

# Chromatin Preparation and Shearing

*A Presentation of Chromatin Applications Enabled by AFA-energetics®*

Covaris®

## Table of Contents

Mapping the Epigenome .....	2
How does Covaris Technology Support Precise Mapping of the Epigenome? .....	2
Which Covaris Instrument Suits Your Sample Requirements Best?.....	3
Chromatin Immunoprecipitation (ChIP).....	3
The Covaris Solution for ChIP from Fresh Frozen Tissue.....	6
The Covaris Solution for ChIP from Formalin-fixed Paraffin-embedded (FFPE) Tissue .....	8
Covaris Chromatin Solutions for Non-Mammalian Input Materials.....	8
NEXSON- Nuclei Extraction by SONication.....	10
RELACS (Restriction Enzyme-based LAbeling of Chromatin in Situ).....	11
Epigenomics Applications Beyond ChIP .....	12
High-resolution Chromosome Conformation Capture: Hi-C.....	13
Chromosome Conformation Capture Using Biotinylated Oligos – Capture-C.....	15
HiC Chromatin Immunoprecipitation – Hi-ChIP .....	17
Chromatin Interaction Analysis by Paired-End Tag Sequencing – ChIA-PET.....	19
Chromatin Isolation by RNA Purification – ChIRP .....	20
Formaldehyde-Assisted Isolation of Regulatory Elements – FAIRE.....	22
Affinity-based Enrichment Analyses of DNA Methylation MChIP, MeDIP, hMeDIP .....	24

## Mapping the Epigenome

Deciphering the epigenetic landscape holds huge potential to a better understanding of phenotypic alterations during development and disease progression. Therefore, researchers widely employ precise mapping of posttranslational histone modifications, transcription factors, epigenetic regulators, ncRNAs and DNA methylation together with 3D structure and chromatin accessibility. Jointly these datasets have improved our understanding of epigenetic gene regulation during various processes and revealed common marks of regulatory regions in the genome.

Furthermore, the dynamic nature of epigenetic modifications provides a valuable tool for the cell to integrate environmental cues and respond to them by establishing adapted gene expression programs. However, misregulation of epigenetic gene regulation has been extensively linked to a wide-range of diseases including cancer. Mapping the epigenome can shed light into mechanisms underlying diseases and their progression, identify biomarkers for certain disease stages and provide druggable targets for epigenetic therapy to restore “normal” expression levels of the regulated genes.

## How does Covaris Technology Support Precise Mapping of the Epigenome?

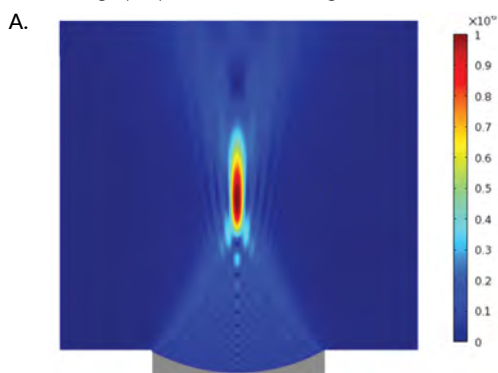
Standardized, reproducible and gentle sample preparation is the key for reliably uncovering the underlying regulatory mechanisms of gene expression on a genome-wide scale. Therefore, it is essential to minimize experimental variability between different samples, researchers and laboratories. One key challenge is reproducible isolation of nuclei from fixed material followed by complete chromatin shearing with a tight size distribution.

Covaris [Adaptive Focused Acoustics® \(AFA®\)](#) is a unique technology that delivers focused acoustic energy to precious samples (**Figure 1A**). This method of energy delivery allows the generated shear force to be precisely focused into the sample vessel meaning that every processed sample will receive the exact same energy which minimizes experimental variations. Established shearing conditions for one sample type can be used over and over with variable cell numbers, i.e. 5 to 30M cell and 100K to 3M cells can be processed with the same settings, reducing experimental biases especially from primary cells, minimizing optimization efforts.

Furthermore, this focused energy delivery dramatically minimizes the required input energy which in turn enables isothermal sample processing (**Figure 1B**). Avoiding heat generation during chromatin preparation and shearing ensures optimal epitope preservation and avoids reversal of cross-links during the shearing procedure due to heating of the sample. This maintenance of sample integrity during chromatin shearing is especially important when working with scarce sample inputs or when mapping low abundant chromatin interactors with potentially short ON-time on chromatin and also increases the signal-to-noise ratio in genome wide datasets.

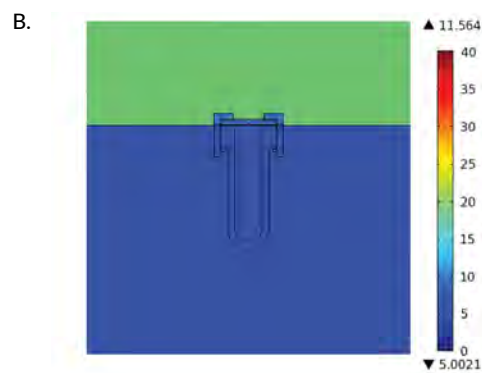
### Pressure Profile

- Focused transducer
- Non-contact processing
- Highly reproducible shearing



### Heat Profile

- Isothermal processing
- Optimal epitope and crosslink preservation






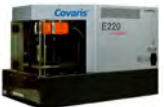


**Figure 1.** Covaris AFA allows focused energy supply which results in very reproducible chromatin shearing under isothermal processing conditions leading to optimal epitope and cross-link preservation.

## Which Covaris Instrument Suits Your Sample Requirements Best?

Covaris Focused-ultrasonicator instruments are available with different power transducers and different sample throughput options. The low power instruments, the M220 (single sample) and the ME220 (8 samples) are suitable to perform ChIP applications from mammalian cells and cultured cells from other origins such as drosophila S2 cells. These instruments are not suited for working with complex organisms as input material. In addition, more complex crosslinking procedures involving DSG, ESG or glutaraldehyde would also favor a high-power instrument to enable proper nuclei isolation from heavily fixed samples.

The Covaris high power Focused-ultrasonicator instruments from the S- and E-series are highly flexible in regards to the input sample types including organoids, tissues, plants, nematodes, flies, yeast and many more. Furthermore, the high-power instruments allow to process up to 30M cells in one shearing reaction very reproducibly while the low power instruments are limited to 15M cells/ reaction. While the S220 is a single sample instrument, the E220*evolution* allows processing of up to 8 samples and the E220 up to 96 samples. However, all three instruments have the same power meaning that settings which have been established on the S220 can be transferred to the E-series and vice versa making a serial upgrading as sample throughput increases very easy. The LE220-plus is a higher throughput instrument processing up to 96 samples, with simultaneous processing of 8 samples at the time, for faster processing. The instrument operates at an intermediate power level per sample and is therefore suited for similar applications like the M-series.

