



Multiplex Chromatin Co-occupancy Profiling with Hi-Plex CUT&Tag

Hi-Plex CUT&Tag

Comprehensive Epigenomic Mapping. Powering Precision Medicine.

Hi-Plex CUT&Tag Overview

Hi-Plex CUT&Tag uses uniquely barcoded antibodies to simultaneously profile multiple chromatin-associated proteins in a single experiment, capturing protein co-occupancy on chromatin to efficiently decipher complex gene regulatory networks.

Key Advantages

Comprehensive

A comprehensive view of chromatin protein–DNA interactions

Reveals genome-wide protein–DNA interactions and chromatin co-occupancy.

Innovative

Define novel cis-regulatory modification panels

Characterizes unexplored chromatin modification combinations at cis-regulatory elements, enabling new discoveries.

High Throughput

Profile up to 30 targets in a single experiment

Rapid profiling of up to 30 targets in one assay

Accurate

Direct detection of chromatin co-occupancy

Directly measures multi-target co-occupancy—no inference required.

Sample-Efficient

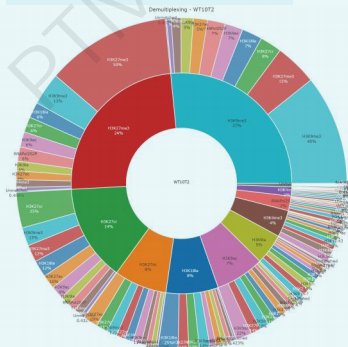
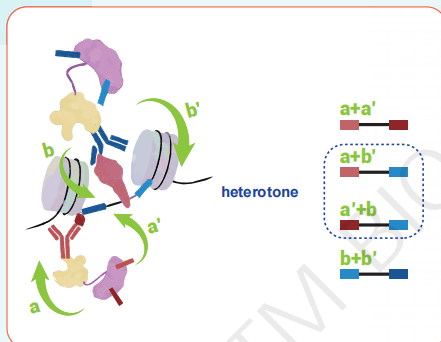
Comprehensive profiling from as few as 100,000 cells
30-target chromatin profiling from as few as 100,000 cells—ideal for precious samples.

Technology Highlights

Heterotone Reveal Authentic Protein Co-occupancy

Co-occupancy brings antibody-bound Tn5 transposases into proximity, enabling distinct antibody-specific adapters to tag both ends of the same DNA fragment and generate heterotones. Heterotones directly reveal protein co-occupancy at the single-molecule level, resolving combinatorial chromatin states beyond conventional approaches.

Example: Heterotone Analysis from 10-Target HCT Experiment^[1]



H3K27ac	RNAPol2S2P
H3K27me3	H3K4me1
H3K27cr	H3K18la
H3K4me3	H3K9la
H3K9me3	H3K9ac

Heterotone reads comprise 66.7% of total reads, enabling confident identification of authentic protein co-occupancy.

