

HiC Chromatin Immunoprecipitation - Hi-ChIP

Scientific Relevance

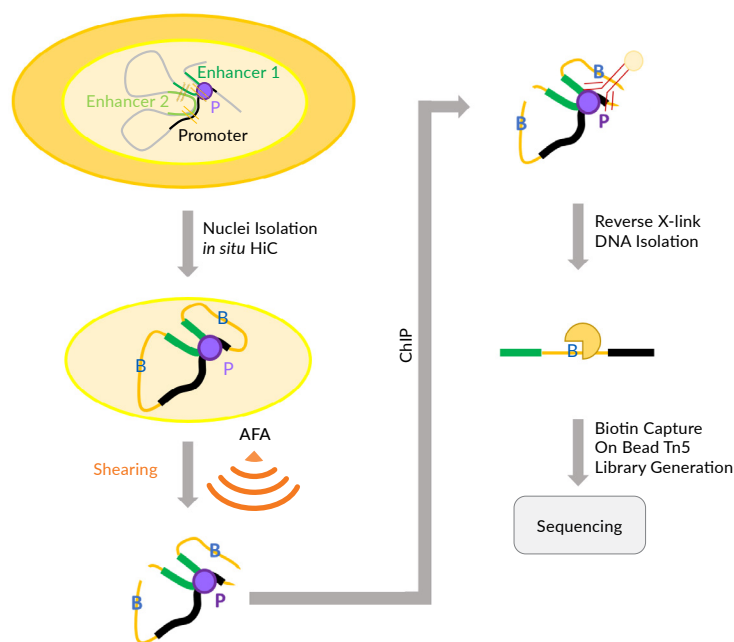
- Three-dimensional chromatin organization regulates gene expression ¹
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ²
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ³
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations ^{4,5,6}
- HiChIP provides a powerful tool to uncover proteins involved in 3D chromatin organization especially suited for low input samples ⁷

Challenges

- Reproducible chromatin shearing with a tight size distribution is required to efficiently capture all binding sites in the vicinity of a biotin contact
- Generation of comprehensive 3D interaction maps involving the protein of interest depends on efficient enrichment of low as well as high affinity sites and therefore requires good epitope preservation during chromatin shearing

Workflow

Fixed Cells



Schematic representation of Hi-ChIP workflow ⁸ Nuclei are isolated from fixed cells and HiC contacts are generated *in situ*. Nuclei are isolated and chromatin is sheared followed by ChIP for the protein of interest. The precipitated DNA is reverse crosslinked and purified to capture HiC contacts using biotin bead pull-down. Tn5 library preparation is performed on beads and samples are subjected to sequencing.

Advantages of Adaptive Focused Acoustics® (AFA®)

[AFA technology](#) is a very gentle, reproducible, and tuneable shearing method.

- Good epitope preservation allows for comprehensive enrichment of sequences bound by the protein of interest
- The tight size distribution reduces the pull-down of non-specific interactions
- Reproducible shearing allows reliable comparison of samples from different origins such as cancer subtypes or different stages of progressive diseases
- Reproducibility and efficiency of shearing allows for small input amounts

Suggested Covaris Products

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

Citations

- [Mumbach et al. HiChIP: Efficient and sensitive analysis of protein-directed genome architecture. Nat Methods. \(2016\)](#)
- [Rowley et al. Evolutionarily Conserved Principles Predict 3D Chromatin Organization. Mol Cell. \(2017\)](#)
- [Pelikan et al. Enhancer histone-QTLs are enriched on autoimmune risk haplotypes and influence gene expression within chromatin networks. Nat Nature Communications. \(2018\)](#)