# Revolutionizing Epigenetics with Direct Multiomic Mapping of DNA Methylation and Chromatin Proteins

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## Gene expression is controlled by crosstalk via DNA methylation and chromatin proteins

- **DNA methylation** (DNAme) and **chromatin proteins** (e.g. histone PTMs and transcription factors) oversee gene expression
- These factors are associated with specific genomic features (Figure 1)
- Current methods are low-resolution or rely on indirect parallel assays
- **Improved technologies are needed to directly resolve DNAme / chromatin protein crosstalk**

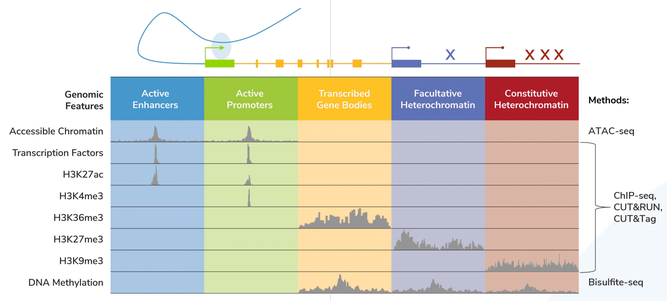


**Figure 1.** Chromatin proteins and DNAme define genomic features and reveal important regulatory mechanisms governing gene expression; however, currently available assays are correlative.

## CUT&RUN-EM simultaneously profiles direct DNAme / chromatin protein interactions

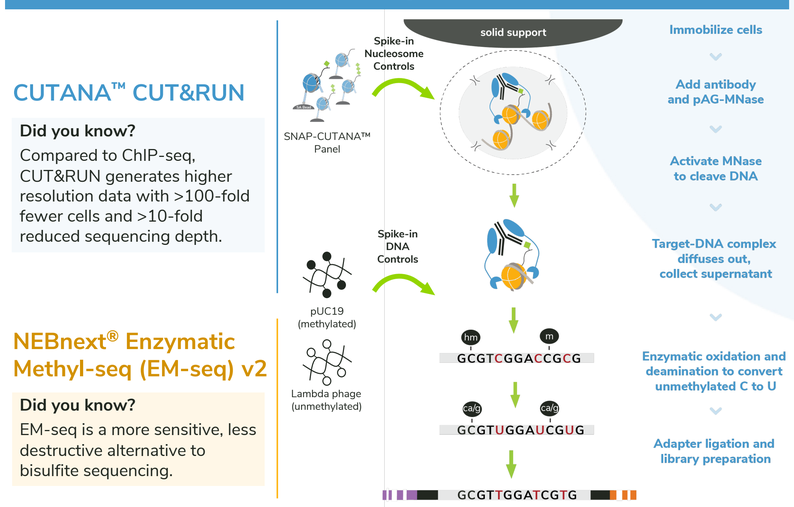


**Figure 2.** CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq v2. CUT&RUN isolates DNA associated with chromatin proteins of interest. EM-seq is then used to enzymatically convert unmethylated cytosines to uracils. Sequencing the resulting libraries **enables CpG resolution of DNAme co-occuring with the chromatin mark of interest**.

## CUT&RUN-EM is highly reproducible across targets, sequencing depths, and DNA inputs

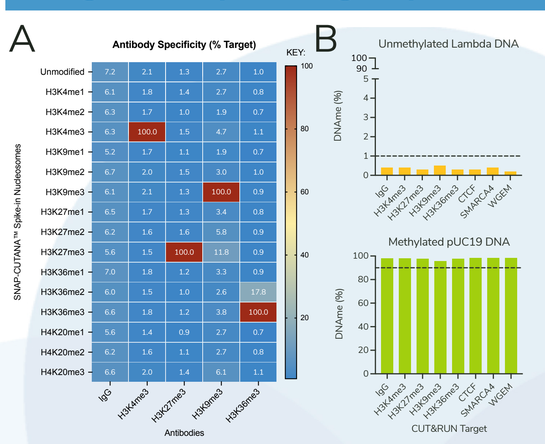


**Figure 3**. Defined spike-in controls demonstrate that CUT&RUN-EM shows high specificity (**A**; <20% cross-reactivity to off-target PTMs) and high conversion of unmethylated cytosines (**B**; >99% conversion efficiency).

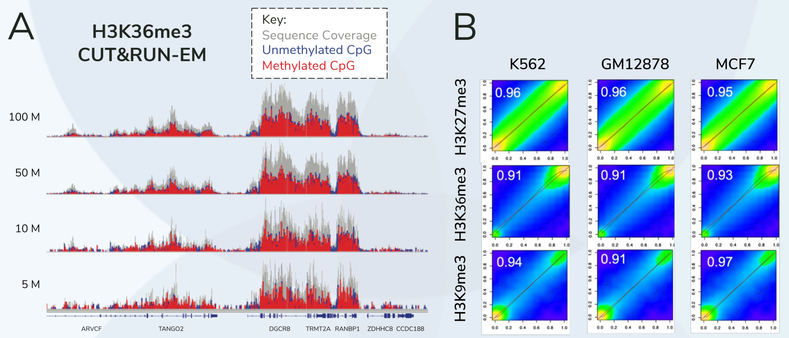


**Figure 4.** CUT&RUN-EM assays are highly reproducible across various sequencing depths (**A**; downsampled genome browser tracks) and replicates (**B**; Pearson correlation coefficients).

