diagenode

A Hologic Company

iDeal ChIP-seq kit for Transcription Factors

Cat. No. C01010054 (10 rxns)
C01010055 (24 rxns)
C01010170 (100 rxns)

USER GUIDE

Version 5 04_2023

Please read this manual carefully
before starting your experiment

Contents

Introduction

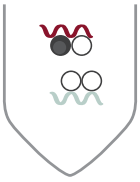
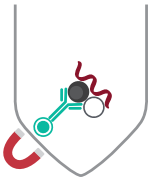
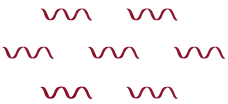
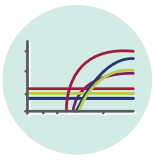
Association between proteins and DNA has a major influence on gene transcription, epigenetic silencing and other nuclear processes which control many vital cellular functions. It is crucial to understand these interactions and the mechanisms by which they control and guide gene regulation pathways and cellular proliferation. Chromatin immunoprecipitation (ChIP) is a technique to analyze the association of proteins with specific genomic regions in intact cells. ChIP can be used to study changes in epigenetic signatures, chromatin remodeling and transcription regulator recruitment to specific genomic sites.

The iDeal ChIP-seq kit for Transcription Factors provides a robust ChIP protocol suitable for investigation of transcriptional factors associated with chromatin from mammalian cells, tissues and yeast. The protocol involves protein-DNA cross-linking with formaldehyde, followed by cell lysis and fragmentation of the cross-linked chromatin. The subsequent immunoprecipitation of chromatin is performed with an antibody (user supplied) specific to a target protein. Protein A magnetic beads are used to isolate the protein-DNA complexes of interest. The immunoprecipitated DNA is eluted and purified using unique IPure magnetic beads. The eluted DNA is ideal for qPCR analysis and ChIP-seq library preparation. Additionally, the kit includes a negative (IgG) and a positive (CTCF) control antibody and primer pairs amplifying CTCF positive and negative loci.

The iDeal ChIP-seq kit for Transcription Factors offers unique benefits:

- Highly optimized protocol for ChIP-seq from cells and tissues
- Most complete kit available (covers all steps, including the control antibodies and primers)
- Validated for ChIP-seq with multiple transcription factors and non-histone targets
- Magnetic beads make ChIP easy, fast and more reproducible
- Combination with Diagenode ChIP-seq antibodies provides high yields with excellent specificity and sensitivity
- Eluted DNA suitable for any downstream application
- Easy-to-follow protocol

Kit method overview & time table

			Time needed	Day
STEP 1		Cell or tissue collection and DNA-protein cross-linking	30 minutes	1
STEP 2		Cell lysis and chromatin shearing	1 to 2 hours	1
STEP 3		Magnetic immunoprecipitation	Overnight	1-2
STEP 4		Elution, decross-linking and DNA purification	5 hours	2
STEP 5	 qPCR	Quantitative PCR and data analysis prior to library preparation and Next-Generation Sequencing	2 to 3 hours	2

LEGEND

Kit materials

The iDeal ChIP-seq kit for Transcription Factors contains enough reagents to perform the number of chromatin preparations, chromatin immunoprecipitations and DNA purifications, as described in Table 1. Reagents for chromatin size assessment of each batch of chromatin are also included.

However, additional supplies are needed for yeast and tissue workflow. Please check the list of requited material not provided.

Table 1. Number of reactions included in the different references of the iDeal ChIP-seq kit for Transcription Factors

Kit reference	Number of chromatin preparations	Number of ChIP reactions	Number of DNA purifications
C01010054	2	10	14
C01010055	4	24	32
C01010170	17	100	134

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

Table 2. Components supplied with the iDeal ChIP-seq kit for Transcription Factors

Description	Qty (x10)	Qty (x24)	Qty (x100)	Storage
Protease inhibitor cocktail	38 µl	80 µl	325 µl	-20°C
5% BSA (DNA free)	380 µl	800 µl	3.25 ml	-20°C
Rabbit IgG	4 µg	8 µg	35 µg	-20°C
ChIP-seq grade CTCF antibody	4 µg	8 µg	35 µg	-20°C
ChIP-seq grade H19 imprinting control region primer pair (human)	42 µl	96 µl	400 µl	-20°C
ChIP-seq grade Myoglobin exon 2 primer pair (human)	42 µl	96 µl	400 µl	-20°C
Carrier	32 µl	72 µl	300 µl	-20°C
Glycine	4.4 ml	8.8 ml	38 ml	4°C
Shearing Buffer iS1b	3.4 ml	6.7 ml	29 ml	4°C

