diagenode

Innovating Epigenetics Solutions

Chromatin EasyShear Kit - Low SDS

Previous name: *Chromatin Shearing Optimization Kit - Low SDS*
Cat. No. C01020013

Compatible with:
iDeal ChIP-seq for Transcription Factors Kit
iDeal ChIP-qPCR Kit
iDeal ChIP-FFPE Kit

USER GUIDE

Version 4 06_12_2019

Please read this manual carefully
before starting your experiment

Contents

Introduction

Chromatin shearing is a crucial step for the success of ChIP experiments. It is important to establish optimal conditions to shear cross-linked chromatin to get the correct fragment sizes needed for ChIP. A successful chromatin preparation relies on the optimization of **cross-linking**, **cell lysis** and **sonication** itself. All 3 steps are interconnected and need to be optimized individually for different experimental settings. Our **Chromatin EasyShear Kits (previous name: Chromatin Shearing Optimization Kits)** together with **the Bioruptor** combine **efficient cell lysis** and **chromatin shearing** leading to **consistent results**.

The Chromatin EasyShear Kits are recommended for:

- The optimization of the chromatin shearing of a new cell line/ new sample type prior to ChIP using Diagenode's ChIP kits as described at page 5
- The optimization of the chromatin shearing and/or chromatin preparation prior to ChIP for other protocols

Each Chromatin EasyShear Kit provides optimized reagents and a thoroughly validated protocol according to your specific experimental needs. SDS concentration is adapted to each workflow taking into account target-specific requirements.

Choose an appropriate kit for your specific experimental needs and get consistent results.

	Chromatin EasyShear Kit Ultra Low SDS	**Chromatin EasyShear Kit** Low SDS	**Chromatin EasyShear Kit** for Plant	**Chromatin EasyShear Kit** High SDS
Cat. No.	C01020010	C01020013	C01020014	C01020012
Sample type	Cells, tissue	Cells, tissue	Plant tissue	Cells - low amount
Target	Histones	Transcription Factors and histones	Histones	Histones
Nuclei isolation	Yes	Yes	Yes	No
SDS concentration	‹ 0.1%	0.2%	0.5%	1%
Corresponding to shearing buffers from	iDeal ChIP-seq Kit for Histones ChIPmentation Kit for Histones	iDeal ChIP-seq Kit for Transcription Factors iDeal ChIP qPCR Kit iDeal ChIP-FFPE Kit	Universal Plant ChIP-seq Kit	True MicroChIP Kit

Kit method overview

The Chromatin EasyShear Kit - Low SDS is validated for the chromatin preparation from cells, tissues and FFPE samples. Please refer to a corresponding section in the protocol.

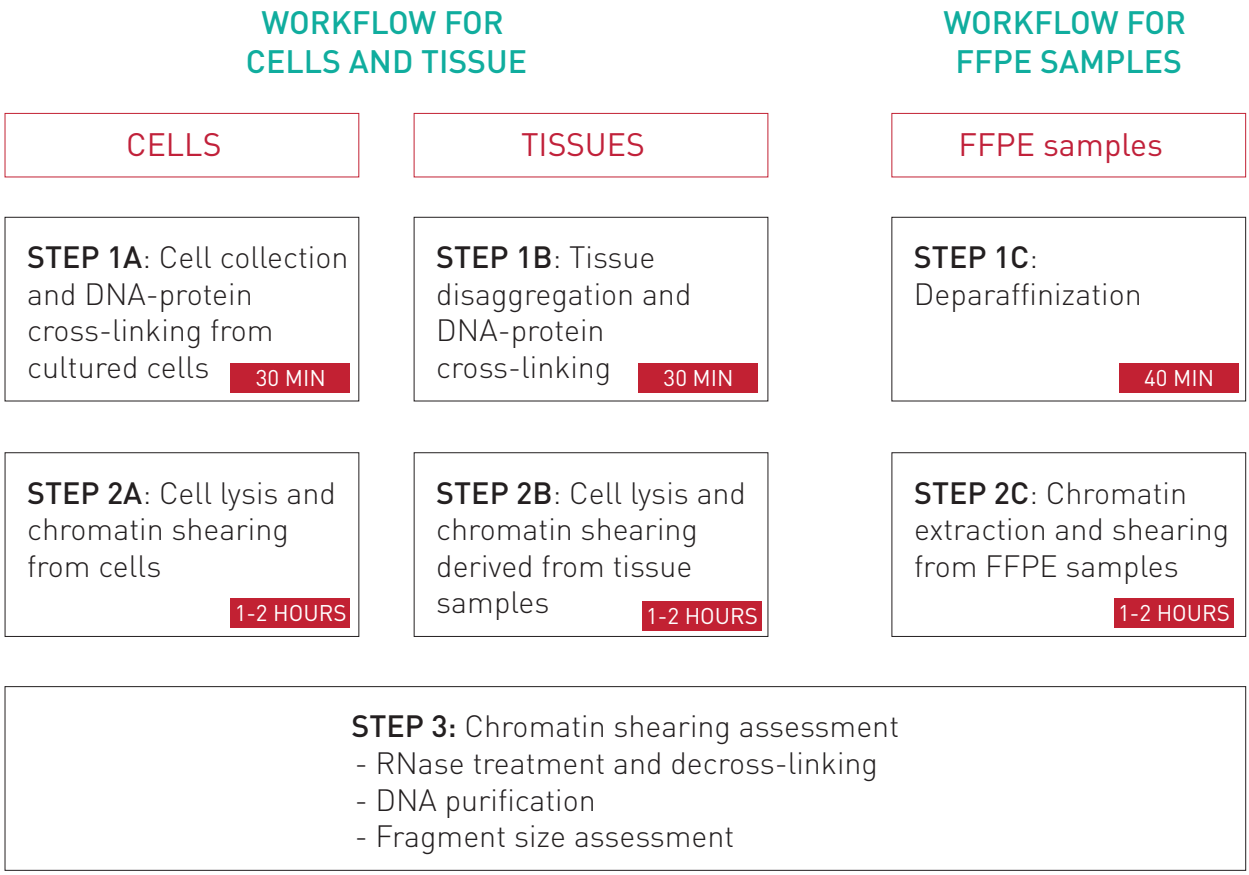


Figure 1. Kit method overview

The kit is also recommended for the optimization of chromatin preparation prior to ChIP performed with the following Diagenode kits:

ChIP kit	Manual version	Automated version
iDeal ChIP-seq Kit for Transcription Factors	C01010054 C01010055 C01010170	C01010058 C01010172
iDeal ChIP-qPCR Kit	C01010180	C01010181
iDeal ChIP-FFPE Kit	C01010190	n/a

Kit materials

The Chromatin EasyShear Kit - Low SDS contains enough reagents to perform the number of chromatin preparations and samples as described in the Table 1. Some components of the kit are not used for chromatin preparation from FFPE samples and some of them will be in excess.

This kit **does not contain** reagents for DNA purification required for shearing assessement. For DNA purification we highly recommend the IPure kit v2 (Diagenode, Cat. No. C03010014) or MicroChIP DiaPure columns (Diagenode, Cat. No. C03040001), which can be purchased separately.

Table 1. Number of reactions included in the Chromatin EasyShear Kit - Low SDS

Samples	Number of chromatin preparations	Material amount per one chromatin preparation	Number of samples/ sonication conditions
Cells	4	Up to 25x10e6 cells	24
Tissues	4	Up to 200 mg of tissue	24
FFPE samples	10	Up to 6 sections of up to 10 µm thickness	10

The kit content is described in Table 2. Upon receipt, store the components at the indicated temperatures.

