Multiomic sequencing technology reveals crosstalk between chromatin proteins and DNA methylation in neurological disease

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Gene expression is controlled by complex molecular crosstalk between DNA methylation and chromatin proteins

- **Precise regulation** of DNA methylation (DNAme) and chromatin proteins underlies many neurobiological gene expression programs
- DNAme and chromatin proteins are **associated with specific genomic features** (**Figure 1**)
- **Studies of DNAme - chromatin protein crosstalk are limited** to low-resolution or indirect methods
- **Improved technologies are needed** to directly resolve DNAme - chromatin protein crosstalk

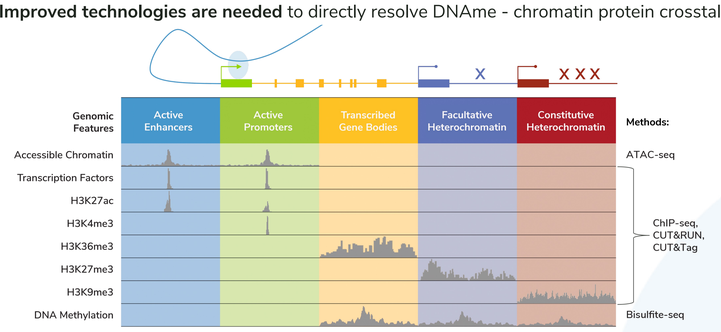


Figure 1. Chromatin proteins and DNAme define genomic features and reveal important regulatory mechanisms governing gene expression.

CUT&RUN-EM is a powerful multiomic assay to simultaneously profile DNAme and chromatin proteins

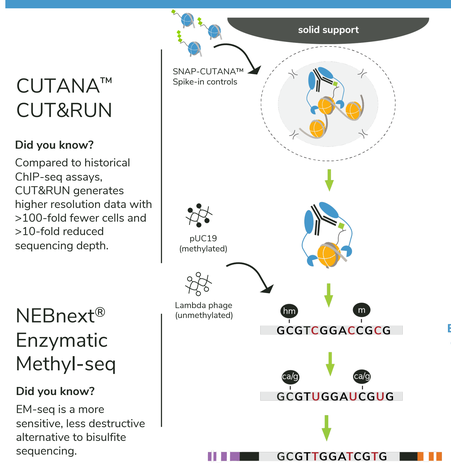


Figure 2. CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq to deliver a direct multiomic assay enabling the simultaneous profiling of DNAme and chromatin proteins. CUT&RUN releases antibody-bound chromatin into solution, which is easily separated from bead-immobilized cells, thus eliminating a major source of background. Isolated DNA is enzymatically converted, distinguishing unmethylated and methylated cytosines in next-generation sequencing data. Defined nucleosome and DNA controls enable assay standardization.

One assay, countless insights: benchmarking CUT&RUN-EM to CUT&RUN and EM-seq

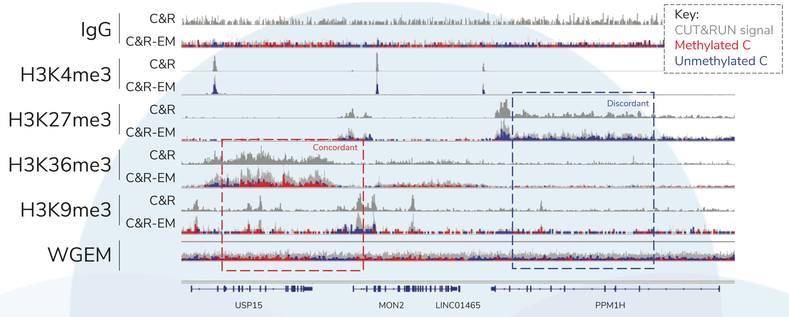
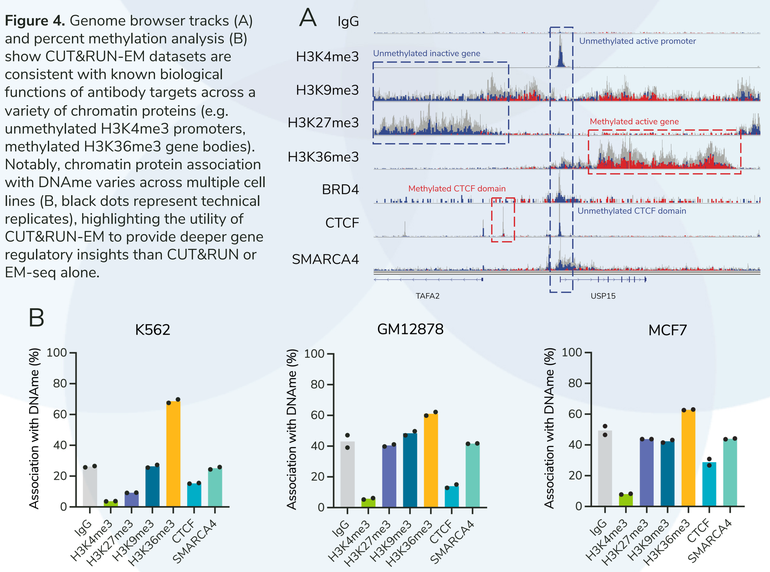


