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

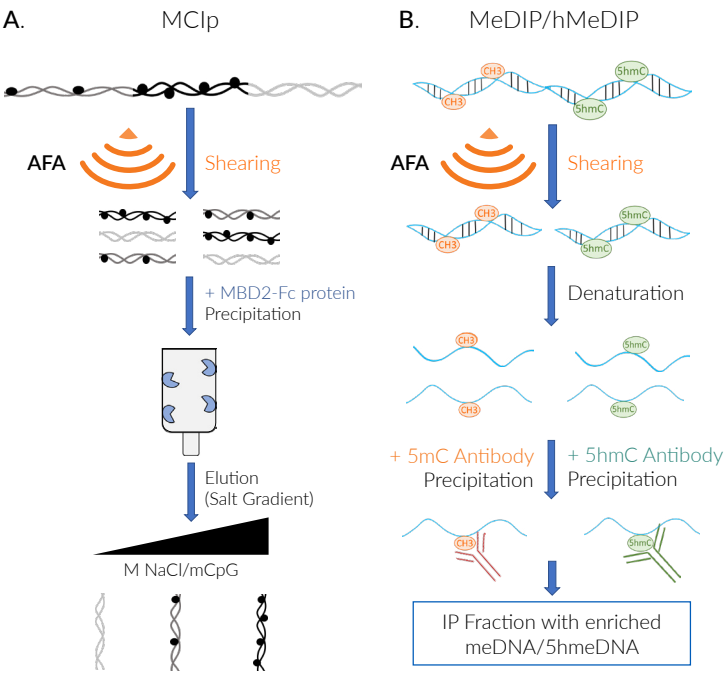
Scientific Relevance

- Cytosine methylation/hydroxmethylation 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 [9] 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 Plcγ1 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 Methylome Profiling. PLoS One (2012)

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Part Number: M020091 | Rev A | Edition August 2018 |