Table 2. Components supplied with the Chromatin EasyShear Kit - Low SDS

Description	Quantity	Storage
Glycine*	10 ml	4°C
Shearing Buffer iS1b	7.5 ml	4°C
Elution Buffer iE1	1.25 ml	4°C
Elution Buffer iE2	100 µl	4°C
TE Buffer	500 µl	4°C
DNA precipitant*	250 µl	4°C
Fixation buffer*	6.4 ml	4°C
Lysis Buffer iL1b**	115 ml	4°C
Lysis Buffer iL2**	68 ml	4°C
DNA co-precipitant*	125 µl	-20°C
Protease inhibitor cocktail	38 µl	-20°C

* components not used in FFPE workflow

** components in excess for FFPE workflow

The composition of the buffers is proprietary.

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- RNase/DNase-free 1.5 ml tubes
- Formaldehyde, 37%, Molecular Grade (not required for FFPE workflow)
- Phosphate buffered saline (PBS) buffer
- Cell culture scraper (for adherent cells)

Equipment

- Bioruptor® sonication device and the associated microtubes:
  - Bioruptor Pico (Diagenode, Cat. No. B01060010) and 1.5 ml Bioruptor Microtubes with Caps (Cat. No. C30010016) or
  - Bioruptor Plus (Diagenode, Cat. No. B01020001) and 1.5 ml TPX Microtubes (Cat. No. C30010010)
- Refrigerated centrifuge for 1.5 ml tubes
- Thermomixer
- Vortex
- DiaMag Rotator (Rotating wheel) (Diagenode, Cat. No. B05000001)
- DNA sizing equipment (agarose gel electrophoresis or an automated capillary electrophoresis instrument, e.g. Fragment Analyzer (Agilent) and High Sensitivity NGS Fragment Analysis Kit (Agilent, DNF-473))

Reagents for DNA purification

- IPure Kit (Diagenode, Cat. No. C03010015) or
- MicroChIP DiaPure columns (Diagenode, Cat. No. C03040001) or
- Phenol-chloroform extraction (Phenol/chloroform/isoamyl alcohol (25:24:1), Chloroform/isoamyl alcohol (24:1), 100% Ethanol, 70% Ethanol
- RNase cocktail (e.g. Ambion, AM2286A)

Additional supplies for tissue protocol

- Protease inhibitor cocktail (Diagenode, Cat. No. C12010011 or C12010012) (100 µl per chromatin preparation)
- Fixation buffer (Diagenode, Cat. No. C01019002, 10 ml)
- Equipment for tissue disruption and homogenization: Dounce homogenizer with loose and tight pestles (2 ml) and TissueLyser (Qiagen) with 2 ml tubes and stainless steel beads, 5 mm (Qiagen, Cat. No.69989)
- Scalpel blades
- Petri dishes
- Qubit® Fluorometer (ThermoFisher Scientific)

Additional supplies for FFPE protocol

- Heptane, molecular grade
- Methanol, molecular grade
- Ethanol, molecular grade
- Equipment for tissue disruption and homogenization: Dounce homogenizer with loose and tight pestles (2 ml) and TissueLyser (Qiagen) with 2 ml tubes and stainless steel beads, 5 mm (Qiagen, Cat. No.69989)
- Qubit® Fluorometre (ThermoFisher Scientific) and dsDNA HS Assay Kit
- DiaFilters (Diagenode)

Optional supplies

- ChIP Cross-link Gold - for efficient fixation of proteins that are not directly bound to the DNA (Diagenode, Cat. No. C01019027)

Remarks before starting

A high quality chromatin sample combines two main features: a **suitable fragment size range** and the **availability of cross-linked epitopes** for ChIP. A perfect sonication profile is a profile in which the highest specific signal and the lowest background are generated.

There is no one universal size range generally applicable for ChIP. As a rule, fragments between **100-600 bp** are suitable for the majority of ChIP experiments and can be used as a starting point. This range can be fine-tuned for particular experimental requirements depending on the specificity of the target (histones or non-histone proteins) and the required downstream analysis (ChIP-qPCR or ChIP-seq). Generally, a tighter fragment distribution is recommended for ChIP-seq than for ChIP-qPCR. Note that **100-300 bp** is compatible (but not absolutely necessary) with **histone ChIP-seq** while a **broader fragment range** is more suitable for **non-histone ChIP-seq** (transcriptional factors and proteins that are not bound directly to DNA and for long-distance interacting proteins). With the recent evolution of sequencing technologies, it is common to perform ChIP-seq experiments from chromatin with a distribution in the 100–800 bp range.

Before starting the ChIP, the chromatin should be sheared to a desired size. Sonication should be optimized for each ChIP project since samples are different in their resistance to sonication. The following parameters should be considered during the chromatin shearing optimization:

1. Starting amount of material (cells, tissues or FFPE slices)
2. Fixation
3. Shearing optimization (followed by shearing assessment)

1. Starting amount of material

Cell number

The protocol describes the preparation of a batch of chromatin that is sufficient for **6 samples**. This allows to test up to 6 different sonication conditions or prepare up to 6 chromatin samples to be used for immunoprecipitation. The starting amount of cells per batch depends on a target to be studied: histone marks require less starting material than transcription factors. We recommend using approximately 1 million cells per sample for histone marks and 4 million cells per sample for transcription factors and other non-histone proteins. Scale the amount of cells per batch according to the experimental plan. Start with a batch of cells up to 25 million for experiment for transcription factors (the protocol presented in the next sections of this manual). Use a smaller cell batch (up to 10 million cells) for histones marks. The sample volume per sonication is 250 µl. Please note that shearing efficiency depends on the sample volume and it should be kept consistent to ensure the reproducible results.

The described protocol uses a prompt direct fixation in a cell culture plate. Fixed and scraped cells cannot be accurately counted. This means that for **adherent cells** you need to use an approximate estimation of cell number per plate. Alternatively, an additional parallel plate for counting can be prepared. **Suspension cells** can be counted before the fixation.

Depending on the experiment requirements (the abundance of the target, the specificity of the antibody, and the number of cells available) it may be possible/necessary to scale up or down the number of cells per sample and/or start with a smaller or a bigger batch of cells.

If starting with an number of cells different from the standard protocol, first determine the number of cells that you will use per sample and the total number of samples per one chromatin preparation. Fix cells as described in the standard protocol. For cell collection and lysis, scale up or down the volume of iL1b and iL2 buffers using **1 ml** of **iL1b** and **0.6 ml** of **iL2b** per **1 million cells**. Define the volume of Shearing Buffer iS1b taking into account that you will need 250 µl of iS1b Buffer per sample. Resuspend the cells in the required volume of Shearing Buffer iS1b and proceed to the sonication step.

Please note that cell density is a key factor for efficient shearing. Sample viscosity may hamper the cavitation process leading to less efficient shearing. Dense cell suspension will require more extensive sonication. Adapt sonication time accordingly.

Tissue amount

The protocol describes the preparation of a batch of chromatin that is sufficient for **6 samples**. This allows to test up to 6 different sonication conditions or prepare up to 6 chromatin samples to be used for immunoprecipitation. The starting amount of tissue per batch depends on a target to be studied: histone marks require less starting material than transcription factors. Scale the amount of tissue per batch accordingly to the experimental plan. Start with a batch of tissue up to up to 200 mg for experiment on transcription factors. Use a smaller cell (up to 40 mg) for histones mark. The sample volume is 250 µl. Please note that shearing efficiency depends on the sample volume and it should be kept consistent to ensure the reproducible results.

Tissue samples have to be homogenized mechanically before sonication. Soft tissues (e.g. liver or brain) can be successfully homogenized using a Dounce homogenizer while it is preferable to use the TissueLyser (Qiagen, or a similar system) for hard fibrous tissues (e.g. muscles). Please note that the TissueLyser workflow is only compatible with frozen tissues since the grinding of fresh tissue will not be efficient with this protocol.

Depending on the experiment requirements (abundance of the target, the specificity of the antibody and the amount of tissue available) it may be possible/necessary to scale up or down the amount of tissue per sample and/or start with a smaller or a bigger batch of tissue.

