EpiFinder™ cNUC

Epigenica

Multiplexed Epigenetic Profiling of Circulating Nucleosomes in Colorectal Cancer Using EpiFinder™ cNUC

Executive Summary

This application note demonstrates integrated, multiplexed epigenetic profiling of circulating nucleosomes from plasma using EpiFinder™ cNUC.

- 24 plasma samples (12 colorectal cancer, 12 controls) analyzed in a single multiplexed workflow
- 200 µL EDTA plasma input per sample
- Parallel profiling of four histone modifications and DNA methylation
- Integrated fragmentomics and copy--number analysis from the same Input library
- Clear CRC vs control separation by unsupervised clustering and PCA
- Gene-level identification of CRC-associated regulatory programs

These findings demonstrate robust multiplexed epigenomic profiling with EpiFinder™ cNUC, enabling biologically meaningful insight from limited liquid biopsy input in a clinically relevant cohort.

Beyond Genetics: Liquid Biopsies yield insight into Epigenetic Regulation

Epigenetic regulation plays a central role in controlling gene expression and cellular identity and is extensively altered in cancer and other diseases. These regulatory changes are encoded in genome-wide patterns of histone post-translational modifications (hPTMs) and DNA methylation, which are preserved on circulating nucleosomes released into the bloodstream.

While circulating cell-free DNA (cfDNA)–based liquid biopsy approaches have transformed cancer research and diagnostics, they primarily capture genetic alterations and fragmentomics features, leaving the regulatory epigenetic layer largely unexplored. Circulating nucleosomes (cNUCs) retain their chromatin context, providing direct insight into cell-of--origin regulatory programs and chromatin states.

Here we present a pilot study using EpiFinder™ cNUC, a high throughput multiplexed platform for profiling multiple epigenetic marks from biobanked blood plasma. Using only 200 µL plasma per sample, we profiled 24 human plasma samples across four histone marks and DNA methylation, demonstrating robust data quality, biologically meaningful chromatin patterns, and the ability to uncover cancer-associated epigenetic signatures.

Why Circulating Nucleosome Epigenomics?

Current liquid biopsy genomics approaches predominantly focus on sequence variants, copy-number alterations, or DNA methylation in isolation. While powerful, these methods provide a limited view of disease biology and often require multiple independent workflows to interrogate different regulatory layers.

Circulating nucleosomes preserve the native chromatin context of their cell of origin, including histone modifications and DNA methylation on the same DNA fragments. Profiling these features enables direct access to regulatory states such as promoter activity, enhancer usage, Polycomb-mediated repression, and heterochromatin organization, key processes underlying cellular identity and disease.

EpiFinder™ cNUC uniquely enables multiplexed epigenetic profiling from plasma and serum, allowing multiple histone marks and DNA methylation to be measured simultaneously from the same sample. This integrated view captures multi-layered regulatory information, which cannot be accessed by single-modality liquid biopsy assays, and provides a scalable foundation for biomarker discovery, disease monitoring, precision medicine, companion diagnostics and early disease detection.

What EpiFinder™ cNUC Offers

- Multiplexed epigenetic profiling from plasma without increasing sample input
- Integrated epigenetics, fragmentomics and copy-number analysis within a single workflow
- Quantitative cross-sample comparison enabled by pooled barcoding and processing
- Complementary biological insight from parallel profiling of multiple histone marks and DNA methylation
- Discovery of disease-associated chromatin signatures across distributed regulatory features
- Informative profiling independent of tumor fraction or CNA status

Together, these capabilities establish EpiFinder™ cNUC as a scalable and integrated platform for next-generation liquid biopsy epigenomics.

EpiFinder™ cNUC Workflow Overview

EpiFinder™ cNUC is built around a single-run, multiplexed workflow for up to 24 samples, enabling genome-wide epigenetic profiling of circulating nucleosomes directly from plasma or serum (Figure 1).

