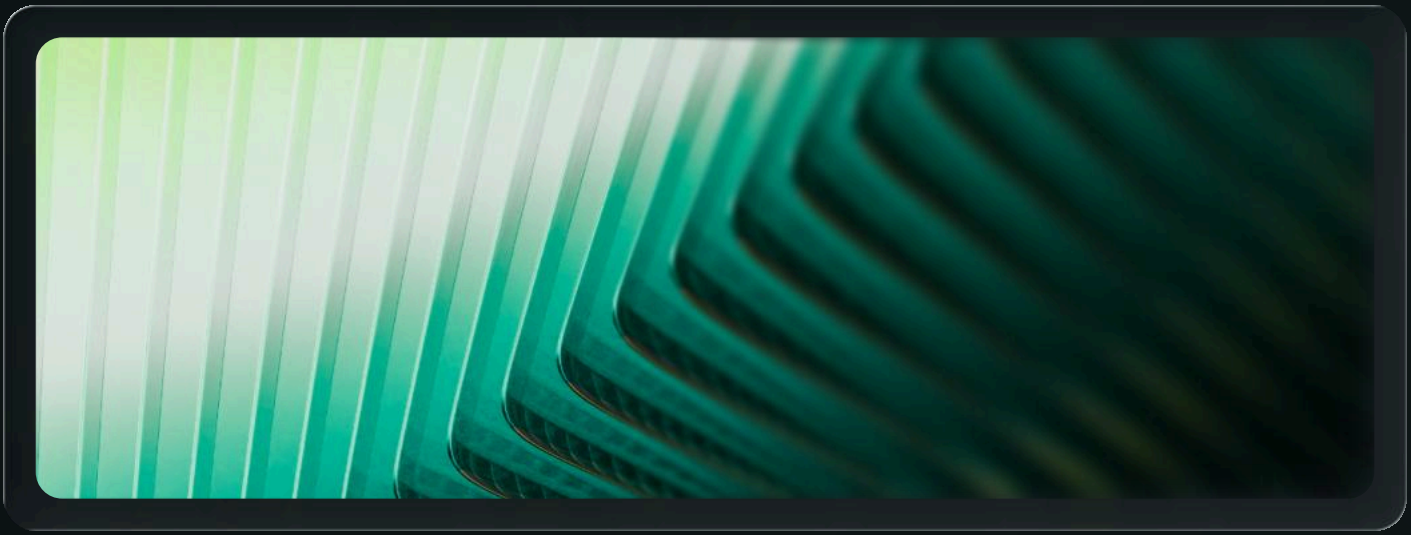
RENEW

Case Study



PCR-Free Whole-Genome Microbial Profiling with dWMS

Species-level accuracy, methylation stability, and the limitations of 16S-based approaches

Summary

Renew Biotechnologies' Direct Whole Methylome Sequencing (dWMS) platform delivers robust, PCR-free microbial profiling that combines species-level quantification with genome-wide methylation detection in a single, scalable workflow. Unlike conventional 16S-based methods constrained by PCR-induced bias, copy number variation, and poor primer coverage, dWMS enables taxonomic and epigenetic discovery directly from native DNA, even under low-input conditions. This case study demonstrates how dWMS outperforms targeted methods in species resolution, functional depth, and data fidelity, positioning it as a high-impact alternative for microbial community profiling and microbiome research.

Study Objectives

Microbial community profiling is foundational to microbiome research, pathogen surveillance, and environmental monitoring. Yet conventional 16S-based methods rely on PCR amplification, leaving them prone to primer mismatch, species dropout, compositional distortion, and an inability to capture epigenetic signatures[1,2].