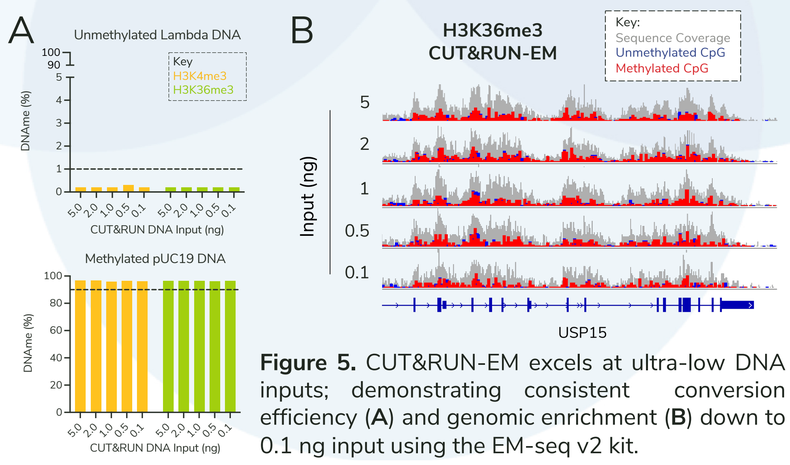


**Figure 5.** CUT&RUN-EM excels at ultra-low DNA inputs; demonstrating consistent conversion efficiency (**A**) and genomic enrichment (**B**) down to 0.1 ng input using the EM-seq v2 kit.

## CUT&RUN-EM reveals distinct DNAme profiles at defined genomic features

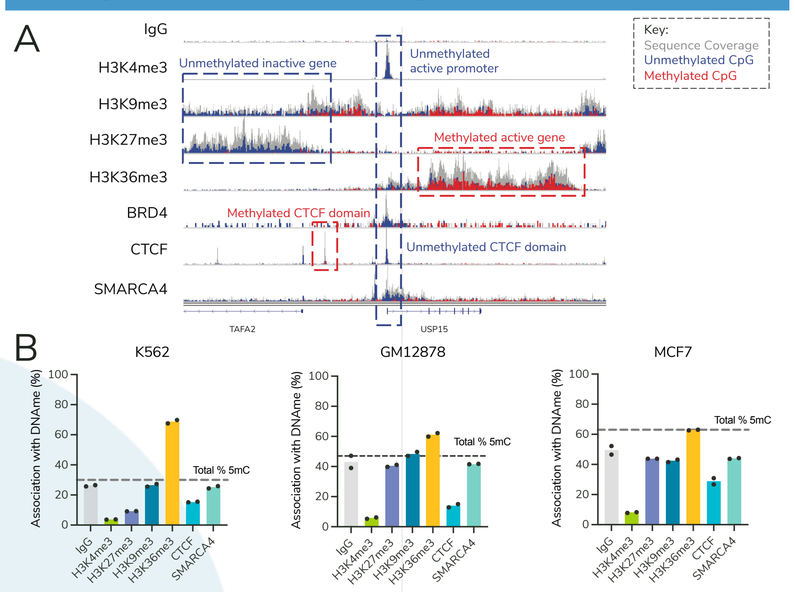


**Figure 6.** Representative genome browser tracks (**A**) demonstrate that CUT&RUN-EM reflects known biological functions of chromatin proteins (e.g. unmethylated H3K4me3 promoters, methylated H3K36me3 gene bodies) when overlaid with DNAme. The association of chromatin proteins with DNAme varies across cell lines (**B**), highlighting the utility of CUT&RUN-EM to provide deep gene regulatory insights.

## CUT&RUN-EM deconvolutes epigenetic crosstalk that is masked in correlative assays

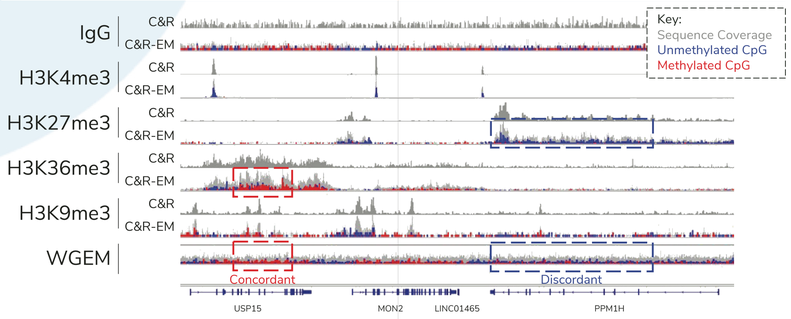


**Figure 7.** CUT&RUN-EM stratifies subpopulations of cells with unique PTM-DNAme crosstalk signatures that are obscured in whole genome EM-seq (WGEM). IGV genome browser comparison of CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and WGEM in K562 cells. C&R-EM loci with concordant results (**red box**) recapitulate the findings of WGEM, while discordant loci (**blue box**) resolve PTM-DNAme specific signatures.