If starting with a tissue amount different from the standard protocol, first determine the amount of tissue that you will use per sample and the total number of samples per one chromatin preparation. Follow the standard protocol for tissue fixation, collection and lysis. Do not scale Lysis Buffers iL1b and iL2. Define the volume of Shearing Buffer iS1b taking into account that you will need **250 µl** of **iS1b buffer** per sample. Resuspend the sample in the required volume of Shearing Buffer iS1b and proceed to the sonication step.

Please note that sample density is a key factor for efficient shearing. Sample viscosity may hamper the cavitation process leading to less efficient shearing. Dense cell suspension will require more extensive sonication. Adapt sonication time accordingly.

We recommend quantifying DNA for a rough estimation of chromatin yield obtained from tissue. The recommended amount of chromatin to be used per immunoprecipitation with iDeal ChIP-seq Kit for Transcription Factors protocol and iDeal ChIP-qPCR should be within 3-15 µg range. Perform a pilot experiment to determine the optimal amount of tissue resulting in a required amount. Once determined, it should be kept consistent between experiments.

FFPE slices

The protocol describes the preparation of chromatin from up to **6 FFPE sections** (up to 10 µm in thickness). The total volume of the sheared chromatin per one chromatin preparation is about 600 µl. Please keep in mind that the chromatin yield varies depending on samples (tissue type, tissue section size/areas, fixation, storage). The minimum DNA amount recommended per one IP is about 300 ng. However, a higher starting amount is recommended when possible. Therefore, the exact amount of sections giving a sufficient chromatin yield should be optimized by the user.

2. Fixation optimization

The described protocol uses a prompt direct fixation by formaldehyde which is essential for transcription factors.

Formaldehyde is the most commonly used cross-linking reagent ideal for two molecules which interact directly. The fixation time can depend on your target of interest and might require an additional optimization (usually between 10 and 20 minutes for transcription factors and 8 - 10 minutes for most histone proteins). Please note that a longer fixation may lead to chromatin resistant to sonication. Adapt sonication time accordingly.

However, for higher order and/or dynamic interactions, other cross-linkers should be considered for efficient protein-protein fixation, e.g. Diagenode ChIP Cross-link Gold, an innovative dual cross-linking ChIP

fixation reagent (Diagenode, Cat. No. C01019027). It is important to note that use of two crosslinking agents can make chromatin more resistant to sonication and de-crosslinking.

If different fixation time will be tested, we recommend starting with a corresponding number of chromatin preparations and testing different sonication settings per each preparation.

Deparaffinization (for FFPE samples)

Tissue sections must be first deparaffinized prior to chromatin extraction. The described protocol uses heptane as a fast and less toxic alternative to a conventional xylene-based approach. Deparaffinized sections do not require additional re-hydratation removing laborious washes with serial dilutions of ethanol. Please note that conventional deparaffinization described in a separate section is also compatible with the further protocol.

Antigen retrieval (for FFPE samples)

While the crosslinking is indispensable for ChIP, the extensive crosslinking of FFPE samples affects the chromatin yield, solubility and antigen availability. A mild controlled antigen retrieval achieved by the heating at 65°C (step 2C, point 2.7 in the protocol) helps to overcome this issue while preserving the chromatin complex. The incubation time at 65°C might require an additional optimization depending on a sample type. The incubation from 30 min up to 2 h can be tested.

3. Shearing optimization

The length of sonication time depends on many factors (cell type, cell density, sample volume, fixation time). Hence it is important to optimize the sonication conditions for each new ChIP project.

The protocol is used in a combination with the Bioruptor (Pico or Plus). Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation.

Choose the protocol which is adapted to your device:

- When using the Bioruptor Pico, an initial time-course experiment of 5-10-12 sonication cycles 30'' ON/30'' OFF is recommended.

- When using the Bioruptor Plus, an initial time-course experiment of 10-20-30 sonication cycles [30 seconds "ON", 30 seconds "OFF"] at High power is recommended.

During the sonication, the mean size of DNA fragments will decline progressively approaching a lower limit of 100-150 bp (mean size of the smear). It is recommended to choose a sonication time before reaching this lower limit. **As best practice, choose the shortest sonication time resulting in a satisfactory shearing and ChIP efficiency (highest recovery/lowest background)**. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments, especially when non-histones proteins are to be evaluated by ChIP (Figure 2).

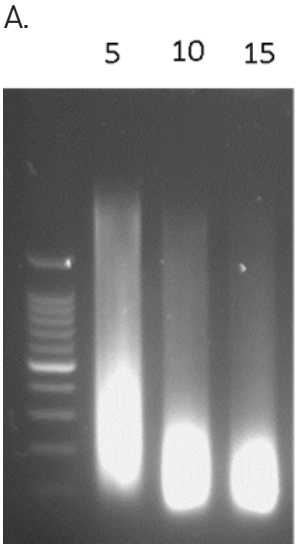
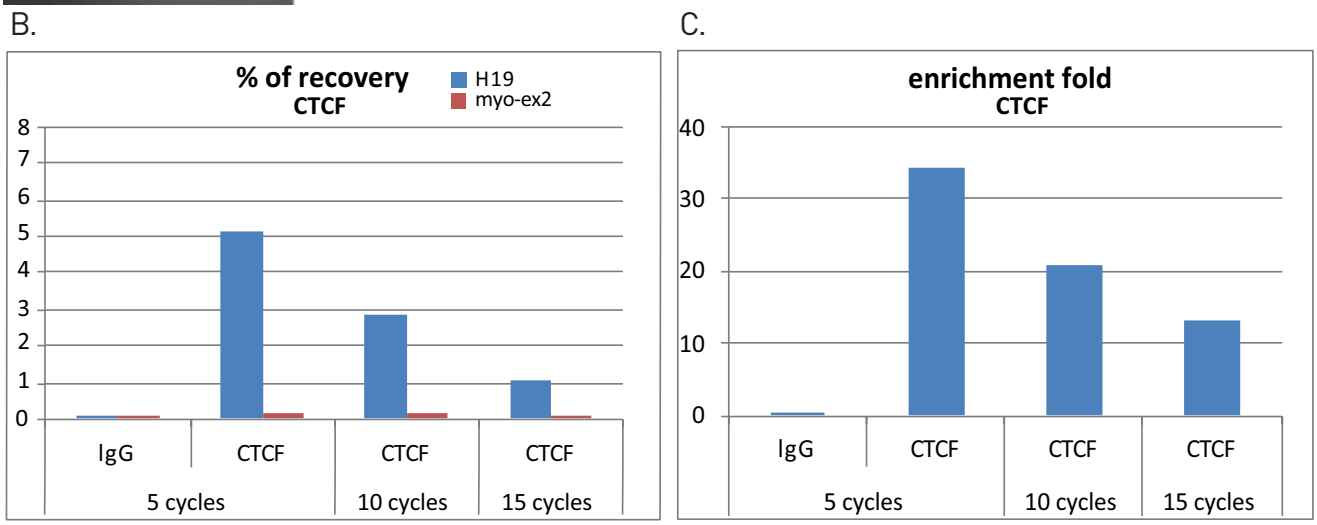


Figure 2. Optimal chromatin shearing profile.

HeLa cells were fixed with formaldehyde for 10 min and chromatin was prepared according to Diagenode's Chromatin EasyShear Kit - Low SDS (Cat. No. C01020013). Samples were sonicated for 5-10-15 cycles of 30" ON/30" OFF as indicated with Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016) followed by de-crosslinking and DNA purification. The fragment size was assessed using agarose gel electrophoresis. A 100 bp ladder was loaded as the size standard (panel A).



Sheared chromatin has been used for immunoprecipitation with CTCF and IgG (negative control) antibodies. Quantitative PCR was performed with positive (H19) and negative (Myoglobine exon 2) control regions. The Figure 2 shows the recovery expressed as % of input (the relative amount of immunoprecipitated DNA compared to input DNA (panel B) and as enrichment fold of positive locus over negative (panel C).