To address these challenges, Renew Biotechnologies (Renew) evaluated long-read, whole-genome sequencing for high-resolution, scalable microbial profiling. This case study investigates Renew's Direct Whole Methylome Sequencing (dWMS) platform, a PCR- and bisulfite-free method based on Oxford Nanopore Technologies (ONT), in two applications: (1) full-genome taxonomic profiling and (2) bioinformatically targeted 16S/18S analysis from dWMS reads. These workflows were compared against ONT's long-read 16S barcoding kit and Illumina short-read 16S sequencing to assess species-level accuracy, PCR bias, low-pass performance, and the added value of native methylation detection.

Methods

Samples & Processing

The ZymoBIOMICS Microbial Community DNA Standard (10 microbial genomes: eight bacteria, two yeasts) were used as the benchmarking sample. gDNA composition provided by Zymo reflected 12% for each bacterial species and 2% for each yeast.

Available Illumina 16S sequencing data from Zymo were used as a short-read comparison (250 bp paired-end). The ONT 16S Barcoding Kit (SQK-16S114) was used to generate a 12-plex full-length 16S library. gDNA was prepared for whole genome dWMS sequencing in alignment with Renew's dWMS protocol.

Sequencing & Bioinformatics

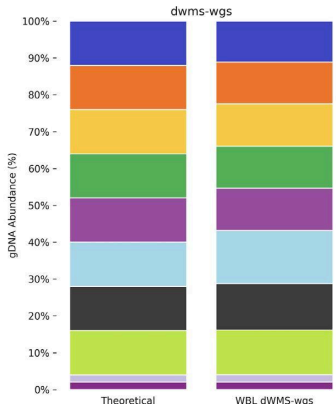
16S/18S aligned reads from ONT 16S Kit and dWMS protocols were aligned to Zymo's curated reference database. Whole-genome alignments and methylation calling were performed using custom scripts developed using NCBI RefSeq genomes, Minimap2 for alignment, and SAMtools for processing. Data were first filtered to include only primary aligned reads then downsampled to 90% through 1.25%.

Methylation calls were generated using modkit with extract and motif-bed arguments to specify modification calling at defined CpG regions. This process was iterated in parallel at the WGS and each downsampling level. Visualizations and analysis were conducted in Python using the pandas, numpy, matplotlib, seaborn, re, glob, and os libraries.

Key Findings

dWMS Accurately Reflects Community Composition

A single replicate of the microbial standard on a single PromethION flow cell generated 142 Gb of raw data, with 127 Gb passing quality filters (Q9). All 10 expected species were correctly identified, with relative abundances only deviating by an average of 5.7% from provided values (Figure 1). This highlights dWMS's precision for whole-genome microbial reconstruction and quantification.

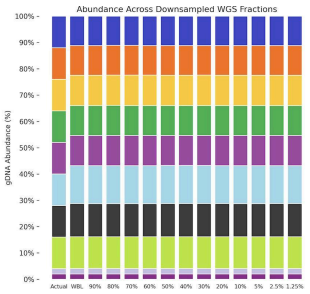


	Species	Avg. GC (%)	Gram Stain	Theoretical Abun. (gDNA%)	Actual Abun. (Reads)	xCov. WGS	xCov. 16S
	Pseudomonas aeruginosa	66.2	-	12	11.15	1981.34	9.0
	Escherichia coli	56.8	-	12	11.3	440.28	4.0
	Salmonella enterica	52.2	-	12	11.53	2695.28	7.0
	Lactobacillus fermentum	52.8	+	12	11.37	6255.74	16.0
	Enterococcus faecalis	37.5	+	12	11.44	3294.41	15.0
	Staphylococcus aureus	32.7	+	12	14.45	5150.74	18.0
	Listeria monocytogenes	38.0	+	12	12.58	3499.99	4.0
	Bacillus subtilis	43.8	+	12	12.08	558.31	8.0
	Saccharomyces cerevisiae	38.4	Yeast	2	2.07	1136.32	3.0
	Cryptococcus neoformans	48.2	Yeast	2	2.02	833.17	3.0

Figure 1. dWMS accurately recapitulates the theoretical composition of a 10-species microbial community.

dWMS Maintains Fidelity Under Downsampling

To test scalability, dWMS data were downsampled across 12 tiers. Even at 1.25% of the full dataset, abundance estimates remained consistent with expected values, demonstrating stable taxonomic resolution under reduced coverage (Figure 2).