### Covaris Focused-ultrasonicators and Sample Types:

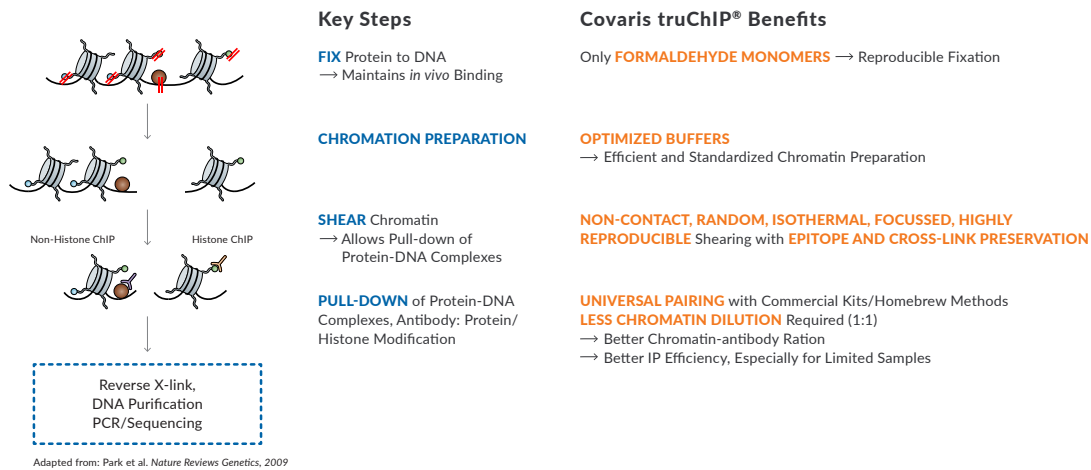
M220	S220	ME220	E220 <i>evolution</i>	E220	LE220-plus
					
<ul style="list-style-type: none"> <li>• Circular transducer</li> <li>• Single sample processing</li> <li>• Up to 75W of power</li> </ul>	<ul style="list-style-type: none"> <li>• Circular transducer</li> <li>• Single sample processing</li> <li>• Up to 500W of power</li> </ul>	<ul style="list-style-type: none"> <li>• Disk-shaped transducer</li> <li>• Processes 1 to 8 samples per run</li> <li>• Up to 75W of power</li> </ul>	<ul style="list-style-type: none"> <li>• Circular transducer</li> <li>• Processes 1 to 8 samples per run</li> <li>• Up to 500W of power</li> <li>• Can be upgraded to E220 or LE220-plus</li> </ul>	<ul style="list-style-type: none"> <li>• Circular transducer</li> <li>• Processes 96 samples per run</li> <li>• Up to 500W of power</li> <li>• Can be upgraded to LE220-plus</li> </ul>	<ul style="list-style-type: none"> <li>• Linear transducer</li> <li>• Processes 96 samples per run (8x faster)</li> <li>• Up to 500W of power</li> </ul>
<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Yeast</li> <li>• Plant</li> <li>• C. Elegans</li> <li>• Drosophila - Tissue/Embryos</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Yeast</li> <li>• Plant</li> <li>• C. Elegans</li> <li>• Drosophila - Tissue/Embryos</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Yeast</li> <li>• Plant</li> <li>• C. Elegans</li> <li>• Drosophila - Tissue/Embryos</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Mammalian Cells</li> <li>• Drosophila - Cultured Insect Cells</li> </ul>

### Chromatin Immunoprecipitation (ChIP):

Epigenetic mapping holds great promise for the identification of biomarkers and targets for epigenetic therapy. However, existing ChIP workflows are largely labor-intensive, require time-consuming optimizations for the cell or tissue type of interest, and therefore require fine-tuned and reproducible sample preparation.

#### Covaris truChIP® kit for standardized ChIP sample preparation

Covaris technology enables the standardization of chromatin preparation and shearing from virtually any sample input with consistent results. AFA technology in combination with optimized reagents provides researchers an easy, fast, reproducible, and scalable workflow to retrieve high-quality sheared chromatin to analyze the epigenetic make-up of their sample on genome-wide scale. Chromatin preparation and shearing conditions can be optimized within less than a week and will remain the same for the respective sample type from experiment to experiment with the [truChIP Kit](#).



**Figure 2.** Schematic representation of key steps in a ChIP workflow highlighting the Covaris truChIP benefits.

### Fixation:

To reliably map the epigenome via ChIP assays, first, the *in vivo* situation is maintained by formaldehyde fixation. The chemical integrity of the fixing agent is key for reliable and reproducible ChIP assays. Since formaldehyde oxidizes when exposed to oxygen and forms oligomers and polymers with altered crosslinking capacity, we provide single use formaldehyde in our truChIP kit to ensure reproducible fixation efficiency between samples and experiments.

### Chromatin Preparation:

For reproducible nuclei isolation and chromatin preparation we provide a multi buffer sample preparation solution. Our final shearing buffer is very mild and contains only 0.1% SDS thereby contributing to an optimal sample integrity during chromatin shearing. Furthermore, our shearing buffer is optimized to work efficiently with our Focused-ultrasonicators allowing for the most efficient energy transfer during the shearing process which minimizes shearing times.

### Chromatin Shearing:

For reproducible and efficient pull-down during the IP step, a complete shearing of chromatin with a tight size distribution is key as fragments of different length precipitate with different efficiencies and non-sheared chromatin fractions would result in an incomplete assessment of the sample. The high reproducibility of chromatin shearing with Covaris Focused-ultrasonicators is key for reliably mapping the epigenome and comparing different sample types. The highly efficient chromatin preparation and shearing allows for lower sample input which is especially important when working with scarce clinical samples because minimal material is lost during the chromatin preparation due to inefficient nuclei isolation or chromatin shearing. In addition, the isothermal processing using Covaris technology leads to unique epitope and cross-link preservation especially important when working with scarce samples or less abundant chromatin binders. To this end, fixation times can often be reduced which avoids adverse effects of epitope masking.

### IP Step:

Chromatin prepared and sheared with the Covaris truChIP kit can be universally subjected to any IP workflow including commercial and homebrew methods. Due to the composition of our shearing buffer, one only needs to dilute the chromatin 1:1 with Covaris dilution buffer to adjust to a physiological salt concentration and sequester the remaining SDS. The low dilution effect ensures an optimal antibody chromatin ratio during the IP step.