Multidimensional Analysis of Protein Co-occupancy

Combinatorial Chromatin States

DNA Sequence Features at Co-occupied Regions

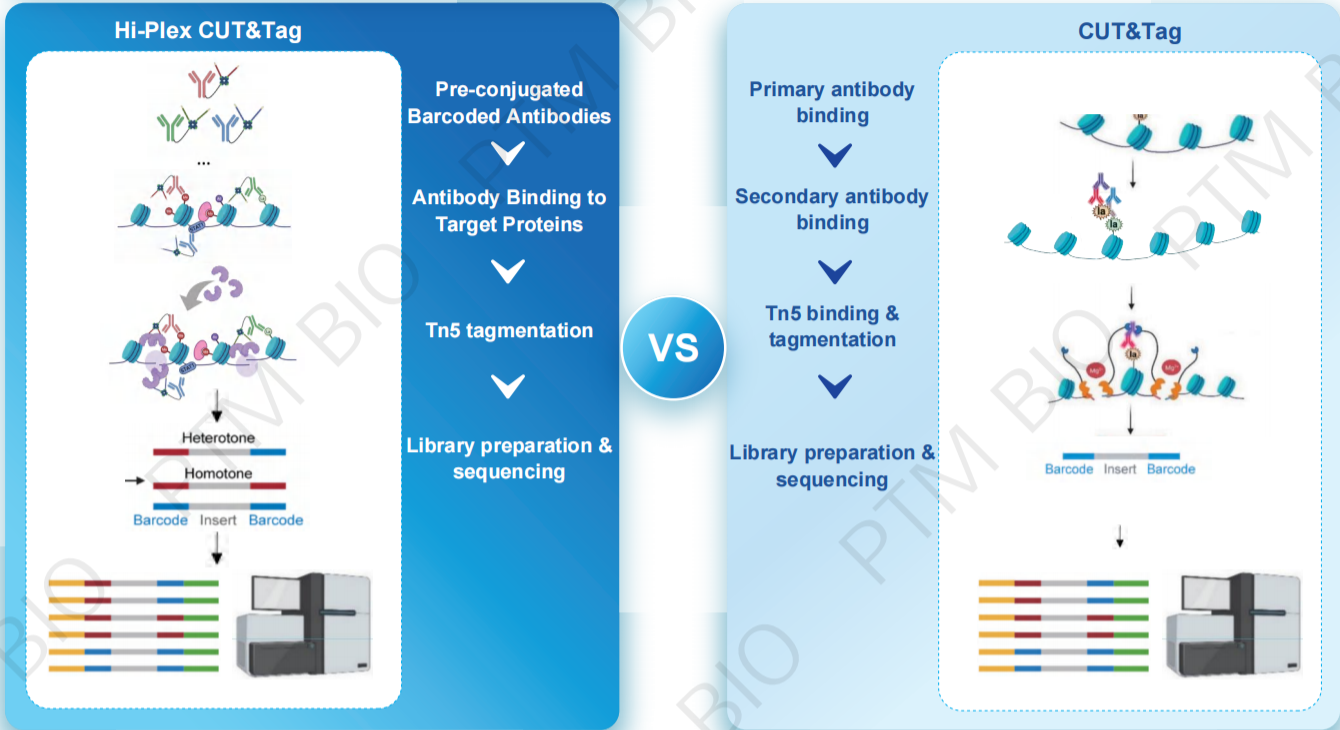
Co-occupancy Distance Informs Potential Regulatory Relationships

References

- [1] Sneha Gopalan et al. Simultaneous profiling of multiple chromatin proteins in the same cells. 2021. *Molecular Cell*.
- [2] Eva Galle et al. H3K18 lactylation marks tissue-specific active enhancers. 2022. *Genome Biology*.

Hi-Plex CUT&Tag VS CUT&Tag

Workflow Comparison



Performance Comparison

Metric	Hi-Plex CUT&Tag	CUT&Tag
Targets Profiled per Run	3 / 5 / 10 / 20 / 30	1
Throughput	High	Low
Required Cell Input	~100,000 cells	~100,000 cells
Signal-to-Noise Ratio	Higher	High
Barcode Loss Rate	Low	Higher
Co-localization Information	Same-fragment co-occupancy indicated by heterotone reads	Assessed by comparing separately generated single-target profiles

Application Value Comparison

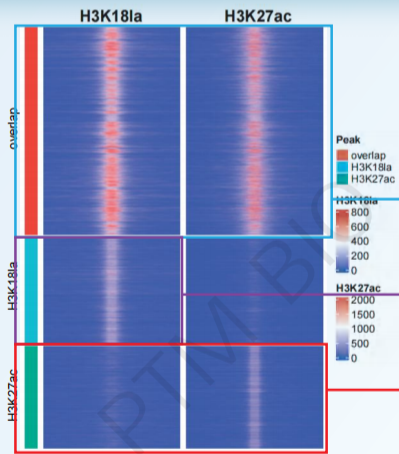
Hi-Plex CUT&Tag	CUT&Tag
Multiplex protein co-localization profiling High-throughput analysis of limited or precious samples Accelerates biological discovery Establishes a new benchmark for chromatin co-localization analysis	High-resolution profiling of individual chromatin targets

