From Genome to Phenome: Revolutionizing Agricultural Epigenetics with Direct Multiomic Mapping of DNA Methylation and Chromatin Proteins

Andrea L Johnstone[1], Vishnu U Kumary[1], Allison Hickman[1], Anup Vaidya[1], Sriharsa Pradhan[2], Michael-Christopher Keogh[1] and Bryan J. Venters[1]

[1] EpiCypher Inc., Durham, NC, USA; [2] New England Biolabs, Ipswich, MA, USA

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**

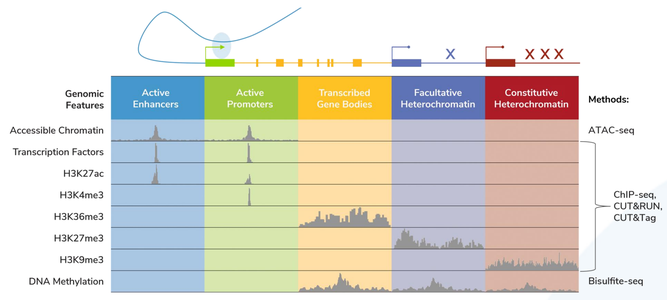


Figure 1. Chromatin proteins and DNAme define genomic features and reveal important regulatory mechanisms governing gene expression; however, currently available assays are correlative. Enrichment profiles shown are characteristic for mammals; some differences exist in plants.

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

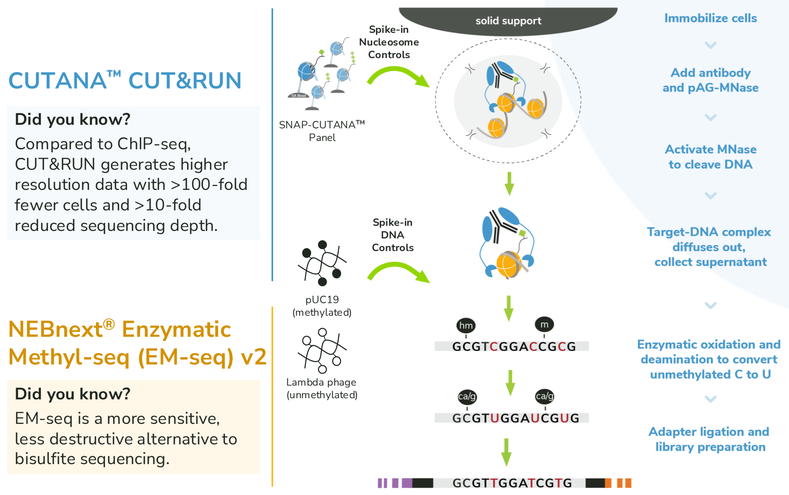
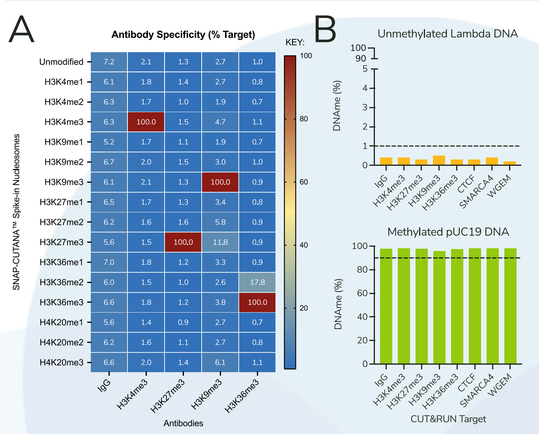


Figure 2. CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq v2 kit. CUT&RUN isolates DNA associated with chromatin proteins of interest. EM-seq is then used to enzymatically convert unmethylated cytosines to uracils. Sequencing the resulting libraries **enables CpG resolution of DNAme co-occuring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