DiaMag protein A-coated magnetic beads	300 µl	720 µl	3 ml	4°C DO NOT FREEZE
Wash buffer iW1	3.5 ml	8.4 ml	35 ml	4°C
Wash buffer iW2	3.5 ml	8.4 ml	35 ml	4°C
Wash buffer iW3	3.5 ml	8.4 ml	35 ml	4°C
Wash buffer iW4	3.5 ml	8.4 ml	35 ml	4°C
ChIP-seq grade water	14 ml	26,6 ml	110 ml	4°C
Elution Buffer iE2	64 µl	144 µl	600 µl	4°C
Fixation buffer	4 ml	8 ml	34 ml	4°C
Wash buffer 1 w/o iso-propanol*	900 µl	2 ml	8 ml	4°C
Wash buffer 2 w/o iso-propanol*	900 µl	2 ml	8 ml	4°C
Buffer C	700 µl	1.6 ml	6.7 ml	4°C
IPure Beads v3	180 µl	400 µl	1.67 ml	4°C DO NOT FREEZE
Elution Buffer iE1	1,5 ml	3.4 ml	14.5 ml	4°C
5x ChIP Buffer iC1b	3.4 ml	6.9 ml	29 ml	4°C
Lysis Buffer iL1b	50 ml	100 ml	425 ml	4°C
Lysis Buffer iL2	30 ml	60 ml	255 ml	4°C

*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.

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- RNase/DNase-free 1.5 ml tubes
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS) buffer
- 100% isopropanol
- qPCR SYBR® Green Mastermix
- ChIP/ ChIP-seq grade antibodies – www.diagenode.com
- Fluorescence-based assay for DNA concentration measurement, e.g. the Qubit High Sensitivity assay (Life Technologies #Q32851)

- Cell culture scraper (for adherent cells)
- (optional) Liquid nitrogen (required for tissue homogenization with the TissueLyser)

Additional supplies for tissue protocol:

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

Additional supplies for yeast protocol

- Fastprep bead beater Bio101 (MP Biomedicals)
- 2 ml screw cap tubes for Fastprep bead beater (MP Biomedicals, cat# 5076-200 and 5065-002)
- Sterile glass beads 425-600 µm (Sigma cat# G8772)
- 32% paraformaldehyde (Electron Microscopy Sciences)
- 20 mM Tris-HCl pH 7.4, 150 mM NaCl

Equipment

- Magnetic rack for 1.5 ml tubes
- Bioruptor® sonication device and the associated microtubes:
    - Bioruptor® Plus (Diagenode, Cat. No. B01020001) and 1.5 ml TPX Microtubes (Cat. No. C30010010) or
    - Bioruptor® Pico (Diagenode, Cat. No. B01060001) and 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
- Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
- Rotator (Rotating wheel)
- Vortex
- Thermomixer
- Qubit® Fluorometer (ThermoFisher Scientific)
- qPCR cycler

Optional supplies

- Chromatin EasyShear kit - Low SDS (Diagenode, Cat. No. C01020013)
- 1M Sodium butyrate (NaBu) (Diagenode, Cat. No. C12020010)
- ChIP Cross-link Gold (Diagenode, Cat. No. C01019027)
- RNase cocktail (e.g. Ambion, AM2286A), required for chromatin shearing assessment
- MicroPlex Library Preparation™ kit (Diagenode, Cat. No. C05010012, Cat. No. C05010001), or TAG kit for ChIPmentation (Diagenode, Cat. No. C01011030]

Remarks before starting

The **iDeal ChIP-seq Kit for Transcription Factors** is suitable for chromatin preparation and immunoprecipitation from mammalian cultured cells, tissues and yeast culture. Please refer to an appropriated protocol.

1. Cell number

The protocol describes the preparation of a batch of chromatin from approximately 25 million cells which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 4 million cells per IP reaction are used in this standard protocol. The protocol is optimized for the use of 250 µl of sheared chromatin in a total volume of ChIP reaction equal to 350 µl. It is crucial to keep these volumes consistent for optimal results.

Please note that 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 abundance of the target, the specificity of the antibody, and the amount of cells available, it may be possible to scale up and down the amount of cells per IP and/or start with a smaller or a bigger batch of cells.