KEY WORKFLOW PRINCIPLES INCLUDE:

- Direct barcoding and pooling of circulating nucleosomes from liquid biopsy material, enabling high-throughput processing while preserving quantitative relationships between samples and minimizing technical variation
- Parallel profiling of multiple epigenetic marks from the same pooled sample
- Integrated Input control library, enabling copy-number analysis, fragmentomics, and tumor DNA fraction estimation
- Sequencing-based unified bioinformatic processing, delivering analysis-ready epigenomic data

This integrated and pooled design enables accurate comparisons across all samples and epigenetic marks without the need for external spike-in controls.

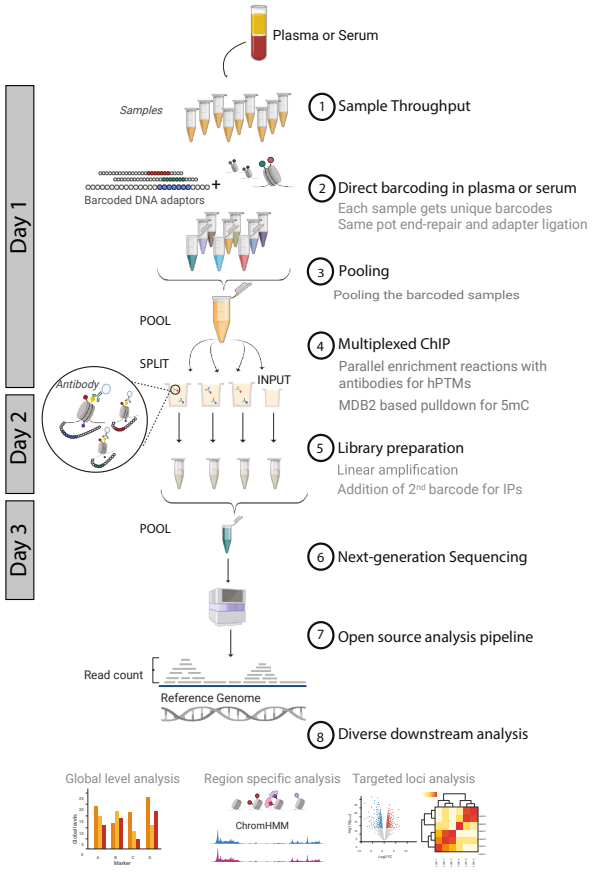

Figure 1. The schematic illustrates the workflow of EpiFinder cNUC. Schematic representation of the single-run, multiplexed workflow for circulating nucleosome profiling from plasma or serum.

Study Design

The EpiFinder™ cNUC workflow was applied to a case–-control plasma cohort comprising 12 colorectal cancer (CRC) patients and 12 non-cancer controls (Figure 2). For each individual, 200 µL of EDTA plasma was processed, and all 24 samples were analyzed within a single multiplexed workflow. Four histone modifications and CpG DNA methylation were profiled, as summarized in Table 1. An Input control library was generated in parallel to enable copy-number analysis, tumor DNA fraction estimation, and fragmentomic assessment.

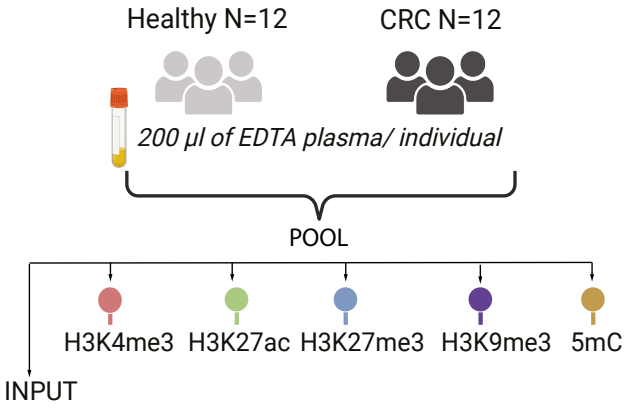

Figure 2. Graphical illustration of the experimental setup. Twenty-four plasma samples,12 colorectal cancer patients (CRC) and 12 healthy controls were processed in a single pooled workflow to profile histone modifications, DNA methylation, and an input control using EpiFinder™ cNUC.