## MeCP2-targeted CUT&RUN generates ultra-sensitive, global DNA methylation profiles at low cost

### Modified CUT&RUN excises methylDNA to reduce seq depth

### Higher quality, bp resolution data using reduced cells & sequencing vs. MeDIP

### MeCP2 captures high-density 5mC with 34x less sequencing than WGEM

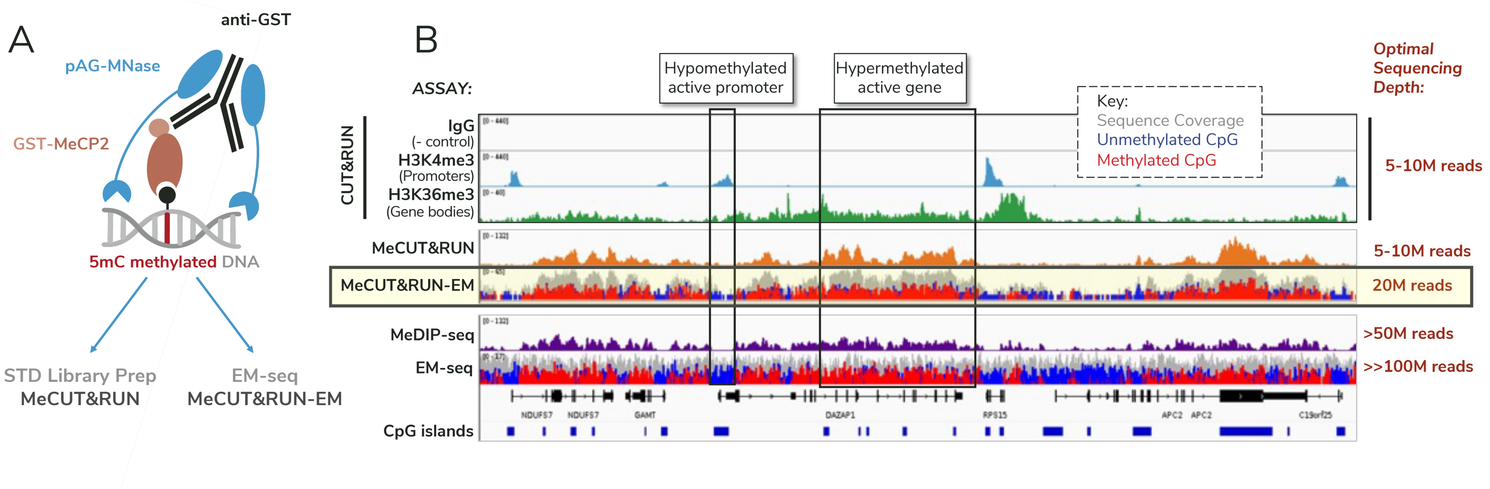


**Figure 8.** (**A**) Schematic representation of a GST-tagged MeCP2 methyl binding domain used in lieu of an antibody in traditional CUT&RUN (MeCUT&RUN) to enrich methylated DNA, Sequencing libraries can be prepared via standard library prep to provide a snapshot of genome-wide DNAme at 150 bp resolution, or unmethylated cytosines can be converted with EM-seq to achieve CpG resolution. (**B**) **MeCUT&RUN** (**orange**) with 500k K562 cells shows high concordance with Methyl-DNA Immunoprecipitation sequencing (MeDIP-seq; **purple**) at >2.5-fold reduced sequencing depth with 10-fold fewer cells. **MeCP2 CUT&RUN-EM** ( yellow boxed track ) generates similar DNA methylation profiles compared to whole genome EM-seq (bottom track). (**C**) Sankey plot of the binned human genome (200 bp) comparing the presence of 5mC in WGEM and MeCUT&RUN-EM. MeCP2 enriches for regions of high 5mC concentration with 34x less sequencing than WGEM, greatly reducing sequencing costs.

## Conclusions

- **CUT&RUN-EM** enables a direct interrogation of DNAme and chromatin protein crosstalk
- **MeCUT&RUN** provides fast and efficient mapping of global DNA methylation levels with ~150 bp resolution
- **MeCUT&RUN-EM** resolves base pair resolution of DNA methylation with 34x less sequencing than WGEM

## See EpiCypher assays in action

- **AML therapy selection** San José-Enériz, *Nat Commun* 2024 (PMID: 38956053)
- **Aging & chromatin opening** Patrick, *Cell Metab* 2024 (PMID: 38959897)
- **Epigenomics of stress** Torres-Berrío, *Neuron* 2024 (PMID: 38959894)
- **iPSC differentiation by BRD4** Padmanabhan, *Nat Cardio Res* 2024 (PMID: 39196112)
- **SCA1 disease mechanism** Coffin, *Neuron* 2023 (PMID: 36577402)
- **H3.3K36M disrupts DNAme** Sinha, *Mol Cell* 2024 (PMID: 39368466)

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