In addition to classic ChIP protocols from mammalian cells, Covaris provides optimized protocols for specific input requirements such as:

- [Ultra-low Input Kit](#), optimized for very scarce input samples to perform efficient chromatin shearing of 100,000 cells or less.
- [Native ChIP Kit](#), optimized for non X-link ChIP and can be used as an alternative to difficult to control enzymatic micrococcal nuclease (MNase) digestion of native chromatin (non-crosslinked) in preparation for ChIP-based applications.

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## The Covaris solution for ChIP from fresh frozen tissue

The Tissue ChIP kit was developed to allow efficient ChIP from tissue samples and provides the same overall advantages as described above for the truChIP kit. The buffers are optimized for efficient nuclei extraction from tissue samples which is why the chromatin shearing buffer contains 0.25% SDS and requires a higher dilution (1:2) before the IP step.

For successful ChIP from tissue samples, the tissue sample must be efficiently disintegrated beforehand to make sure that the inner and outer parts of the sample are accessible for downstream processing with the same level of efficiency. The Covaris cryoPREP system allows contact-free, isothermal and reproducible dry pulverization of the tissue sample and is the ideal preparation method to reliably and reproducibly extract biomolecules from tissue samples. The exact mode of action can be watched in [this video](#).

To summarize, the tissue sample is placed into a Covaris tissueTUBE which can stand very harsh and cold processing due to the Kapton® material. After flash freezing the sample in liquid nitrogen, the researcher can choose between 6 different impacting settings on the cryoPREP instrument depending on the composition of the tissue and cryofracture the specimen. The resulting fine tissue powder is simply transferred into a processing tube and nuclei are isolated using the [truChIP tissue kit](#). The prepared chromatin is sheared on a Covaris ultrasonicator and subjected to IP. Every tissue sample from the same source can then be disintegrated with the same efficiency in the future by applying the same settings, enabling high reproducibility from a heterogenous input sample like tissue.

### cryoPREP Tissue Pulverization for ChIP from Tissue



**Figure 3.** Processing of tissue samples with the cryoPREP dry pulverizer enables fast and easy processing without sample heating or cross contamination. After cryofracturing, standardized chromatin preparation is performed using Covaris truChIP tissue kit followed by Chromatin shearing on a Covaris Focused-ultrasonicator such as the S220.

Tissue pulverization prior to ChIP assays enables optimal chromatin extraction from the entire tissue sample as it allows for efficient nuclei extraction from the whole organ and not just subparts. Scientists have adapted the same workflow for hard to lyse cuticle organisms such as nematodes (6). This improved sample preparation has also been adopted for other workflows beyond ChIP which require nuclei isolation from non-fixed fresh frozen tissue such as ATACseq ([K. Ramachandran, 2019](#)).

Additionally, homogenous pulverization of the entire organ sets ideal standards for multiOMIC applications where the tissue powder can be partitioned for several downstream assays including epigenomics, transcriptomics and proteomics. The sub-samples can be dissolved in the optimal buffer for the respective downstream assay (1).


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## The Covaris Solution for ChIP from Formalin-fixed Paraffin-embedded (FFPE) Tissue

Deciphering alterations that occur during the onset and progression of diseases is critical to characterizing human malignancies to help predict which therapeutic interventions are most likely to improve clinical outcomes. To leverage information available from collections of malignant tissue as well as respective healthy controls existing in tissue banks, we provide a solution to perform ChIP from FFPE samples.

The Covaris [FFPE ChIP kit](#) enables ChIP from FFPE samples and was developed in collaboration with a research group in France, see [Application Note](#). The Covaris FFPE ChIP workflow works reliably for histone marks and CTCF from FFPE material and can be adapted for other marks and factors with our [application support](#). The truChIP FFPE Chromatin Shearing Kit utilizes an AFA-based active paraffin emulsification process prior to chromatin shearing. This AFA-based emulsification of chromatin is contact-free and does not require any toxic, organic solvent such as Xylene and therefore no time-consuming re-hydration steps. Furthermore, the improved solubilization and rehydration makes epitopes better accessible for subsequent IP.

Before AFA	After AFA	AFA-based Deparaffinization
• FFPE section in buffer	• Deparaffinized sample • Emulsified paraffin • Rehydrated sample	• Better deparaffinization • Hands-free • Aqueous buffer, no organic solvents • Compatible with all FFPE inputs
		AFA-based Tissue Rehydration
		• Better tissue digestion by Proteinase K • Improved extraction of DNA/RNA

20 µm section in microTUBE-130

Such active deparaffinization and rehydration via Covaris technology works equally well for other biomolecules such as [DNA and RNA from the same input material or proteins](#).



## Covaris Chromatin Solutions for Non-Mammalian Input Materials

Chromatin shearing with Covaris devices is the method of choice for plethora of researchers when working with model organisms beside mammals. AFA enables reproducible shearing also from difficult to lyse input material with good epitope preservation. While chromatin shearing from mammalian samples can be optimized within less than one week with any Covaris device we suggest our high power instruments for more complex organisms such as yeast, nematodes, plants and *Drosophila*.

In collaboration with German researchers we have been able to optimize an efficient workflow for chromatin shearing from a limited amount of *Drosophila* embryos which is described in more detail in this [Application Note](#). Furthermore, please refer to the following scientific publications for efficient ChIP from *Drosophila* samples as well as other organisms and reach out to the [Covaris Application team](#) for support to optimize sample preparation for the input material of choice.

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#### ChIP – *C.elegans*

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## NEXSON- Nuclei EXtraction by SONication

NEXSON provides a simple but highly reproducible technique for efficient nuclei isolation, ensuring the generation of comparable chromatin maps from different sample types. Especially for hard to lyse sample types such as PBMCs, several primary cells as well as very mesenchymal cells supporting chemical lysis with mechanical lysis using Covaris AFA improves sample preparation for epigenomic applications such as ChIP and HiC. Settings can be found in our [truChIP manual](#) ([Appendix C, page 15](#)).

