

High resolution epigenetics: Direct multiomics simultaneously maps DNA methylation and chromatin proteins

Andrea Johnstone¹, Vishnu U. Sunitha Kumary¹, Allison Hickman¹, Anup Vaidya¹, Jonathan M. Burg¹, Louise Williams², Chaithanya Ponnaluri², Hang Geong Chin², Pierre-Olivier Esteve², Isaac Meek², Sriharsa Pradhan², Zu-Wen Sun¹, Martis W. Cowles¹, Bryan J. Venters¹, & Michael-Christopher Keogh¹



¹ EpiCypher Inc, Durham, NC 27709, USA; ² New England Biolabs, Ipswich, MA 01938, USA

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

- DNA methylation (DNAm) & chromatin proteins (histone PTMs, transcription factors) coordinate to oversee gene expression
- These factors are associated with specific genomic features (Figure 1)
- Current methods to study DNAm/protein interactions rely on indirect parallel assays
- Improved technologies are needed to resolve DNAm / chromatin protein crosstalk

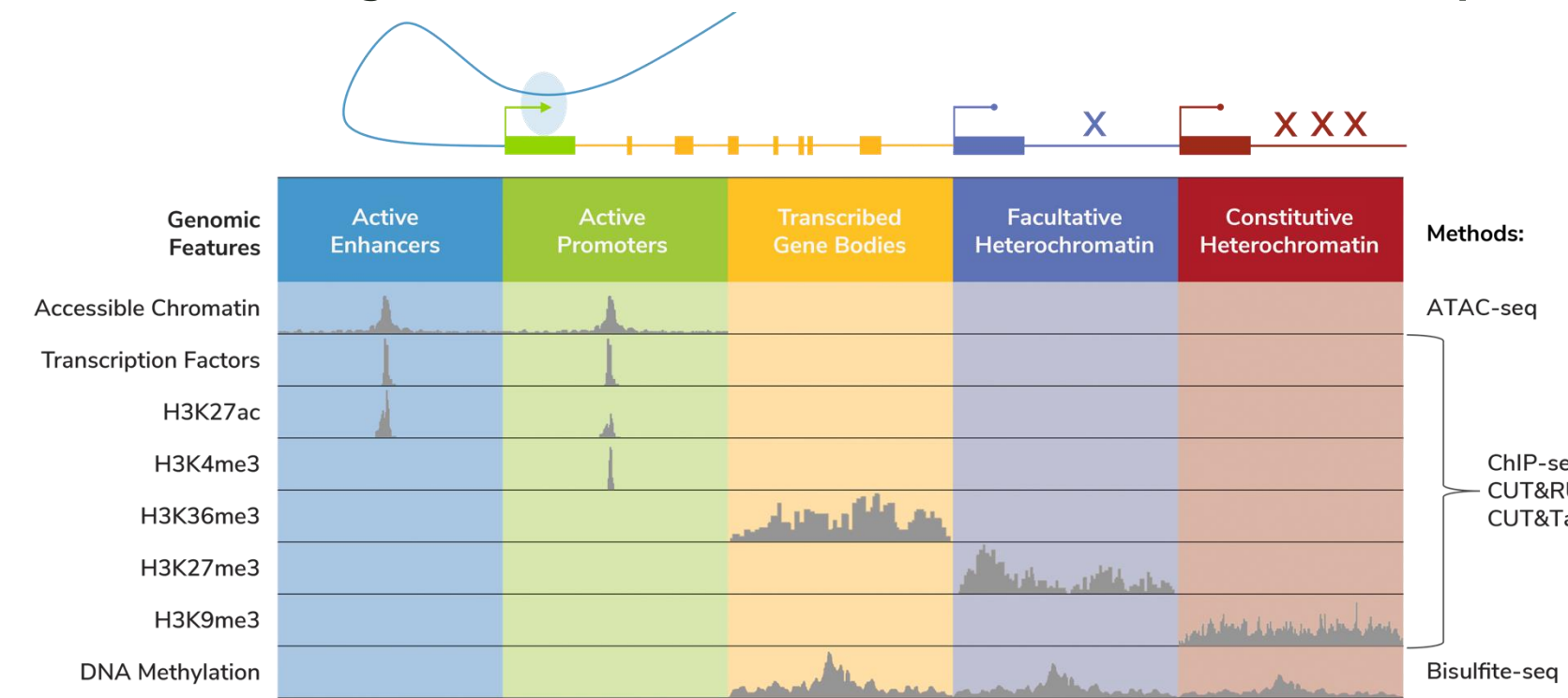


Figure 1. Chromatin proteins and DNAm define genomic features and govern gene expression; however, currently available assays are correlative.