For using lower amounts of cells per IP, you can start with a batch of 25 millions of cells (as for a standard protocol) and follow the protocol up to the chromatin shearing step. Then simply dilute the sheared chromatin in shearing buffer iS1b before adding it to the IP reaction. The final volume of diluted chromatin containing the desired amount of cells should be 250 µl per IP reaction.

If starting with an amount of cells different from the standard protocol or if you want to use a higher amount of cells per IP, first determine the number of cells that you will use per IP and the total number of IPs. 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 sheared chromatin (containing a desired amount of cells) per IP reaction
- 2.5 µl of sheared chromatin per input
- 50 µl of sheared chromatin for chromatin shearing assessment
- Add 5% excess of iS1b

Resuspend the cells in the required volume of shearing buffer iS1b and follow the standard protocol.

Please note that an increased or decreased cell concentration in the shearing buffer may impact the shearing efficiency and an additional optimization of the shearing conditions may be required.

2. Tissue amount

The protocol describes the preparation of a batch of chromatin from approximately 200 mg of tissue which is sufficient for 6 IP reactions, 1 input sample and 1 sample for chromatin shearing assessment. Approximately 30 mg of tissue per IP reaction are used in this standard protocol. The protocol is optimized for use of 250 µl of sheared chromatin in a total volume of ChIP reaction equal to 350 µl. It is crucial to keep these volumes constant for optimal 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 abundance of the target, the specificity of the antibody and the amount of tissue available, it may be possible to scale up and down the amount of tissue per IP and/or start with a smaller or a bigger

batch of tissue.

For using lower amounts of tissue per IP, start with 200 mg of tissue (as for a standard protocol) and follow the protocol up to the chromatin shearing. Then simply dilute the sheared chromatin in shearing buffer iS1b before adding it to the IP reaction. The final volume of diluted chromatin containing a desired amount of tissue should be 250 µl per IP reaction.

If starting with a tissue amount different from the standard protocol or if you want to use a higher amount of tissue per IP, first determine the amount of tissue that you will use per IP and the total number of IPs. Fix the cells as described in the standard protocol. 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 sheared chromatin (containing the desired amount of tissue) per IP reaction
- 2.5 µl of sheared chromatin per input
- 50 µl of sheared chromatin for chromatin shearing assessment
- Add 5% excess of iS1b

Resuspend the tissue in the required volume of shearing buffer iS1b and follow the standard protocol.

Please note that the increased cell concentration in the shearing buffer may impact the shearing efficiency and an additional optimization of the shearing conditions may be required.

When harvesting cross-linked chromatin from tissue samples, the yield of chromatin can vary significantly between tissue types. Usually, the amount of chromatin to be used per IP is 3-10 µg. We recommend performing a pilot experiment to determine the optimal amount of tissue. Once determined, it should be kept consistent between experiments.

3. Yeast cell culture

The protocol describes the successful chromatin preparation from 50

ml culture (OD 0.6 – 1.0 at A600) enough for 1 immunoprecipitation reaction. Scale accordingly to the number of reactions.

Depending on the abundance of the target, the specificity of the antibody, and the number of cells available, it may be possible to scale up and down the amount of cells per chromatin preparation and IP. Grow a desired volume of overnight culture until reach the required number of cells and proceed with the protocol. Please note that an increased or decreased cell concentration in the shearing buffer may impact the shearing efficiency and an additional optimization of the shearing conditions may be required.

We recommend including 1 input per series of experiments/strain type. If different stains (e.g., whild type vs mutant) are studied, we recommend include a corresponding input.

Please note that some additional reagents should be purchased separately to the kit to fit the yeast workflow. Moreover, included primer pairs are not suitable for yeast samples. Specific primers should be design by the user.

4. Fixation optimization

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). Please note that a longer fixation may lead to chromatin resistant to sonication.

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).

5. Shearing optimization

Chromatin shearing is one of the most critical steps for a successful ChIP experiment. Chromatin fragments between 100-600 bp are ideal for ChIP experiments. The optimal time of sonication depends on many factors,

like cell type, cell density, sample volume, fixation time, etc. Hence it is important to optimize the sonication conditions for each new ChIP project. For optimization of the shearing conditions, we recommend using the Chromatin EasyShear Kit - Low SDS (Cat. No. C01020013) which contains all buffers needed for chromatin preparation compatible with the iDeal ChIP-seq kit for TF. The reagents included in this kit allow preparing chromatin using already optimized shearing settings only; it does not contain sufficient reagents for optimization of chromatin shearing.