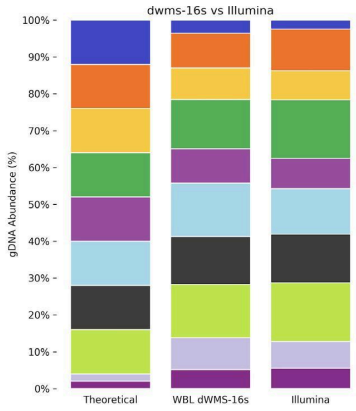


	Species	90% WGxCov	80% WGxCov	70% WGxCov	60% WGxCov	50% WGxCov	40% WGxCov	30% WGxCov	20% WGxCov	10% WGxCov	5% WGxCov	2.5% WGxCov	1.25% WGxCov
	Pseudomonas aeruginosa	1502.69	1335.08	1168.14	1001.17	833.81	666.93	500.6	334.25	166.92	83.22	41.72	20.98
	Escherichia coli	2313.12	2055.9	1799.27	1541.33	1285.18	1027.78	770.3	513.89	257.16	128.61	64.16	32.19
	Salmonella enterica	2577.45	2291.39	2004.92	1718.9	1431.02	1144.0	857.74	571.43	286.13	143.18	71.43	35.93
	Lactobacillus fermentum	4621.11	4107.46	3595.69	3081.76	2568.96	2052.99	1541.58	1029.28	515.59	257.75	129.55	64.68
	Enterococcus faecalis	3636.39	3232.68	2829.48	2424.17	2019.46	1616.48	1212.05	808.22	404.64	202.42	101.14	50.69
	Staphylococcus aureus	5060.9	4499.37	3937.46	3375.62	2812.88	2250.05	1686.38	1124.01	561.8	280.64	139.83	69.9
	Listeria monocytogenes	4344.11	3859.25	3376.04	2894.16	2410.59	1929.89	1446.83	964.78	481.86	241.09	120.35	60.1
	Bacillus subtilis	2649.78	2355.19	2060.31	1764.82	1471.03	1177.14	882.01	586.95	293.15	146.09	73.08	36.54
	Saccharomyces cerevisiae	174.34	155.02	135.54	115.91	96.48	77.29	57.81	38.8	19.39	9.74	4.89	2.51
	Cryptococcus neoformans	105.33	93.65	81.88	70.2	58.51	46.86	35.11	23.46	11.73	5.9	2.96	1.49

Figure 2. dWMS composition accuracy maintained at low coverage.

Bioinformatically Targeted 16S from dWMS Mirrors Illumina Bias

Bioinformatically extracted 16S/18S reads from dWMS sequencing mirrored skew observed in the short-read Illumina data, likely reflecting a combination of rRNA gene copy number variation, conserved sequence overlap, and mapping ambiguity (Figure 3). This reinforces the limitations of single-marker profiling, even when embedded in whole-genome

