An enrichment-based nucleotide-resolution approach to measure DNA methylation

Nathaniel Chapin, Joseph Fernandez, Jason C. Poole, Benjamin Delatte* (bdelatte@activemotif.com)

Active Motif, Advanced Research lab, Carlsbad, CA

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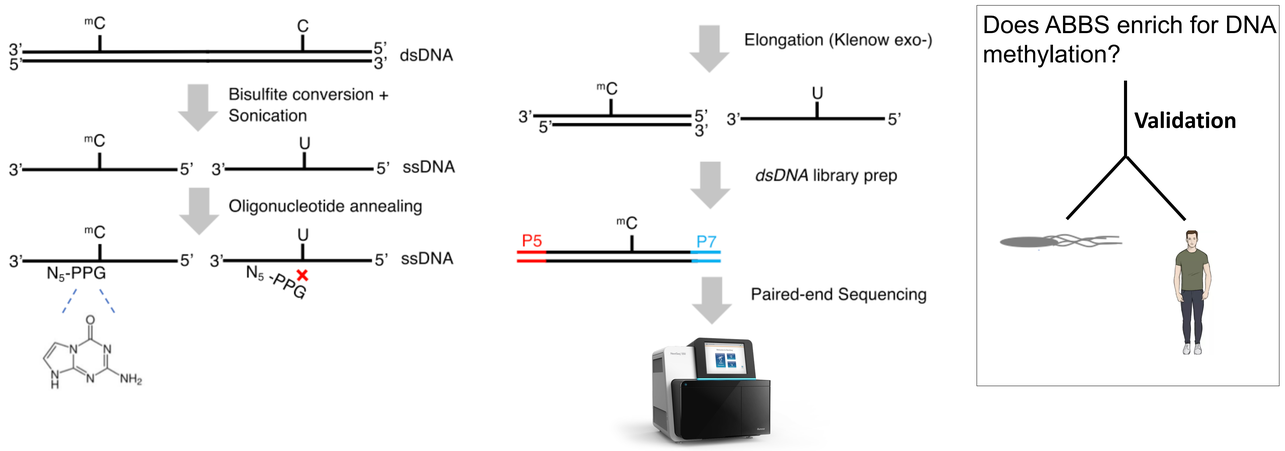
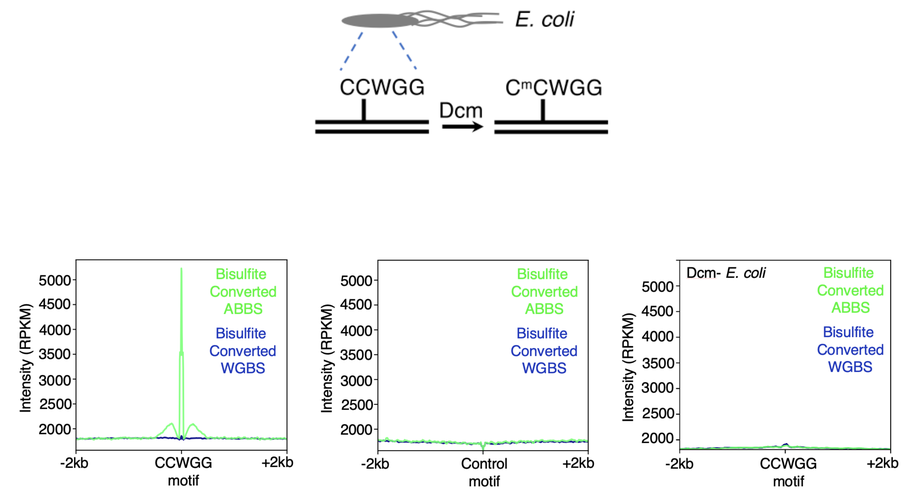
5-Methylcytosine (5-mC) is the most abundant epigenetic DNA modification in eukaryotes. The presence of 5-mC alters gene expression and genome metabolism, and its deregulation is associated with many human diseases. It is therefore a central area of focus in biomedical research. Widely-used methods to measure DNA methylation include Methylated DNA immunoprecipitation followed by sequencing (MeDIP-seq), where 5-mC-specific antibodies capture methylated DNA fragments, and Whole Genome Bisulfite Sequencing (WGBS), which uses bisulfite to chemically convert unmethylated cytosines to uridines while leaving methylated cytosines untouched. MeDIP-seq is relatively affordable but provides semi-quantitative detection of 5-mC with a limited (~100 bp) resolution. By contrast, WGBS offers quantitative measurement of 5-mC and single-base resolution but is costly due to its requirement for high sequencing depth. Hence, there currently is a need for genome-wide approaches to affordably detect 5-mC in a quantitative manner and at single-base resolution. Here we present Anchor-Based Bisulfite Sequencing (ABBS). ABBS uses an anchored primer and bisulfite treatment of DNA to focus sequencing power to methylated regions of the genome, thereby decreasing sequencing depth and cost. We show that ABBS accurately determines DNA methylation levels in *Escherichia coli* and in humans at single-base resolution, while requiring up to 15-20 times fewer sequencing reads than WGBS. ABBS performs well on cell lines, tissues, as well as cell-free DNA (cfDNA), and can detect differences in methylation between healthy and diseased tissues.