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



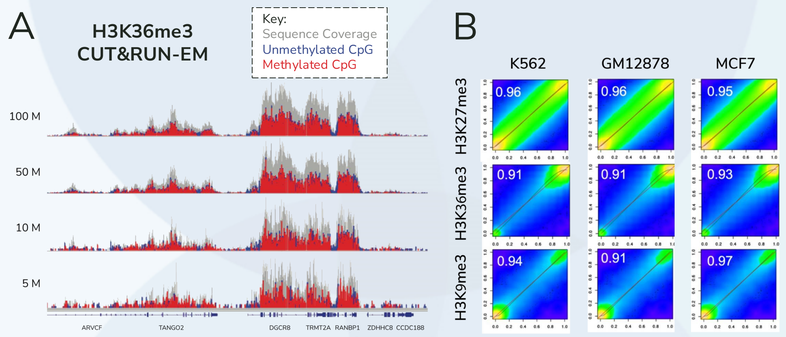


Figure 4. CUT&RUN-EM assays are highly reproducible across various sequencing depths (**A**; downsampled genome browser tracks) and replicates (**B**; Pearson correlation coefficients).

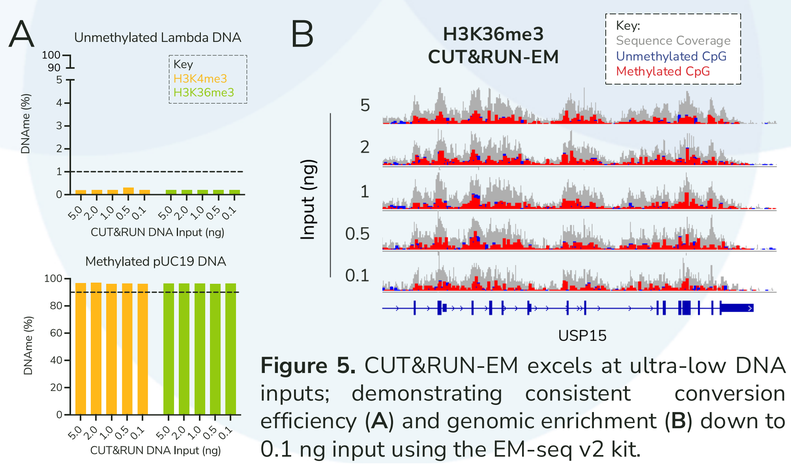


Figure 5. CUT&RUN-EM excels at ultra-low DNA inputs; demonstrating consistent conversion efficiency (**A**) and genomic enrichment (**B**) down to 0.1 ng input using the EM-seq v2 kit.

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

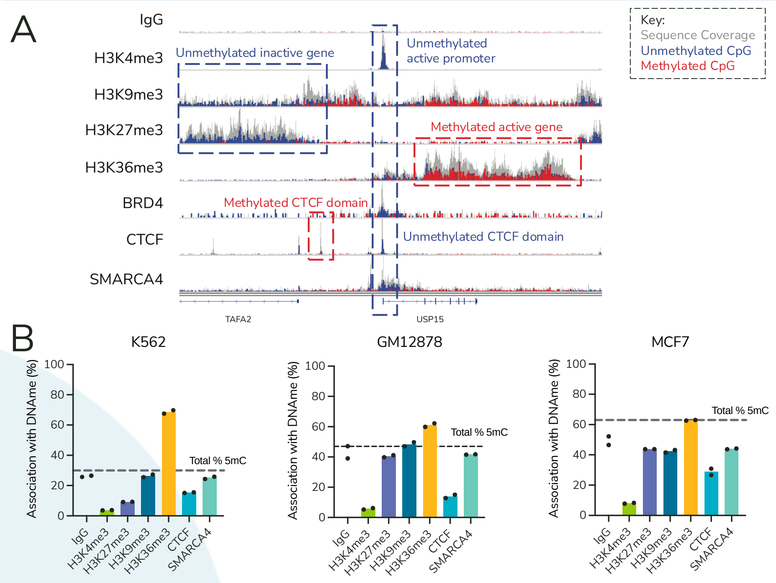


Figure 6. Representative genome browser tracks (**A**) demonstrate that CUT&RUN-EM reflects known biological functions of chromatin proteins (e.g. unmethylated H3K4me3 promoters, methylated H3K36me3 gene bodies) when overlaid with DNAme. The association of chromatin proteins with DNAme varies across 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