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

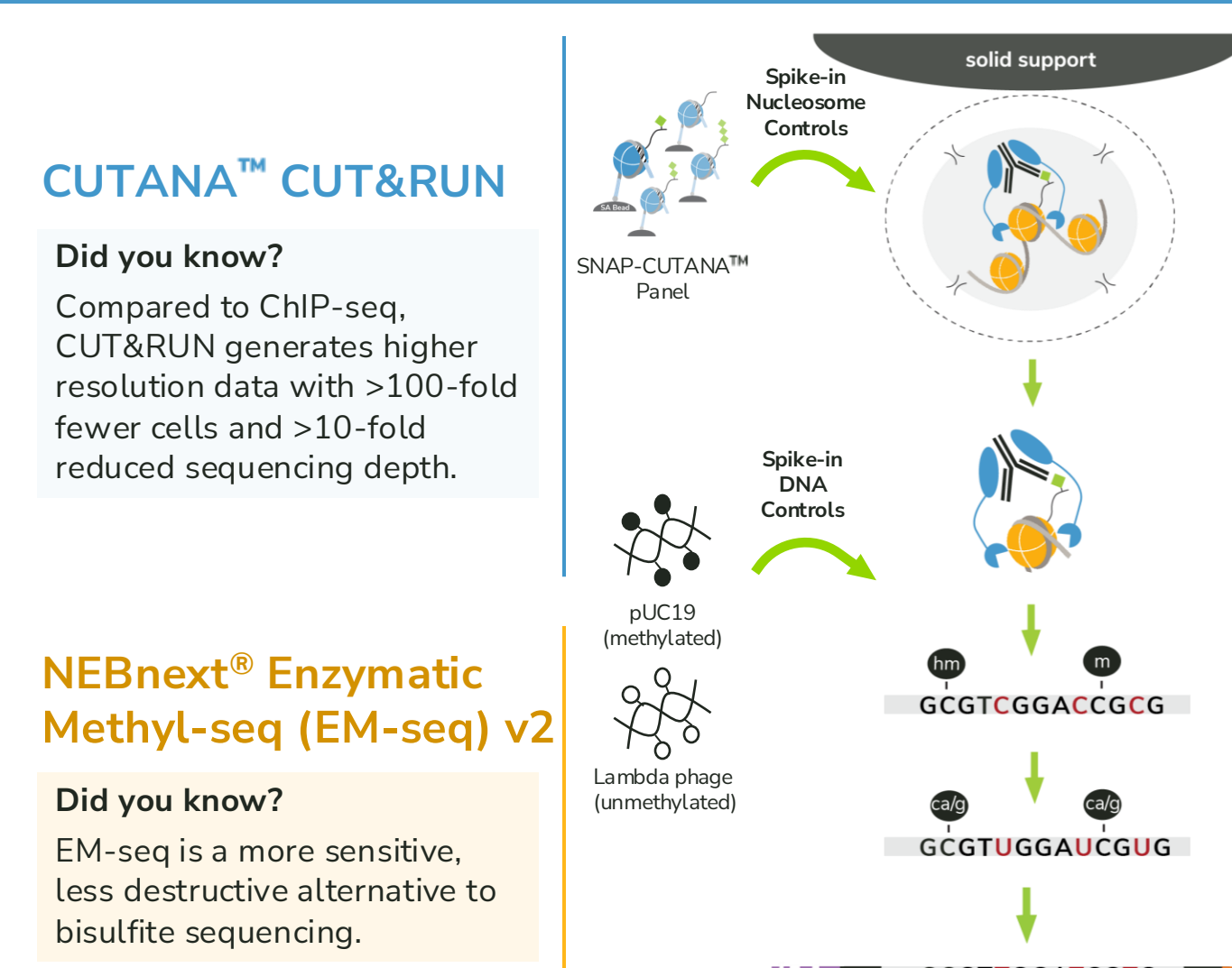


Figure 2. CUT&RUN-EM integrates EpiCypher CUT&RUN with NEB EM-seq v2. CUT&RUN isolates DNA associated with chromatin proteins of interest. EM-seq then enzymatically converts unmethylated cytosines to uracils. Sequencing the resulting libraries enables CpG resolution of DNAm co-occurring with the chromatin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

