CUT&RUN-EM: An Ultra-Sensitive Multiomic Method that Directly Links Chromatin Features to DNA Methylation

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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 gene expression programs
- DNAme and chromatin proteins are associated with specific genomic features (Figure 1)
- Current methods are low-resolution or rely on correlating parrallel assays
- **Improved technologies are needed to directly resolve DNAme - chromatin protein crosstalk**

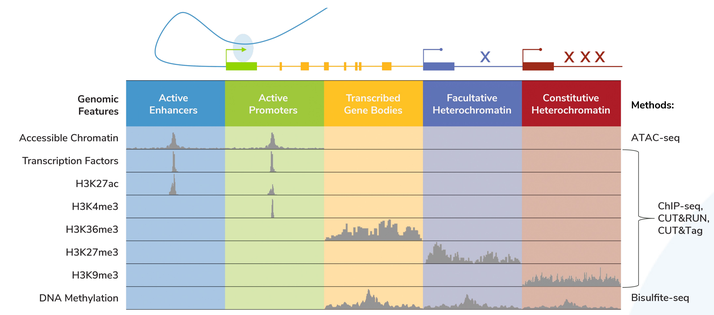


Figure 1. Chromatin proteins and DNAme define genomic features and reveal important regulatory mechanisms governing gene expression; however, currently available assays are correlative.

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

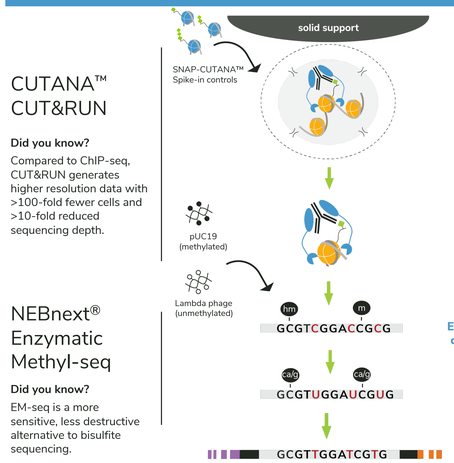


Figure 2. CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq to deliver a direct multiomic assay enabling the simultaneous profiling of DNAme and chromatin proteins. CUT&RUN has significantly improved signal to noise compared to ChIP-seq, resulting in reduced cellular input and sequencing requirements. Unmethylated cytosines from the isolated DNA is enzymatically converted, **enabling CpG resolution of DNA methylation that co-occurs with the chromatin protein of interest**.

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

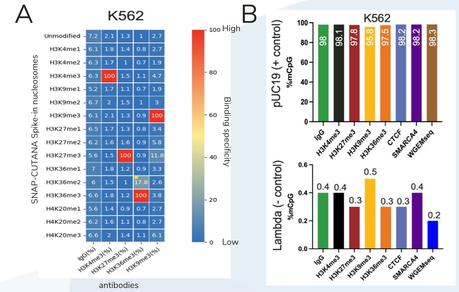


Figure 3. CUT&RUN-EM shows high specificity and percent conversion for a variety of chromatin proteins as determined using defined spike-in controls. (**A**) Heatmap of SNAP-CUTANA™ Spike-in nucleosomes showing the percent enrichment of each antibody tested in CUT&RUN. Red and blue indicate high and low binding specificity, respectively. (**B**) Methylated and Unmethylated EM-seq spike-in DNAs were used to monitor enzymatic conversion of unmethylated cytosines. As expected, pUC19 was found to be >95% methylated and Lambda DNA methylation was <0.5%, consistent with the standard for whole genome EM-seq (WGEMseq).