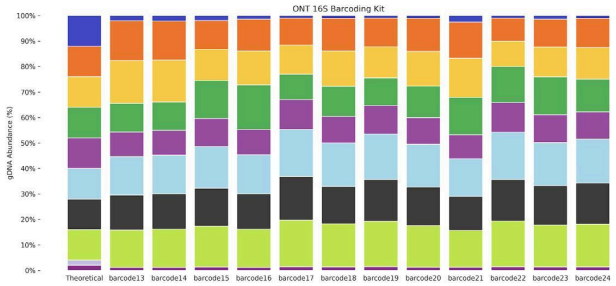


	Species	Avg. GC (%)	Gram Stain	gDNA Abun. (%)	dWMS 16s xCov.	Illumina 16s xCov.
	Pseudomonas aeruginosa	66.2	-	12	9.0	0.146
	Escherichia coli	56.8	-	12	4.0	0.066
	Salmonella enterica	52.2	-	12	7.0	0.104
	Lactobacillus fermentum	52.8	+	12	16.0	0.316
	Enterococcus faecalis	37.5	+	12	15.0	0.301
	Staphylococcus aureus	32.7	+	12	18.0	0.29
	Listeria monocytogenes	38.0	+	12	4.0	0.05
	Bacillus subtilis	43.8	+	12	8.0	0.104
	Saccharomyces cerevisiae	38.4	Yeast	2	3.0	0.041
	Cryptococcus neoformans	48.2	Yeast	2	3.0	0.04

Figure 3. Limitations of 16S profiling across methods.

ONT 16S Amplicons Show PCR-Related Variability

The ONT 16S barcoding kit delivered full-length reads but showed compositional skew similar to other 16S methods (Figure 4). Notably, S. cerevisiae failed to amplify in half of replicates, highlighting risks of primer dropout and PCR bias.



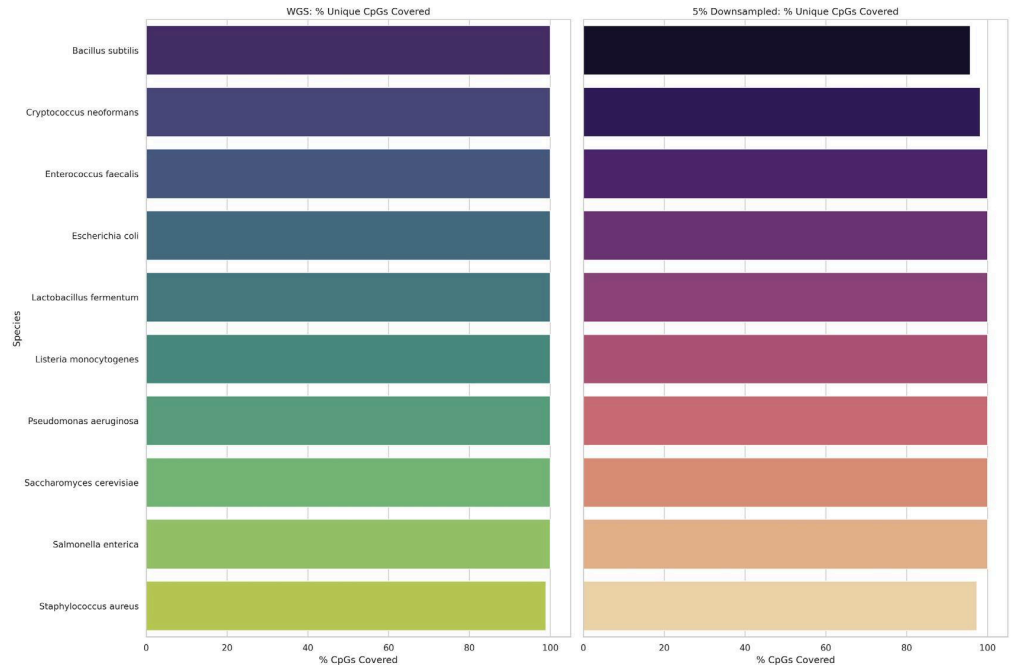
	Species	Avg. GC (%)	Gram Stain	gDNA Abun. (%)	Avg. 16s xCov
	Pseudomonas aeruginosa	66.2	-	12	0.37
	Escherichia coli	56.8	-	12	3.71
	Salmonella enterica	52.2	-	12	4.51
	Lactobacillus fermentum	52.8	+	12	10.32
	Enterococcus faecalis	37.5	+	12	5.89
	Staphylococcus aureus	32.7	+	12	9.63
	Listeria monocytogenes	38.0	+	12	8.2
	Bacillus subtilis	43.8	+	12	6.32
	Saccharomyces cerevisiae	38.4	Yeast	2	PCR failed
	Cryptococcus neoformans	48.2	Yeast	2	0.11

Figure 4. 16S variability from long-read PCR amplicons.