Application Scenarios

CUT&Tag	Hi-Plex CUT&Tag
Single-target profiling of protein occupancy on genomic DNA	Exploratory Applications: Systematic Mechanistic Studies Integrating Epigenomics and Proteomics Conventional epigenomic or proteomic studies often identify numerous differential targets but provide limited mechanistic insight. Hi-Plex CUT&Tag enables direct investigation of protein co-localization, helping bridge the gap between omics discovery and mechanistic validation Identification of Cis-Regulatory Elements Directly determine whether multiple enhancer-associated markers co-occupy the same genomic region, enabling accurate identification of active and functional cis-regulatory elements. Rapid Mechanistic Investigation Following Antibody or Omics Screening Following candidate discovery through proteomics, epigenomics, or antibody-based screening, Hi-Plex CUT&Tag enables rapid mechanistic validation by directly profiling chromatin co-localization, eliminating the need for multiple rounds of sequential WB or IF validation. Screening Novel Acylation Modification Panels Construct customized panels of emerging protein acylation modifications for high-throughput profiling, enabling investigation of metabolic regulation, downstream target genes, and phenotype-associated molecular mechanisms. Validation Applications: Phenotype-Driven Mechanistic Validation Starting from genes associated with specific phenotypes, Hi-Plex CUT&Tag enables reverse mapping of upstream epigenetic regulators using PTM BIO's customized modification panels and high-throughput chromatin profiling platform.

Bioinformatics Analysis & Data Interpretation

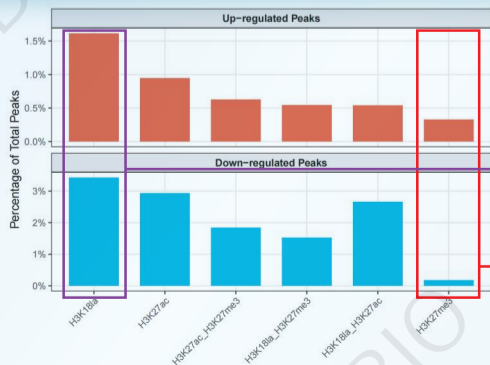
Shared and Unique Peaks Across Two Epigenetic Marks



Applications: Visualize shared and mark-specific peaks across chromatin features

- Shared peaks indicate H3K27ac–H3K18la co-occupancy
- H3K18la-specific peaks indicate unique occupancy
- H3K27ac-specific peaks indicate unique enrichment

Differential Peak Distribution Analysis

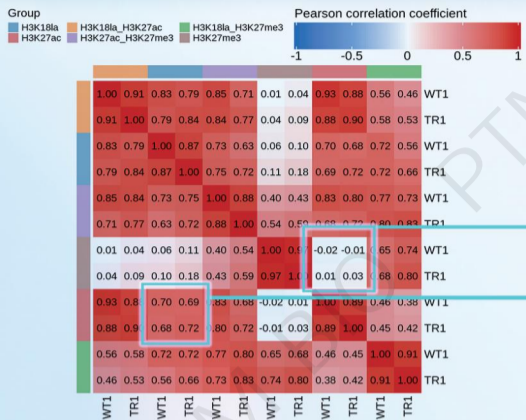


Applications: Comparing CR and WT groups identifies the most responsive chromatin marks, guiding downstream mechanistic studies

- H3K18la shows the largest treatment-associated changes, highlighting its dynamic regulation and potential as a priority target
- H3K27me3 shows the smallest changes, indicating relatively stable chromatin occupancy

