diagenode

Innovating Epigenetic Solutions

Chromatin EasyShear Kit High SDS

Previous name: Chromatin shearing optimization kit - High SDS

Cat. No. **C01020012**

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Content

MANUAL

Introduction

The first critical step of a successful Chromatin Immunoprecipitation (ChIP) experiment is the preparation of sheared chromatin. We therefore suggest the use one of our optimized Shearing ChIP kits.

- Chromatin Shearing Optimization kit - **Low** SDS (Cat. No. C01020010)
- Chromatin Shearing Optimization kit - **Medium** SDS (Cat. No. C01020011)
- Chromatin Shearing Optimization kit - **High** SDS (Cat. No. C01020012)
- Chromatin Shearing Optimization kit - **Low** SDS (for iDeal ChIP-seq kit for TFs) (Cat. No. C01020013)

Our Chromatin Shearing Optimization kits are used in combination with the Bioruptor® in order to ensure a highly reproducible chromatin shearing and make sure you obtain the right fragment size needed for your experiment. Establish optimal conditions required for shearing cross-linked chromatin (protein-DNA) is usually laborious; the protocol of the Chromatin Shearing Optimization kits is fast, easy-to-use and optimized to get the best results possible.

The ChIP assay offers great potential to improve knowledge about the regulation of gene expression. This technique is now used in a variety of life science disciplines including cellular differentiation, tumor suppressor gene silencing, and the effect of histone modifications on gene expression.

Isolation and shearing of the chromatin after formaldehyde fixation is the first step in the ChIP method. The following two steps are: 1/ specific immunoprecipitation of chromatin fragments using antibody against the protein of interest and 2/ analysis of the immunoprecipitated DNA fragment. With the present kit, the first step of the ChIP method is guaranteed, providing you with the best results.

Using standard protocols and kits leads to a significant decrease in potential variables that can occur from shearing to ChIP, which makes interpretation and analysis from experiment to experiment much more accessible.

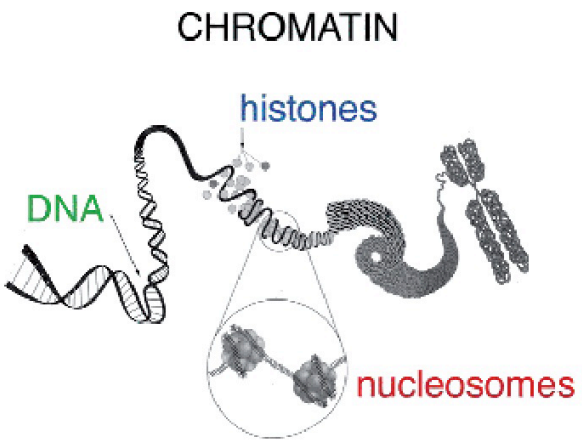

Figure 1

Representation of the chromatin

Chromatin is that portion of the cell nucleus which contains all the DNA of the nucleus in animal or plant cells. DNA in chromatin is organised in arrays of nucleosomes. Two copies of histone proteins are assembled into an octamer that has 145-147 base pairs (bp) of DNA wrapped around it to form a nucleosome core. The nucleosome, in its role as the principal packaging element of DNA within the nucleus, is the primary determinant of DNA accessibility.

(Luger et al. 1997)

Kit method overview & time table

Table 1 : Protocol overview

Step		Time needed
1	Cell collection and DNA-protein crosslinking	1 hour
2	Cell lysis and Chromatin shearing	1 hour
3	Sheared chromatin analysis	1 day

Kit Content

Table 2: Kit content and storage

This kit contains enough reagents for the shearing of chromatin from 100 million cells. These components are identical to the ones included in the LowCell# ChIP kit (Cat. No. C01010070, C01010072, C01010071 and C01010073) and True MicroChIP kit (Cat.No C01010130).