When using the Bioruptor® Pico, an initial time-course experiment of 5-10-15 sonication cycles 30'' ON/30'' OFF is recommended. Please refer to the Guide for successful chromatin preparation using the Bioruptor® Pico (https://www.diagenode.com/files/protocols/bioruptor-pico-chromatin-preparation-guide.pdf)

When using the Bioruptor Plus, an initial time-course experiment of 10-20-30 sonication cycles 30'' ON/30'' OFF at High Power is recommended. Please refer to The Ultimate Guide for Chromatin Shearing Optimization with Bioruptor® Standard and Plus (https://www.diagenode.com/files/protocols/The_Ultimate_Guide_for_Chromatin_Shearing_Optimization_with_Bioruptor_protocol.pdf)

Choose the shortest sonication time resulting in an efficient chromatin shearing. Avoid over-sonication, as it may lead to a drop in efficiency in ChIP experiments, especially when non-histone proteins are to be evaluated by ChIP.

6. Magnetic beads

This kit includes DiaMag Protein A-coated magnetic beads and IPure beads v3. Make sure the beads do not dry during the procedure as this will result in reduced performance. Keep the beads homogenously in suspension at all times when pipetting. Variation in the amount of beads will decrease reproducibility. Do not freeze the beads.

DiaMag Protein A-coated magnetic beads are suitable for immunoprecipitation of rabbit polyclonal antibodies, mouse IgG2a, IgG2b and IgA, guinea pig IgG, dog IgG, pig IgG.

7. ChIP/ChIP-seq grade antibodies

The quality of antibodies used in ChIP/ChIP-seq is essential for success. It is recommended to use only validated antibodies, specifically recognizing the target. Diagenode offers extensively validated and high-performance antibodies, confirmed for their specificity in ChIP/ChIP-seq. Each batch is validated, and batch-specific data are available on the website www.diagenode.com

8. Input

The input sample corresponds to whole DNA which went through the full ChIP procedure without any immunoselection. The input sample is used as a reference to calculate the recovery at the end of the ChIP procedure. It is also used by most of the bioinformatics tools for analysis of ChIP-seq data where it serves to determine the bias which may result from experimental conditions. We recommend including one input for each chromatin preparation.

9. Negative and positive controls

The kit contains a negative (IgG) and a positive (CTCF) control antibody to monitor the efficiency of the IP on the same chromatin as the one used with the antibody of interest. We recommend including one negative IgG control in each series of ChIP reactions. We also recommend using the positive control ChIP-seq grade CTCF antibody at least once.

The kit also contains qPCR primer pairs for amplification of a positive and negative control target for CTCF in Human (H19 imprinting control region and Myoglobin exon 2, respectively).

10. Quantification

After the ChIP, determine the concentration of the IP'd DNA with a highly sensitive method such as the dsDNA HS Assay Kit on the Qubit® system from ThermoFisher Scientific. PicoGreen® is also suitable but UV spectrophotometric methods such as the NanoDrop are usually not sufficiently sensitive. In most cases it is sufficient to use approximately 10% of the IP'd material for quantification. The DNA yield will be dependent

on different factors such as cell type, quality of the antibody used and antibody target. The expected DNA yield obtained with the positive control CTCF antibody on 4,000,000 HeLa cells is approximately 20 ng.

11. Quantitative PCR analysis

Prior to the sequencing, we recommend analysing the input and immunoprecipitated samples by SYBR® Green qPCR using at least 1 positive and 1 negative control region to determine the enrichment. The kit contains two primer pairs targeting two regions which are positive (H19 imprinting control region) and negative (Myoglobin Exon 2) for the control antibody provided in the kit (CTCF ChIP-seq grade antibody). Each specific antibody will require specific control primers designed by the user. For each primer pair, run the input DNA alongside the immunoprecipitated samples. In order to have sufficient DNA left for sequencing, we recommend not using more than 10% of the total IP'd DNA for qPCR. You can dilute the DNA (1/10 or more) to perform sufficient PCR reactions. PCR reactions should be performed at least in duplicate although performing in triplicate is recommended to be able to identify potential outliers.

