

Chromatin Isolation by RNA Purification - ChIRP

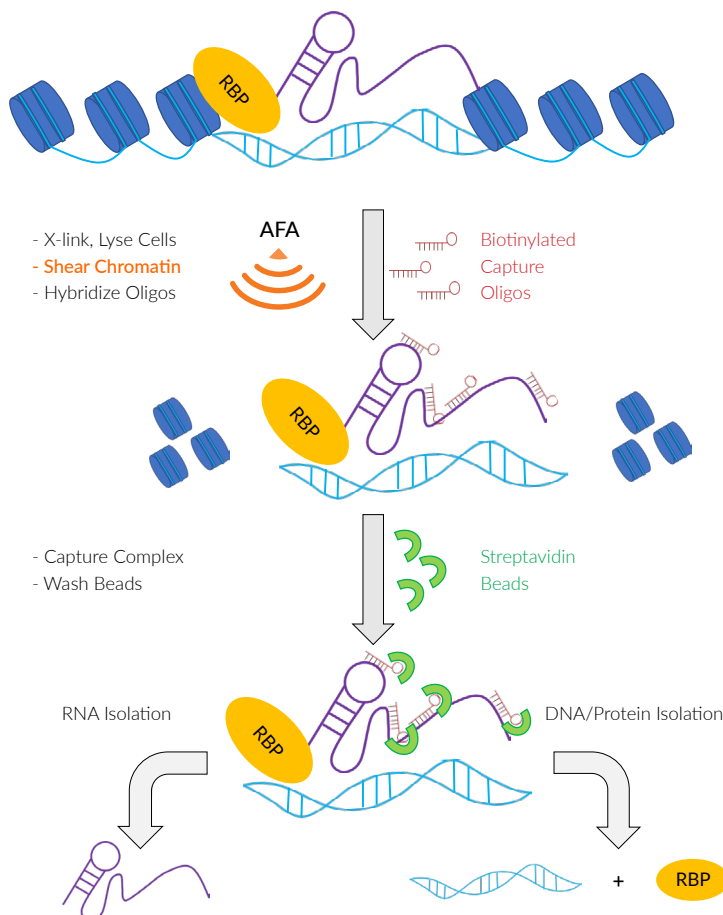
Scientific Relevance

- A huge repertoire of non-coding transcripts is highly essential for proper gene regulation involved in many biological processes ¹
- Long non-coding RNAs (lncRNAs) regulate chromatin states ^{2,3,4} and serve as landing platforms for chromatin modifying complexes ⁵
- ChIRP provides a powerful tool to map lncRNA binding sites on chromatin and can be combined with sequencing methods for a genome-wide resolution

Challenges

- lncRNAs display a focal, interspersed, and gene-selective binding making the identification of binding sites challenging and therefore requires optimal fixation and shearing parameters
- Gentle shearing conditions are required to restore complete complexes that use lncRNAs as landing platforms on chromatin
- Proper shearing of chromatin is required to identify genome-wide high resolution binding patterns for lncRNAs as well as binding motifs

Workflow



Schematic representation of ChIRP workflow adapted from [Chu et al.](#)

Chromatin is crosslinked using the thermo-stable crosslinker glutaraldehyde to preserve *in vivo* interactions with target lncRNAs and sheared applying Covaris AFA technology. Biotinylated tiling probes capturing the lncRNA of interest are added and chromatin complexes associated to the target lncRNA are pulled down using streptavidin beads. From the eluted fraction RNA as well as bound DNA and protein can be purified.

Advantages of Adaptive Focused Acoustics® (AFA®)

[AFA technology](#) is a very gentle and tunable shearing method.

- Glutaraldehyde fixation time can be reduced allowing for shorter shearing times with better epitope preservation
- Big complexes bound to chromatin are more likely to be restored with all subunits
- lncRNAs with very few distinct binding sites are detected better because of good epitope preservations

Suggested Covaris Products

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

Citations

- [Quinn et al. Rapid evolutionary turnover underlies conserved lncRNA genome interactions. Genes Dev. 30, 191–207 \(2015\).](#)