TABLE 1. EPIGENETIC FEATURES PROFILED IN THIS STUDY

Epigenetic Marks	Biological Association	Functional Context
H3K4me3	Active promoters	Marks transcription start sites of actively transcribed genes
H3K27ac	Active enhancers and promoters	Associated with active regulatory elements and gene activation
H3K27me3	Polycomb-mediated repression	Linked to developmentally regulated and silenced genes
H3K9me3	Constitutive heterochromatin	Associated with repetitive elements and stable gene silencing
DNA methylation (5mC)	DNA methylation at CpG-rich regions	Regulates gene expression and chromatin accessibility

Primary Data Quality and Assay Performance

ROBUST GENOME-WIDE PROFILING FROM LIMITED PLASMA INPUT

Across all 24 samples and five libraries (four histone marks plus Input), EpiFinderTM cNUC generated sufficient high-quality, genome-wide reads from only 200 µL plasma sample to perform a variety of downstream analyses and identify disease-associated alterations.

INPUT LIBRARY AS A MULTI-PURPOSE READOUT

The pooled Input library represents cfDNA from all 24 samples and enables parallel analysis of:

- Estimation of tumor DNA fraction
- Detection of large-scale copy-number alterations (CNAs)
- Fragmentomic profiling

In this cohort, a subset of samples displayed characteristic CNAs, confirming that the Input library can serve as a classical cfDNA analysis layer alongside epigenetic profiling. By integrating tumor fraction estimation, copy-number analysis, and fragmentomic characterization within the same workflow, EpiFinder™ cNUC delivers a unified view of circulating DNA biology within a single workflow.

FRAGMENTOMIC PROFILING REVEALS TUMOR-ASSOCIATED DNA FRAGMENTATION PATTERNS

To evaluate whether fragmentomic information is preserved within the pooled workflow, we analyzed cfDNA fragment size distributions derived from the Input library (Figure 3).

Median fragment size was significantly reduced in colorectal cancer (CRC) samples compared to healthy controls ($p = 0.017$), consistent with established ctDNA biology (Figure 3A).

Fragment size distribution curves demonstrated canonical mono-nucleosome peaks (~145–170 bp) across all samples, confirming preservation of nucleosomal structure (Figure 3B). Notably, CRC samples showed increased representation of sub-nucleosomal fragments (<140 bp, Figure 3C), a known characteristic of tumor-derived cfDNA.

Together, these results confirm that early barcoding and pooled processing preserve biologically meaningful fragmentomic signals, enabling simultaneous analysis of copy-number alterations and DNA fragmentation patterns within the same assay.

