Direct multi-omics for the masses: Linking DNA methylation to chromatin targets via TEM-seq

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Molecular crosstalk between DNA methylation and other chromatin features

DNA methylation (DNAme) is an epigenetic mark that includes the modification of cytosine resides (5mC) within CpG islands. In addition to well characterized roles regulating gene expression, imprinting and silencing parasitic DNA elements, the misregulation of DNAme is implicated in multiple diseases. Evidence is emerging that DNAme is not an independent epigenetic mark but rather closely linked to the post translational modification (PTM) of histone proteins. However, examining the direct relationships between 5mC and PTMs are hampered by correlated analyses of separate assays that cannot establish a direct mechanistic linkage. Furthermore, the traditional approach to measure 5mC relies upon harsh bisulfite chemical conversation of DNA, which introduces DNA breaks and systemic biases.

To address these limitations, we developed a Targeted Enzymatic Methylation-sequencing (TEM-seq) approach, an ultra-sensitive multi-omic genomic mapping technology that delivers high resolution DNAme profiles at epitope-defined chromatin features. Importantly this assay is capable of examining the direct molecular link between 5mC and histone PTMs and/or chromatin associated proteins (ChAPs).

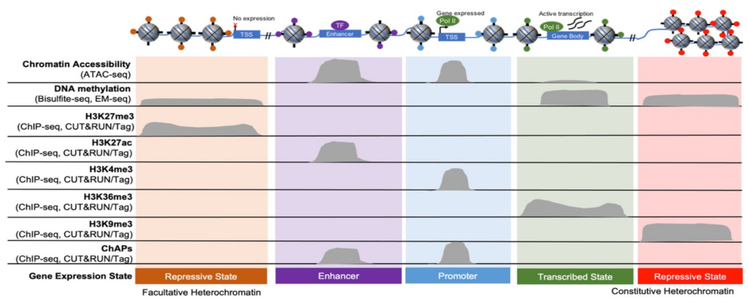


Figure 1: Gene expression is regulated by chromatin structure and accessibility. PTMs provide useful markers of chromatin elements, such as promoters, enhancers, and gene bodies. Adapted from (1).

TEM-seq is a powerful multi-omic assay that directly links 5mC to PTMs and/or ChAPs

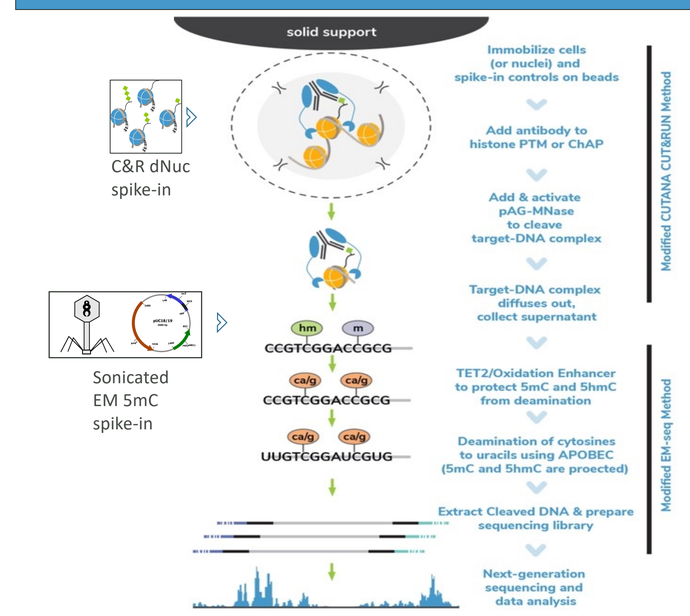


Figure 2: Workflow for TEM-seq (Targeted Enzymatic Methylation-sequencing).

TEM-seq reveals distinct DNAme levels linked to different histone PTMs and ChAPs

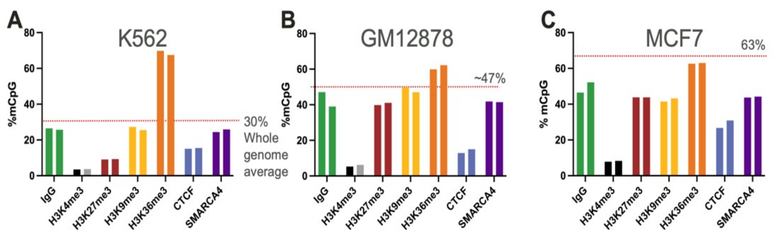


Figure 3: Percent methylation analysis from TEM-seq data sets are consistent with known biological functions of antibody targets. TEM-seq was performed using a set of antibodies to different histone PTM or chromatin targets. For each data set, the percent of methylated CpGs was calculated to provide a global view of relative methylation directly linked to each antibody target.