Description	Quantity	Storage
Glycine	17.25 ml	4°C
Lysis Buffer tL1*	3.75 ml	4°C
Elution Buffer tE1	15 ml	4°C
Elution Buffer tE2	1200 µl	4°C
TE buffer	1.5 ml	4°C
Precipitant tP1	3 ml	4°C
Co-precipitant tCP1	750 µl	-20°C
Protease inhibitor cocktail	75 µl	-20°C

*** Lysis Buffer tL1** is equivalent to the **Buffer B** from the LowCell#ChIP kit

Required Materials Not Provided

Reagents

- Formaldehyde (37% stock)
- Ice-cold PBS buffer
- Agarose and TAE buffer
- DNA molecular weight marker
- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- 100% Ethanol
- 70% Ethanol

- TE buffer
- Cell culture medium (protocol A compatible with True MicroChIP kit)
- Hank's balanced salt solution (HBSS) (protocol A compatible with True MicroChIP kit)

Equipment

- Cell counter
- Centrifuges (at 4°C) for 1.5 ml tubes and 15 ml tubes
- Bioruptor® from Diagenode (with recommended consumables)
- Agarose gel apparatus
- 30°C incubator or water bath
- Thermomixer (37°C and 65°C)
- Vortex

General remarks before starting

Before starting the ChIP, the chromatin should be sheared to fragments in the 100 to 600 bp range. Our kits and protocols are optimized for Chromatin shearing using the Bioruptor® (Pico, Plus and Standard).

The present Chromatin Shearing optimization kit – High SDS allows optimising the chromatin preparation compatible with Diagenode True MicroChIP kit (Cat. No. C01010130) (protocol A) or LowCell#ChIP kit (C01010072) (protocol B). Please refer to a corresponding protocol in the manual.

Please note that **Lysis Buffer tL1** is equivalent to the **Buffer B** from the LowCell#ChIP kit.

We recommend optimizing sonication conditions for each cell type before processing large quantities of cells or samples. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation.

Please refer to tables below to choose appropriated tubes corresponding to your experimental setting (a sample volume and a model of Bioruptor)

Recommended sonication tubes and sample volume for the Bioruptor Pico®

Description	Cat. No.	Minimum volume per tube	Maximum volume per tube
0.1 ml Bioruptor® Microtubes	C30010015	5 µl	50 µl
0.65 ml Bioruptor® Microtubes	C30010011	100 µl	100 µl
1.5 ml Bioruptor® Microtubes with Caps	C30010016	100 µl	300 µl
15 ml Bioruptor® Tubes & sonication beads	C01020031	500 µl	2 ml

Recommended sonication tubes and sample volume for the Bioruptor Plus and Standard®

Description	Cat. No.	Minimum volume per tube	Maximum volume per tube
0.5 ml Bioruptor® Plus Microtubes	C30010013	50 µl	100 µl
1.5 ml Bioruptor® Plus TPX microtubes	C30010010-1000	100 µl	300 µl
15 ml Bioruptor® Plus TPX tubes	C30010009	500 µl	2 ml

Remarks before starting: protocol A (Compatible with True MicroChIP kit)

This protocol has been optimized for shearing of 10 000 cells in 100 µl using the Diagenode's Bioruptor® and then subsequent immunoprecipitation on 10 000 cells in 200 µl. Determine the number of IP you will perform and start with fixation of a unique batch of chromatin. For example, if you would like to perform 4 ChIP on the same chromatin, start with fixation of 40 000 cells. Add also an extra chromatin preparation to use for the input.

Due to the low amount of starting material it is critical to avoid sample loss throughout the experiment to ensure reproducible and consistent results. Avoid pipetting up and down when adding buffers to samples. It is also recommended to use low retention Eppendorf tubes (except for sonication step which should be done using specific Bioruptor tubes) at each step of the protocol to minimize sample lost.

The use of an automated cell counter is also recommended to reduce variations in the amount of the starting cell number. The protocol is also compatible with higher cell numbers. Efficient shearing has been validated in a cell range from 10 000 cells to 100 000 cells to allow performing ChIP assay on 10 000 to 100 000 cells. Determine the number of cells you would like to use per ChIP reaction (between 10 000 and 100 000 cells) and perform shearing on that cell number. Fixation can be done on larger cell numbers (scale accordingly volumes of Lysis Buffer tL1 and HBSS to use) and cell lysate will then be split into 100 µl aliquots (corresponding to the number of cells that will be use per IP reaction) before shearing. Then sheared chromatin will be diluted two times with ChIP Buffer tC1 before performing the immunoprecipitation.

Nevertheless analysis of shearing efficiency is not obvious when working with 10 000 cells due to the low amount of DNA recovered after sonication and crosslinking reversion for subsequent analysis on agarose gels. Therefore at least 6 replicates should be performed to check the shearing efficiency and pooled before loading onto agarose gel.

