative potential of dWMS in providing high-quality, unbiased methylation data across a wide range of plant and animal genomes. By overcoming traditional challenges such as DNA degradation, synthetic biases, and genome complexity, dWMS enables researchers to explore methylation dynamics with unprecedented resolution. From deciphering epigenetic regulation in model organisms like Arabidopsis thaliana and Mus musculus and beyond, Renew's innovative approach empowers breakthroughs in gene regulation, environmental adaptation, and evolutionary biology. These findings highlight dWMS as a versatile and indispensable tool for advancing epigenomic research across diverse taxa.

Additional Services

Genome Assembly

To meet diverse research objectives, Renew offers tiered genome assembly services, ranging from foundational gene discovery to comprehensive genomic and functional analyses.

Three Tiers of Renew's Genome Assembly

Renew Biotechnologies provides three scalable options: moderate-coverage draft genomes for gene discovery, ultra-long-read chromosome-level assemblies, and advanced functional insights integrating RNA sequencing and native methylation data.

Tier	Offering	Description
01	Draft Genome	Moderate-coverage assemblies for gene annotation and genome size estimation.
02	Chromosome-Level Assembly	Ultra-long reads for resolving repetitive regions.
03	Enhanced Functional Insights (In Development)	Direct RNA and methylation data integrated with genome assembly.

Genome Assembly Coverage Guidelines

Coverage is key to achieving high-quality genome assemblies, as it directly impacts the resolution of repetitive regions, structural variants, and overall genome continuity. Tailoring coverage levels to your research goals ensures optimal results. The table below summarizes coverage recommendations and expected outputs for Renew's genome assembly tiers.

Attribute	Draft Genome	Chromosome-Level Assembly	Enhanced Functional Insights**
Coverage*	High (30-50X)	Draft Genome	High (>50X)
Assembly Output*	Fragmented Contigs	Chromosome-Level Scaffolds	Chromosome-level scaffolds with integrated gene expression data
Technology	Long Reads	Chromosome-Level Scaffolds	Long reads | Ultra-Long Reads Direct RNA sequencing
Continuity*	Limited, Due to Unresolved Repeats	Full continuity with repeat resolution & epigenetic mapping	Full continuity with repeat resolution and epigenetic mapping
Use Cases	Gene Discovery Genome Size Estimation	Comprehensive Genomic, Epigenomic, & Structural Studies	Functional genomics, transcriptomics, and epigenetics

* Dependent on sample quality and quantity. ** In Development

Unlock The Full Potential Of Your Genomic Research With Renew Biotechnologies.

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