TEM-seq allows sensitive profiling to relate DNAme to Histone PTMs and ChAPs

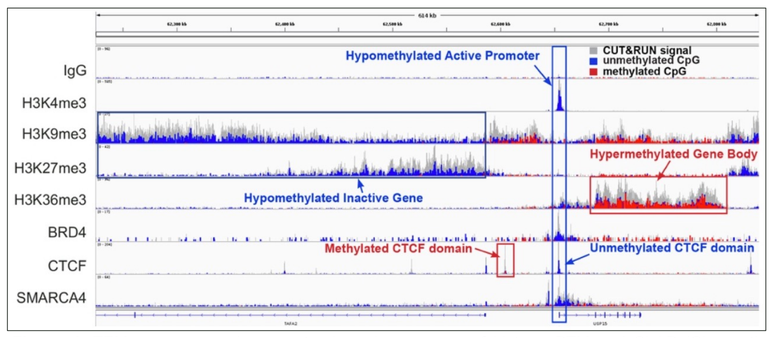


Figure 4: TEM-seq genome browser tracks using a range of histone PTM and ChAP (Chromatin Associated Protein) targets. Composite histone PTM or ChAP signal (*i.e.* CUT&RUN data) is in gray. Blue = unmethylated CpG; Red = methylated CpG. TEM-seq generates high quality genomic maps for both histone PTMs and ChAPs and also reveals differential DNAme at various genomic regions, (*e.g.* hypermethylation at gene bodies / hypomethylation at promoters).

High reproducibility and sensitivity of TEM-seq at low seq depths

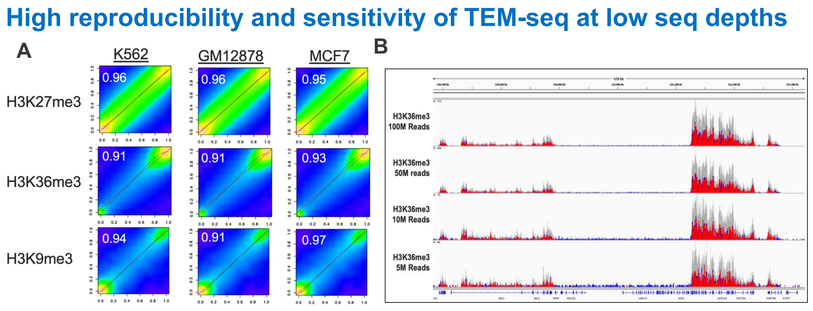


Figure 5: (**A**) TEM-seq assays are highly reproducible. Biological replicate analysis in K562, GM12878, and MCF7 cells shows that TEM-seq assays are highly reproducible (r > 0.91). (**B**) TEM-seq genome browser tracks for H3K36me3 were randomly downsampled / analyzed at represented sequence depths and similar DNAme distribution observed using as few as 5M reads.