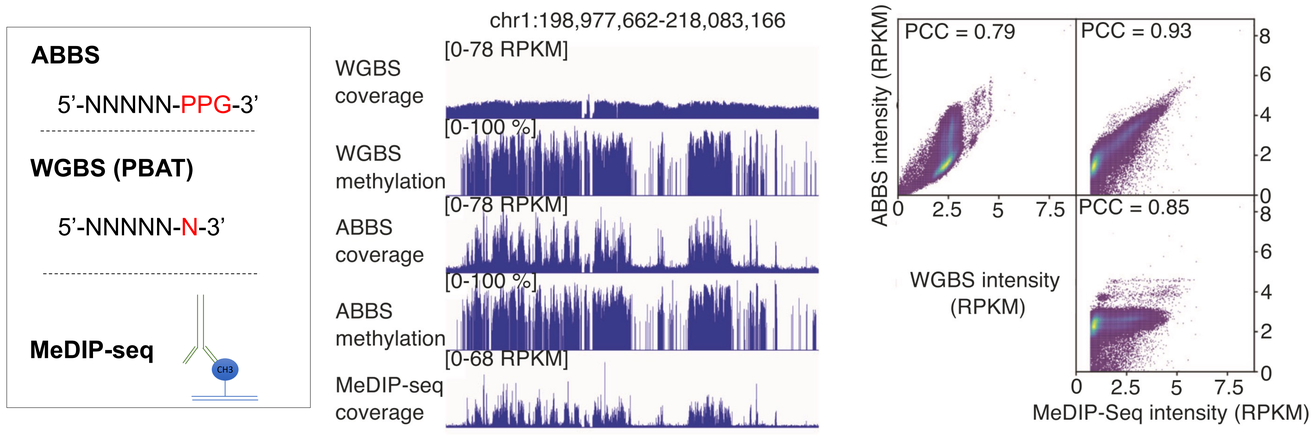
1. Principle of ABBS (Anchor-Based Bisulfite Sequencing)