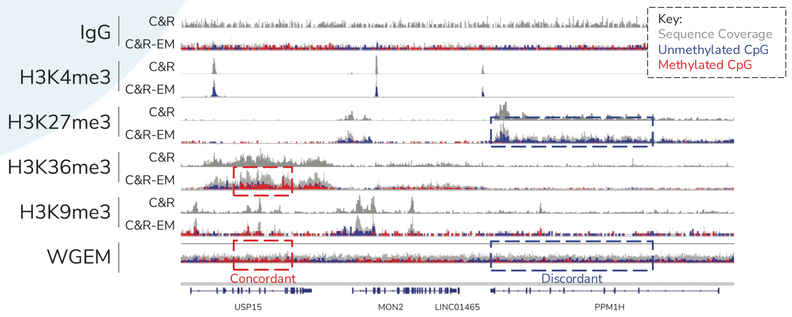


Figure 7. CUT&RUN-EM stratifies subpopulations of cells with unique PTM-DNAme crosstalk signatures that are obscured in whole genome EM-seq (WGEM). IGV genome browser comparison of CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and WGEM in K562 cells. C&R-EM loci with concordant results (**red box**) recapitulate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

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

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

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

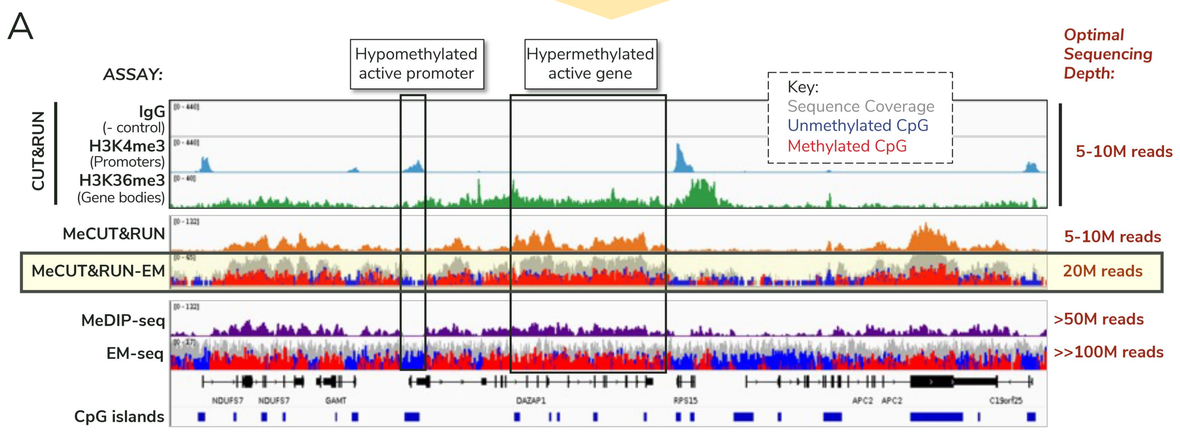


Figure 8. (**A**) GST-tagged MeCP2 methyl binding domain used in lieu of an antibody in traditional CUT&RUN ("**MeCUT&RUN**"; **orange**) with 500k K562 cells provides ~150bp resolution of genome-wide DNAme. MeCUT&RUN shows high concordance with Methyl-DNA Immunoprecipitation sequencing (MeDIP-seq; **purple**) at >2.5-fold reduced sequencing depth. To achieve CpG resolution of DNAme, MeCP2 CUT&RUN was followed by EM-seq ("**MeCP2 CUT&RUN-EM**"; yellow boxed track). MeCP2 CUT&RUN-EM generates similar DNA methylation profiles compared to whole genome EM-seq (bottom track). (**B**) Sankey plot of the binned human genome (200 bp) comparing the presence of 5mC in WGEM and MeCUT&RUN-EM. 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**

See CUT&RUN/Tag in Ag Research

- **Arabidopsis imprinting** León, *Nucleic Acids Res* 2024 (PMID: 38967011)
- **Porcine development** Innis, *Epigen Chrom* 2024 (PMID: 38773546)
- **Maize heat tolerance** Li, *Plant Cell* 2024 (PMID: 38573521)
- **Arabidopsis development** Zhang, *Mol Plant* 2024 (PMID: 38825830)
- **Rice fungal infection** Xue, *Plant Cell Rep* 2024 (PMID: 38393406)
- **Wheat regeneration** Liu, *Nat Plants* 2023 (PMID: 37142750)
- **Chicken development** Sun, *Poult Sci* 2023 (PMID: 36764138)
- **Cotton methods** Tao, *Plant Methods* 2020 (PMID: 32884577)

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