PROTOCOL

Protocol

The iDeal ChIP-seq kit for transcription factors is suitable for chromatin preparation and immunoprecipitation from from mammalian cells, tissues and yeast. The protocol for Step 1 "DNA-protein cross-linking " and Step 2 "Cell lysis and chromatin shearing " differs for cells and tissues. Please refer to the corresponding section. Starting from Step 3 "Magnetic immunoprecipitation ", the protocol is identical for both sample types.

The protocol below describes the chromatin preparation (formaldehyde cross-linking, cell collection, lysis and chromatin shearing) from a batch of approximately 25 million cells or 200 mg of tissue. This will be sufficient for 6 ChIP reactions (using approximately 4 million cells or 30 mg of tissue per IP), 1 input and 1 sample for chromatin shearing assessment.

The yeast protocol describes the successful chromatin preparation from 50 ml culture (OD 0.6 – 1.0 at A600) enough for 1 immunoprecipitation reaction. Scale accordingly to the desired number of reactions. We recommend including 1 input per series of experiments/strain type. If different stains (e.g. whild type vs mutant) are studied, we recommend include a corresponding input.

Please note that 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 cells number per plate. Alternatively, an additional parallel plate for counting can be prepared. Suspension cells can be counted before the fixation.

If using a different amount of starting material and/or different amount of material per IP, please refer to "Remarks before starting".

STEP 1

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

1 *Day 1* *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.

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 2

Cell lysis and chromatin shearing from cells

1 *Day 1* *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® Plus use 1.5 ml TPX Microtubes (Cat. No. C30010010)
- When using the Bioruptor® Pico use 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)

***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® Plus, shear for 10 to 30 cycles [30

seconds “ON”, 30 seconds “OFF”] each at High power setting.

- When using the Bioruptor® Pico shear for 5 to 12 cycles [30 seconds “ON”, 30 seconds “OFF”].

***NOTE**: We recommend performing pilot experiments for each new sample type using the Chromatin EasyShear Kit - Low SDS (Cat. No. C01020013).*

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 protocol is described in a separate section “Protocol for chromatin shearing analysis”.

***NOTE**: We recommend analysing the shearing for each batch of chromatin. This step can be omitted when optimal sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimised previously. The analysis of chromatin shearing can be done in parallel with the following steps of the protocol or can be included in the main workflow starting from Step 4.2 (decross-linking). Store the chromatin aliquot at -20°C until analysis.*

2.11. Use the chromatin for immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months.

STEP 1

Tissue disaggregation and DNA-protein cross-linking

1 *Day 1* *30 minutes*

FOR TISSUES

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.

1.4. Chop the tissue into small pieces (between 1-3 mm^3) using a scalpel blade and disaggregate sample using a Dounce homogenizer or TissueLyzer.

For Dounce homogenizer:
Transfer tissue pieces into a Dounce homogenizer. Add 1 ml of formaldehyde diluted in Fixation buffer.

Immediately disaggregate the tissue using a Dounce homogenizer (loose fitting pestle) 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.

Transfer the tissue suspension into a 15 ml tube. Rinse the Dounce homogenizer (or 2 ml tubes for Tissue Lyser option) with an additional 1 ml of diluted formaldehyde and pool with the sample in the same 15 ml tube.

For Tissue Lyser: 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 pre-cooled TissueLyser Adaptors (at least 8 hours at -80°c) and operate the TissueLyser for 2-4 minutes at 25-30 Hz.

Add 2 ml of the cross-linking solution directly to the tissue lysate and start deducing the fixation time from this point, 15 minutes for the total fixation time.

1.5. Incubate for a total time of 15 minutes 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".*

1.6. 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 2

Cell lysis and chromatin shearing derived from tissue samples

1 *Day 1* *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 a 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.**

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**.

2.8. Incubate at 4°C with gentle mixing on a 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 and incubate on ice 10 minutes.

Optional: *if the suspension is not homogenious after pippeting, an additionla homogenization usinga Dounce homogenizer or Tissue Lyser could be performed. Homogenize using a dounce homogenizer (tight pestle) or TissueLyser.*

For Dounce homogeniser*, transfer the suspension to the homogeniser, perform several strokes to get a homogeneous suspension and proceed with the sonication as described below.*

For TissueLyser*, transfer the suspension to 2 ml tubes, supplemented with 2 stainless steel beads (5 mm) pre-cooled at 4°C. Insert 2 ml tubes into TissueLyser Adaptors and operate the TissueLyser for 2-4 minutes at 25 Hz. Proceed with the sonication as described below.*

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

a. When using the Bioruptor® Plus use 1.5 ml TPX Microtubes (Cat. No. C30010010)

b. When using the Bioruptor® Pico use 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)

***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®. Choose the protocol which is adapted to your device:

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

***NOTE**: We recommend performing pilot experiments for each new sample type using the Chromatin EasyShear Kit - Low SDS (Cat. No. C01020013).*

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. Pool the supernatants which contain the sheared chromatin.