The microfluidics technology (Fragment Analyzer from Advanced Analytical or Agilent BioAnalyzer 2100) is more sensible alternative to an agarose gel. The shearing efficiency from as little as 10.000-20.000 cells can be analysed. However, some inconsistencies between agarose gel and BioAnalyzer 2100 profiles have been documented (over-estimation of high molecular weight fragments using BioAnalyzer).

Remarks before starting: protocol B (Compatibles with LowCell#ChIP kit)

This protocol has been optimized for shearing of 1 million to 100,000 cells in 130 µl Buffer tL1 using the Diagenode's Bioruptor®. The sheared chromatin is diluted 10x with buffer A (included in LowCell#ChIP kit) and used for subsequent immunoprecipitation on 10.000-100.000 cells in 100 µl.

Please check table for number of cells needed per IP.

Note that another one hundred thousand to one million cells are needed to check shearing efficiency.

	Number of cells per IP						
Number of cells per Shearing		100,000	50,000	20,000	10,000	5,000	1,000
	1 million	Buffer B (130 µl) Buffer A (870 µl) **10 IP's**	Buffer B (130 µl) Buffer A (1,870 µl) **20 IP's**	Buffer B (130 µl) Buffer A (4,870 µl) **50 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**	-	-
	500,000	Buffer B (50 µl) Buffer A (450 µl) **5 IP's**	Buffer B (50 µl) Buffer A (870 µl) **10 IP's**	Buffer B (50 µl) Buffer A (2,370 µl) **25 IP's**	Buffer B (50 µl) Buffer A (4,870 µl) **50 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**	-
	100,000	-	-	Buffer B (50 µl) Buffer A (450 µl) **5 IP's**	Buffer B (50 µl) Buffer A (450 µl) **10 IP's**	Buffer B (50 µl) Buffer A (450 µl) **20 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**

Example: If you shear 1 million cells and if you want to perform your ChIP with chromatin equivalent to 50,000 cells, we recommend to shear your cells in 130 µl **Buffer B** with Protease Inhibitor and to dilute the sheared chromatin with 1,870 µl **Buffer A** with Protease Inhibitor (20 IPs can be performed with this dilution).

Short protocol for experienced users

a. Protocol A (compatible with True MicroChIP kit)

The protocol below is for use with 10 000 cells per ChIP. To perform ChIP with higher cell numbers refer to « Remarks Before Starting ».

STEP 1a. Cell collection and DNA-protein crosslinking

1a. Harvest and count the cells.

2a. Add medium to cells to a final volume of 1 ml.

3a. Add 27 µl of 36, 5 % formaldehyde per 1 ml sample. Invert tube and incubate 10 minutes at RT.

4a. Add 115 µl of Glycine to the sample. Invert the tube and incubate 5 minutes at RT.

5a. Work on ice from this point onwards.

6a. Centrifuge at 300 x g for 10 minutes at 4°C. Aspirate the supernatant slowly.

7a. Wash cells with 1 ml ice-cold HBSS with inhibitors. Invert the tube to resuspend the cells and centrifuge at 300 x g for 10 minutes at 4°C. Aspirate the supernatant and keep the cell pellet on ice.

STEP 2a. Cell lysis and chromatin shearing

8a. Add 25 µl of complete Lysis Buffer tL1 (Lysis Buffer tL1 + Protease Inhibitor Cocktail - PIC) per 10 000 cells and agitate manually the bottom of the tube to resuspend the cells.

9a. Incubate on ice for 5 minutes.

10a. Add 75 µl of complete HBSS (HBSS + PIC) per 10 000 cells and sonicate aliquots of 10 000 cells (in 100 µl) for 1 to 5 runs of 5 cycles of: [30 seconds "ON", 30 seconds "OFF"] using the Bioruptor®. Optimization is needed depending on the cell type and the Bioruptor® model used.

11a. Centrifuge at 14,000 x g for 10 minutes and collect the supernatant.

12a. Assess shearing efficiency.

b. Protocol B (compatible with LowCell#ChIP kit)

STEP 1b. Cell collection and DNA-protein crosslinking

1b. Add inhibitors before cell harvest and mix gently. Harvest, wash, and then count cells.

2b. Add PBS (including inhibitors) to cells to a final volume of 500 µl.