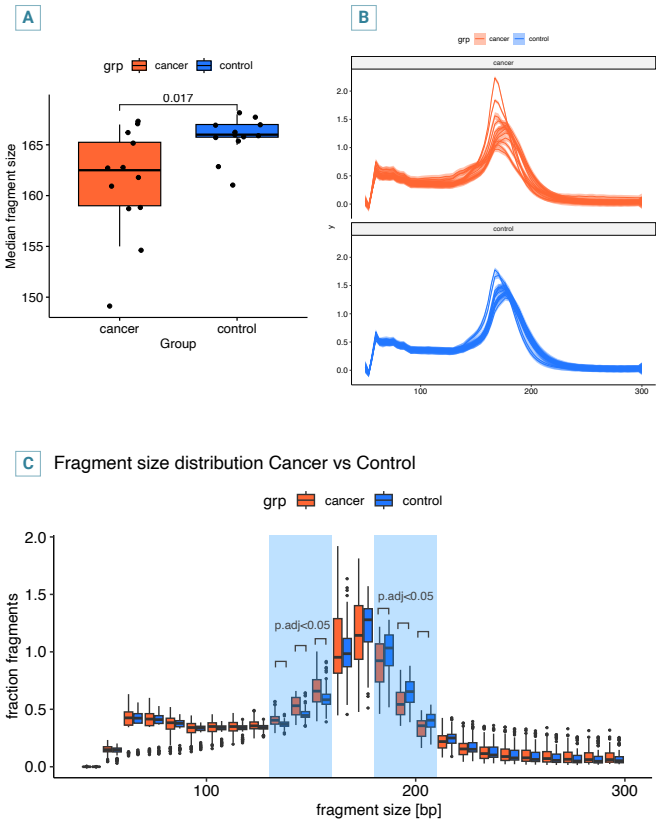

Figure 3. Fragmentomic analysis from the pooled Input library. (A) Boxplots represent median cfDNA fragment size in colorectal cancer (CRC) and healthy control samples. (B) Fragment size distribution profiles display canonical mono-nucleosome peaks (~145–170 bp) and increased sub-nucleosomal fragments (<140 bp) in CRC samples. (C) Boxplots display relative fragment abundance across size bins in colorectal cancer (CRC) and control samples. Adjusted p-values (p.adj < 0.05) indicate significant enrichment of sub-nucleosomal fragments and depletion of longer fragments in CRC.

Genome-wide Epigenetic Landscapes in Plasma

EXPECTED CHROMATIN ENRICHMENT PATTERNS

Metagene and locus-aggregated analyses revealed canonical enrichment patterns across all profiled epigenetic marks (Figure 4).

- H3K4me3 and H3K27ac showed strong enrichment at transcription start sites (TSS), consistent with active promoters and enhancers.
- H3K27me3 was enriched at Polycomb--repressed regions.
- H3K9me3 displayed characteristic enrichment over repeat-rich and heterochromatic regions.
- DNA methylation (MBD2 pulldown) showed strong enrichment at CpG islands.

Together, these patterns confirm that circulating nucleosomes retain biologically meaningful chromatin information in plasma and that EpiFinder™ cNUC preserves mark-specific signal fidelity across divers regulatory elements.