2.15. 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".

***NOTE**: We recommend analysing the shearing for each batch of chromatin. This step can be omitted when optimal sonication settings for given experimental conditions (cell type, cell number and fixation) have been optimised previously. The analysis of chromatin shearing can be done in parallel with the following steps of the protocol or can be included in the main workflow starting from Step 4.2 (decross-linking). Store the chromatin aliquot at -20°C until analysis.*

2.16. Use the chromatin in immunoprecipitation (Step 3 Magnetic immunoprecipitation) or store it at -80°C for up to 2 months.

STEP 1

Cell collection and DNA-protein cross-linking from yeast

FOR YEAST

1.1. Grow an overnight culture (50 ml) of yeast cells in appropriate liquid medium until the A600 reaches OD 0.6 – 1.0.

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

1.3. Prepare the cross-linking solution in a fume hood by mixing **1.7 ml of 32% paraformaldehyde** to **3.3 ml of Fixation Buffer** and add these 5 ml of cross-linking reagent to the yeast culture.

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 **5.5 ml of Glycine** to the yeast culture (proportion of 1:10 to stop the fixation). Incubate for 5 minutes at room temperature with gentle shaking. Proceed to the next step immediately.

1.5. Centrifuge in a 50 ml falcon tube for 5 minutes at 4000 x g and remove supernatant.

1.6. Resuspend the cell pellet in **25 ml cold (4°C) TBS buffer** (20 mM Tris-HCl pH 7.4, 150 mM NaCl).

1.7. Centrifuge in a 50 ml falcon tube for 5 minutes at 4000 x g and remove supernatant.

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 2 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 centrifugation at 500 x g for 5 minutes and 4°C and discard the supernatant. Store the pellet at -80°C.*

STEP 2

Cell lysis and chromatin shearing from yeast

FOR YEAST

2.1. Resuspend the cells in **3 ml of ice-cold Lysis Buffer iL1b** by pipetting up and down several times.

2.2. Incubate at 4°C for 20 minutes with gentle mixing on a rotator. Pellet the cells by centrifugation at 4000 x g for 5 minutes at 4°C and discard the supernatant.

2.3. Resuspend the cell pellet in **2 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 rotator.

2.4. Pellet the cells again by centrifugation for 5 minutes at 4000 x g and 4°C and discard supernatant.

2.5. Add **1.5 µl of 200x protease inhibitor cocktail to 298.5 µl of Shearing Buffer iS1b**. This is a complete Shearing Buffer needed for 1 sample. Keep on ice.

2.6. Transfer the liquid into a 2 ml screw cap tubes for Fastprep bead beater containing 300 µl of sterile glass beads (425-600 µm).

2.7. Break cells in a Bead beater Fastprep-24® device at 4°C (3 cycles of 30 sec at maximum speed. Put the tubes in ice for 2 minutes after each cycle).

2.8. Fix the Fastprep tubes at the top of 15 ml tubes. Punch a hole at the bottom of the Fastprep tubes using a needle. Centrifuge at 900 x g for 1 minute at 4°C to collect the liquid in the 15 ml tubes.

2.9. Transfer into a 1.5 ml Bioruptor sonication tubes and shear by sonication. Chose the protocol which is adapted to your device and tubes:

When using the Bioruptor Pico and 1.5 ml Bioruptor Microtubes (cat # C30010016), sonicate for 5-12 cycles 30 '' ON/30'' OFF

When using the Bioruptor Plus and 1.5 ml TPX Bioruptor Microtubes (cat # C30010010-300), sonicate for 10-30 cycles 30 '' ON/30'' OFF at High Power.

***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.*

2.10. Gently spin down sample at low speed and transfer the supernatant in a new 1.5 ml tube.

2.11. Centrifuge 10 minutes at 16000 x g and 4°C, then transfer the supernatant in a new 1.5 ml tube. The supernatant contains the sheared chromatin that can be used for immunoprecipitation and stored at -80°C for several weeks.