3b. Add 13.5 µl of 36.5% formaldehyde per 500 µl sample. Vortex gently and incubate 8 minutes at RT.

4b. Add 57 µl of 1.25 M glycine to the sample. Vortex gently and incubate 5 minutes at RT.

5b. Work on ice from this point onwards.

6b. Centrifuge at 470 x g for 10 minutes at 4°C. Aspirate the supernatant slowly – leave 30 µl behind.

7b. Wash cells twice with 0.5 ml ice-cold PBS with inhibitors. Gently vortex and centrifuge at 470 x g for 10 minutes at 4°C.

STEP 2b. Cell lysis and chromatin shearing

8b. Aspirate the supernatant and leave 10-20 µl behind.

9b. Make complete Buffer tL1 by adding protease inhibitor and NaBu. Add 130 µl of complete Buffer B to cells and resuspend.

10b. Sonicate using the Bioruptor®.

11b. Assess shearing efficiency.

MANUAL

Detailed protocol

a. Protocol A (compatible with True MicroChIP kit)

The protocol below is for use with 10 000 cells per ChIP. To perform ChIP with higher cell numbers refer to « Notes Before Starting ».

STEP 1a. Cell collection and DNA-protein crosslinking

1a. Prepare and harvest cells as follows:

ii. Place PBS, cell culture medium and trypsin-EDTA at room temperature (RT).

iii. If using adherent cells, discard medium to remove dead cells. Wash cells by adding 10 ml PBS.

4a. Detach cells by trypsinization. Collect cells by adding culture medium and transfer the medium with cells in a 15 ml centrifugation tube. Use culture medium containing serum (you can the same medium as the one used for culturing the cells). Centrifuge 5 minutes at 1,300 rpm.

5a. Keep the cell pellet and discard the supernatant.

6a. If using suspension cells, centrifuge for 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant.

7a. Resuspend the cells in cell culture medium. You should have at least 10 000 cells per ml of cell culture medium. Count the cells.

8a. Label new 1.5 ml tube(s). Add medium to a final volume of 1 ml after the cells have been added. To determine the amount of cells to use for fixation, determine the number of immunoprecipitation you will perform and start fixation of a unique batch of chromatin (see also notes before starting).

9a. Add 27 µl of 36.5% formaldehyde per 1 ml of sample (final concentration should be ~1%) and invert tubes immediately two to three times to ensure complete mixing.

10a. Incubate for 10 minutes at room temperature to enable fixation with occasional manual agitation. Optimization of fixation time may be required depending cell type, it could be 8-10 minutes.

11a. Add 115 µl of Glycine to the sample.Mix by inversion of the tube four to five times. Incubate for 5 minutes at room temperature to stop the fixation. Work on ice from this point onwards.

12a. Centrifuge at 300 x g for 10 minutes at 4°C.

- We recommend the use of a swing-out rotor with soft settings for deceleration.

13a. Aspirate the supernatant slowly and leave approximately 30 µl of the solution. Do not disturb the pellet. Take care to not remove these cross-linked cells. Wash the cross-linked cells with 1 ml of ice cold HBSS containing protease inhibitor cocktail (PIC, 200x; final concentration 1x).

- Add 1 ml of HBSS and invert the tube four to five times to resuspend the cells.
- When working with higher cell numbers (100 000 cells and more) you should gently vortex to completely resuspend the cells.
- Centrifuge at 300 x g for 10 minutes at 4°C (in a swing-out rotor with soft settings for deceleration).

14a. Discard the supernatant and keep the cell pellet on ice. Proceed directly to cell lysis or, if desired, the cell pellets can be store at -80°C for up to 2 months.

STEP 2a. Cell lysis and chromatin shearing

15a. Prepare Lysis Buffer. Add protease inhibitor cocktail (1x final concentration) to Lysis Buffer tL1 (RT). This is the complete Lysis Buffer tL1. Keep the buffer at room temperature until use. Discard what is not used within a day.

Make sure that there are no crystals in the Lysis Buffer tL1 before using. Gently heat and mix until crystals disappear.

16a. Add complete Lysis Buffer tL1 to the cells. Use 25 µl of complete Lysis Buffer tL1 per 10 000 cells. Agitate manually the bottom of the tube to resuspend the cells and allow bubbles to form. Scale accordingly when using higher numbers of cells.

