

Chromosome Conformation Capture Using Biotinylated Oligos - Capture-C

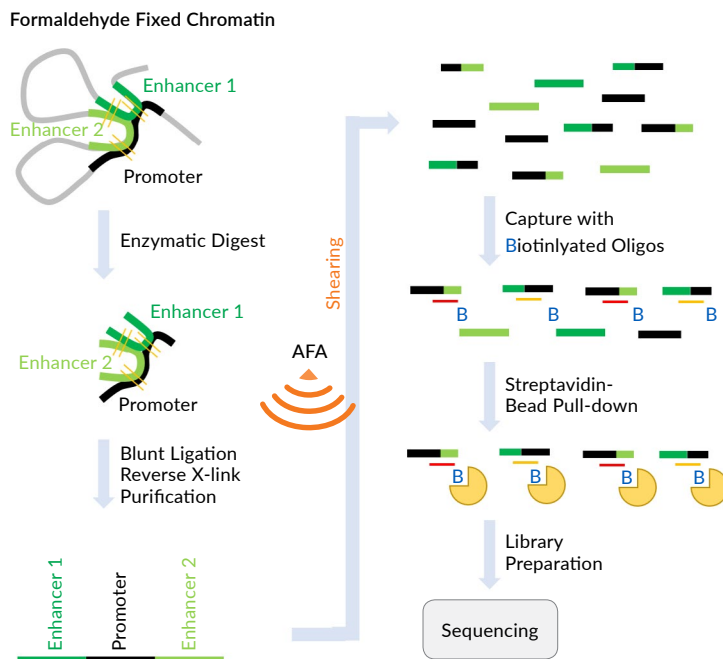
Scientific Relevance

- Three-dimensional chromatin organization regulates gene expression¹
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms²
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes³
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations^{4,5,6}
- Capture-C⁷ and its refinement NG Capture-C⁸ provides a powerful tool to study long-range interactions with high sensitivity allowing for relatively low input amounts

Challenges

- Protocol requires several replicates to retrieve reliable 3D-conformation data, good sets of controls and optimizations are essential
- Unbiased, reproducible shearing with a tight DNA fragment size distributions is required to capture all chromosomal interactions of interest especially in low input derivatives of the method^{8,9}

Workflow



Schematic representation of Capture-C workflow: Crosslinked chromatin is digested and ligated. Unbiased and reproducible shearing by AFA allows for efficient capture with Biotinylated Oligos which are enriched by Streptavidin-Bead pull-down and subjected to sequencing.

Advantages of Adaptive Focused Acoustics® (AFA®)

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

- Random shearing guarantees an unbiased fragmentation of ligation products which reduces PCR biases and cost per sample
- The tight size distribution ensures comprehensive representation of all ligation junctions in the sequencing library
- 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

- [Hughes et al. Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. Nature Genetics, \(2014\)](#)
- [Davies et al. Multiplexed analysis of chromosome conformation at vastly improved sensitivity. Nature Methods, \(2016\)](#)
- [Oudelaar et al. Robust detection of chromosomal interactions from small numbers of cells using low-input Capture-C. NAR, \(2017\)](#)