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

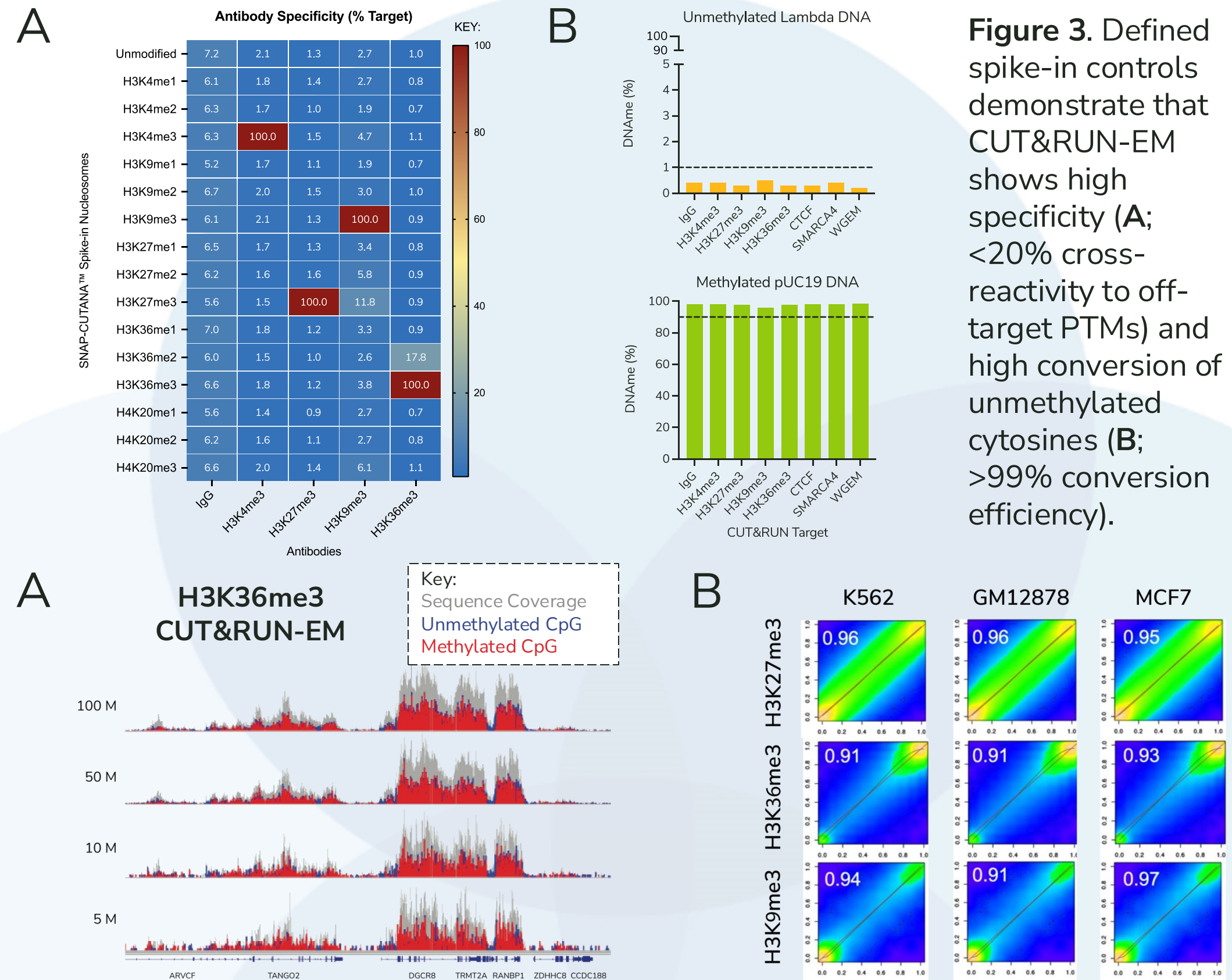


Figure 4. CUT&RUN-EM is highly reproducible across various sequencing depths (A; downsampled genome browser tracks) and replicates (B; Pearson correlation coefficients).