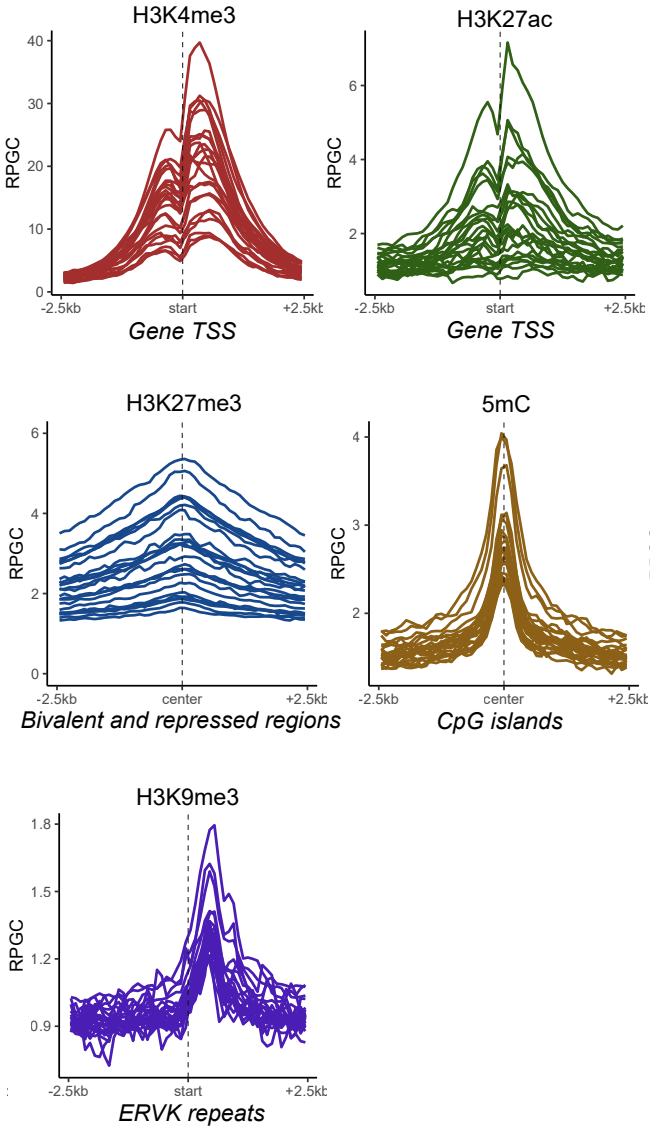

Figure 4. Genome-wide chromatin enrichment patterns from plasma-derived circulating nucleosomes. Metagene profiles show expected enrichment of histone modifications and DNA methylation at transcription start sites, Polycomb-repressed regions, repeat elements, and CpG islands across individual plasma samples.

Disease-Associated Epigenetic Signatures

DIFFERENTIAL ANALYSIS AND FEATURE SELECTION

Disease-associated regulatory changes are often distributed across many genomic loci rather than confined to a small number of highly significant regions. To capture this complexity, we applied a multi-step analysis pipeline:

1. Differential region calling between CRC and control groups
2. Selection of top distinguishing loci (30–1000 features)
3. Dimensionality reduction using principal component analysis (PCA)

UNSUPERVISED CLUSTERING REVEALS DISEASE--DRIVEN STRUCTURE

Unsupervised hierarchical clustering based on 500 H3K4me3-enriched regions selected in an unsupervised manner from genome-wide 5kb windows revealed strong separation of colorectal cancer (CRC) and healthy samples (Figure 5). Samples clustered predominantly according to disease status, indicating that global epigenetic similarity reflects underlying biological differences rather than technical variation.

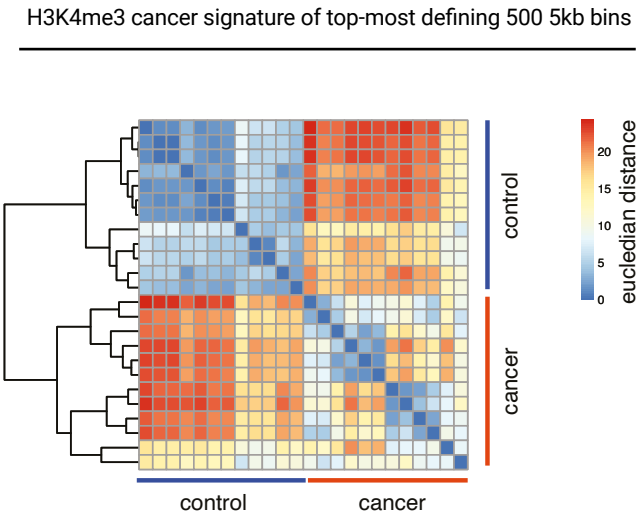

Figure 5. Unsupervised hierarchical clustering of plasma epigenetic profiles. Heatmap of pairwise sample similarity based on promoter-associated H3K4me3 regions. Hierarchical clustering separates colorectal cancer (CRC) and healthy control samples into distinct groups.

CLEAR SEPARATION OF SAMPLE GROUPS

Dimensionality reduction using PCA further demonstrated clear separation between CRC and healthy samples (Figure 6). Importantly, this separation did not rely on a small number of highly significant loci but instead emerged from the integration of hundreds of weakly distinguishing features distributed across the genome.

This pattern reflects the biological complexity of disease-associated regulatory changes, which are typically widespread rather than driven by single dominant markers. The PCA therefore illustrates a key capability of EpiFinder™ cNUC: robust integration of distributed epigenetic signals enabled by multiplexed profiling.