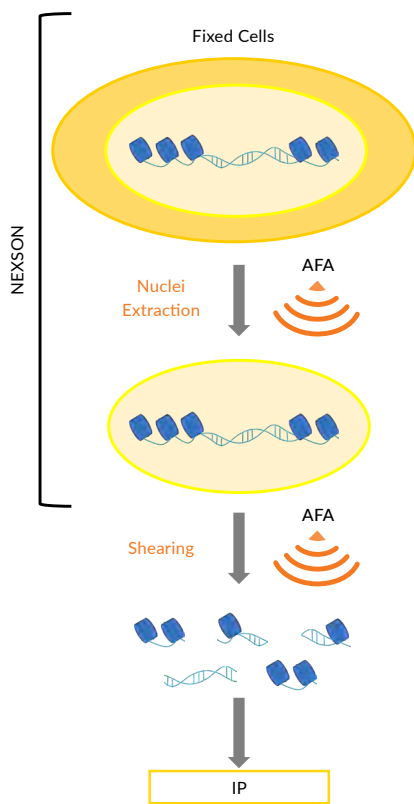
### Scientific Relevance

- ChIP-Seq is a powerful tool to map DNA binding proteins as well as histone modifications across the genome ([A. Barki, 2007](#), [D. Johnson, 2007](#))
- To allow the comparison of different studies and different input materials standardization of the sample preparation steps are required, which has been challenging ([L. Arrigoni, 2015](#), [T. Furey, 2012](#))
- NEXSON provides a simple but highly reproducible technique for efficient nuclei isolation, ensuring the generation of comparable chromatin maps from different sample types ([L. Arrigoni, 2015](#))

### Challenges

- Huge variations in sample preparation for ChIP make data comparison challenging
- In particular, the chromatin shearing step is tedious to optimize and standardize for different input materials and across different laboratories
- Insufficient chromatin shearing compromises the quality of chromatin and sequencing results
- Shearing optimization is labor-intensive, material-consuming, expensive, and especially difficult when working with precious samples such as clinical specimens

### Workflow



**Schematic representation of NEXSON workflow:** Nuclei from fixed cells are efficiently isolated applying tuneable AFA. Pure fractions of isolated nuclei are subjected to ChIP workflow. In brief, chromatin is sheared using AFA and subjected to IP for histone modifications or transcription factors of interest.

### Advantages of Adaptive Focused Acoustics (AFA)

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

- The high efficiency of nuclei isolation allows the scale down of input material to 10,000 cells/histone ChIP and 100,000 cells/transcription factor ChIP
- Increased robustness permits comparison across different tissues, cell types, disease stages, and laboratories
- The highly reproducible and efficient isolation of nuclei allows for novel high-throughput derivatives of ChIP technology

## References

### NEXSON for ChIP:

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3. Castex J, Willmann D, Kanouni T, et al. Inactivation of Lsd1 triggers senescence in trophoblast stem cells by induction of Sirt4. *Cell Death Dis*. 2017;8(2):e2631. DOI: [10.1038/cddis.2017.48](https://doi.org/10.1038/cddis.2017.48)
4. Borisova ME, Voigt A, Tollenaere MAX, et al. p38-MK2 signaling axis regulates RNA metabolism after UV-light-induced DNA damage. *Nat Commun*. 2018;9(1):1017. DOI: [10.1038/s41467-018-03417-3](https://doi.org/10.1038/s41467-018-03417-3)
5. Durek P, Nordström K, Gasparoni G, et al. Epigenomic Profiling of Human CD4 T Cells Supports a Linear Differentiation Model and Highlights Molecular Regulators of Memory Development. *Immunity*. 2016;45(5):1148-1161. DOI: [10.1016/j.immuni.2016.10.022](https://doi.org/10.1016/j.immuni.2016.10.022)

### NEXSON for Hi-C:

1. Ramírez F, Bhardwaj V, Arrigoni L, et al. High-resolution TADs reveal DNA sequences underlying genome organization in flies. *Nat Commun*. 2018;9(1):189. DOI: [10.1038/s41467-017-02525-w](https://doi.org/10.1038/s41467-017-02525-w)
2. Salhab A, Nordström K, Gasparoni G, et al. A comprehensive analysis of 195 DNA methylomes reveals shared and cell-specific features of partially methylated domains. *Genome Biol*. 2018;19(1):150. DOI: [10.1186/s13059-018-1510-5](https://doi.org/10.1186/s13059-018-1510-5)
3. Renschler G, Richard G, Valsecchi CLK, et al. Hi-C guided assemblies reveal conserved regulatory topologies on X and autosomes despite extensive genome shuffling. *Genes Dev*. 2019;33(21-22):1591-1612. DOI: [10.1101/gad.328971.119](https://doi.org/10.1101/gad.328971.119)

In collaboration with a scientist at Bayer we could further optimize this workflow for cell types such as primary hepatocytes and adipocytes which store a lot of lipids that can interfere with AFA. This highly reproducible workflow is outlined in this [Application Note](#).

## RELACS (Restriction Enzyme-based LABELing of Chromatin in Situ)

The team from the MPI in Freiburg has developed a high-throughput version of ChIP based on the NEXSON protocol called RELACS (restriction enzyme-based labeling of chromatin in situ). This method efficiently extracts nuclei from fixed material using AFA during the NEXSON step. Isolated nuclei are swelled and chromatin is digested *in-situ* using restriction enzymes and barcoded by hairpin adapter ligation. Then, nuclei of different samples (harboring different barcodes) are pooled and nuclei are lysed mechanically by applying AFA. The released pooled chromatin can now be subjected to a single pull-down reaction which offers the advantage that any experimental bias during the IP step is the same for every initial sample and furthermore the combined IP step saves a lot of reagents and hands-on time.

Using this method, the authors have been able to produce robust and reproducible ChIP profiles from 100 cells for histone marks and 1000 cells for transcription factors. This method is not limited to mammalian cells but works equally well for mouse tissues.