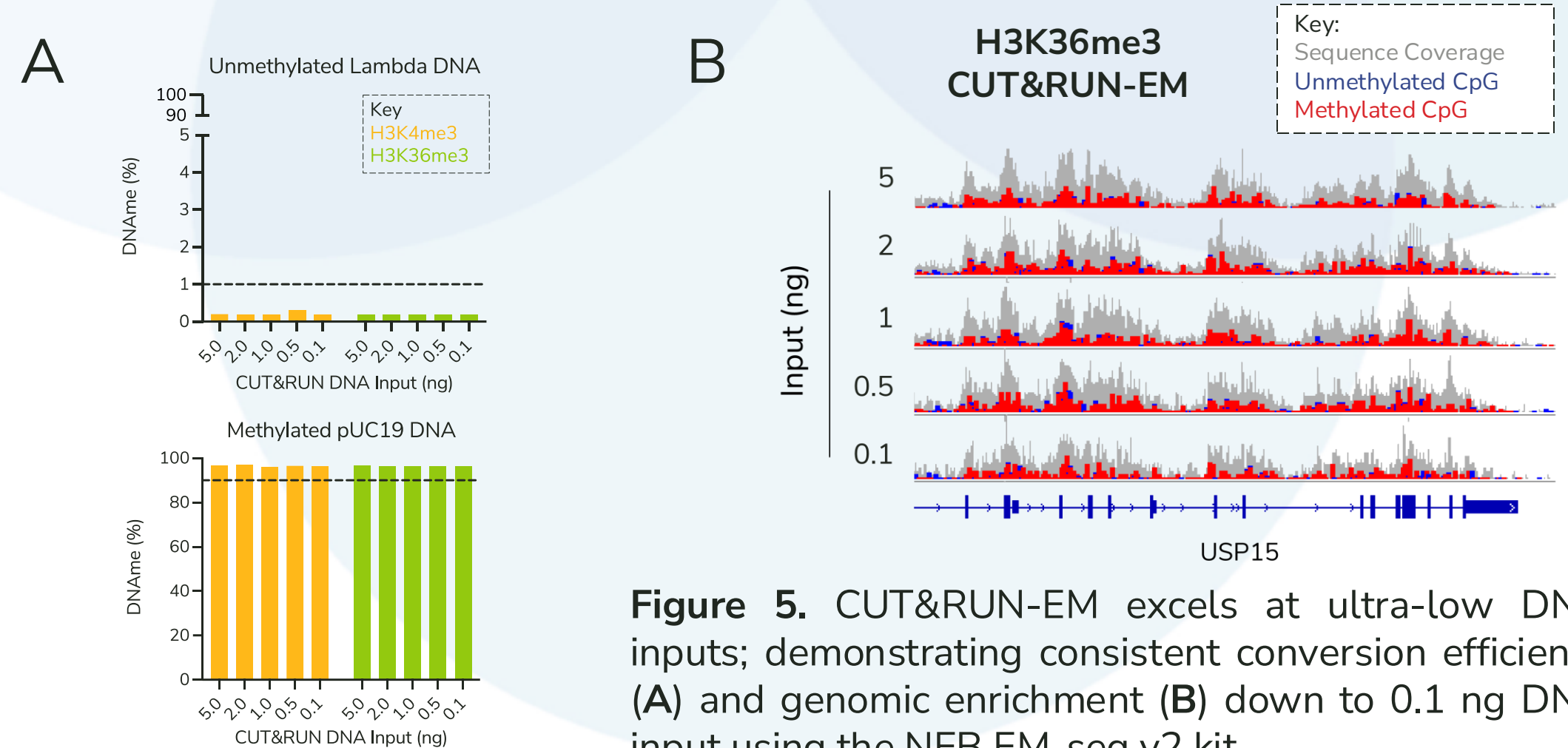


Figure 5. CUT&RUN-EM excels at ultra-low DNA inputs; demonstrating consistent conversion efficiency (A) and genomic enrichment (B) down to 0.1 ng DNA input using the NEB EM-seq v2 kit.