Analysis of the results from Figure 2.

Fragments suitable for ChIP experiments with transcription factors are generated after 5 cycles (panel A). The fragment size distribution from 100-600 bp is compatible with requirements for all ChIP-seq experiments together with the best enrichment obtained transcription factor (CTCF) (panel B and C).

Chromatin sheared for 10 cycles (100 - 300 bp, panel A) is suboptimal for ChIP-seq for transcriptional factors due to a drop of ChIP efficiency (panel B and C).

Chromatin after 15 cycles with fragment size distribution of 100 - 200 bp might be over-sheared: while fragment size is suitable for ChIP-seq experiment (100 - 200 bp, panel A), a significant drop of ChIP efficiency is observed for transcription factor marks (panel B and C).

In some situations, it is preferable to re-shear the purified de-crosslinked DNA after immunoprecipitation rather than over-sonicate. Re-shearing enables the enrichment of fragments in the desired optimal size range suitable for next-generation sequencing. Please refer to the following protocol

www.diagenode.com/files/protocols/ChIP-re_shearing-protocol.pdf

Ensure that only the recommended tubes are used for sonication. It is important to note that sonication tubes recommended for the Bioruptor Pico are different from the tubes recommended for the Bioruptor Plus and Bioruptor Standard. Using the wrong tubes will lead to inefficient shearing. Please refer to the following guide.

www.diagenode.com/files/organigram/bioruptor-organigram-tubes.pdf

Be aware that sonication efficiency may differ depending on a type of tubes used. Switching to another type of tubes (e.g. from 1.5 ml to 15 ml tubes) will require an additional optimization.

Ensure that the sample volume per sonication is in the recommended range. Any deviations from this recommended range will lead to inefficient shearing and lack of reproducibility.

DNA purification and chromatin shearing assessment

For accurate size determination of the chromatin fragments, reverse crosslinking, including RNase treatment followed by DNA purification, is advised. Size estimation of chromatin fragments without reverse cross-linking is not precise. The presence of the crosslinks retards electrophoretic migration resulting in a misinterpretation of fragment size.

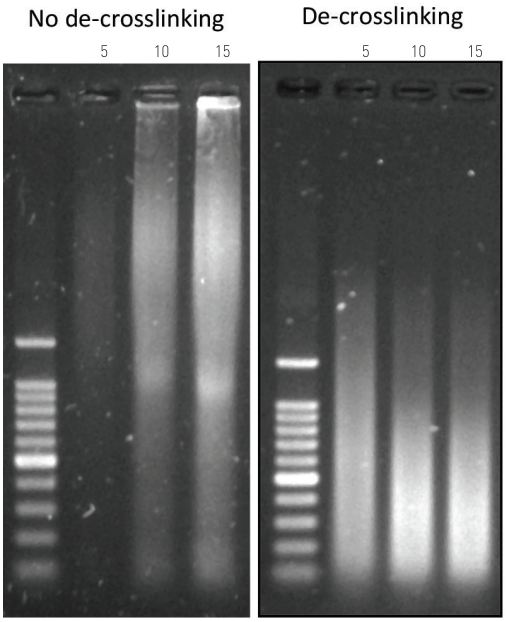


Figure 3. Reversing crosslinks is necessary for accurate size estimation.

HeLa cells were fixed with formaldehyde and chromatin was prepared accordingly to Diagenode's Chromatin Shearing Optimization Kit – Medium SDS (Cat. No. C01020011). Samples were sonicated for 5, 10 and 15 cycles of 30" ON/30" OFF as indicated with the Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (C30010016). A 100 bp ladder was loaded as size standard. Left panel: non de-crosslinked chromatin. Right panel: de-crosslinked chromatin. RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing.

RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing. The presence of degraded RNA in the sample might lead to mis-interpretation of the shearing. Smear below 100 bp is due to degraded RNA but not over-sheared DNA.

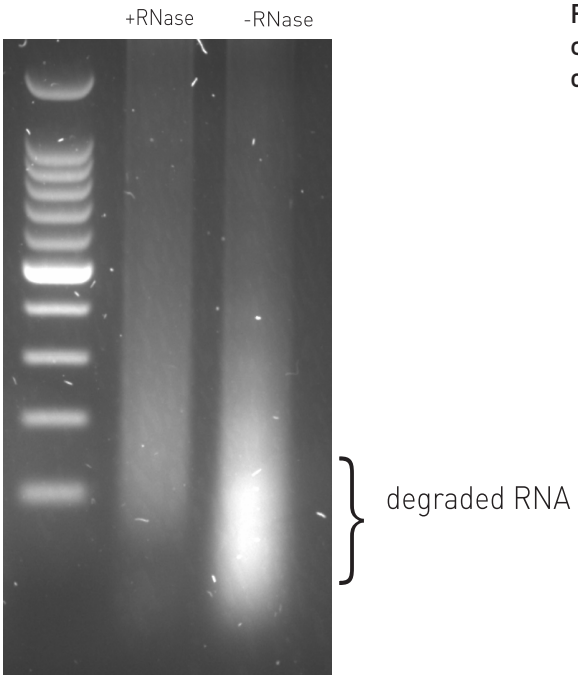


Figure 4. RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing.

Chromatin from HeLa cells was prepared according to Diagenode's protocol. Samples were sonicated for 5 cycles of 30" ON/30" OFF with Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016) followed by de-crosslinking and DNA purification in the presence or absence of RNase as indicated. The fragment size was assessed using agarose gel electrophoresis. A 100 bp ladder was loaded as the size standard.

This protocol is compatible with different DNA purification methods:

- Magnetic beads purification (e.g. IPure beads from Diagenode) ,
- Columns-based DNA clean-up (e.g. MicroChIP DiaPure columns from Diagenode)
- Conventional phenol-chloroform extraction.

Please note that reagents for DNA purification are not included in the kit and should be provided by user.

DNA derived from FFPE samples should be purified using MicroChIP DiaPure columns. Eluted DNA is enough concentrated to be analysed using the Fragment Analyzer (Agilent) and High Sensitivity NGS Fragment Analysis Kit (Agilent, DNF-473). The agarose gel is not sensitive enough to visualize the low amount of DNA obtained from FFPE samples.

For the size assessment of sheared chromatin we recommend using an agarose gel analysis or the Fragment Analyzer (Agilent).

If using an **agarose gel**, the sheared chromatin should be analyzed on a 1.2 - 1.8% agarose gel. The optimal DNA amount from sheared chromatin is around 300 ng per lane. A serial dilution from 100 ng to 500 ng could be run. Do not overload the gel as the migration of large quantities of chromatin on an agarose gel can lead to poor quality pictures which do not reflect the real DNA fragmentation. The minimum amount of sheared chromatin that can be visualized in an agarose gel corresponds to 60,000 cells equivalent. Both the pre- and post-staining of the agarose gel with ethidium bromide or SybrSafe dye can be used for visualization of sheared fragments. Some slight differences might be observed between post- and pre-stained gels. Post-staining eliminates any possibility that the dye interferes with the migration and ensures an even background noise. However, pictures are usually less clear and bright with some background noise. If pre-stained agarose gels are used, it is advised that the electrophoresis buffer contains the stain in the same concentration as in the gel. If the stain is present in the gel but not in the buffer, the gel will result in uneven staining because the free dye migrates towards the top of the gel leaving the bottom part with no stain. Therefore, the background noise becomes non-uniform.

Using **Fragment Analyzer** (Agilent), please follow the manufacturer's instruction.

If using the Agilent BioAnalyzer, please keep in mind that traces are log-based, so a large distribution of higher molecular weight fragments are compacted into a much smaller area of the trace as compared to the smaller size fragments leading to a visual misinterpretation of fragment distribution.