Bioinformatics Results & Interpretation

Target Correlation Heatmap Across Samples

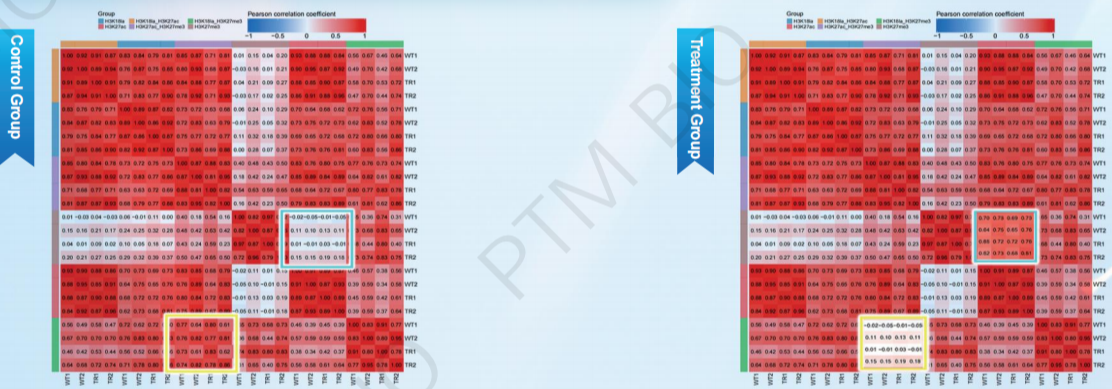


Application: Target Distribution Correlation
Identifying new co-localized target combinations

H3K27ac (active) and H3K27me3 (repressive) show low distributional correlation in this system.

H3K27ac and H3K18la (active) show high correlation, indicating potential co-localization.

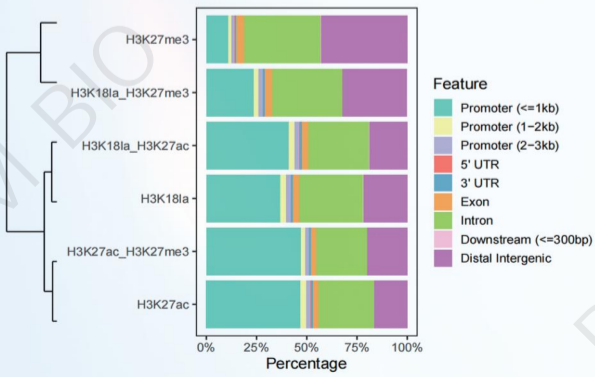
Target Correlation Heatmap Across Samples



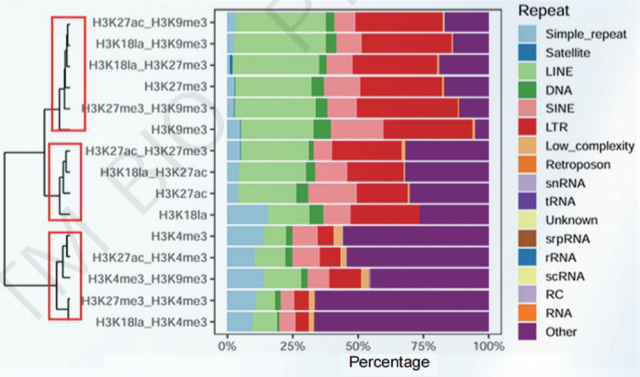
Applications: Compare correlation patterns between control and treatment groups to identify marker combinations exhibiting the greatest changes in correlation, providing valuable candidates for phenotype-associated mechanistic studies