From a translational perspective, this dimensionality reduction provides a practical framework for:

- Discovery of disease-associated chromatin signatures
- Sample stratification and subgroup identification
- Development of downstream classification models

Importantly, similar discriminative signatures were identified independently across multiple histone modifications and DNA methylation, highlighting the complementary nature of each epigenetic layer and the added value of multiplexed measurement (representative data shown for H3K4me3 and 5mC).

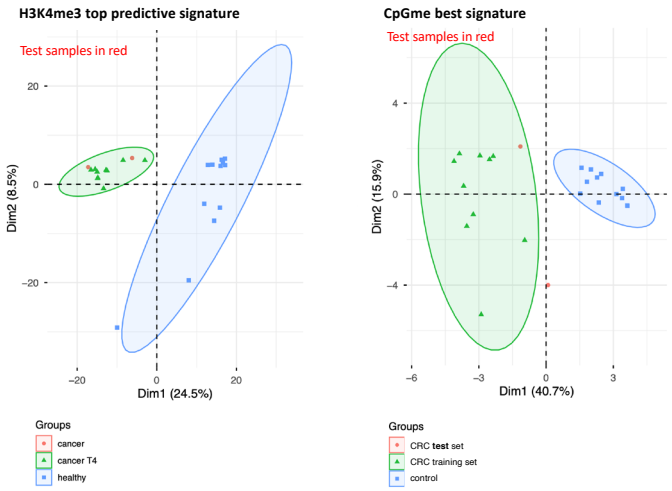

Figure 6. Distinct epigenetic signatures distinguish CRC and healthy samples. Principal component analysis (PCA) based on promoter-associated epigenetic profiles demonstrates clear separation between colorectal cancer (CRC) and healthy control samples. Representative PCA plots for H3K4me3 and DNA methylation (5mC) show consistent and independent discrimination of sample groups.

Differentially Marked Regions Reveal CRC-Associated Regulatory Programs

To assess locus-level differences, epigenetic signals were compared between colorectal cancer samples with higher tumor DNA fraction and healthy controls. For this analysis, four CRC samples with the highest estimated tumor DNA fraction were selected to maximize sensitivity for detecting tumor-associated regulatory changes. This analysis identified genes with significant differential marking across multiple epigenetic layers, including H3K4me3, H3K27me3, H3K9me3, and DNA methylation (5mC) (Figure 7).

Differential signals were also observed in the input dataset, reflecting shared copy-number alterations (CNAs) in colorectal cancer samples. Comparison between immunoprecipitation libraries and the input control revealed that repressive marks (H3K27me3 and H3K9me3) and DNA methylation partially overlapped with CNA-associated signals, while H3K4me3-associated changes showed no overlap with input, indicating regulation driven by transcriptional activity rather than copy-number variations.