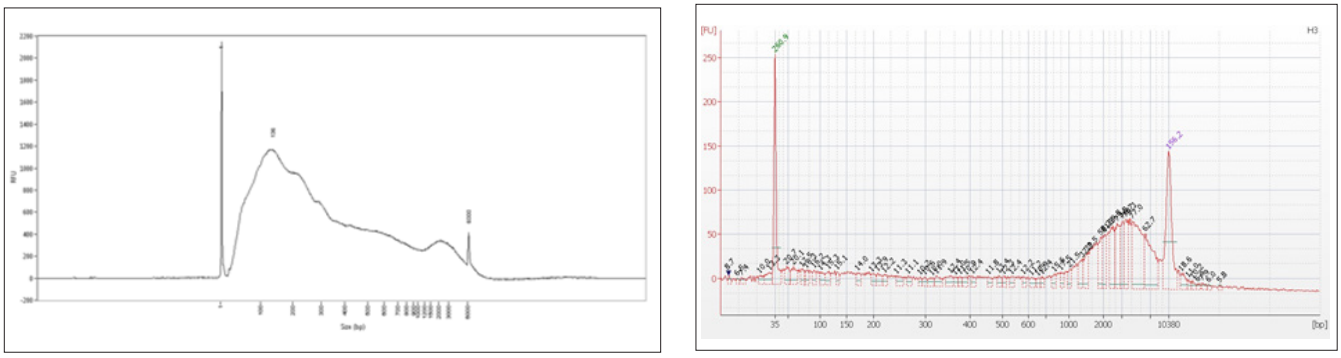


Figure 5. An appropriate method should be used for the shearing assessment

HeLa cells were fixed with formaldehyde for 8 min and chromatin was prepared according to Diagenode's Chromatin EasyShear Kit - Ultra SDS (Cat. No. C01020010). Samples were sonicated for 10 cycles of 30" ON/30" OFF with Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016) followed by de-crosslinking and DNA purification. The fragment size was assessed using Fragment Analyzer (left) and BioAnalyzer (right). The BioAnalyzer trace is biased towards the high molecular weight fragments.

If high molecular weight fragments are present, it is recommended estimating a molar ratio between fragments in a desired range and higher molecular weight fraction. The molarity allows estimating a number of molecules in a particular range. The presence of high molecular weight fragments up to 15% - 20% (molar ratio) is acceptable (Figure 6).

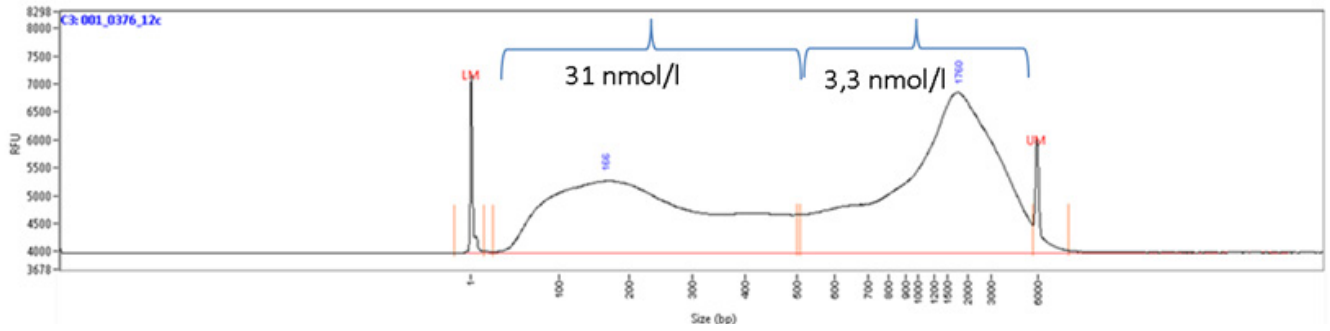


Figure 6. A molar ratio between desired fragments range and high molecular weight fraction should be estimated.

HeLa cells were fixed with formaldehyde for 10 min and chromatin was prepared according to Diagenode's Chromatin EasyShear Kit - Low SDS (Cat. No. C01020013). Samples were sonicated for 12 cycles of 30" ON/30" OFF with Bioruptor Pico using 1.5 ml Bioruptor microtubes with caps (Cat. No. C30010016) followed by de- crosslinking and DNA purification. The fragment size was assessed using Fragment Analyzer. The molar content of fragments in the range 100-500 bp and 500-5.000 bp was estimated showing that large fragment do not exceed 15%.

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PROTOCOL FOR CHROMATIN PREPARATION FROM CULTURED CELLS

STEP 1A

Cell collection and DNA-protein cross-linking from cultured cells

30 minutes

FOR CULTURED CELLS

1.1. Equilibrate the Fixation Buffer to room temperature before use.

1.2. Prepare the cross-linking solution in a fume hood by adding **formaldehyde** to the **Fixation Buffer** to a final concentration of **11%** (e.g. add 0.596 ml of 37% formaldehyde to 1.407 ml of Fixation Buffer). Add the **diluted formaldehyde** directly to the cell culture medium in a proportion of 1:10. For a 20 ml cell culture you will need 2 ml of cross-linking solution.

***NOTE**: Use always fresh formaldehyde.*

1.3. Incubate the cells for 15 minutes at room temperature with gentle shaking.

***NOTE**: The fixation time might require an additional optimization. Please refer to the "Remarks before starting".*

1.4. Add **Glycine** to the cell culture medium in a proportion of 1:10 to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking. Proceed to the next step immediately.

***NOTE**: We strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP. If not possible, the fixed cells can be stored at -80°C for up to 4 months. To freeze the cells, remove the medium, wash the cells once with 20 ml of PBS. Add another 5 ml of PBS, collect the cells by scrapping and/or centrifugation at 500 x g for 5 minutes and 4°C and discard the supernatant. Store the cell pellet at -80°C.*

STEP 2A

Cell lysis and chromatin shearing from cells

1 to 2 hours

FOR CULTURED CELLS

For adherent cells:

2.1. Remove the medium and wash the cells once with **20 ml of PBS**. Discard the PBS. Keep everything at 4°C or on ice from now on.

2.2. Add **5 ml of cold Lysis Buffer iL1b** to the plate. Collect the cells by scraping and transfer them into a 50 ml tube.

2.3. Rinse the flask with **20 ml of Lysis Buffer iL1b** and add this to the 50 ml tube. The total volume of Lysis Buffer iL1b should be **25 ml per 25 million cells** (for up or down scaling use 1 ml of buffer iL1b per million of cells). Proceed immediately with step 2.4.

For suspension cells:

2.1. Transfer cells into a 50 ml tube. Pellet the cells by centrifugation at 500 x g and 4°C for 5 minutes. Discard the cell culture medium.

2.2. Resuspend the cells in **20 ml of ice-cold PBS**, centrifuge at 500 x g and 4°C for 5 minutes and gently discard the supernatant. Keep everything at 4°C or on ice from now on.

2.3. Resuspend the cells in **1 ml of ice-cold Lysis Buffer iL1b** by pipetting up and down several times. Add **24 ml of buffer iL1b** to obtain a total

volume of **25 ml per 25 million cells** (for up or down scaling use 1 ml of iL1b per 1 million cells). Proceed immediately with step 2.4.

2.4. Incubate at 4°C for 20 minutes with gentle mixing on a DiaMag Rotator. Pellet the cells by centrifugation at 500 x g and 4°C for 5 minutes and discard the supernatant. Resuspend the cell pellet in **1 ml of ice-cold Lysis Buffer iL2** by pipetting up and down several times. Add **14 ml of buffer iL2** and incubate for 10 minutes at 4°C with gentle mixing on a DiaMag Rotator (for up or down scaling, use 600 µl of buffer iL2 per 1 million of cells).

2.5. Pellet the cells again by centrifugation for 5 minutes at 500 x g and 4°C and discard supernatant.

2.6. Add 8.4 µl of **200x protease inhibitor cocktail** to **1.67 ml of Shearing Buffer iS1b**. This is a complete Shearing Buffer needed for 25 million cells. Keep on ice.