Clustering Analysis of Target Genomic Distribution

Genomic Feature-Based Clustering

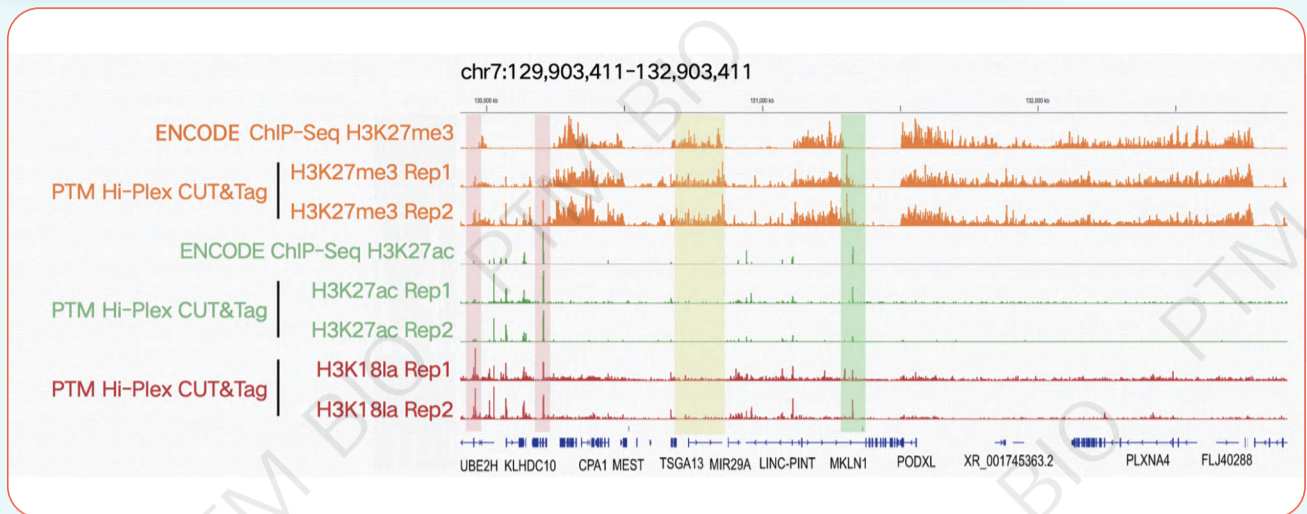


DNA Sequence Element-Based Clustering



Applications: Infer the potential biological functions of uncharacterized chromatin marks or novel combinatorial epigenetic signatures by leveraging the known functions of established chromatin modifications.

IGV Visualization: Cross-Validation with Public Single-Marker Datasets



PTM BIO Hi-Plex CUT&Tag profiles showed strong concordance with ENCODE ChIP-seq data for H3K27me3 and H3K27ac (yellow and green boxes). H3K18la partially overlapped with H3K27ac in K562 cells (red boxes), consistent with previous studies. These results validate the accuracy and reliability of the platform.

Heterotone Analysis Reveals Multi-Target Co-occupancy



H3K27ac-H3K18la heterotone signals closely matched homotone profiles, with enrichment at co-localized regions (purple boxes) but not at non-overlapping regions (red boxes), consistent with previous findings. These results demonstrate that three-target Hi-Plex CUT&Tag faithfully captures histone modification co-localization patterns.

Project Background

H3K27ac marks active gene regulatory elements, whereas the functions of emerging acylation marks such as H3K27cr remain unclear. Whether H3K27cr is exclusively activating remains debated. Five-target Hi-Plex CUT&Tag enabled systematic profiling of H3K27cr in its native chromatin context.

Study Design

Hi-Plex CUT&Tag maps H3K27cr alongside established histone marks, directly revealing its spatial relationships, chromatin regula-
tory network, and functions.

Study Overview

•Sample & Experimental Design

Untreated control vs. CR-treated cells

Inducing H3K27cr dynamics to profile genomic distribution and regulatory functions

•Target Panel

Target	Description
H3K27cr	Histone crotonylation mark investigated in this study.
H3K27me3	A canonical Polycomb Repressive Complex 2 (PRC2)-mediated repressive histone mark enriched at gene promoters and associated with facultative heterochromatin.
H3K27ac	A hallmark of active enhancers and promoters, widely used to identify transcriptionally active regulatory elements.
H3K4me3	A well-established marker of active promoters, highly enriched around transcription start sites (TSSs) of actively transcribed or poised genes.
H3K9me3	A classical constitutive heterochromatin mark primarily associated with pericentromeric, telomeric, and repetitive genomic regions, contributing to chromatin stability.

Key Findings

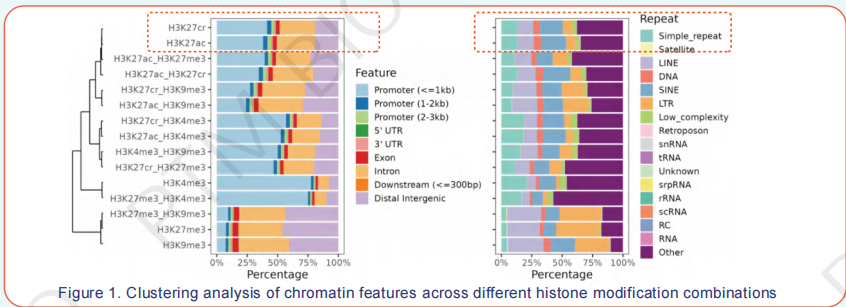


Figure 1. Clustering analysis of chromatin features across different histone modification combinations

Finding 1 | H3K27cr Displays a Chromatin Distribution Pattern Closely Associated with Active Histone Marks

Interpretation: H3K27cr consistently clustered with H3K27ac across genomic features and repeat elements, indicating enrichment in transcriptionally active regions.