Figure 3. IGV genome browser comparison of CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and whole genome EM-seq (WGEM) in K562 cells. IgG is shown as negative control. Histone PTM-defined loci are concordant (red box) or discordant (blue box) from DNA methylation signal, highlighting the power of CUT&RUN-EM to characterize multiple epigenomic features.

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

Figure 4. Genome browser tracks (A) and percent methylation analysis (B) show CUT&RUN-EM datasets are consistent with known biological functions of antibody targets across a variety of chromatin proteins (e.g. unmethylated H3K4me3 promoters, methylated H3K36me3 gene bodies). Notably, chromatin protein association with DNAme varies across multiple cell lines (B, black dots represent technical replicates), highlighting the utility of CUT&RUN-EM to provide deeper gene regulatory insights than CUT&RUN or EM-seq alone.



CUT&RUN-EM provides direct multiomic insights into patient-identified Rett syndrome alleles

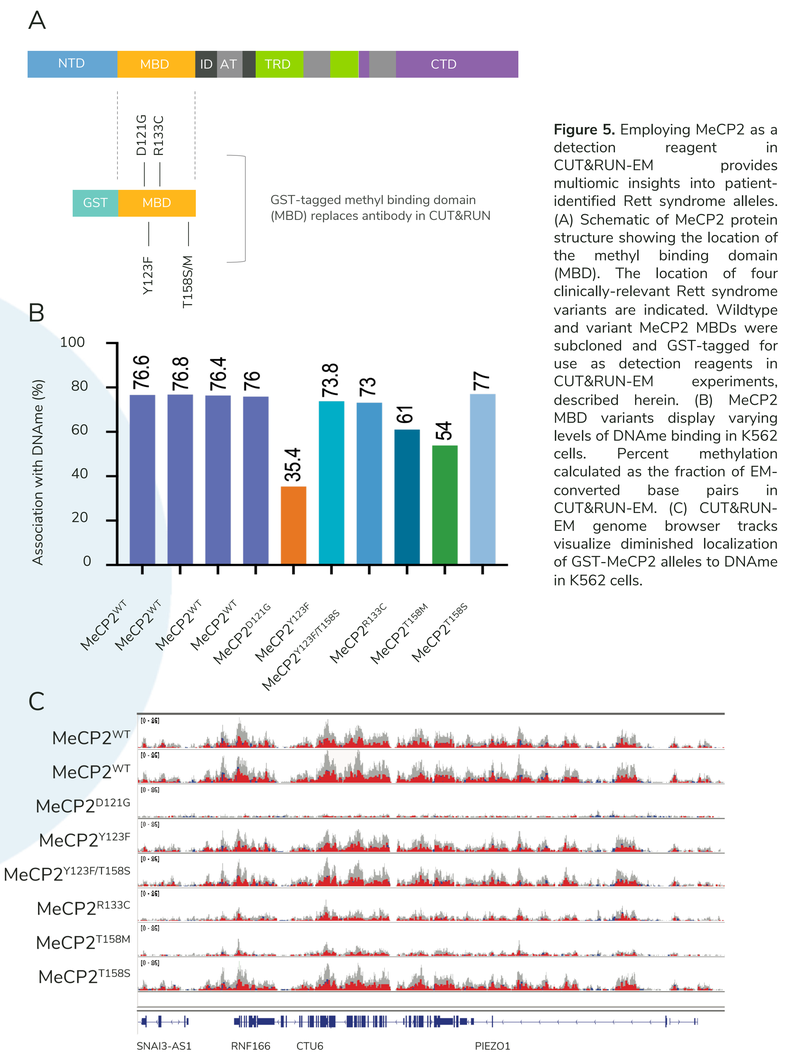
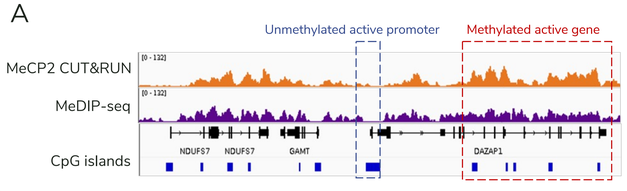


