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 and influences transcription factor binding affinities.
- Such modifications are essential for proper gene expression states during development and often altered in diseases involving cancer development and metastasis.
- DNA methylation marks have huge potential as biomarkers and display druggable targets.
- 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 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

A. MCIp

1. AFA Shearing
2. + MBD2-Fc Protein Precipitation
3. Elution (Salt Gradient)
4. M NaCl/mCpG

B. MeDIP/hMeDIP

1. AFA Shearing
2. Denaturation
3. + 5mC Antibody Precipitation
4. + 5hmC Antibody Precipitation
5. IP Fraction with enriched meDNA/5hmeDNA

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