Methylation Insights

We benchmarked CpG coverage from full dWMS data versus 5% downsampling across the ten microbial species. Despite a 95% reduction in sequencing depth, all species retained at least 97% of unique CpG sites detected at ≥1× coverage (Figure 5). Eight of ten species maintained 100% of CpG site detection, underscoring the robustness of ONT-based methylation calling—even in low-input contexts.

Together, these findings reinforce dWMS as a robust, PCR-free platform for accurate microbial reconstruction and reliable epigenetic profiling, scalable across input levels and sequencing depths.



Percent of Unique CpG Sites Covered at Least Once (1×)

Species	WGS	5% Downsample
Bacillus subtilis	100	95.7
Cryptococcus neoformans	100	98.1
Enterococcus faecalis	100	100
Escherichia coli	100	100
Lactobacillus fermentum	100	100
Listeria monocytogenes	100	100
Pseudomonas aeruginosa	100	100
Saccharomyces cerevisiae	100	100
Salmonella enterica	100	100
Staphylococcus aureus	98.9	97.4

Figure 5. Proportion of unique CpG sites detected at ≥1× coverage per species, comparing full and 5% downsampled dWMS data.

Conclusion

Renew's Direct Whole Methylome Sequencing (dWMS) platform offers a high-resolution, multi-omic alternative to conventional microbial profiling workflows. By combining accurate species quantification with genome-wide methylation detection in a single, PCR- and bisulfite-free workflow, dWMS enables comprehensive analysis of microbial identity, function, and regulatory state, even under significantly reduced data conditions.

Conventional 16S-based methods rely on PCR amplification, making them prone to primer mismatch, species dropout, compositional distortion, and an inability to capture epigenetic signals. These limitations were evident across short-read 16S, long-read amplicons, and WGS-derived 16S profiles, all of which showed consistent taxonomic skew and dropout effects. Where 16S-based approaches fall short due to amplification bias, copy number variation, and lack of regulatory resolution, dWMS delivers whole-genome context and native DNA methylation signals with the flexibility to support a wide range of study designs. In contrast to 16S workflows, dWMS accurately recapitulated expected microbial abundances and preserved epigenetic architecture, even at 5% of total sequencing depth.

Beyond microbial profiling, dWMS applications extend to host epigenetics, enabling integrated investigations of how microbial communities influence—and are influenced by —host regulatory dynamics. In parallel human analyses, dWMS demonstrated broader CpG island coverage compared to microarray approaches, low batch variability, and high-resolution tissue-of-origin detection[3], supporting use in studies of mucosal inflammation, gut–brain axis signaling, immune imprinting, and epigenetic remodeling during infection[4]. These capabilities open new avenues for understanding host–microbe interactions and other applications in contexts such as inflammatory and metabolic diseases[5,6], antimicrobial response[7], and microbiome-mediated immunomodulation[8], areas for both basic research and translational discovery.

Together, these findings establish dWMS as a discovery-ready platform that unifies taxonomic profiling and epigenetic insight. From infectious disease surveillance to translational microbiome research, dWMS opens new avenues for whole-genome and whole-epigenome applications in systems biology, with the flexibility and scalability to support both discovery research and applied microbiome studies. dWMS enables researchers to move beyond taxonomic snapshots into full-genome, regulatory, and systems-level discovery.

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Partnering with Renew Biotechnologies

Renew offers end-to-end microbial profiling solutions leveraging long-read sequencing and advanced bioinformatics. Our dWMS platform supports infectious disease, microbiome, and environmental studies with unmatched accuracy and multi-omic depth.

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