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

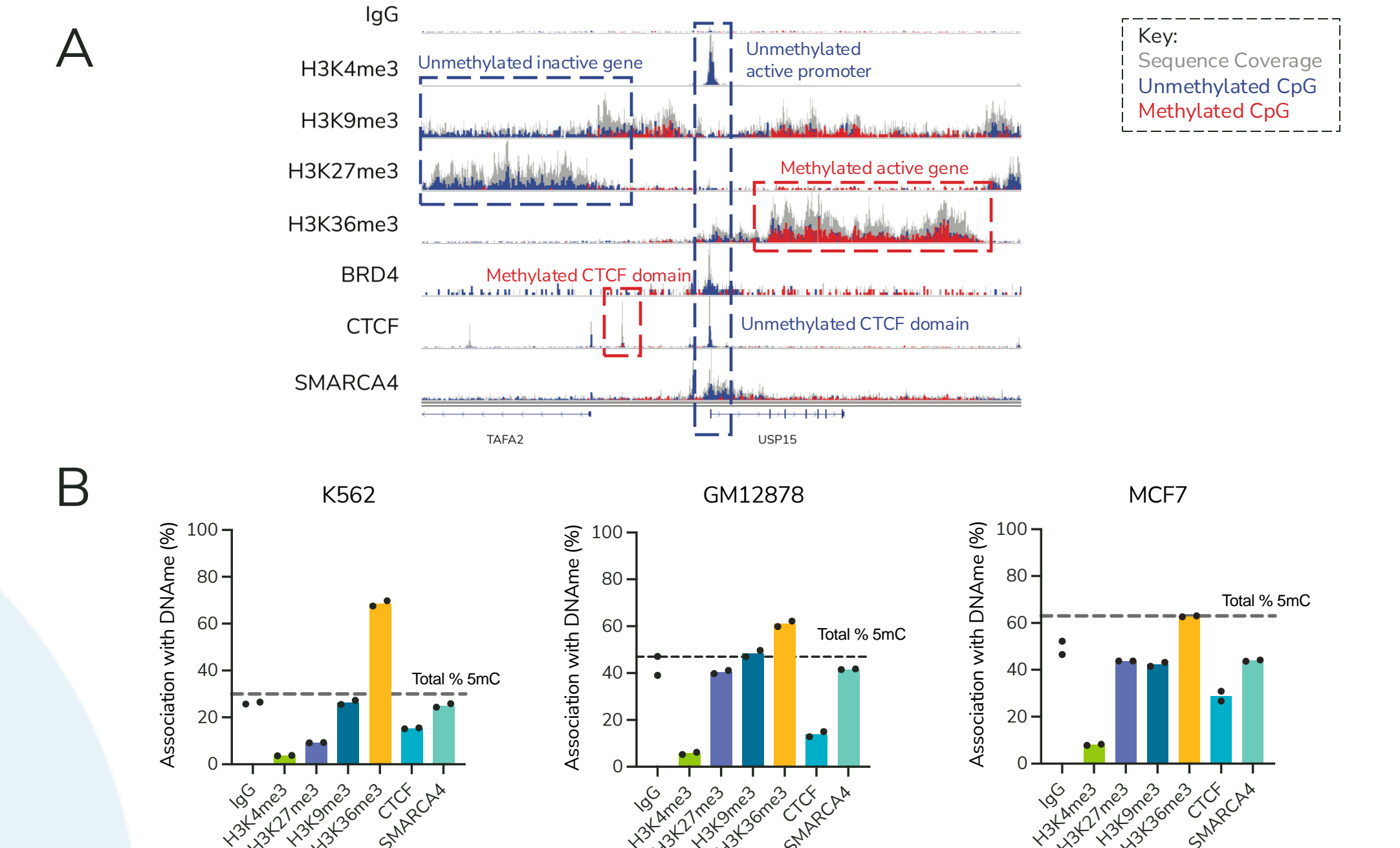


Figure 6. Representative genome browser tracks (A) demonstrate that CUT&RUN-EM reflects known biological functions of chromatin proteins (e.g. unmethylated H3K4me3 promoters, methylated H3K36me3 gene bodies) when overlaid with DNAm. The association of chromatin proteins with DNAm varies across cell lines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