Key Takeaway: H3K27cr is predominantly associated with active chromatin. Under crotonic acid treatment, a subset of H3K27cr–H3K9me3 heterotone peaks decreased, warranting further study of a potential repressive role.

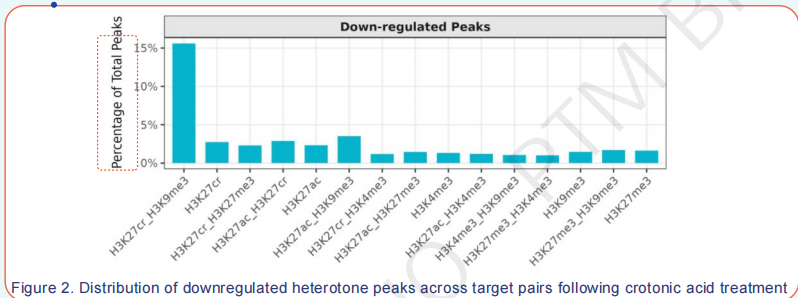


Figure 2. Distribution of downregulated heterotone peaks across target pairs following crotonic acid treatment

Finding 2 | H3K27cr-H3K9me3 Heterotone Peaks Show the Largest Downregulated Fraction After Crotonic Acid Treatment

Interpretation: H3K27cr–H3K9me3 showed the highest downregulated heterotone fraction (>15% of total peaks) after crotonic acid treatment. This locus-specific pattern is consistent with published evidence for repressive H3K27cr contexts, but does not establish repressive function. (Liu et al., Molecular Cell, 2023; PMID: 37311463).

CUT&Tag Maps the Chromatin Context of H3K27cr

Technical Validation

High Reproducibility |
Pearson $r > 0.95$

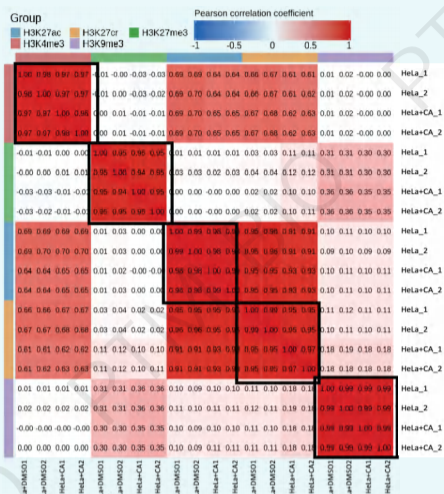


Figure 3. Sample Correlation Analysis

High Sensitivity |
median >25,000 peaks per target

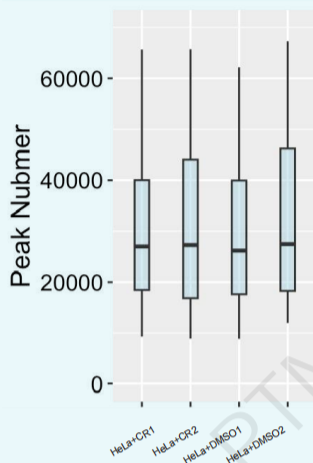


Figure 4. Peak Count Distribution

High Signal-to-Noise Ratio |
median FRiP >15%

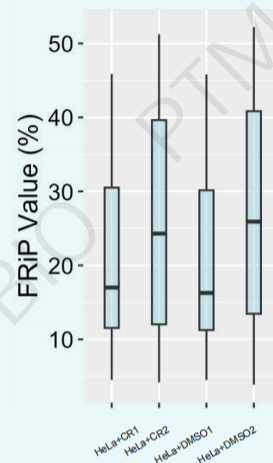


Figure 5. FRiP Score

Reproducibility Assessment

Sample correlation analysis showed excellent reproducibility across homotone datasets (Pearson $r > 0.95$; black boxes). Biologically related histone marks showed consistently high correlations, while unrelated marks showed lower correlations. These results confirm the robustness and reliability of the Hi-Plex CUT&Tag dataset.