Benchmarking TEM-seq to CUT&RUN and EM-seq

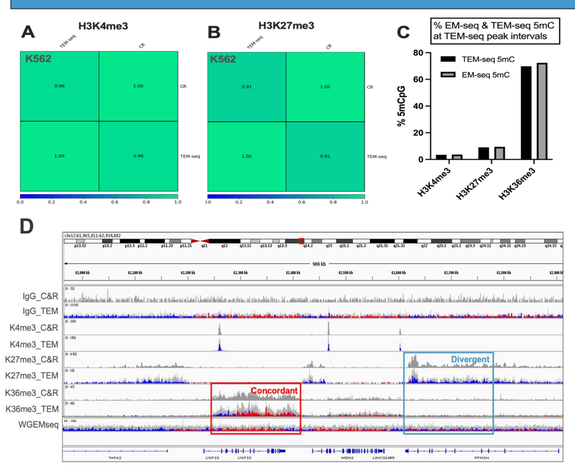


Figure 6: TEM-seq benchmarking to CUT&RUN and EM-seq. (**A-B**) H3K4me3 and H3K27me3 CUT&RUN peaks were compared with TEM-seq peaks by using Pearson Correlation (PC). Both H3K4me3 and H3K27me3 showed a PC (R) > 0.9. (**C**) Percent methylation analysis (% 5mCpG) was compared for TEM-seq and EM-seq at TEM-seq peak intervals. These data show that the % DNAme is nearly identical at called peak intervals, demonstrating that EM-seq and TEM-seq datasets are highly concordant. (**D**) Comparison of CUT&RUN (C&R), TEM-seq, and whole genome EM-seq (WGEM-seq) datasets (K562 cells) using IGV genome browser. IgG was included as a negative control. Red box labels a genome locus where DNA methylation levels as determined by TEM-seq and EM-seq are concordant. Blue box labels a genome locus where DNA methylation levels as determined by TEM-seq and EM-seq are divergent.

TEM-seq spike-ins provide quantitative metrics for rigorous assay monitoring

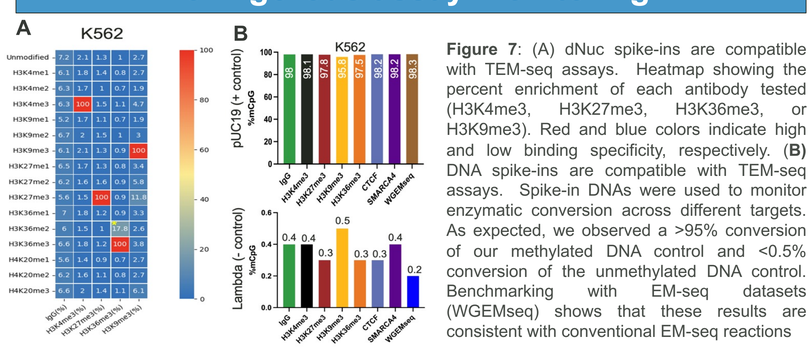


Figure 7: (A) dNuc spike-ins are compatible with TEM-seq assays. Heatmap showing the percent enrichment of each antibody tested (H3K4me3, H3K27me3, H3K36me3, or H3K9me3). Red and blue colors indicate high and low binding specificity, respectively. (**B**) DNA spike-ins are compatible with TEM-seq assays. Spike-in DNAs were used to monitor enzymatic conversion across different targets. As expected, we observed a >95% conversion of our methylated DNA control and <0.5% conversion of the unmethylated DNA control. Benchmarking with EM-seq datasets (WGEMseq) shows that these results are consistent with conventional EM-seq reactions

TEM-seq excels at sub-nanogram amounts of input DNA

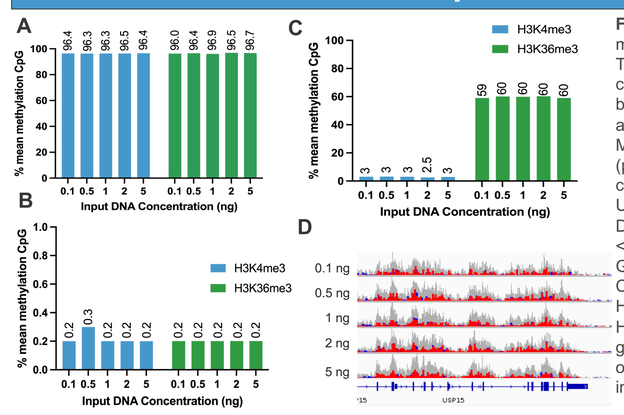


Figure 8: Percent methylation analysis from TEM-seq data sets are consistent with known biological functions of antibody targets. **A)** Methylated spike-in DNA (pUC19) showing > 95% conversion, **B)** Unmethylated spike-in DNA (Lambda) showing <5% conversion, **C)** Global view of methylated CpGs in the context of H3K4me3 and H3K36me3 **D)** TEM-seq genome browser tracks of H3K36me3 at different input DNA concentration

