Simultaneous genetic and epigenetic profiling of the human genome at single-base resolution with PacBio HiFi sequencing

Abstract # 7064F

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Native base modification detection with HiFi

- HiFi sequencing profiles polymerase kinetic signals (pulse width and inter-pulse duration).
- Changes in kinetic signals correlate with nucleotide base modifications [1].
- 5mC occurs within the CpG context in mammalian genomes and correlates with gene regulation.
- 6mA occurs in prokaryotes and can be exogenously added to eukaryotic DNA to profile open chromatin.

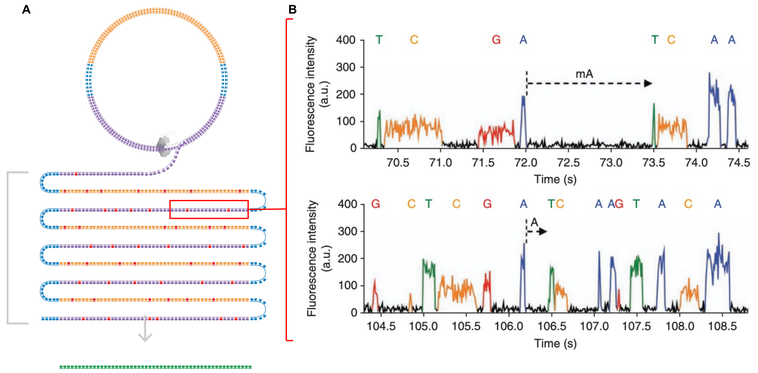
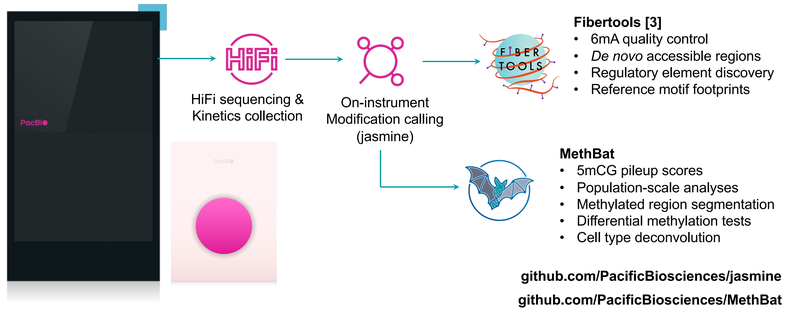


Figure 1. **Kinetics-based modification detection. (A)** Circular consensus sequencing provides per-base reading of subread pulse-widths and inter-pulse durations. **(B)** Modified bases (6mA shown here) affect kinetics that translate to changes in polymerase kinetics measured by the fluorescent dye

HiFi epigenetics solutions



Fibertools [3]
- 6mA quality control
- *De novo* accessible regions
- Regulatory element discovery
- Reference motif footprints

MethBat
- 5mCG pileup scores
- Population-scale analyses
- Methylated region segmentation
- Differential methylation tests
- Cell type deconvolution

github.com/PacificBiosciences/jasmine
github.com/PacificBiosciences/MethBat
github.com/fiberseq/fibertools-rs

Deep learning modification prediction models for 5mC

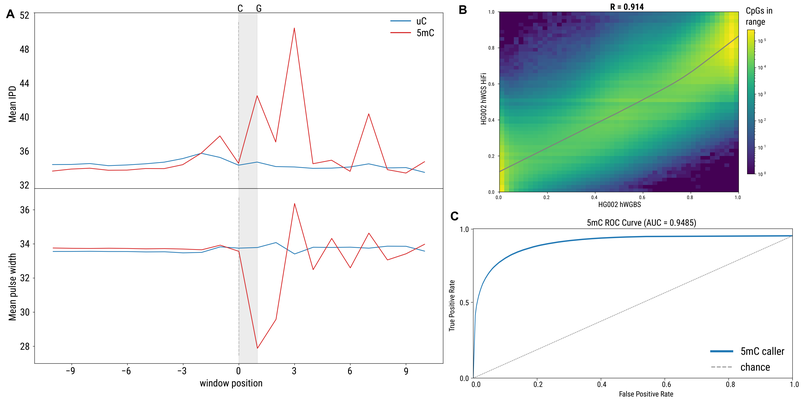


Figure 2. **Overview of the 5mC model and performance. (A)** Effect of 5mC modification on inter-pulse duration (IPD) and pulse widths downstream of CpG bases, contrasting unmethylated Cs (uC) and 5-methylcytosines (5mC) **(B)** Genome-wide comparison of CpG methylation levels in HG002, measured using HiFi sequencing at 30x coverage and whole-genome bisulfite sequencing (WGBS) **(C)** 5mC ROC curve in labeled control data

