

Affinity-based Enrichment Analyses of DNA Methylation MCIp, MeDIP, hMeDIP

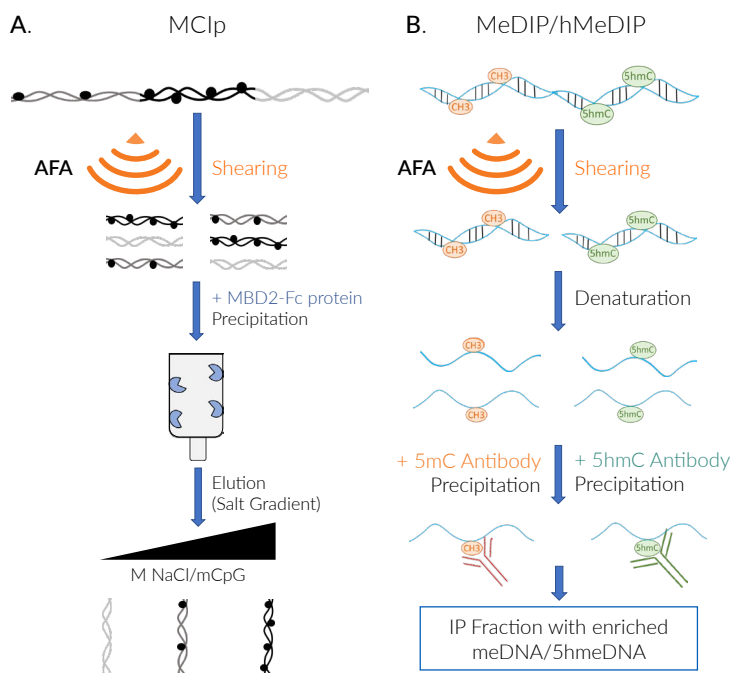
Scientific Relevance

- Cytosine methylation/hydroxymethylation on DNA represent an important epigenetic regulatory layer that alters chromatin structure ¹ and influences transcription factor binding affinities ^{2,3}
- Such modifications are essential for proper gene expression states during development ⁴ and often altered in diseases involving cancer development and metastasis ⁵
- DNA methylation marks have huge potential as biomarkers ⁶ and display druggable targets ^{7,8}
- Precipitation based methods provide a fast and easy tool to analyze genome-wide DNA methylation profiles and identify differentially methylated regions

Challenges

- Affinity-based enrichment methods display an inherent sequence bias ² and therefore require unbiased and reproducible DNA shearing for optimal resolution and coverage
- Sequences of varying fragment length will precipitate with different efficiency and therefore require tight DNA fragment size distributions

Workflow



Schematic representation of precipitation based methods to detect DNA methylation. **A:** Methyl-CpG immunoprecipitation (MCIp) ¹⁰ relies on shearing of genomic DNA followed by immobilisation of methylated DNA on a MBD2-Fc protein A-Sepharose matrix. Applying increasing salt concentrations DNA fractions with different degree of DNA methylation are eluted. **B:** Methylated DNA immunoprecipitation (MeDIP) ¹¹ as well as hydroxymethylated DNA immunoprecipitation (hMeDIP) require DNA shearing and denaturation followed by immunoprecipitation with monoclonal 5mC/5hmC specific antibody.

Advantages of Adaptive Focused Acoustics® (AFA®)

[AFA technology](#) is known as the gold standard for DNA shearing which is tunable and ensures the utmost reproducibility and efficiency.

- Random fragmentation ensures unbiased representation of genomic regions
- Enables comparison of different samples e.g. tumour vs. healthy tissue or time course of follow-up samples

AFA technology enables solubilization of [RNA and DNA](#) as well as [chromatin from FFPE tissues](#) and therefore allows for genome-wide DNA methylation profiling from FFPE tissue.

Suggested Covaris Products

- [Covaris Focused-ultrasonicator](#) (M-Series, S-Series, E-Series, or LE-Series)
- [truXTRAC FFPE](#)

Citations

- [Reizel et al. Postnatal DNA demethylation and its role in tissue maturation. Nat Commun. \(2018\)](#)
- [Heilmann et al. Genome-wide screen for differentially methylated longnoncoding RNAs identifies Esrp2 and lncRNA Esrp2-as regulated by enhancer DNA methylation with prognostic relevance for human breast cancer. Oncogene. \(2017\)](#)
- [Schnöder et al. Epo-induced erythroid maturation is dependent on Plcy1 signaling. Cell Death and Differentiation. \(2015\)](#)
- [Clark et al. A Comparison of the Whole Genome Approach of MeDIP-Seq to the Targeted Approach of the Infinium HumanMethylation450 BeadChip® for Methylation Profiling. PLoS One \(2012\)](#)