Formaldehyde-Assisted Isolation of Regulatory Elements - FAIRE

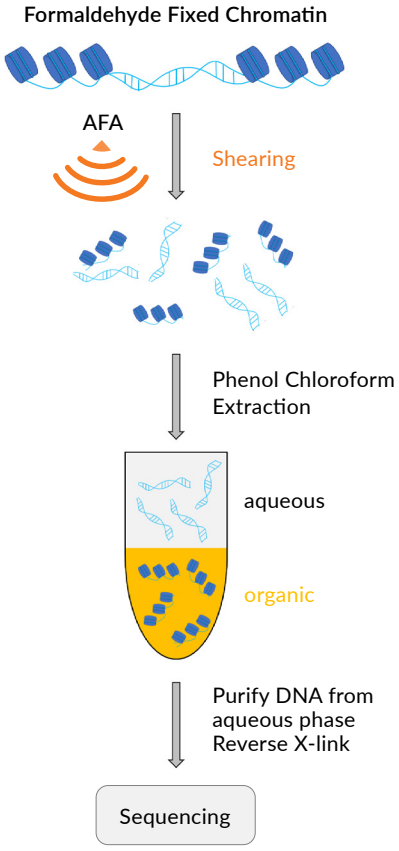
Scientific Relevance

- Nucleosome positioning determines availability of TF binding sites and has significant regulatory functions affecting transcription, DNA repair, replication, and recombination (1)
- Changes in chromatin accessibility accompany biological processes such as cell differentiation (2, 3), environmental signalling (4), and disease development (5, 6)
- FAIRE provides a streamlined method for isolation and identification of functional regulatory elements (7, 8)

Challenges

- Reproducible chromatin shearing with a tight size distribution, is key to allowing efficient isolation of regulatory regions embedded in open chromatin
- Insufficient chromatin shearing causes high signal-to-noise-ratios and inefficient capture of regulatory elements

Workflow



Schematic representation of FAIRE workflow (7, 8). Crosslinked chromatin is sheared using unbiased and reproducible AFA and subjected to phenol-chloroform extraction. Here DNA that is heavily bound by proteins such as heterochromatic regions migrate to the organic phase while protein-free DNA fragments such as open chromatin embedded regulatory elements reside in the aqueous phase. DNA is purified from the aqueous phase, the cross-link is reversed and the material is sequenced.

Advantages of Adaptive Focused Acoustics® (AFA®)

AFA technology is a very gentle, reproducible, and tuneable shearing method.

- Tight size distribution ensures comprehensive representation of regulatory regions
- Random shearing guarantees an unbiased fragmentation and sufficient capture of regulatory elements
- 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

- Rodriguez-Gil et al., The CCR4-NOT complex contributes to repression of Major Histocompatibility Complex class II transcription. Scientific Reports, (2017)
- Rodriguez-Gil et al., Formaldehyde-assisted Isolation of Regulatory Elements to Measure Chromatin Accessibility in Mammalian Cells. J Vis Exp., (2018)

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