17a. Incubate on ice for 5 minutes to ensure complete cell lysis.

18a. Add HBSS containing protease inhibitor cocktail (PIC, 200x; final concentration 1x) to the cell lysate. Use 75 µl of complete HBSS per 10 000 cells. Scale accordingly when using higher numbers of cells.

19a. Dispense 100 µl of cell lysate (equivalent to 10 000 cells) into 1,5 ml microtubes. If cell lysis was performed on more than 10 000 cells, make sure that there are no precipitate before spliting cell lysate into 100 µl aliquots. Otherwise gently heat until crystals disappear.

20a. Split into aliquots of 100 to 300 µl and transfer the cell suspension to 1.5 ml TPX microtubes (Cat. No.C30010010-1000) when using the Bioruptor Standard or Plus or to 1.5 ml Bioruptor® Microtubes with Caps (Cat No C30010016) optimized for chromatin shearing with the Bioruptor® Pico.

21a. Shear the chromatin by sonication using the Bioruptor®. When using the Bioruptor Standard or Plus, shear for 1 to 3 runs of 10 cycles [30 seconds "ON", 30 seconds "OFF"] each at high power setting. Briefly vortex and spin between each run. Shear for 5-10 cycles [30 seconds "ON", 30 seconds "OFF"] when using the Bioruptor Pico. These shearing conditions will work excellent for many cell types. However, depending on the cell type and Bioruptor® system used, optimisation may be required.

22a. Transfer sheared samples to a new 1.5 ml standard tube and centrifuge at 14,000 x g (13,000 rpm) for 10 minutes and collect the supernatant which contains the sheared chromatin.

23a. Assess shearing efficiency. The remaining sheared chromatin can be used directly in ChIP experiments or stored for up to 2 months at -80°C.

- Do not freeze/thaw.

STEP 3a. Sheared chromatin analysis

A minimum of 60 000 cells is needed to be visualized on agarose gel. If each 100 µl of sheared chromatin correspond to 10 000 cells, then perform 6 reactions in parallel and pool the DNA pellets obtained at Step 37a during respuspension in TE.

When using a microfluidic system (Fragment Analyser, BioAnalyser), 10.000-20.000 cells are enough to be visualized.

24a. Take an aliquot of 100 µl of sheared chromatin and spin the chromatin at 14,000 x g (13,000 rpm) for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.

25a. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1 µl of cocktail in 150 µl of water).

26a. Add 2 µl of diluted RNase cocktail to the chromatin.

27a. Incubate 1h at 37°C.

28a. Add 100 µl of the Elution Buffer tE1 and 8 µl of Elution Buffer tE2 to each chromatin sample.

29a. Mix thoroughly and incubate samples at 65°C for 4 hours (or overnight).

30a. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the samples at RT for 10 minutes on a rotating wheel.

31a. Centrifuge for 2 minutes at 14,000 xg (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

32a. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the samples at RT for 10 minutes on a rotating wheel.

33a. Centrifuge for 2 minutes at 14,000 x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

34a. Precipitate the DNA by adding precipitant tP1 (1/10 of the volume) and 2 µl of co-precipitant tCP1. (OPTIONAL: when DNA is isolated from low cell amounts, add also 2 µl of co-precipitant tCP2). Add 1 ml of cold 100% ethanol. Vortex and incubate at -80°C for 30 minutes.

35a. Centrifuge for 25 minutes at 14,000 x g (13,000 rpm) at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70 % ethanol to the pellet.

36a. Centrifuge for 10 minutes at 14,000 x g (13,000 rpm) at 4°C. Carefully remove the supernatant, leave tubes opened for 30 minutes at RT to evaporate the remaining ethanol.

37a. For agarose gel analysis: re-suspend 6 DNA pellets in 10 µl of TE. Run samples (10 µl of DNA + 2 µl of 6x loading dye) in a 1.5% agarose gel along with DNA size marker to visualise shearing efficiency. For microfluidic analysis: re-suspend DNA pellet in 1-2 µl of TE and follow microfluidic instrument instructions for analysis. Use high sensitivity kit.

b. Protocol B (compatible with LowCell#ChIP kit)

STEP 1b. Cell fixation and collection

1b. Prepare and harvest cells as follows:

- Immediately before cell harvesting, if necessary, add any desired inhibitors (eg. HDAC inhibitors such as 20mM NaBu) to the culture medium and mix gently. If adding sodium butyrate* (NaBu) from a 1M stock solution, dilute to reach a final concentration of 20mM. The protocol below includes the use of NaBu-PBS for histone ChIPs. The complete PBS mentioned below refers to PBS with inhibitor(s). *** HDAC inhibitor (available separately; Cat. No. C12020010).**
- Place PBS at room temperature (RT).
- If using adherent cells, discard medium to remove dead cells. Wash cells by adding 10 ml PBS. Harvest cells by trypsinization using trypsin with inhibitor(s). Transfer cells in a tube containing 10 ml PBS (RT), and centrifuge 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant. Optionally, before transferring, the trypsin can be inactivated by adding serum and inhibitors (if necessary. Then wash the cells in complete PBS and discard medium.
- If using suspension cells, centrifuge for 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant. Wash cells by adding 10 ml PBS (RT) containing 20 mM sodium butyrate (NaBu-PBS) or any other inhibitor.

2b. Count the cells (e.g. determine the number of cells in about 200 µl of your sample).

3b. Label new 1.5 ml tube(s). Add PBS to a final volume of 500 µl after the cells have been added. If necessary add inhibitors. Then, transfer cells and wash the pipette tip thoroughly in the sample.

4b. In order to preserve the cells, use either a 1000 µl pipette tip or a smaller tip that has been cut in order to increase the opening.

5b. Add 13.5 µl of 36.5% formaldehyde per 500 µl of sample (final concentration should be ~1%).

6b. Mix by gentle vortexing. Incubate for 8 minutes at room temperature to enable fixation. Optimization of fixation time may be required depending cell type, it could be 8-10 minutes.

7b. Add 57 µl of 1.25 M Glycine to the sample.

8b. Mix by gentle vortexing. Incubate for 5 minutes at room temperature to stop the fixation. Work on ice from this point onwards.

9b. Centrifuge at 470 x g for 10 minutes at 4°C.

- We recommend the use of a swing-out rotor with soft settings for deceleration.

10b. Aspirate the supernatant slowly and leave approximately 30 µl of the solution. Do not disturb the pellet. Take care to not remove these cross-linked cells.

11b. Wash the cross-linked cells twice with 0.5 ml of ice cold PBS with 20mM final concentration NaBu and/or any other inhibitor of choice.

- Add the NaBu-PBS solution, gently vortex and centrifuge at 470 x g (in a swing-out rotor with soft settings for deceleration) for 10 minutes at 4 °C.
- For 100,000 cells or more you might need to resuspend with a pipette to ensure cells are thoroughly washed.
- Smaller cell numbers are more easily washed and resuspended by vortexing.
- Make sure that cells are in suspension before proceeding to the next point.

STEP 2b. Cell lysis and chromatin shearing

Place the buffer tL1 at room temperature (RT) before use.
Work on ice unless otherwise stated.After the last wash, aspirate the supernatant, and leave 10 to 20 µl behind to avoid material loss.

12b. Prepare Buffer B. Add protease inhibitor (1x final concentration) and if necessary, NaBu (20mM final concentration) to Buffer B (RT). This is the complete Buffer B. Keep the buffer at room temperature until use. Discard what is not used within a day.

Make sure that there are no crystals in the Buffer B. Gently heat and mix until crystals disappear.

13b. Add complete buffer tL1 to cells. See Table 4 in Step 2 "Cell collection and DNA-protein cross-linking." Refer to Table for the volume of complete buffer tL1 to add to cells. Vortex until resuspended. Incubate for 5 minutes on ice and transfer the cell suspension to 1.5 ml TPX microtubes (Diagenode cat. No. C30010010-1000) when using the Bioruptor Standard or Plus or to 1.5 ml Bioruptor® Microtubes with Caps (Cat No C30010016) optimized for chromatin shearing with the Bioruptor® Pico.

14b. Sonicate samples to shear the chromatin using the Bioruptor® Shear the chromatin by sonication using the Bioruptor®. When using the Bioruptor Standard or Plus, shear for 1 to 3 runs of 10 cycles [30 seconds "ON", 30 seconds "OFF"] each at high power setting. Briefly vortex and spin between each run. Shear for 5-15 cycles [30 seconds "ON", 30 seconds "OFF"] when using the Bioruptor Pico. These shearing conditions will work excellent for many cell types. However, depending on the cell type and Bioruptor® system used, optimisation may be required.