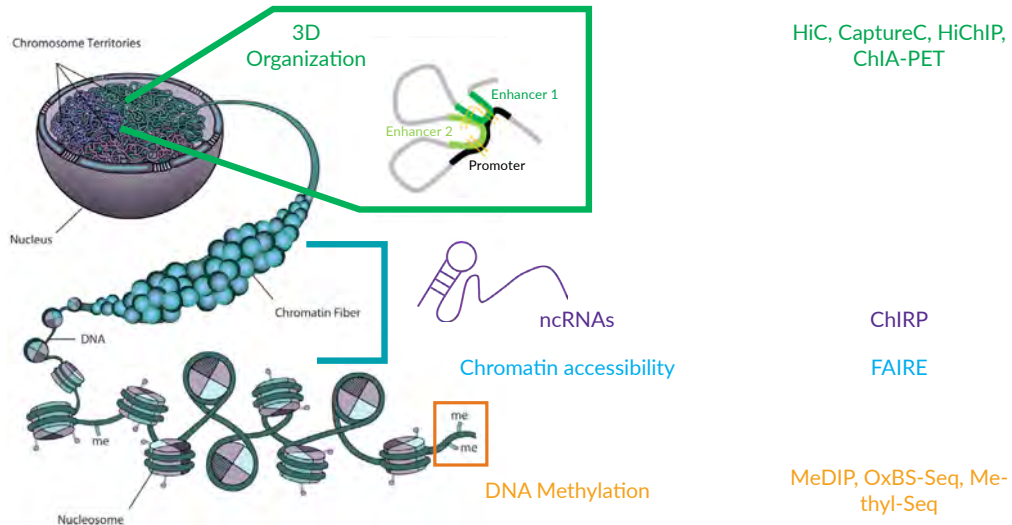
One thing to keep in mind is that the chromatin fragmentation is enzymatic which might limit the application to a certain subset of scientific questions as certain parts of the genome might be over or underrepresented in comparison to mechanical fragmentation. However, for the marks investigated, the overall analysis compares well to published datasets.

**Citation:** Arrigoni, L., Al-Hasani, H., Ramírez, F. et al. RELACS nuclei barcoding enables high-throughput ChIP-seq. *Commun Biol* 1, 214 (2018). DOI: [10.1038/s42003-018-0219-z](https://doi.org/10.1038/s42003-018-0219-z)

## Epigenomic Applications Beyond ChIP

There are additional layers in epigenetic gene regulation which complement mapping of histone modifications and transcription factor landscape. Among these are 3D-organization, chromatin accessibility, DNA methylation and ncRNA binding to chromatin.

These regulatory layers are precisely mapped by scientists through highly reproducible and standardized sample preparation powered by Covaris AFA. Understanding the impact of 3D organization as well as protein complexes involved in this regulatory network enhances our understanding of gene modulation in various contexts.



## High-resolution Chromosome Conformation Capture: Hi-C

### Scientific Relevance

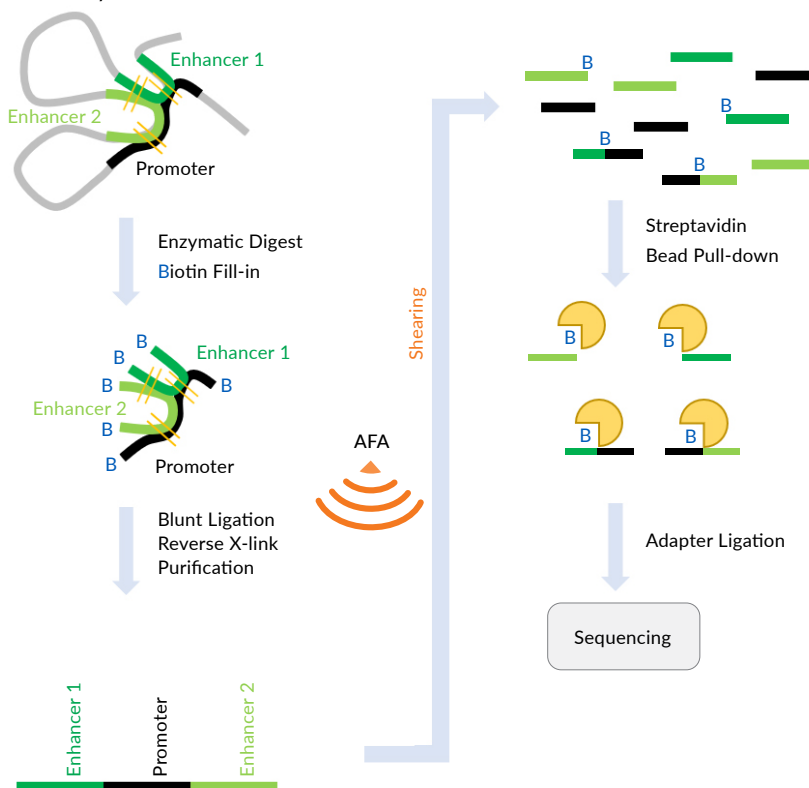
- Three-dimensional chromatin organization regulates gene expression ([G. Li, 2011](#))
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ([R. Jia, 2017](#))
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ([M. Rousseau, 2014](#))
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations ([J. Loven, 2013](#)), ([S. Ember, 2017](#)), ([J. Pulikkan, 2018](#))
- Hi-C provides a powerful tool to better characterize 3D chromatin organization and helps to uncover the impact of cancer risk-associated SNPs ([N. Dryden, 2014](#)), ([M. Du, 2016](#))

### 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 distribution is required to capture all chromosomal interactions especially in low input derivatives of the method

### Workflow

#### Formaldehyde Fixed Chromatin



#### Advantages of Adaptive Focused Acoustics (AFA)

- Random shearing guarantees an unbiased fragmentation of ligation products
- 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

**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.

## References

### Hi-C Publications with Covaris:

- Kaaij LJT, Van der weide RH, Ketting RF, De wit E. Systemic Loss and Gain of Chromatin Architecture throughout Zebrafish Development. Cell Rep. 2018;24(1):1-10.e4. DOI: [10.1016/j.celrep.2018.06.003](https://doi.org/10.1016/j.celrep.2018.06.003)
- Van berkum NL, Lieberman-aiden E, Williams L, et al. Hi-C: a method to study the three-dimensional architecture of genomes. J Vis Exp. 2010;(39) DOI: [10.3791/1869](https://doi.org/10.3791/1869)
- Ramani et al. Mapping three-dimensional genome architecture through in situ DNase Hi-C. Nat Protoc, (2016) DOI: [10.1038/nprot.2016.126](https://doi.org/10.1038/nprot.2016.126)
- Elphege et al. Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell, (2017) DOI: [10.1016/j.cell.2017.05.004](https://doi.org/10.1016/j.cell.2017.05.004).
- Szabo Q, Jost D, Chang JM, et al. TADs are 3D structural units of higher-order chromosome organization in . Sci Adv. 2018;4(2):aar8082. DOI: [10.1126/sciadv.aar8082](https://doi.org/10.1126/sciadv.aar8082)
- Kaaij LJT, Van der weide RH, Ketting RF, De wit E. Systemic Loss and Gain of Chromatin Architecture throughout Zebrafish Development. Cell Rep. 2018;24(1):1-10.e4. DOI: [10.1016/j.celrep.2018.06.003](https://doi.org/10.1016/j.celrep.2018.06.003)

## Chromosome Conformation Capture Using Biotinylated Oligos – Capture-C

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.