In vitro characterization of Rett syndrome MeCP2 mutants with Luminex assay

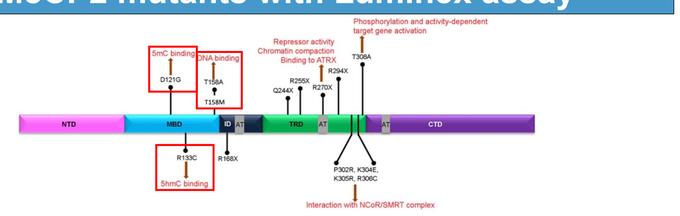
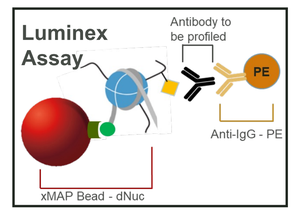
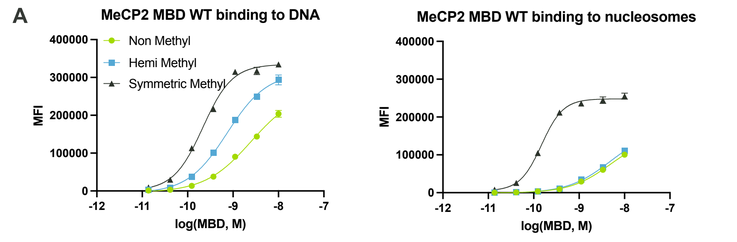


Figure 9: Schematic of MeCP2 structure. MeCP2 contains a Methyl CpG binding domain (MBD), a transcription repression domain (TRD), a C terminal domain (CTD) and AT-like hooks (DNA-binding elements). Adapted from (8).

Rett syndrome Box: X-linked neurodegenerative disorder resulting from mutation of the DNAme reader **MeCP2 (8)**, a transcriptional repressor that binds methylated CpG residues via its Methyl Binding domain (MBD).



MeCP2 titration on Luminex



Specificity of MeCP2 (WT & Rett syndrome alleles) for symmetric 5mC (salt dependence)

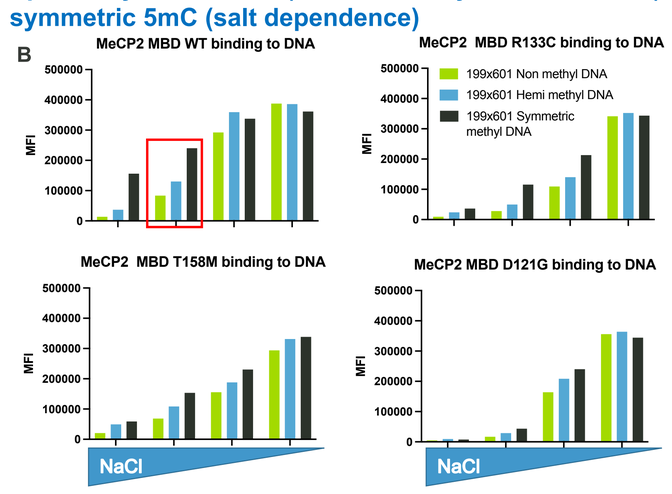
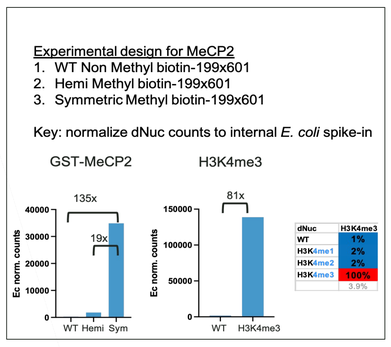


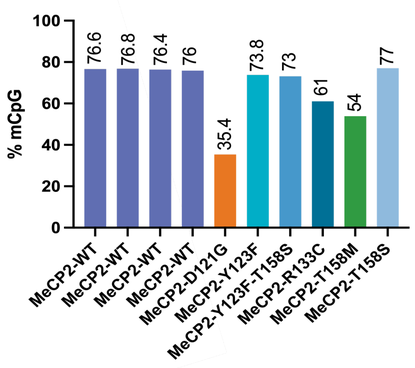
Figure 10. Luminex characterization of MeCP2 and Rett syndrome mutations. (**A**) GST-MeCP2 MBD WT shows a strong preference for symmetrically methylated 5mC, most particularly in the nucleosome context in a buffer containing 200mM NaCl (right panel). (**B**) Impact of Rett syndrome mutations on binding of MeCP2 MBD to free DNA (left) under different NaCl concentrations. Arrow indicates conditions chosen for reader TEM-seq studies with GST-MeCP2 MBD WT (see **Figure 11**).