2.7. Add **the complete Shearing Buffer iS1b** to the cell pellet. The cell concentration in the shearing buffer should be 1.5 million of cells per 100 µl of iS1b. Resuspend the cells by pipetting up and down several times and incubate on ice for 10 minutes. Split the cell suspension into aliquots of maximum 300 µl by transferring it to the appropriate sonication microtubes:

- When using the Bioruptor Pico use 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
- When using the Bioruptor Plus use 1.5 ml TPX Microtubes (Cat. No. C30010010)

***NOTE**: The maximum volume for shearing with the Bioruptor is 300 µl per 1.5 ml Microtube. The use of correct type of microtubes is essential for the efficiency of sonication.*

2.8. Shear the chromatin by sonication using the Bioruptor. Choose the protocol which is adapted to your device:

- When using the Bioruptor Pico shear for 5 to 12 cycles [30 seconds "ON", 30 seconds "OFF"].
- When using the Bioruptor Plus or Standard, shear for 10 to 30 cycles [30 seconds "ON", 30 seconds "OFF"] each at High power setting.

***NOTE**: Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of water.*

2.9. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Pool the supernatants which contain the sheared chromatin.

2.10. Take an aliquot of 50 µl of sheared chromatin for the shearing assessment. The remaining chromatin can be stored it at -80°C for up to 2 months and used further for ChIP experiments.

PROTOCOL FOR CHROMATIN PREPARATION FROM TISSUES

STEP 1B

Tissue disaggregation and DNA-protein cross-linking

30 minutes

FOR TISSUES

***NOTE**: If TissueLyser will be used for homogenization, pre-cool 2 ml tubes, adaptors and stainless steel beads at -80°C. Only frozen tissue could be proceeded with TissueLyser.*

1.1. Equilibrate the Fixation Buffer to room temperature before use.

1.2. Prepare the cross-linking solution in a fume hood by adding **54 µl** of **37% formaldehyde** to **2 ml of Fixation Buffer** to a final concentration of 1%. Use 2 ml of Fixation Buffer for one chromatin preparation.

1.3. Put **200 mg** of fresh or frozen **tissue** in a petri dish on ice. Keep samples on ice at all times and minimize the time of manipulation to prevent sample degradation.

***NOTE**: Use always fresh formaldehyde.*

1.4. Use about **200 mg** of **tissue** per preparation and homogenize using one of the following options: TissueLyser or a Dounce homogenizer. Minimize the time of manipulation until the fixation step to prevent sample degradation.

***NOTE**: The exact amount of tissue per one preparation might vary depending on a tissue type. Choose an appropriate method of homogenization. Please refer to the "Remarks before starting"*

- **TissueLyser:** Place frozen tissue in a petri dish on dry ice and chop it into small pieces (between 1-3 mm^3) using a scalpel blade. Transfer tissue pieces to 2 ml tubes, supplemented with 2 stainless steel beads (5 mm) and immerse in liquid nitrogen for a few minutes. Insert 2 ml tubes into TissueLyser Adaptors and operate the TissueLyser for 2 minutes at 25 Hz. Add immediately 1 ml of formaldehyde diluted in Fixation Buffer and transfer the sample to the Dounce homogenizer. Disaggregate sample to get a homogeneous suspension. Set the timer for a total fixation time of 15 minutes and start deducting the fixation time from this point.
- Alternatively, use a **Dounce homogenizer** with a loose fitting pestle: place fresh or frozen tissue in a petri dish on ice and chop it into small pieces (between 1-3 mm^3) using a scalpel blade. Transfer tissue pieces to the homogenizer and add 1 ml of formaldehyde diluted in Fixation Buffer. Disaggregate the sample to get a homogeneous suspension. Set the timer for a total fixation time of 15 minutes and start deducting the fixation time from this point.

1.5. Transfer the tissue suspension into a 15 ml tube. Rinse the Dounce homogenizer with an additional **1 ml of diluted formaldehyde** and pool with the sample in the same 15 ml tube.

1.6. Incubate for a total time of 15 minutes (starting at step 1.4) at room temperature with gentle rotation on a DiaMag Rotator.

***NOTE**: The fixation time might require an additional optimization. Please refer to the "Remarks before starting".*

1.7. Add **200 µl** of **Glycine** to the tissue suspension to stop the fixation. Incubate for 5 minutes at room temperature with gentle mixing on a DiaMag Rotator. Proceed to the next step immediately.

***NOTE**: We strongly recommend using freshly fixed tissue for preparation of sheared chromatin prior to ChIP. If not possible, the fixed tissue can be stored at -80°C for up to 4 months. To freeze the tissue, remove the medium and wash the tissue once with 2 ml of PBS. Store the tissue pellet at -80°C.*

STEP 2B

Cell lysis and chromatin shearing derived from tissue samples

1 to 2 hours

FOR TISSUES

***NOTE**: An additional 100 µl of protease inhibitors cocktail is required per chromatin preparation – available separately from Diagenode.*

2.1. Centrifuge samples at 850 x g for 5 minutes at 4°C. Gently discard the supernatant and keep the pellet.

2.2. Wash the pellet with **10 ml of ice-cold PBS** and centrifuge samples at 850 x g for 5 minutes at 4°C. Gently discard the supernatant. Keep everything at 4°C or on ice from now on.

2.3. Add **50 µl of 200x protease inhibitor cocktail** to **10 ml of ice-cold Lysis buffer iL1b**. This is the complete Lysis Buffer iL1b needed for 200 mg of tissue.

2.4. Add **1 ml of ice-cold complete Lysis Buffer iL1b** to the pellet and resuspend by pipetting up and down several times. Add **the remaining amount of complete buffer iL1b** (for up or down scaling use 1 ml of iL1b per 20 mg of tissue).

2.5. Incubate at 4°C for 20 minutes with gentle mixing on a DiaMag Rotator.

2.6. Pellet the cells by centrifugation at 850 x g for 5 minutes at 4°C and discard the supernatant.

2.7. Add **50 µl of 200x protease inhibitor cocktail** to **10 ml of ice-cold Lysis Buffer iL2**. This is a complete Lysis Buffer iL2 needed for 200 mg of tissue. Add **1 ml of ice-cold complete Lysis Buffer iL2** to the cell pellet and resuspend the cells by pipetting up and down several times. Add **the remaining amount of complete buffer iL2** (for up or down scaling use 0.6 ml of iL1b per 20 mg of tissue).

2.8. Incubate at 4°C with gentle mixing on a DiaMag Rotator for 10 minutes.

2.9. Pellet the cells again by centrifugation at 850 x g for 5 minutes at 4°C and discard supernatant.

2.10. Add **8.4 µl of 200x protease inhibitor cocktail** to **1.67 ml of Shearing Buffer iS1b**. This is a complete Shearing Buffer needed for 200 mg cells. Keep on ice.

2.11. Add **the complete Shearing Buffer iS1b** to the pellet. Resuspend the cells by pipetting up and down several times.

Optionally: *The suspension can be re-homogenized using a Dounce homogenizer with a loose fitting pestle. The most reproducible and efficient shearing is achieved with homogenous suspension.*

2.12. Split the cell suspension into 6 aliquots of maximum 300 µl by transferring it to the appropriate 1.5 ml microtubes:

- When using the Bioruptor Pico use 1.5 ml Bioruptor Microtubes with Caps (Cat. No. C30010016)
- When using the Bioruptor Plus use 1.5 ml TPX Microtubes (Cat. No. C30010010)

NOTE*: The maximum volume for shearing with the Bioruptor is 300 µl per 1.5 ml Microtube. The use of correct type of microtubes is essential for the efficiency of sonication.*

2.13. Shear the chromatin by sonication using the Bioruptor. Perform a shearing time-course experiment using 2 samples per condition. Choose the protocol which is adapted to your device:

- When using the Bioruptor Pico shear for 5 to 12 cycles [30 seconds "ON", 30 seconds "OFF"].
- When using the Bioruptor Plus or Standard, shear for 10 to 30

cycles [30 seconds "ON", 30 seconds "OFF"] each at High power setting.