### Scientific Relevance

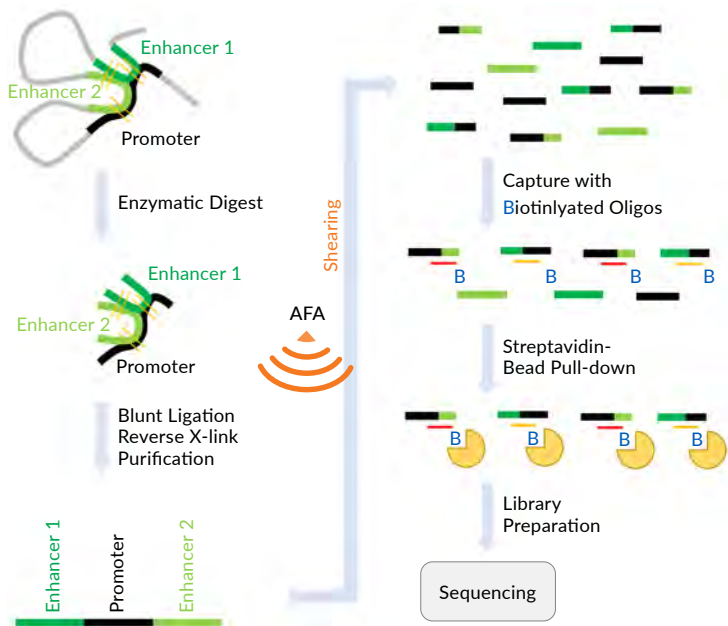
- Three-dimensional chromatin organization regulates gene expression ([G. Li, 2011](#))
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ([R. Jia, 2017](#))
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ([M. Roaousseau, 2014](#))
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations ([J. Loven, 2013](#), [S. Ember, 2020](#), [J. Pulikkan, 2018](#))
- Capture-C ([J. Hughes, 2014](#)) and its refinement NG Capture-C ([J. Davies, 2016](#)) 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 ([J. Davies, 2016](#), [A.M. Oudelaar, 2017](#))

### Workflow

#### Formaldehyde Fixed Chromatin



**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)

- 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.



## References

### Capture-C

- Hughes et al. Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. *Nature Genetics*, 2014. DOI: [10.1038/ng.2871](https://doi.org/10.1038/ng.2871)
- Davies et al. Multiplexed analysis of chromosome conformation at vastly improved sensitivity. *Nature Methods*, 2016. DOI: [10.1038/nmeth.3664](https://doi.org/10.1038/nmeth.3664)
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- Espeso-gil S, Halene T, Bendl J, et al. A chromosomal connectome for psychiatric and metabolic risk variants in adult dopaminergic neurons. *Genome Med*. 2020;12(1):19. DOI: [10.1186/s13073-020-0715-x](https://doi.org/10.1186/s13073-020-0715-x)

## HiC Chromatin Immunoprecipitation – Hi-ChIP

Hi-ChIP provides a powerful tool to uncover proteins involved in 3D chromatin organization especially suited for low input samples.

### Scientific Relevance

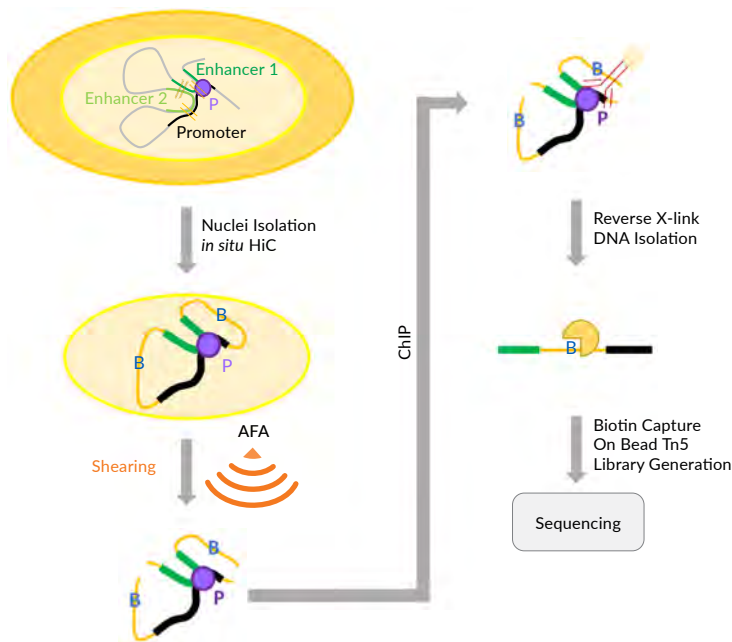
- Three-dimensional chromatin organization regulates gene expression ([G. Li, 2011](#))
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ([R. Jia, 2017](#))
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ([R Jia, 2017](#))
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations ([J. Loven, 2013](#), [S. Ember, 2017](#), [J. Pulikkan, 2018](#))
- HiChIP provides a powerful tool to uncover proteins involved in 3D chromatin organization especially suited for low input samples ([Mumbach, 2016](#))

### 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** <sup>8</sup> 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)

- 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

## References

### Hi-ChIP

- Flavahan WA, Drier Y, Johnstone SE, et al. Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. *Nature*. 2019;575(7781):229-233. DOI: [10.1038/s41586-019-1668-3](https://doi.org/10.1038/s41586-019-1668-3)
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- Mumbach et al. HiChIP: Efficient and sensitive analysis of protein-directed genome architecture. *Nat Methods*. 2016. DOI: [10.1038/nmeth.3999](https://doi.org/10.1038/nmeth.3999)
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## Chromatin Interaction Analysis by Paired-End Tag Sequencing – ChIA-PET

ChIA-PET provides a powerful tool to uncover proteins involved in 3D chromatin organization.