TEM-seq provides multi-omic insights into Rett syndrome

(A) *EpiCypher* dNucs can be used as spike in controls to monitor specific enrichment in reader TEM-seq



(B) TEM-seq reveals differences in DNAme levels linked to Rett MeCP2 mutants



(C) Rett MeCP2 mutants display defective genome distribution.

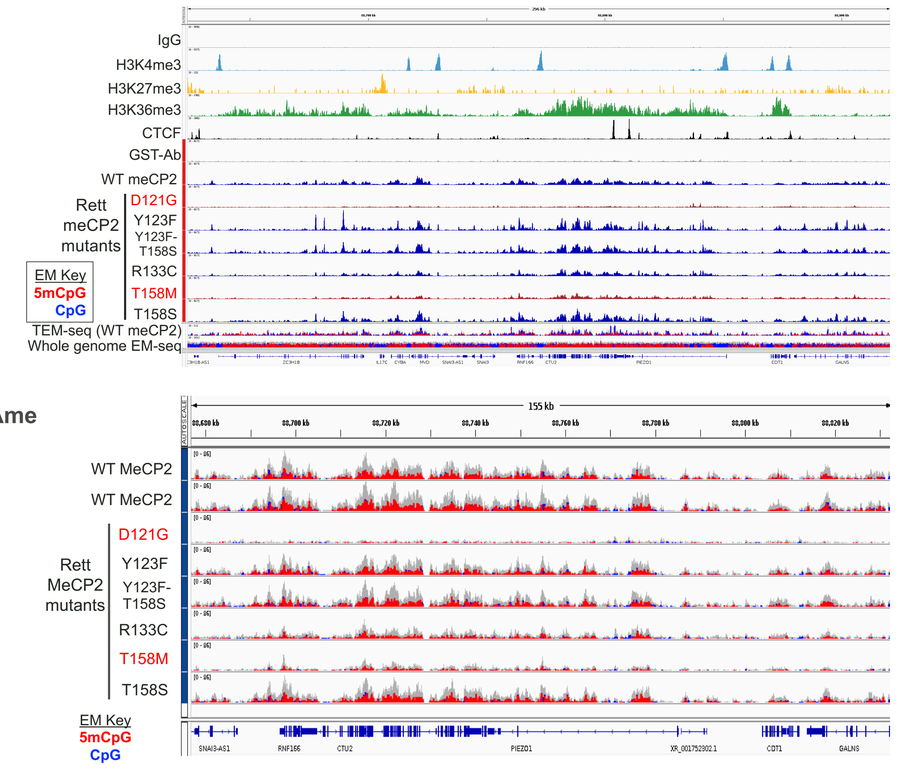


Figure 11. Reader TEM-seq using GST-MeCP2. A) MeCP2 MBD WT was used in a reader TEM-seq experiment with non-methyl, hemi-methyl and symmetric-methyl Nucs [22-N-22; [0x (un) - 3x (hemi) - 6x (symmetric) 5meC in each linker region] as spike in controls. MeCP2 showed preferential enrichment of the symmetric-methyl spike-in compared to non-methyl (135-fold) and hemi-methyl (19-fold) Nucs. Anti-H3K4me3 and SNAP-CUTANA K-MetStat mini-panel (**Figure 2**) positive control showed 81-fold enrichment of H3K4me3 over unmodified Nuc. **(B)** Percent methylation analysis reveals the effect of Rett mutants in the meCP2 binding (**C**) Genome browser tracks (CUT&RUN and TEM-Seq) for GST-MeCP2 MBD using K562 cells show its localization to methyl-CpG (red) over non methyl CpG (blue).

Conclusions

- TEM-seq is a novel multi-omic approach that directly links DNA methylation to chromatin features.
- TEM-seq delivers high quality data at low sequence depths.
- Spike-in controls are important to monitor TEM-seq performance.
- dCypher 3.0 optimizes conditions for reader TEM-seq experiments.
- TEM-seq can be leveraged to gain mechanistic insights into clinically relevant chromatin reader domains (e.g., MDB of MeCP2)

References

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