STEP 3

Magnetic immunoprecipitation

1-2 *Day 1-2* *Overnight*

FOR CELLS, TISSUES AND YEAST

3.1. Determine the amount of IP reactions to be run including the positive and negative control IPs. Take the required amount of DiaMag Protein A-coated magnetic beads and transfer it to a clean 1.5 ml tube. **30 µl of beads** are needed per IP.

3.2. Prepare **4 ml of 1x ChIP buffer iC1b** by mixing the following reagents:

- 3.2 ml ChIP-seq grade water
- 0.8 ml 5x ChIP buffer iC1b
- 80 µl of 5% BSA

Keep the diluted ChIP buffer iC1b on ice.

3.3. Wash the beads 3 times with **1 ml of ice-cold 1x ChIP buffer iC1b**. To wash the beads, add 1 ml of 1x ChIP buffer iC1b directly to the beads suspension, resuspend the beads by pipetting up and down several times and incubate at 4°C with gentle shaking for 5 minutes. Spin the tubes and place them in the magnetic rack. Wait for one minute to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 2 times.

3.4. After the last wash, resuspend the beads in **1x ChIP buffer iC1b** adding the original volume of beads (this means 30 µl per IP).

3.5. Take the required number of 1.5 ml tubes (one tube per IP) and add **30 µl of the washed beads** to each tube.

3.6. Prepare the **ChIP reaction mix** as described below for 1 IP. Scale according to the number of IPs including a small excess (0.5 extra reaction).

***NOTE**: Proceed separately with reaction mixes to be used with different antibodies*

- 6 µl of BSA
- 1.8 µl 200x protease inhibitor cocktail
- 20 µl 5x iC1b buffer
- (42.2 µl –x µl) ChIP-seq grade water
- Add x µl of ChIP-seq grade antibody

x - amount of a ChIP-seq grade antibody

The total volume of the ChIP reaction mix per IP is 70 µl

***NOTE**: The required amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody.*

Use 1 µg of IgG (negative control antibody) for the negative control IP. If a positive control IP is included in the experiment, use 1 µg of the CTCF positive control antibody.

If required, NaBu (20 mM final concentration) or other inhibitors can be added.

3.7. Add **70 µl of ChIP reaction mix** to the individual tubes containing **30 µl** of washed **Protein A-coated magnetic beads**. Incubate the tubes for 2-4 hours at 4°C under constant rotation on the rotator.

3.8. Briefly spin the tubes containing the ChIP reaction mix and add **250 µl of sheared chromatin**. Keep aside **2.5 µl of the sheared chromatin** at 4°C to be used as an **INPUT** starting from step 4.2. Incubate the tubes overnight at 4°C under constant rotation on the rotator.

3.9. Perform the washes as follows: briefly spin the tubes and place them in the magnetic rack. Wait for one minute and remove the supernatant. Add **350 µl of Wash buffer iW1**: gently shake the tubes to resuspend the beads and incubate for 5 minutes on the rotator at 4°C.

3.10. Repeat the washing step as described above once with **Wash buffer iW2, iW3 and iW4**, respectively.

STEP 4

Elution, decross-linking and DNA purification

2 *Day 2* *5 hours*

FOR CELLS ,TISSUES AND YEAST

***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.*

4.1. After removing the last wash buffer, add **100 µl of Elution buffer iE1** to the beads, resuspend the beads pellet and incubate for 30 minutes on the rotator at room temperature.

***NOTE**: If a precipitation is observed in Elution buffer iE1, warm it at 37°C until it becomes clear. This will not impair the reaction.*

4.2. Briefly spin the tubes and place them into the magnetic rack. Wait for one minute, transfer the supernatant to a new 1.5 ml tube and add **4 µl of Elution buffer iE2**. At the same time add **97.5 µl buffer iE1** and **4 µl of buffer iE2** to the **2.5 µl INPUT** sample. Incubate for 4 hours or overnight at 65°C with shaking.