***NOTE**: Never leave empty spaces in the tube holder. Fill the empty spaces with tubes containing the same volume of water.*

2.14. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Keep the supernatants which contain the sheared chromatin.

2.15. Take an aliquot of **50 µl** from each sample and quantify the DNA concentration using dsDNA HS Assay Kit on the Qubit® system from ThermoFisher Scientific. Estimate the total yield of chromatin per 250 µl (corresponding to one IP).

***NOTE**: If the yield is out of recommended range (3-15 µg), the starting amount of tissue should be scaled up/down accordingly.*

2.16. Take an aliquot of 50 µl of sheared chromatin from each sample for the shearing assessment. The remaining chromatin can be stored it at -80°C for up to 2 months and used further for ChIP experiments.

PROTOCOL FOR CHROMATIN PREPARATION FROM FFPE SAMPLES

STEP 1C

Deparaffinization

40 minutes

FOR FFPE SAMPLES

1.1. Cut sections up to 10 µm from FFPE blocks and collect directly in 1.5 ml tube with tweezers. Use up to 6 sections per one chromatin preparation.

***NOTE**: If sections are not used immediately, store the material at -80 °C. The exact numbers and thickness of sections should be defined by user.*

1.2. Under a fume hood, add **1 ml** of **heptane** in the 1.5 ml tube containing the paraffin sections.

1.3. Vortex for 30 seconds and incubate at room temperature for 30 minutes with rotation on the DiaMag Rotator.

1.4. Add **50 µl** of **100% methanol** and vortex for 30 seconds. Centrifuge at 16.000 g for 2 minutes at room temperature.

***NOTE**: Make sure that tiny tissue fragments are not discarded with the supernatant. Repeat the centrifugation step if required.*

1.5. If the paraffin is not completely removed, repeat steps 1.1-1.4 one more time.

1.6. Remove the supernatant carefully and discard.

1.7. Add **500 µl** of **100% ethanol** and vortex for 10 seconds. Centrifuge at 16.000 g for 2 minutes at room temperature.

1.8. Remove as much of the solution as possible and discard. Let the sample dry at room temperature for 1-2 minutes.

STEP 2C

Chromatin extraction and shearing from FFPE samples

1 to 2 hours

FOR FFPE SAMPLES

2.1. Resuspend the sample in **0.6 ml** of ice-cold **Lysis Buffer iL1b** by pipetting up and down several times and carefully transfer it to DiaFilter column using a cut tip. Make sure that all material has been transferred.

***NOTE**: Care should be taken not to damage the DiaFilter with a tip.*

2.2. Incubate at 4°C for 10 minutes with gentle mixing on a DiaMag Rotator. Pellet the sample on the DiaFilter by centrifugation at 8.000 x g for 5 minutes at 4°C and discard the flow-through from the collection tube.

2.3. Resuspend the pellet in **0.6 ml** of ice-cold **Lysis Buffer iL2** by pipetting up and down several times and incubate for 10 minutes at 4°C with gentle mixing on a DiaMag Rotator.

2.4. Pellet the sample again on the DiaFilter by centrifugation at 8.000 x g for 5 minutes at 4°C and discard the flow-through from the collection tube.

2.5. Add **3 µl** of **Protease inhibitor cocktail** to **600 µl** of **Shearing Buffer iS1b**. This is a **complete Shearing Buffer** needed for the preparation of chromatin from up to **6 FFPE slices** (up to 10 µM in thickness). Scale accordingly.

2.6. Add **300 µl** of **complete Shearing Buffer iS1b** to the sample and resuspend by pipetting up and down several time. Homogenize using one of the following options:

- TissueLyser: Transfer the sample to a 2 ml tube containing 2 stainless steel beads, diameter 5 mm. Place the tube in the TissueLyser Adaptor precooled at 4°C. Operate the system for 5 minutes at 25 Hz.
- Alternatively, use a Dounce homogenizer: transfer the sample to the homogenizer and disaggregate it using a tight fitting pestle to get a homogeneous suspension.

2.7. Transfer the sample into 1.5 ml tube and incubate for 1h30 at 65°C with shaking on Thermomixer for antigen retrieval. Add **100 µl of 100% isopropanol** to each sample.

***NOTE**: The incubation time at 65°C might require an additional optimization. The incubation from 30 minutes up to 2 hours can be tested.*

2.8. Transfer the sample to the appropriate sonication microtubes:

- When using the Bioruptor Pico use 1.5 ml Bioruptor Microtubes with Caps (Cat. No. C30010016)
- When using the Bioruptor Plus use 1.5 ml TPX Microtubes (Cat. No. C30010010)

2.9. Sonicate sample using the Bioruptor. Choose the protocol which is adapted to your device:

- When using the Bioruptor Pico shear for 8-10 cycles [30 seconds "ON", 30 seconds "OFF"].
- When using the Bioruptor Plus or Standard, shear for 15-20 cycles [30 seconds "ON", 30 seconds "OFF"] each at High power setting.

***NOTE**: An additional optimization of sonication parameters might be required.*

2.10. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Collect the supernatants to a new 1.5 ml tubes and keep it on ice.

2.11. Resuspend the remaining pellet in **150 µl** of **complete Shearing Buffer**

iS1b, transfer the sample to the appropriate sonication microtube and sonicate for a **second round** using the above sonication settings.

2.12. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Transfer the supernatants to 1.5 ml tubes containing the sheared chromatin from the previous step and keep it on ice.

2.13. Resuspend the remaining pellet in **150 µl** of **complete Shearing Buffer iS1b**, transfer the sample to the appropriate sonication microtube and sonicate for a third round the above sonication settings.

2.14. Briefly spin down the liquid in the samples for 15 seconds. Transfer samples to new 1.5 ml tubes and centrifuge at 16,000 x g at 4°C for 10 minutes. Combine the supernatant with the sheared chromatin from the previous steps in 1.5 ml tube.

2.15. Add **1 µl** of **RNase cocktail** (user-supplied) to the chromatin pool and incubate at 37°C for 15 minutes. Keep everything at 4°C or on ice from now on.

2.16. Precisely estimate the volume of the chromatin pool. Take an aliquot of **5 µl** and quantify the DNA concentration using dsDNA HS Assay Kit on the Qubit® system. Determine the total DNA yield in the chromatin pool.

***NOTE**: Although the DNA quantification before de-crosslinking is not very precise, it can be used for a rough estimation of chromatin yield. The minimum DNA amount required per one IP is about 300 ng. However, a higher starting amount is recommended when possible.*

2.17. From this chromatin pool, take an aliquot of 50 µl of sheared chromatin for the shearing assessment. The protocol for chromatin shearing analysis is described in a separate section "Protocol for chromatin shearing analysis".

2.18. The remaining chromatin can be stored at -80°C for up to 2 months and used for immunoprecipitation later.

Additional protocol

Protocol for deparaffinization and rehydratation using xylene

Required reagents (user-supplied):

- Xylene
- Ethanol 100%
- Ethanol 95%
- Ethanol 70%
- Ethanol 50%
- Ethanol 20%

1. Cut sections up to **10 µm** from FFPE blocks and collect directly in 1.5 ml tube with tweezers. Use up to **6 sections** per **one chromatin preparation**.

NOTE: If sections are not used immediately, store the material at -80 °C. The exact numbers and thickness of sections should be defined by user.

2. Under a fume hood, add **1 ml** of **Xylene** and incubate for 10 minutes at room temperature.

3. Centrifuge at 16.000 x g for 3 minutes at room temperature.

4. Carefully discard the supernatant and repeat Steps 2–3 four more times, total five times.

NOTE: make sure that tiny tissue fragments are not discarded with the supernatant. Repeat the centrifugation step if required.