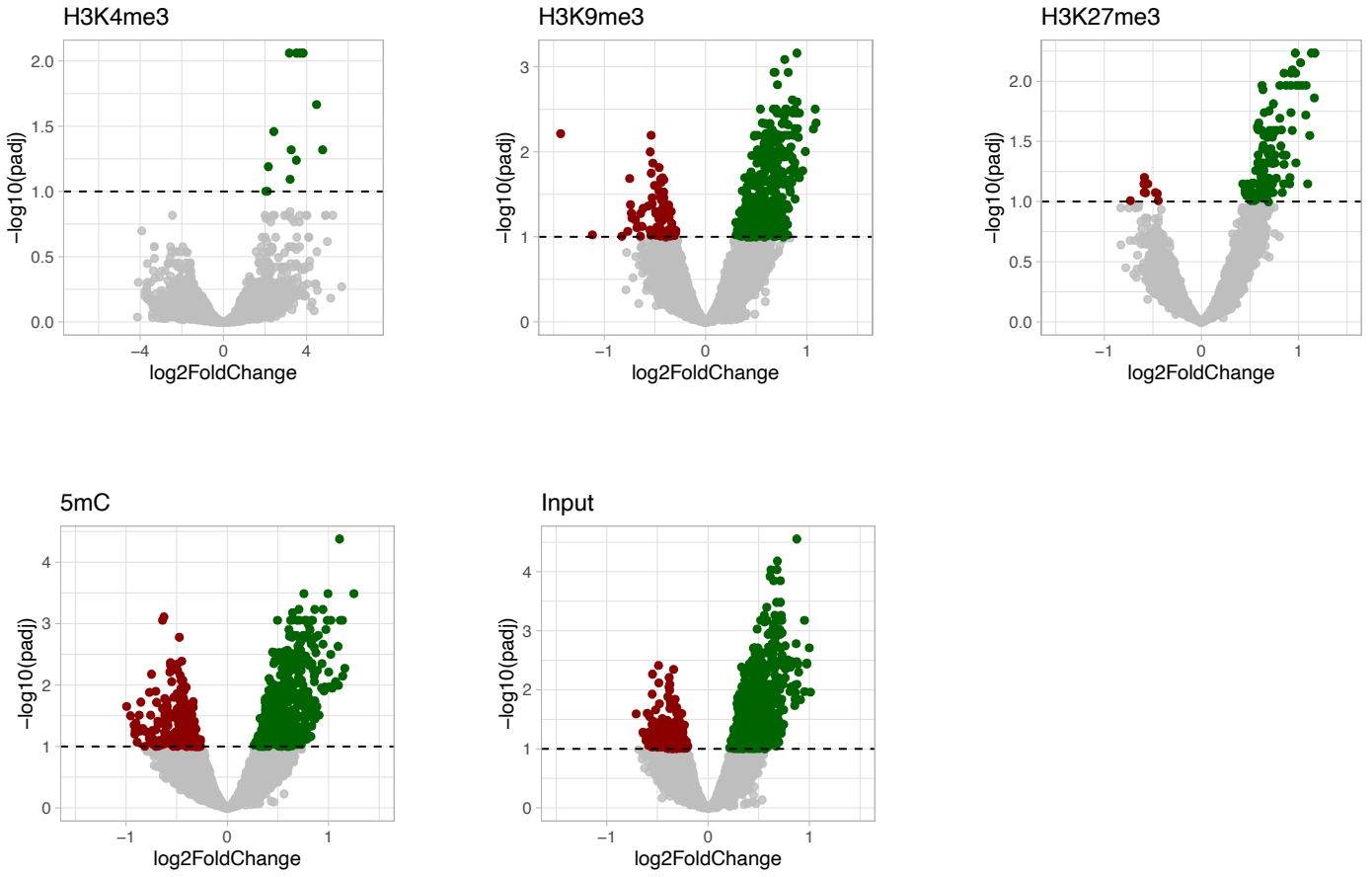

Figure 7. Differentially marked genes across epigenetic marks in colorectal cancer. Volcano plots show differential signals between colorectal cancer (CRC) samples and healthy controls across multiple epigenetic datasets and the input control. Raw read counts over annotated genes were used to assess differential marking between four CRC samples with higher tumor DNA fraction and twelve healthy controls. The −log10 adjusted p-value is plotted against log2 fold change, highlighting genes with increased (green) or decreased (red) signal in CRC samples.

Genes exhibiting increased H3K4me3 signal in colorectal cancer (CRC) samples (top 100 loci) were significantly enriched for pathways associated with colorectal cancer biology, including epithelial differentiation, intestinal development, and tissue-specific transcriptional programs (Figure 8). In contrast, genes showing reduced H3K4me3 enrichment in CRC were predominantly associated with immune and leukocyte-related pathways. This pattern is consistent with a relative decrease in leukocyte-derived nucleosome contribution in samples with higher tumor DNA fraction.