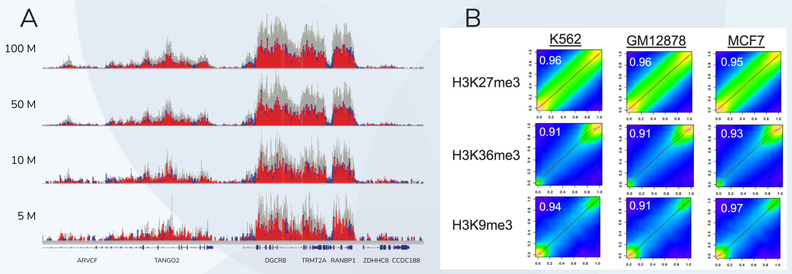


Figure 4. CUT&RUN-EM assays are highly reproducible across sequencing depths and replicates. (**A**) Representative genome browser tracks from H3K36me3 CUT&RUN-EM data, bioinformatically downsampled, shows qualitatively similar DNAme distribution using as few as 5M reads. (**B**) Biological replicates in K562, GM12878, and MCF7 cells shows that CUT&RUN-EM assays are highly reproducible (r > 0.91).

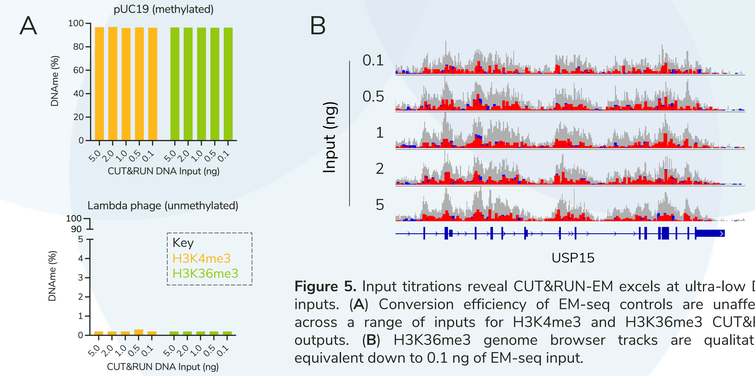
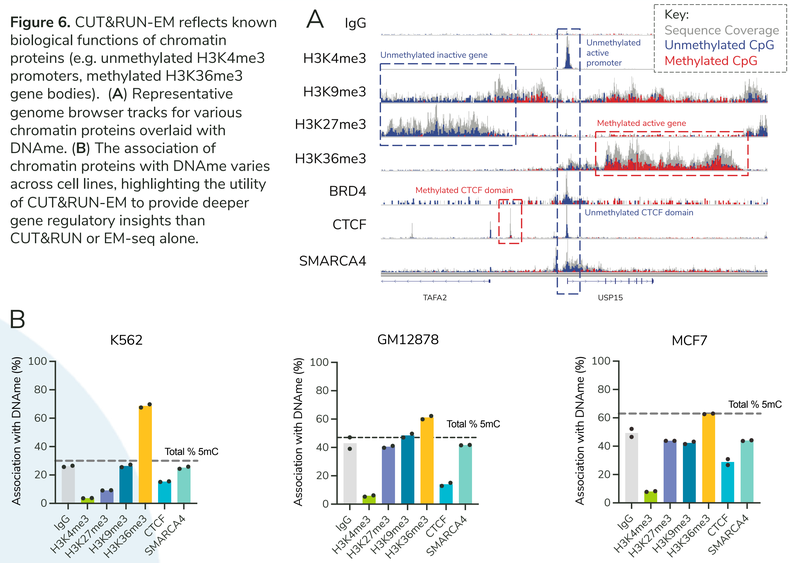


Figure 5. Input titrations reveal CUT&RUN-EM excels at ultra-low DNA inputs. (**A**) Conversion efficiency of EM-seq controls are unaffected across a range of inputs for H3K4me3 and H3K36me3 CUT&RUN outputs. (**B**) H3K36me3 genome browser tracks are qualitatively equivalent down to 0.1 ng of EM-seq input.