15b. Assess shearing efficiency. The remaining sheared chromatin can be used directly in ChIP experiments or stored for up to 2 months at -80°C.

- Do not freeze/thaw.

STEP 3b. Sheared chromatin analysis

16b. Take an aliquot of 100 µl of sheared chromatin and spin the chromatin at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.

17b. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1µl of cocktail in 150 µl of ChIP-seq grade water).

18b. Add 2 µl of diluted RNase cocktail.

19b. Incubate 1h at 37°C.

20b. Add 100 µl of elution buffer tE1.

21b. Add 8 µl of elution buffer tE2, mix thoroughly.

22b. Incubate samples at 65°C for 4h (or overnight).

23b. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the sample at RT for 10 min on a rotating wheel.

24b. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

25b. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.

26b. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

27b. Precipitate the DNA by adding 20 µl DNA precipitant tP1, 5 µl of co-precipitant tCP1 and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.

28b. Centrifuge for 25 min at 13,000rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.

29b. Centrifuge for 10 min at 13,000 rpm at 4°C. Carefully remove the supernatant, leave tubes open for 30 min at RT to evaporate the remaining ethanol.

30b. Re-suspended the pellet in 20 µl of TE buffer.

31b. Run samples (20 µl of DNA + 4 µl of 6x loading dye) in a 1.5% agarose gel.

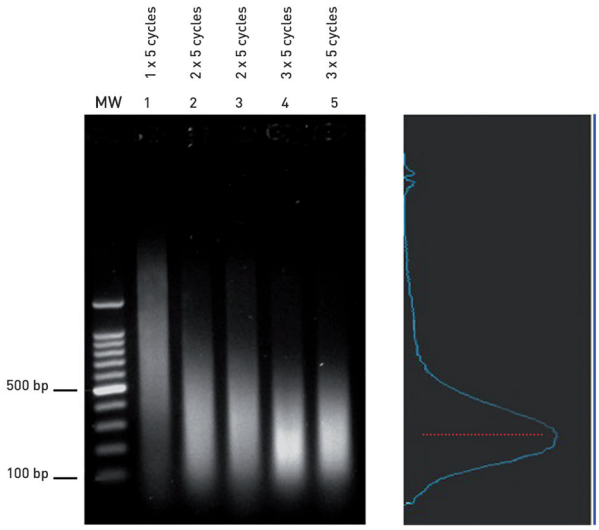

Figure 2

Efficient Chromatin shearing with the Bioruptor® PLUS using the buffers and protocol of Diagenode's Chromatin Shearing Optimization kit – High SDS

HeLa cells are fixed with 1% formaldehyde (for 8 min at RT). 1 million cells are resuspended in 130 µl of buffer B prior to Chromatin shearing. Samples are sheared during 1, 2 or 3 rounds of 5 cycles of 30 sec ON/ 30 sec OFF with the Bioruptor® PLUS combined with the Bioruptor® Water cooler (Cat. No. BioAcc-cool) & Single Cycle Valve (Cat. No. VB-100-0001)at HIGH power setting (position H) using 1.5 ml TPX microtubes (Cat No. M-50001). Sample viscosity may have a major impact on sonication results. Careful homogenization of chromatin sample is strongly recommended before and during sonication processing. For optimal results, samples should be gently vortexed before and after performing 5 sonication cycles, followed by a short centrifugation at 4°C to recover sample volume at the bottom of the tube.

Panel A: The sheared chromatin is then decross-linked prior to agarose gel electrophoresis. 10 µl of DNA are analyzed on a 1.5% agarose gel.The left lane shows the 100 bp DNA molecular weight marker (MW). Lane 1: 1x 5 cycles (each 30 sec ON/30 sec OFF); Lane 2 and 3 (duplicate): 2x 5 cycles (each of 30 sec ON/30 sec OFF); Lane 4 and 5 (duplicate): 3x 5 cycles (each 30 sec ON/30 sec OFF).

Panel B: Gel image was captured with Gel Logic 1500 Imaging System (Kodak). Lane 4 was scanned using Kodak Molecular Imaging Software (v 4.0.5) to estimate the DNA size distribution and concentration.

In the given example, the optimal shearing condition corresponds to 3 rounds of 5 cycles (30 sec ON/30 sec OFF) corresponding to the optimal DNA size range for ChIP-seq analysis (100 - 600 bp).