### Scientific Relevance

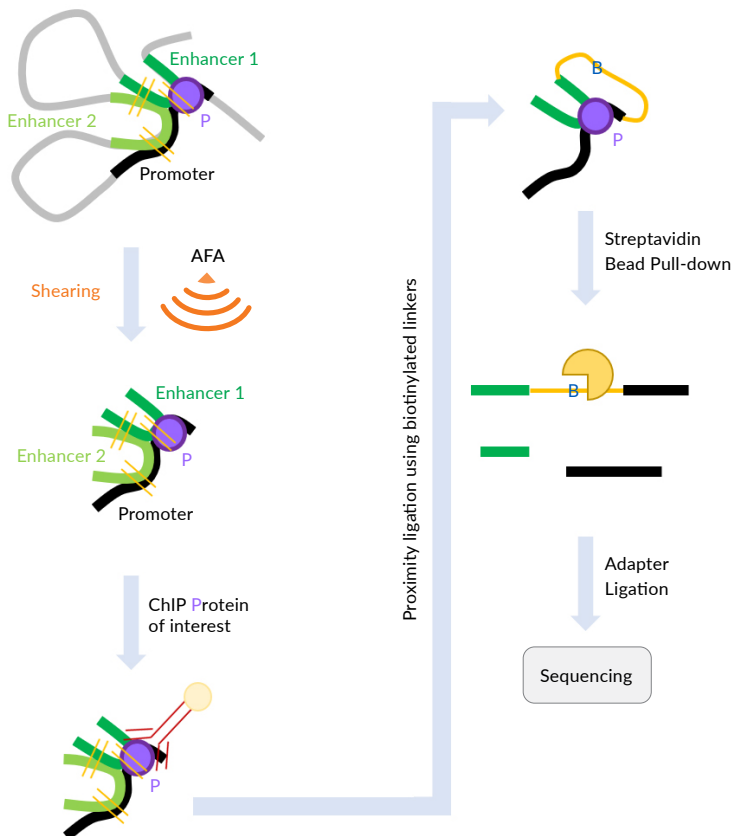
- Three-dimensional chromatin organization regulates gene expression ([G. Li, 2011](#))
- Aberrant chromatin looping causes altered gene regulation in malignancies including solid tumors as well as hematologic neoplasms ([R. Jia, 2017](#))
- Characterization of 3D-chromosomal conformations allows classification of cancer subtypes ([M. Rosseau, 2014](#))
- Cancer progression can be alleviated by inhibiting certain chromatin loop formations ([J. Loven, 2013](#), [S. Ember, 2017](#), [J. Pulikkan, 2018](#))
- ChIA-PET provides a powerful tool to uncover proteins involved in 3D chromatin organization ([M. Fullwood, 2009](#))

### 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)

- 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

### References

#### ChIA-PET

- Kwon et al. Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. *Cell*. 2014. DOI: [10.1016/j.cell.2013.11.039](https://doi.org/10.1016/j.cell.2013.11.039)
- Zhang et al. ChIA-PET analysis of transcriptional chromatin interactions. *Methods*. 2012. DOI: [10.1016/j.ymeth.2012.08.009](https://doi.org/10.1016/j.ymeth.2012.08.009)

## Chromatin Isolation by RNA Purification – ChIRP

ChIRP provides a powerful tool to map lncRNA binding sites on chromatin and can be combined with sequencing methods for a genome-wide resolution.

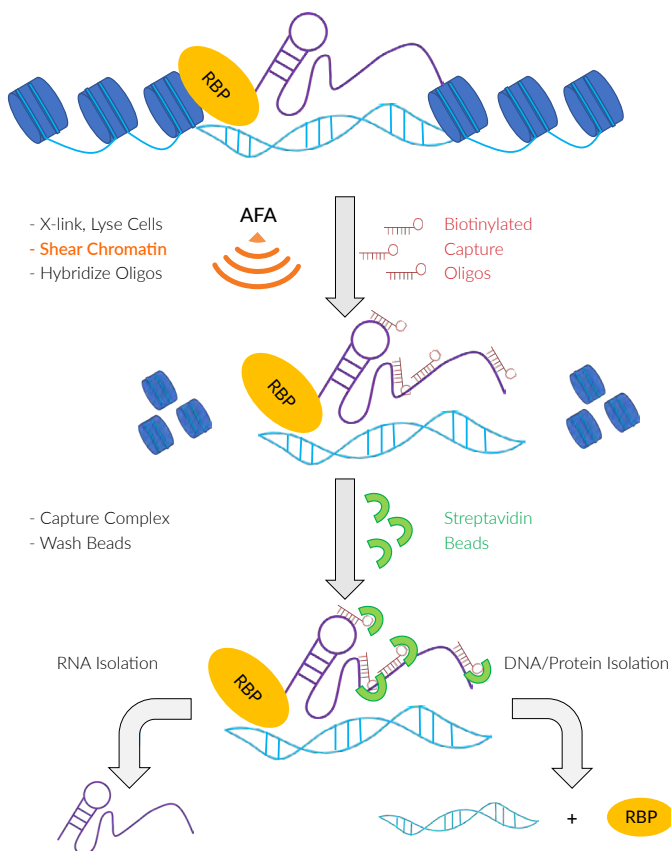
### Scientific Relevance

- A huge repertoire of non-coding transcripts is highly essential for proper gene regulation involved in many biological processes ([D.C. Zappalulla, 2018](#))
- Long non-coding RNAs (lncRNAs) regulate chromatin states ([A. Khalil, 2009](#), [J. Zhao, 2010](#), [C. Davidovich, 2007](#)) and serve as landing platforms for chromatin modifying complexes ([M.C. Tsai, 2010](#))
- 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)

- 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

## References

### ChIRP

- Quinn et al. Rapid evolutionary turnover underlies conserved lncRNA genome interactions. *Genes Dev.* 30, 191–207. 2015. DOI: [10.1101/gad.272187.115](https://doi.org/10.1101/gad.272187.115)
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- Zheng X, Han H, Liu GP, et al. LncRNA wires up Hippo and Hedgehog signaling to reprogramme glucose metabolism. *EMBO J.* 2017;36(22):3325-3335. DOI: [10.15252/embj.201797609](https://doi.org/10.15252/embj.201797609)
- Giakountis A, Moulos P, Zarkou V, et al. A Positive Regulatory Loop between a Wnt-Regulated Non-coding RNA and ASCL2 Controls Intestinal Stem Cell Fate. *Cell Rep.* 2016;15(12):2588-96. DOI: [10.1016/j.celrep.2016.05.038](https://doi.org/10.1016/j.celrep.2016.05.038)

### Hi-ChIRP

- Mumbach MR, Granja JM, Flynn RA, et al. HiChIRP reveals RNA-associated chromosome conformation. *Nat Methods.* 2019;16(6):489-492. DOI: [10.1038/s41592-019-0407-x](https://doi.org/10.1038/s41592-019-0407-x)

## Formaldehyde-Assisted Isolation of Regulatory Elements – FAIRE

FAIRE provides a streamlined method for isolation and identification of functional regulatory elements.