5. Resuspend the deparaffinized tissue in **1 ml** of 100% ethanol.

6. Incubate for 10 minutes at room temperature.

7. Centrifuge at 16.000 x g for 5 minutes at room temperature. Carefully discard the supernatant.

8. Repeat Steps 5-7 using **1 ml** of **95% EtOH**.

9. Repeat Steps 5-7 using **1 ml** of **70% EtOH**.

10. Repeat Steps 5-7 using **1 ml** of **50% EtOH**.

11. Repeat Steps 5-7 using **1 ml** of **20% EtOH**.

12. Let the sample dry at room temperature for 1-2 minutes and proceed to the Step 2C: Chromatin extraction and shearing.

PROTOCOL FOR CHROMATIN SHEARING ANALYSIS

STEP 3

Chromatin shearing assessement

FOR CELLS, TISSUES, FFPE SAMPLES

Workflow for analysis of sheared chromatin:

- RNAse treatment (1h, optional but highly recommended for an accurate size assessment)
- Reverse **cross-linking** (4h or overnight)
- DNA purification
- Fragment size assessment (agarose gel or Fragment Analyzer) (1 h)

RNAse treatment

***NOTE**: RNase cocktail (e.g. Ambion, AM2286A) is not supplied with this kit.*

1. Take **50 µl of sheared chromatin** (step 2.10 for cells or step 2.15 for tissues in the protocol) and transfer to a 1.5 ml microtube.
2. Dilute **1 µl of RNase cocktail** (e.g. Ambion, AM2286A) in **150 µl of ChIP-seq grade water**.
3. Add **2 µl of diluted RNase cocktail** to the aliquot of sheared chromatin.
4. Incubate for 1 hour at 37°C.

Reverse cross-linking

5. Add **50 µl of Elution Buffer iE1** and **4 µl** of **Elution Buffer iE2** to the sample, mix thoroughly.
6. Incubate samples at 65°C for 4 hours (or overnight) with shaking.

7. Purify DNA using a method of choice (reagents not included in the kit):
   - Option A: IPure kit (Diagenode, Cat. No. C03010014)
   - Option B: MicroChIP DiaPure columns (Diagenode, Cat. No. C03040001)
   - Option C: phenol-chloroform extraction

Option A - DNA purification using the IPure Kit v2

***NOTE**: Before the first use of the kit, prepare Wash Buffer 1 and Wash Buffer 2 by adding an equal volume of isopropanol. Wash Buffers 1 and 2 should be stored at 4°C. Never leave the bottle open during storage to avoid evaporation. This option is not recommended for FFPE samples.*

8. Add **2 µl of carrier** to the sample.

9. Add **108 µl of 100% isopropanol** to the samples.

   ***NOTE:** Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.*

10. Resuspend the **IPure beads v2** by vortexing and add **20 µl** to the sample.

11. Incubate samples for 10 minutes at room temperature on the DiaMag Rotator.

12. Briefly spin the tubes, place in the DiaMag1.5 magnetic rack, wait 1 minute and discard the buffer.

13. Add **100 µl of Wash Buffer 1** per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for 30 seconds at room temperature. Briefly spin the tubes, place in the DiaMag1.5 magnetic rack, wait 1 minute and discard the buffer.

14. Add **100 µl of Wash Buffer 2** per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for 30 seconds at room temperature. Briefly spin the tubes and place them into the DiaMag 1.5 magnetic rack, wait 1 minute and discard the buffer.

15. Spin the tubes again and place them on the DiaMag1.5. Discard the remaining Wash Buffer 2 if necessary. Resuspend the beads pellet in

25 µl of Buffer C. Incubate at room temperature for 15 minutes on the DiaMag Rotator.

16. Spin the tubes and place them into the DiaMag 1.5, wait 1 minute and transfer the supernatants into a new 1.5 ml tube. Discard the beads and keep the supernatant containing purified DNA.

17. Analyze the purified DNA on a 1.5% agarose gel. Load around 300 ng of DNA for an optimal separation and visualization. Alternatively, you can use a Fragment Analyzer (Standard Sensitivity NGS Fragment Analysis Kit (DNF-473)). Chose the shortest sonication time resulting in a satisfactory shearing profile. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments, especially for non-histone proteins.

Option B - DNA purification using MicroChIP DiaPure columns

8. Proceed to DNA purification using MicroChIP DiaPure columns. Add 0.5 ml of ChIP DNA Binding Buffer to each sample and mix briefly.

9. Transfer the mixture to a provided spin column in a collection tube and centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through.

10. Add **200 µl** of **DNA Wash Buffer** to the column. Centrifuge at ≤10,000 x g for 30 seconds.

11. Add **200 µl** of **DNA Wash Buffer** to the column. Centrifuge at ≤10,000 x g for 30 seconds.

12. Add **50 µl** of **DNA Elution Buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≤10,000 x g for 30 seconds to elute the DNA.

    NOTE: *Use 6 µl of DNA elution buffer in a case of ChIP-FFPE workflow in order to ensure the sufficient DNA concentration per analysis.*

13. Analyze the purified DNA using an agarose gel electrophoresis or Fragment Analyzer (Standard Sensitivity NGS Fragment Analysis Kit (DNF-473). Chose the shortest sonication time resulting in a satisfactory shearing profile. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments, especially for non-histone proteins.

NOTE: *Fragment Analyzer and High Sensitivity NGS Fragment Analysis Kit (DNF-474) should be used for FFPE samples.*

Option C - DNA purification using phenol-chloroform extraction

8. Under a fume hood, add an **equal volume** of **phenol/chloroform/isoamyl alcohol (25:24:1)** to the sample. Vortex thoroughly for 1 minute.
9. Centrifuge for 2 minutes at 16,000 x g at room temperature. Transfer the top aqueous phase into a new 1.5 ml tube.
10. Add an **equal volume** of **chloroform/isoamyl alcohol (24:1)** to the sample. Vortex thoroughly for 1 minute.
11. Centrifuge for **2 minutes** at 16,000 g at room temperature. Transfer the top aqueous phase into a new 1.5 ml tube.
12. Add **10 µl** of **DNA precipitant**, **5 µl** of **co-precipitant**, and **500 µl** of cold **100% ethanol** to the sample. Vortex thoroughly for 1 minute and incubate at -80 °C for 30 minutes (or at -20°C for 1 hour).
13. Centrifuge for 25 minutes at 16,000 x g at 4°C. Carefully remove the supernatant and add **500 µl** of ice-cold **70% ethanol** to the pellet.
14. Centrifuge for 10 minutes at 16,000 x g at 4°C. Carefully remove the supernatant and let the sample dry at room temperature for 2-5 minutes.
15. Re-suspend the pellet in **20 µl** of **TE Buffer**.

NOTE: *In case of FFPE sample re-suspend the pellet in 6µl of TE Buffer.*

16. Analyze the purified DNA using an agarose gel electrophoresis or Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit (DNF-473).
17. Chose the shortest sonication time resulting in a satisfactory shearing profile. Avoid over-sonication, as it may lead to a drop of efficiency in ChIP experiments, especially for non-histone proteins.

Related products

Product	Cat. No.
ChIP Cross-link Gold	C01019027
Chromatin EasyShear Kit – Low SDS	C01020013
Chromatin EasyShear Kit - Ultra Low SDS	C01020010
Chromatin shearing optimization kit – High SDS	C01020012
Chromatin shearing optimization kit – for Plant	C01020014
IPure Kit v2	C03010014
MicroChIP DiaPure columns	C03040001
iDeal ChIP-seq kit for Transcription Factors	C01010055
iDeal ChIP-qPCR kit	C01010080
iDeal ChIP-FFPE kit	C01010190
Bioruptor Pico	B01080010
Bioruptor Plus	B01020001

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