Sensitivity Assessment

>25,000 median peaks per target or target combination across all four samples, demonstrating high-sensitivity chromatin profiling.

Signal-to-Noise Assessment

Individual targets and combinations consistently achieved median FRiP >15% across all four samples, indicating strong signal enrichment and high signal-to-noise.

High Accuracy | H3K27 Crotonylation Induction Is Consistent with Biological Expectations

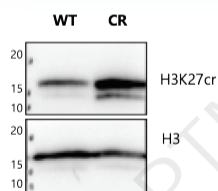


Figure 6. Western Blot Validation Following Crotonic Acid Treatment

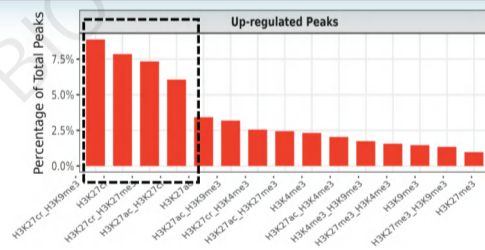


Figure 7. Differential Peak Analysis of Upregulated Peaks Across Target Combinations

Crotonic acid treatment increased H3K27cr abundance, as confirmed by Western blot. Hi-Plex CUT&Tag showed the highest proportion of upregulated peaks in H3K27cr homotone and heterotone datasets, consistent with Western blot results and confirming biological accuracy.

High Accuracy | Canonical Histone Marks Exhibit the Expected Genomic Distribution

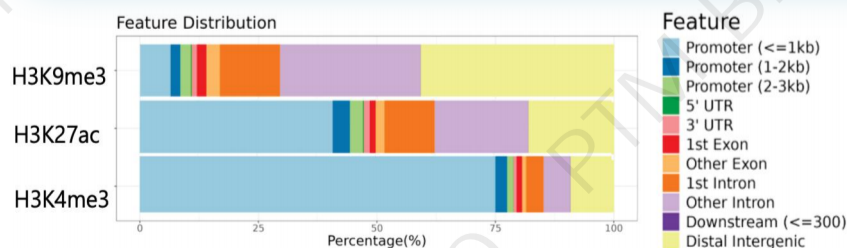


Figure 8. Genomic Feature Distribution of Representative Histone Marks

Target Enrichment Pattern Validation | H3K9me3 was enriched mainly in non-promoter regions, whereas active marks H3K27ac and H3K4me3 were strongly enriched at promoters, consistent with their established functions.

Internal Validation Study | Data Showcase of Ten-Target Hi-Plex CUT&Tag

Project Background

Building on the 5-plex validation study, additional histone marks and regulatory targets were added to characterize H3K27cr. Comparison of 5-plex and 10-plex data with IGV visualization further validated target localization and the robustness of the PTM BIO Hi-Plex CUT&Tag platform.

Project Details

Sample & Experimental Design

Untreated HeLa cells served as controls; crotonic acid treatment induced dynamic H3K27cr changes to assess its regulatory and functional roles.

Target Panel

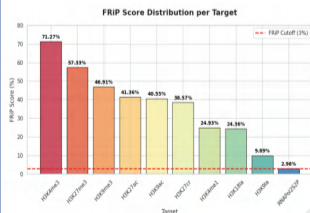
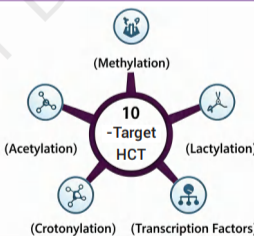
Target	Description
H3K27cr	Target modification investigated in this study
H3K27me3	A canonical repressive histone mark catalyzed by Polycomb Repressive Complex 2 (PRC2), primarily enriched at developmental gene promoters and associated with facultative heterochromatin
H3K27ac	A key active enhancer mark widely used for identifying potential gene regulatory elements
H3K4me3	A prominent active promoter mark, specifically enriched at transcription start sites (TSSs) of actively expressed or poised genes
H3K9me3	A major constitutive heterochromatin mark enriched at repetitive genomic regions, including pericentromeric and subtelomeric regions, contributing to genome stability
H3K4me1 (New)	An enhancer-associated mark enriched at enhancer regions and involved in enhancer activation through interactions with p300/CBP
H3K18la (New)	A lactylation-associated histone modification responsive to metabolic states and enriched at regulatory regions linked to transcriptional regulation
H3K9la (New)	A lactylation-associated modification associated with open chromatin states and transcriptional activation, regulated by cellular lactate metabolism
H3K9ac (New)	An acetylation mark associated with active promoters, promoting chromatin accessibility and gene expression activation
RNAPolS2P (New)	Serine 2 phosphorylation of RNA Polymerase II, a marker of transcription elongation and mRNA processing activity