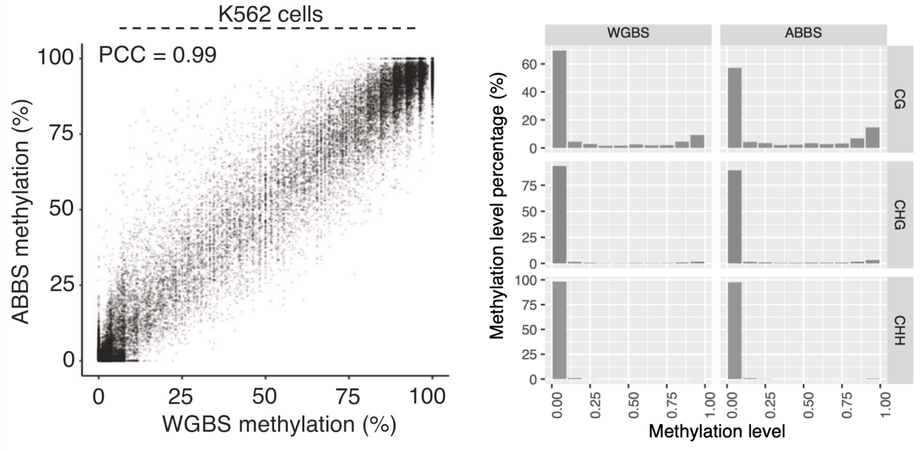
2. Validation of ABBS in *E. coli*



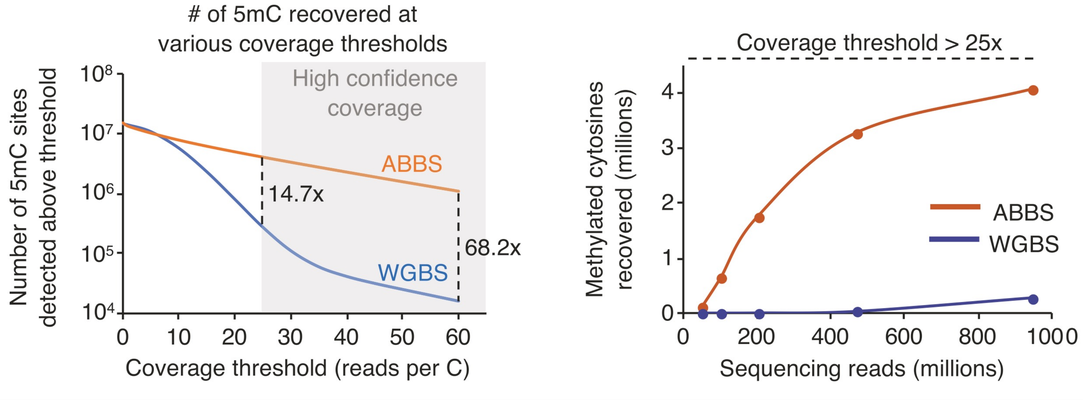
3. Validation of ABBS in human K562 cells (a)



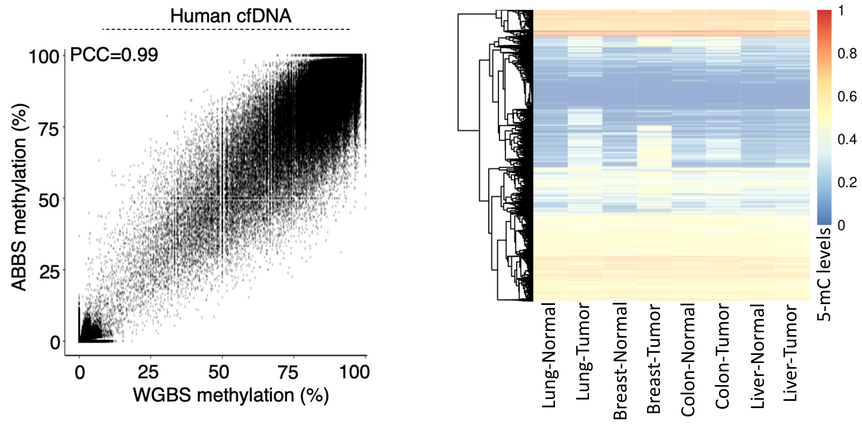
3. Validation of ABBS in human K562 cells (b)



4. ABBS requires fewer reads than WGBS



5. Applications: (a) cfDNA and (b) biomarkers discovery



Perspectives

- **Methylation analyses in RNA**
- **5-hydroxymethylcytosine (5-hmC) analyses**