

Chromatin Interaction Analysis by Paired-End Tag Sequencing - ChIA-PET

Scientific Relevance

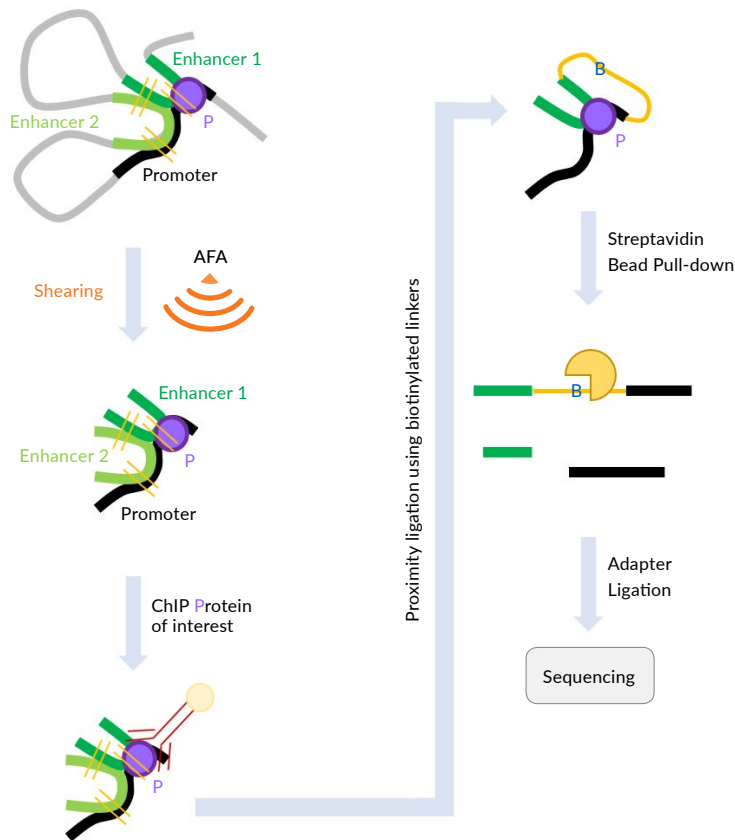
- Three-dimensional chromatin organization regulates gene expression ¹
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ²
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ³
-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

Challenges

- Reproducible chromatin shearing with a tight size distribution is required to minimize the capture of unspecific interactions
- 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

Formaldehyde Fixed Chromatin



Schematic representation of ChIA-PET workflow adapted from [Davies et al.](#)

Crosslinked chromatin is sheared using AFA and binding sites of the protein of interest are enriched using ChIP. After proximity ligation using biotinylated linkers the ligation products are pulled-down using streptavidin beads and 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

Suggested Covaris Products

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

Citations

- [Kwon et al. Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. Cell. \(2014\)](#)
- [Zhang et al. ChIA-PET analysis of transcriptional chromatin interactions. Methods. \(2012\)](#)