1. End-to-End Integration



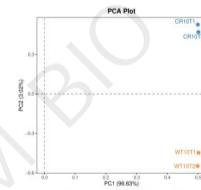
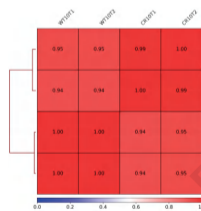
10-Target HCT profiling established, delivering high-quality datasets from wet-lab to bioinformatics

2. Comprehensive 10-Target HCT



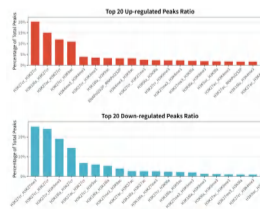
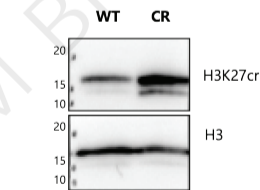
Strong enrichment across all targets, with FRIP scores >3%.

3. High Quality & Credibility



High inter-replicate consistency ($r > 0.8$) with clear replicate clustering by PCA.

4. Biological Validation



Biological Validation
Hi-Plex CUT&Tag closely matched Western blot, with consistent trends for the most responsive targets.

ENCODE
PTM BIO HCT
ENCODE
PTM BIO HCT
ENCODE
PTM BIO HCT
ENCODE
PTM BIO HCT
ENCODE
PTM BIO HCT
ENCODE
PTM BIO HCT

ENCODE_H3K27ac
PTMHCT_H3K27ac
ENCODE_H3K27me3
PTMHCT_H3K27me3
ENCODE_H3K4me3
PTMHCT_H3K4me3
ENCODE_H3K9me3
PTMHCT_H3K9me3
ENCODE_H3K4me1
PTMHCT_H3K4me1
ENCODE_H3K9ac
PTMHCT_H3K9ac

H3K27ac
H3K27me3
H3K4me3
H3K9me3
H3K4me1
H3K9ac

Gene Annotation
CP4A MEST1 RNASSP246 LINC00513 LINC-PINT MKLN1 PODXL EEF1B2P6 NDUFB9P2
CP4A MEST TSGA13 MIR335 KLF14 HAP1 RNJ6-1010P RPL31P36 CEP41 COG20T1 MIR29A COG2 MIR29B1

Six reference marks showed highly concordant peak locations, intensities, and enrichment patterns between HCT and ENCODE ChIP-seq. The remaining four lacked matched ENCODE datasets. These results support robust, stable, and reproducible 10-target HCT profiling, with single-target performance comparable to ChIP-seq for downstream cis-regulatory element identification, chromatin profiling, and gene regulatory network analysis.

Scan QR code to
view all targets



HCT Recommended Target Panel						
H4K12la	H3K18ac	H3K9me2	H3K36me3	H3K27pr	BRD2	H3K79me3
H3K14la	H2AK5ac	H3K56me2	H3K27cr	H3K9pr	PBX1	H3.3
H4K8la	H3K9me1	H3K122me2	H3K4cr	H3K23hib	MBD3	H3K4ac
H3K9la	H3K23me1	H3K27me2	H4K8cr	H3S28ph	U2AF2	H2BK120ac
H3K18la	H3K27me1	H3K4me2	H3K14bu	H2BK120ub	Topoisomerase I	H2AK119ub
H3K27la	H3K79me1	H3K27me3	H3K18bu	H3	HDAC2	CTCF
H3K27ac	H3K4me1	H3K4me3	H3K9bu	c-Jun	STAT1	STAT3
H3K9ac	H4K20me1	H3K9me3	H3K56pr	MYC	GRL	