Troubleshooting guide

Critical steps	Troubles, solutions and comments	
Cross-linking	Cross-linking is too weak.	Make sure you perform the fixation step for the correct period of time, at the right temperature and with the correct formaldehyde concentration. e.g: incubate for 10-20 minutes at room temperature with 1 % formaldehyde final concentration (weight/ volume). Also, use high quality, fresh formaldehyde.
	Cross-linking is too strong.	
	Proteins have unique ways of interacting with the DNA. Some proteins are not directly bound to the DNA but interact with other DNA-associated proteins.	Very short or very long cross-linking time can lead to DNA loss and/or elevated background, therefore the optimal cross-linking time should be found empirically as maximal specificity and efficiency of ChIP.
	Both cross-linking time and formaldehyde concentration are critical.	Cross-linking can affect both efficiency of chromatin shearing and efficiency of specific antigen immunoprecipitation. Shorter cross-linking times (5 to 10 minutes) and/or lower formaldehyde concentrations (<1%, weight/ volume) may improve shearing efficiency while, for some proteins especially those that do not directly bind DNA, this might reduce the efficiency of cross-linking and thus the yield of precipitated chromatin.
	The optimal duration of cross-linking varies between cell type and protein of interest.	It is possible to optimize the fixation step by testing different incubation times: such as 10, 20 and 30 minutes. Do not cross-link for longer than 30 minutes as cross-links of more than 30 minutes can not be efficiently sheared.
	Efficient fixation of a protein to chromatin in vivo is a crucial step for ChIP. The extent of cross-linking is probably the most important parameter.	Two major problems concerning the subsequent immunoprecipitation step should be taken into account: 1/ an excess of cross-linking can result in the loss of material or reduced antigen availability in chromatin, or both. 2/ the relative sensitivity of the antigen epitopes to formaldehyde. It is essential to perform the cross-linking step with care.
	It is essential to quench the formaldehyde.	Use glycine to stop the fixation: quench formaldehyde with 125 mM glycine for 5 minutes at room temperature (add 1/10 volume of 1.25M glycine). Alternatively, wash the fixed cells properly and make sure you get rid of ALL the formaldehyde.
Cell lysis	Temperature is critical.	Perform cell lysis at 4°C (cold room) or on ice. Keep the samples ice-cold at all times during the cell lysis and use ice-cold buffers see STEP 3.
	Protein degradation during lysis can occur.	Add the protease inhibitors to the lysis buffer immediately before use.
Cell type	Kit protocol validation.	HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells) and Kerationocytes have been used to validate the Magnetic ChIP protocol.
Chromatin shearing	Optimal shearing conditions are important for ChIP efficiency.	Shearing conditions for each cell type must be optimized from cell collection, fixation to shearing method (settings of the sonicator apparatus).
	Critical points for shearing optimization.	1) Not to start with a too high concentration of cells (15 x 10e6 cells/ml or less is ok) 2) Keep samples cold (4°C)
	Shear the samples of chromatin using the Bioruptor® Pico from Diagenode.	Maintain temperature of the samples close to 0°C. The chromatin shearing needs to be optimized for each cell type. A troubleshooting guide for Bioruptor-chromatin shearing is available at Diagenode.

Sheared chromatin analysis	Purify the DNA from the sheared chromatin as described in the kit protocol to analyse the shearing.	Extract total DNA from an aliquot of sheared chromatin and run on 1% agarose gel (stain with EtBr). In order to analyse the sheared chromatin on gel, take DNA purified from the sheared chromatin input -prepared at STEP 3 . Some unsheared chromatin can be analysed on gel as well (purify it as done with the input sample (see "6. Additional protocols" section). Chromatin eqvivalent to 100,000 cells, one million cells or more can for sure be visualized on a gel.
	Do not load too much DNA on a gel.	Loading of large quantities of DNA on agarose gel can lead to poor quality pictures, which do not reflect the real DNA fragmentation. The DNA amount to load depends on the size of the well and on the gel size.
	Agarose concentration.	Do not use more than 1-1.5% agarose gel and run slowly (Volt/cm and time depend on the gel size).
	Running buffer concentration.	1x TAE or TBE is preferred to 0.5x TAE, which can lead to smears on agarose gel.

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