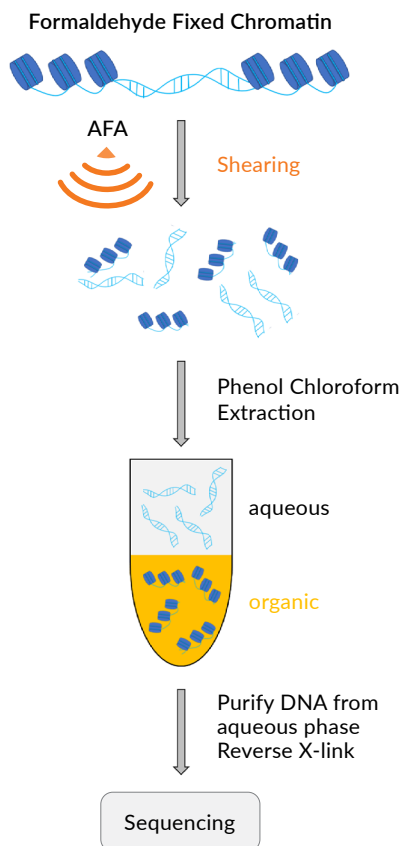
### Scientific Relevance

- Nucleosome positioning determines availability of TF binding sites and has significant regulatory functions affecting transcription, DNA repair, replication, and recombination ([M. Radman-Livajo, 2010](#))
- Changes in chromatin accessibility accompany biological processes such as cell differentiation ([A. Gaspar-Maia, S. Thakurela, 2015](#)), environmental signalling ([A. Badeaux, 2013](#)), and disease development ([D. Hargreaves, 2011](#), [J. Schwartzentruber, 2012](#))
- FAIRE provides a streamlined method for isolation and identification of functional regulatory elements ([P. Giresi, 2007](#), [J. Simon, 2011](#))

### 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



### Advantages of Adaptive Focused Acoustics (AFA)

- 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

**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.

## References

### FAIRE

- Rodriguez-Gil et al., The CCR4-NOT complex contributes to repression of Major Histocompatibility Complex class II transcription. *Scientific Reports*, 2017. DOI: [10.1038/s41598-017-03708-7](https://doi.org/10.1038/s41598-017-03708-7)
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- Chaudhri VK, Dienger-stambaugh K, Wu Z, Shrestha M, Singh H. Charting the cis-regulome of activated B cells by coupling structural and functional genomics. *Nat Immunol*. 2020;21(2):210-220. DOI: [10.1038/s41590-019-0565-0](https://doi.org/10.1038/s41590-019-0565-0)



## Affinity-based Enrichment Analyses of DNA Methylation MCIp, MeDIP, hMeDIP

Precipitation-based methods provide a fast and easy tool to analyze genome-wide DNA methylation profiles and identify differentially methylated regions.

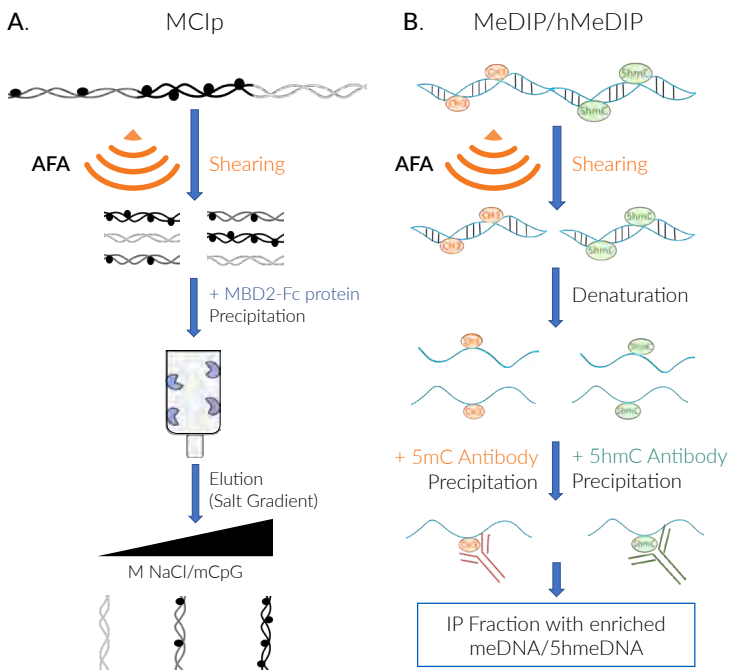
### Scientific Relevance

- Cytosine methylation/hydroxymethylation on DNA represent an important epigenetic regulatory layer that alters chromatin structure ([A. Razin, 1998](#)) and influences transcription factor binding affinities ([H. Zhu, 2016](#), [A. Blattler, 2013](#))
- Such modifications are essential for proper gene expression states during development ([J. Borgel, 2010](#)) and often altered in diseases involving cancer development and metastasis ([A. Portela, 2010](#))
- DNA methylation marks have huge potential as biomarkers ([T. Mikeska, 2014](#)) and display druggable targets ([K. Blum, 2011](#), [L. Gore, 2017](#))
- Precipitation based methods provide a fast and easy tool to analyze genome-wide DNA methylation profiles and identify differentially methylated regions

### Challenges

- Affinity-based enrichment methods display an inherent sequence bias <sup>2</sup> and therefore require unbiased and reproducible DNA shearing for optimal resolution and coverage
- Sequences of varying fragment length will precipitate with different efficiency and therefore require tight DNA fragment size distributions

### Workflow



**Schematic representation of precipitation based methods to detect DNA methylation.** **A:** Methyl-CpG immunoprecipitation (MCIp) <sup>10</sup> relies on shearing of genomic DNA followed by immobilisation of methylated DNA on a MBD2-Fc protein A-Sepharose matrix. Applying increasing salt concentrations DNA fractions with different degree of DNA methylation are eluted. **B:** Methylated DNA immunoprecipitation (MeDIP) <sup>11</sup> as well as hydroxymethylated DNA immunoprecipitation (hMeDIP) require DNA shearing and denaturation followed by immunoprecipitation with monoclonal 5mC/5hmC specific antibody.

### Advantages of Adaptive Focused Acoustics (AFA)

- Random fragmentation ensures unbiased representation of genomic regions
- Enables comparison of different samples e.g. tumour vs. healthy tissue or time course of follow-up samples. AFA technology enables solubilization of RNA and DNA as well as chromatin from FFPE tissues and therefore allows for genome-wide DNA methylation profiling from FFPE tissue.

## References

### MeDIP

- Reizel et al. Postnatal DNA demethylation and its role in tissue maturation. Nat Commun. 2018. DOI: [10.1038/s41467-018-04456-6](https://doi.org/10.1038/s41467-018-04456-6)
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### MCIp

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