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

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



CUT&RUN-EM deconvolutes mechanisms of chromatin protein-DNAme crosstalk that would be masked in correlative assays

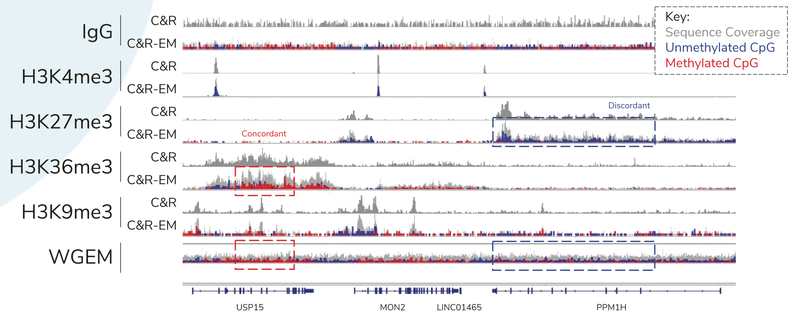
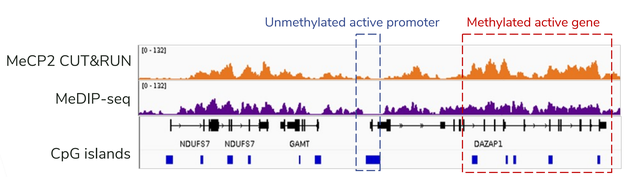


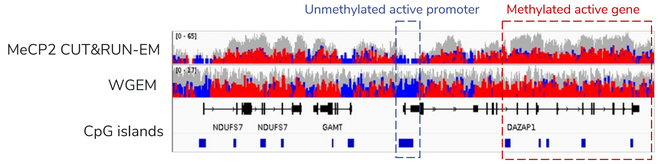
Figure 7. CUT&RUN-EM stratifies specific subpopulations of cells with unique PTM-DNAme crosstalk signatures that are obscured in the global whole genome EM-seq (WGEM) profile. IGV genome browser comparison of CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and WGEM in K562 cells. C&R-EM loci with concordant results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

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

Higher quality data with reduced cells & sequencing vs. MeDIP



Combine with EM-seq for base-pair resolution



MeCP2 captures 83% of 5mC with 34x less sequencing vs. WGEM

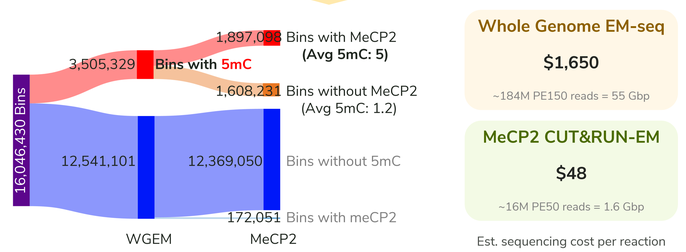


Figure 7. (**A**) GST-tagged MeCP2 methyl binding domain used in lieu of an antibody in traditional CUT&RUN with 500k K562 cells (orange) provides ~150bp resolution of genome-wide DNAme. MeCP2 CUT&RUN showed high concordance with Methyl-DNA Immunoprecipitation sequencing (MeDIP-seq; purple) at >5-fold reduced sequencing depth. (**B**) To achieve CpG resolution of DNAme, MeCP2 was used in CUT&RUN-EM. MeCP2 CUT&RUN-EM generates similar DNA methylation profiles compared to WGEM. (**C**) Sankey plot of the binned human genome (200 bp) comparing the presence of 5mC in WGEM and MeCP2 CUT&RUN. MeCP2 enriches for regions with higher concentrations of 5mC, identifying 83% of 5mCs found in WGEM with **34x less sequencing**, greatly reducing sequencing costs.

Conclusions

- CUT&RUN-EM reveals the direct association of DNAme and chromatin proteins in a single workflow
- CUT&RUN-EM can deconvolute bulk cell heterogeneity through multiomic filtering
- **MeCP2 CUT&RUN-EM provides base pair resolution of global DNA methylation using 34x less sequencing than WGEM.**

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