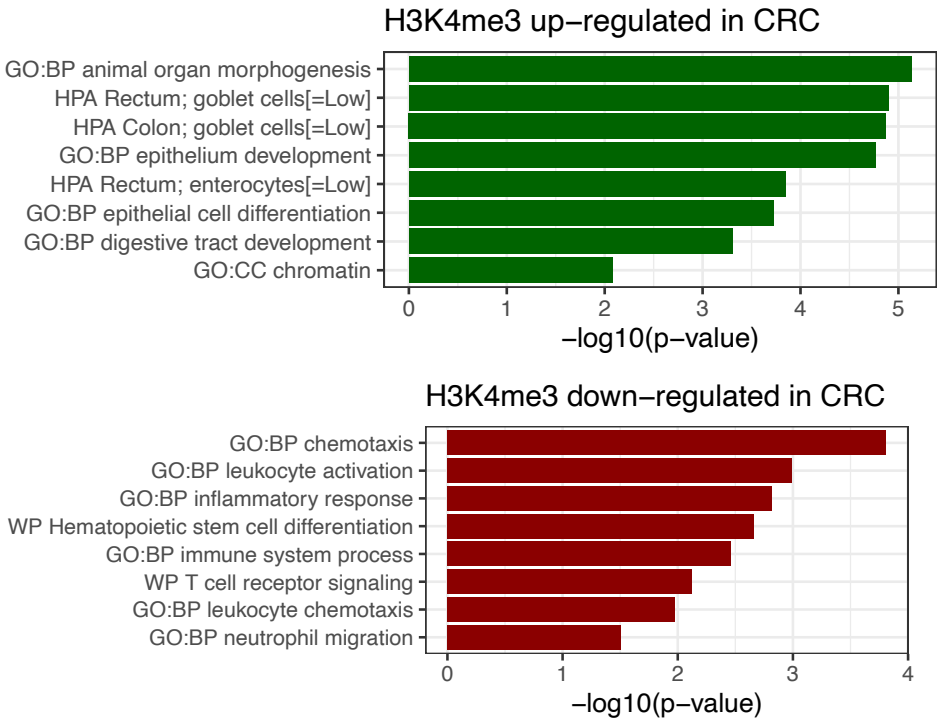

Figure 8. Functional enrichment of genes with differential H3K4me3 marking in colorectal cancer. Bar plots show Gene Ontology biological process enrichment for the top 100 genes with increased (up) or decreased (down) H3K4me3 signal in colorectal cancer samples relative to healthy controls.

Together, these results demonstrate that EpiFinder™ cNUC enables gene-level resolution of disease--associated epigenetic regulation from plasma, while the inclusion of an input control supports interpretation of epigenetic versus copy-number–driven effects.

Key findings

- **Robust genome-wide profiling from 200 µL plasma**
- **Integrated fragmentomics and CNA analysis from the same Input library**
- **Clear CRC vs control separation by clustering and PCA**
- **Significant enrichment of sub-nucleosomal fragments in CRC**
- **Identification of CRC-associated regulatory gene programs**

Conclusion

This pilot study establishes EpiFinder™ cNUC as a powerful and scalable platform for liquid biopsy epigenomics, enabling robust genome-wide profiling of circulating nucleosomes directly from plasma.

By unifying fragmentomics, copy-number analysis, histone modifications, and DNA methylation in a single multiplexed assay, EpiFinder™ cNUC supports analysis across multiple levels of resolution, from global chromatin landscapes and sample stratification to gene-level regulatory changes and biologically meaningful pathway signatures. Together, these capabilities open new dimensions for liquid biopsy-based epigenetic discovery and translational research.

Outlook and Applications

By enabling multiplexed, genome-wide epigenetic profiling directly from plasma or serum, EpiFinder™ cNUC supports a wide range of discovery and translational research applications beyond genetic analysis alone.

Key application areas include discovery and clinical research, oncology, immunotherapy and immune-mediated diseases, inflammatory disorders, aging and age-related studies, and drug response and mechanism-of-action research.

Together, these applications position **EpiFinder™ cNUC** as a versatile platform for advancing liquid biopsy-based epigenetic research across disease areas.

Acknowledgements

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