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

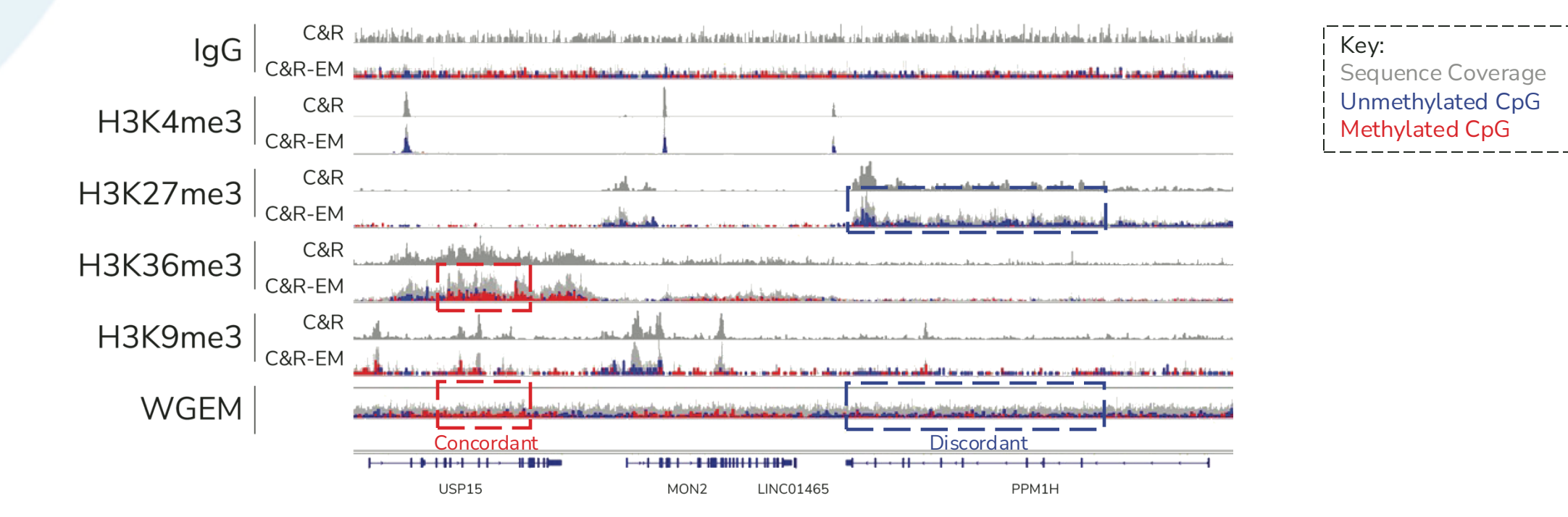


Figure 7. CUT&RUN-EM stratifies subpopulations of cells with unique PTM-DNAm crosstalk signatures that are obscured in whole genome EM-seq (WGEM). IGV genome browser comparison of CUT&RUN (C&R), CUT&RUN-EM (C&R-EM), and WGEM in K562 cells. C&R-EM loci with concordant results (red box) recapitulate the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

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

Modified "meCUT&RUN" workflow enriches methylDNA, reducing seq depth

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

meCUT&RUN captures high-density 5mC with 34x less sequencing than WGEM

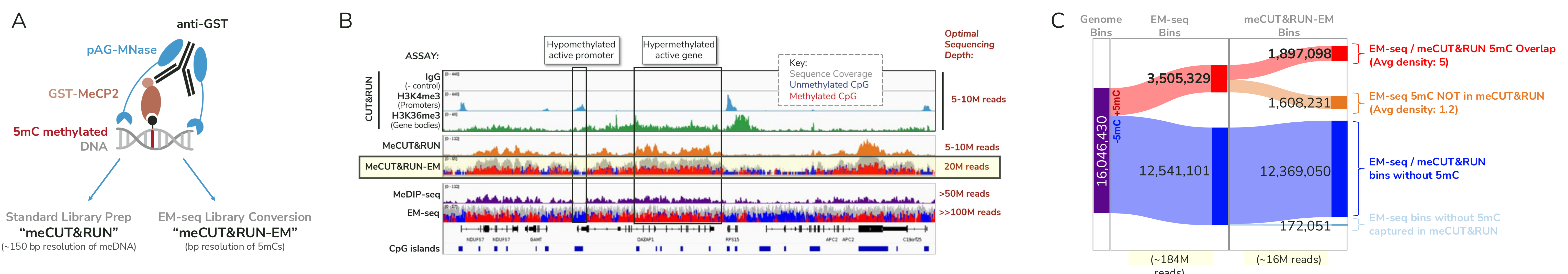


Figure 8. (A) Schematic representation of a GST-tagged MeCP2 methyl binding domain used in lieu of an antibody in traditional CUT&RUN (meCUT&RUN) to enrich methylated DNA. Sequencing libraries can be prepared via standard library prep to provide a snapshot of genome-wide DNAm at 150 bp resolution, or 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 DNAm 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:

Whole Genome EM-seq	vs	MeCP2 CUT&RUN-EM
\$1,650		\$48
~184M PE150 reads = 55 Gbp		~16M PE50 reads = 1.6 Gbp

EpiCypher assays in action

- T-cell activation
Arce, *Nature* 2025 (PMID: 39663454)
- T-cell exhaustion
Ford, *Sci Immunol* 2022 (PMID: 35930654)
- T-cell anticancer activity
Mamedov, *Nature* 2024 (PMID: 37648854)
- CAR T-cell expansion
Battistello, *Mol Cell* 2023 (PMID: 36944333)
- dCas9/Cas9 targeting
Wang, *Nucleic Acids Res* 2022 (PMID: 35849129)
- Epigenome editing
Rohm, *Cell Genom* 2025 (PMID: 39947136)
- iPSC profiling
Schreiber, *Mol Metab* 2021 (PMID: 34352411)
- Immune cell fingerprinting
Immunological Genome Project www.immgen.org

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