Strand-specific models for 6mA detection

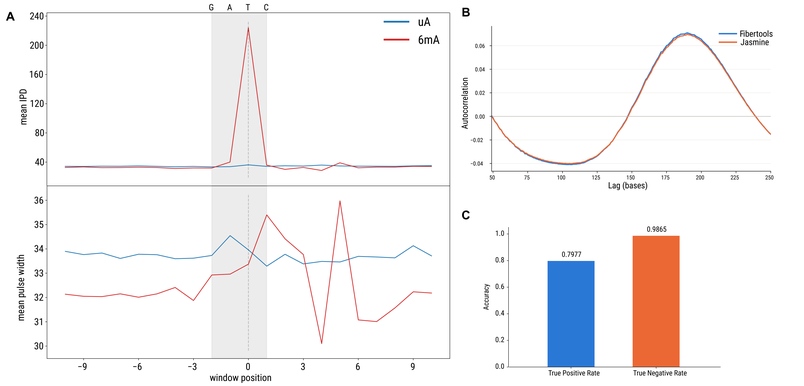


Figure 3. **Overview of the 6mA model and performance. (A)** Effect of 6mA modification in inter-pulse duration and pulse width in the GATC motif, which is unmethylated human DNA (uA) and contains the 6-methyladenine mark (6mA) in *E. coli*. **(B)** 6mA autocorrelation in Fiber-seq data using the Jasmine on-instrument caller and the Fibertools 6mA caller **(C)** Performance of the Jasmine 6mA caller: true positive rate measured in *E. coli* sequencing data and true negative rate measured in human DNA

HiFi support for episignature analysis

HiFi WGS delivers biologically meaningful methylation calls that correlate strongly with bisulfite sequencing. EpiSign METRIC 5-base supports HiFi, and some EpigenCentral signatures correctly classified HiFi samples [4].

Simultaneous profiling of accessibility and methylation with Fiber-seq

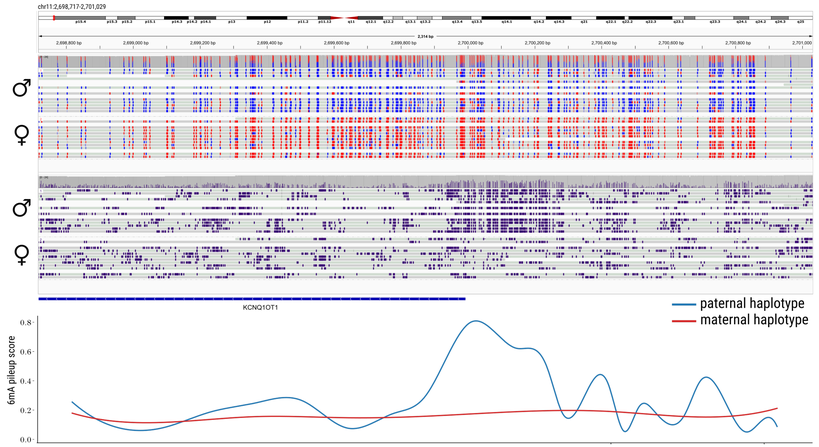


Figure 4. **Simultaneous profiling of methylation and accessibility.** IGV snapshot of a phased Fiber-seq [2] HG002 sample mapped to hg38. Depicted is region chr11:2,698,717-2,701,029, which contains the transcription start site (TSS) for the gene KCNQ1OT1. The phasing shows paternal (♂) allele CpG hypomethylation and accessible TSS (increased 6mA methylation), whereas the maternal (♀) allele shows CpG hypermethylation and limited 6mA labeling (nucleosome condensed DNA)

Conclusion

- PacBio HiFi accurately detects 5mC and 6mA from polymerase kinetics.
- The Fiber-seq assay enables joint profiling of CpG methylation and chromatin accessibility on individual DNA molecules, providing a high-resolution view of the genome sequence and epigenetic landscape.

References

1. Flusberg, Benjamin A., et al. "Direct detection of DNA methylation during single-molecule, real-time sequencing." *Nature methods* 7.6 (2010): 461-465.
2. Bohaczuk, Stephanie C., et al. "Resolving the chromatin impact of mosaic variants with targeted Fiber-seq." *Genome Research* 34.12 (2024): 2269-2278.
3. Jha, Anupama, et al. "DNA-m6A calling and integrated long-read epigenetic and genetic analysis with fibertools." *Genome research* 34.11 (2024): 1976-1986.
4. Cheung, Warren A., et al. "Direct haplotype-resolved 5-base HiFi sequencing for genome-wide profiling of hypermethylation outliers in a rare disease cohort." *Nature Communications* 14.1 (2023): 3090.

Acknowledgements

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