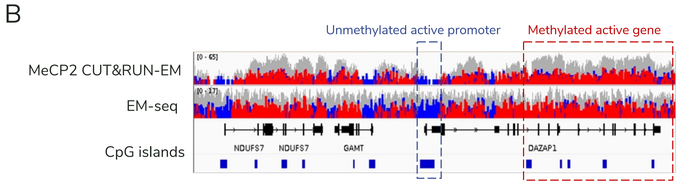
Figure 5. Employing MeCP2 as a detection reagent in CUT&RUN-EM provides multiomic insights into patient-identified Rett syndrome alleles. (A) Schematic of MeCP2 protein structure showing the location of the methyl binding domain (MBD). The location of four clinically-relevant Rett syndrome variants are indicated. Wildtype and variant MeCP2 MBDs were subcloned and GST-tagged for use as detection reagents in CUT&RUN-EM experiments, described herein. (B) MeCP2 MBD variants display varying levels of DNAme binding in K562 cells. Percent methylation calculated as the fraction of EM-converted base pairs in CUT&RUN-EM. (C) CUT&RUN-EM genome browser tracks visualize diminished localization of GST-MeCP2 alleles to DNAme in K562 cells.

Ultra-sensitive, low-cost DNA methylation profiling with MeCP2 CUT&RUN-EM

MeCP2 CUT&RUN provides massive gains vs. MeDIP-seq



Combine with EM-seq for base-pair resolution DNAme profiling



Capture 83% of DNAme with 34x less sequencing

Figure 6. The use of MeCP2 as a DNA methylation sensor in CUT&RUN provides multiple strategies for DNA methylation profiling. (A) Validation of GST-MeCP2 MBD in CUT&RUN (orange), using 500k K562 cells and 10M sequencing reads. To examine DNA methylation enrichment capabilities, MeCP2 CUT&RUN was benchmarked against commonly used methyl-DNA immunoprecipitation (MeDIP-seq) assays, shown in purple. MeCP2 showed high concordance with MeDIP-seq at >5-fold reduced sequencing depth. (B) MeCP2 was used in CUT&RUN-EM to enrich methylated DNA and provide a base-pair resolution readout of DNA methylation (K562 cells). Whole-genome EM-seq was used to benchmark data, which required >100M reads. Notably, MeCP2 CUT&RUN-EM generates similar DNA methylation profiles, while only requiring 16M reads and 500k cells. (C) Genome-wide targeting of DNAme-rich areas using MeCP2 CUT&RUN-EM greatly reduces sequencing cost while maintaining sensitivity.

Conclusions

- CUT&RUN-EM provide a wholistic view of DNAme and chromatin protein **interplay in a single workflow**
- CUT&RUN-EM can be leveraged to **gain mechanistic insights** into patient-identified genetic variants
- MeCP2 CUT&RUN-EM provides **a base pair resolution, low-cost alternative** to genome-wide DNAme assays

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