***NOTE**: if desired, include a chromatin sample for shearing assessment (from step 2.10 for cells or from step 2.15 for tissues) at this step. Perform decross-linking and DNA purification in parallel with the IP and input samples. Please follow the instructions described in a separate section "Protocol for chromatin shearing analysis"*

4.3. Add **2 µl of carrier** to each IP and INPUT sample.

4.4. Add **100 µl of 100% isopropanol** to each IP and INPUT sample.

***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.*

4.5. Resuspend the **IPure beads v3** by vortexing and transfer **10 µl** to each IP and INPUT sample.

4.6. Incubate the IP and INPUT samples for 10 minutes at room temperature on the rotator.

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

4.8. Add **100 µl of Wash buffer 1** (completed with isopropanol) 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 magnetic rack, wait 1 minute and discard the buffer.

4.9. Add **100 µl of Wash buffer 2** (completed with isopropanol) 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 magnetic rack, wait 1 minute and discard the buffer.

4.10. Spin the tubes again and place them on the magnetic rack. 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 rotator.

***NOTE**: Buffer C is compatible with down-stream applications such as qPCR analysis and library preparation for Next-Generation sequencing.*

4.11. Spin the tubes and place them into the magnetic rack, wait 1 minute and transfer the supernatant containing the immunoprecipitated DNA into a new labelled 1.5 ml tubes. Discard the beads.

4.12. Place the DNA on ice and take 2 µl of IP'd DNA to determine the concentration with Qubit® dsDNA HS Assay Kit or a similar method.

4.13. Determine the total number of regions to be analyzed by qPCR for each sample. Take the required volume of INPUT and immunoprecipitated samples for qPCR analysis. Take into account that these samples will be diluted 1/10 and 5 µl will be used per PCR reaction.

***NOTE**: The dilution of samples and the volume per PCR may vary depending on a sensitivity of a Master Mix and qPCR cycler used.*

4.14. Store the remaining DNA at -20°C until further use.

STEP 5

Quantitative PCR analysis

2 *Day 2* *2 to 3 hours*

FOR CELLS ,TISSUES AND YEAST

***NOTE**: For each primer pair, run the INPUT DNA alongside the immuno-precipitated samples and negative IgG control.*

5.1. Take an aliquot of immunoprecipitated DNA and a corresponding INPUT (step 4.13) and dilute them 1/10 using ChIP-seq grade water.

5.2. Prepare the **qPCR mix** as follows (20 µl reaction volume using the provided control primer pairs):

- 10 µl of a 2x SYBR® Green qPCR master mix
- 1 µl of primer pair
- 4 µl of water
- 5 µl of diluted IP'd or INPUT DNA

5.3. Use the following PCR program:

***NOTE**: These conditions may require optimization depending on the type of Master Mix, qPCR system used and user provided primer pair.*

Step	Time/cycles		Temperature
1. Denaturation	3-10 min*		95°C
2. Amplification	30 seconds	40 cycles	95°C
	30 seconds		60°C
	30 seconds		72°C (acquire fluorescence data)
3. Melting curve**	Follow qPCR instrument manufacturer recommendations		

* *Please check carefully supplier's recommendations about Taq polymerase activation time*

** *Include and inspect the melting curves based on the protocols recommended by the qPCR instrument manufacturer to ensure that primer pairs amplify only a single specific product*

5.4. Record the threshold cycles (Ct values) from the exponential phase of the qPCR for the IP'd DNA sample and input for each primer pair.

5.5. Calculate the relative amount of immunoprecipitated DNA compared to INPUT DNA for the control regions (% of recovery) using the following formula:

$$\% \text{ recovery} = 2\text{^}((Ct_{input} - 6.64) - Ct_{sample}) * 100\%$$

- Ct_{sample} and Ct_{input} are the threshold cycles from the exponential phase of the qPCR for the IP'd DNA sample and INPUT, respectively.
- 2 is the amplification efficiency
- 6.64 is a compensatory factor to correct the input dilution

***NOTE**: This equation assumes that the PCR is 100% efficient (amplification efficiency = 2). For accurate results, the amplification efficiency with given primer pair has to be close to 100% meaning that for each cycle the amount of product is doubles (E=2). The real amplification efficiency, if known, should be used. The formula takes into account that 1% of INPUT was used as suggested in the protocol (2.5 µl INPUT vs 250 µl of chromatin per IP). If the amount of INPUT used is different from 1%, an introduction of a compensatory factor in the formula is required to correct the input dilution (x) as follows:*

%recovery = 2^[(Ct(input)-log2(X%) Ct(sample)]x100%

Where: log2(x) accounts for the INPUT dilution

***Example**: If you use an INPUT of 5 µl from 250 µl of chromatin used per IP, it corresponds to 50 X dilution. The compensatory factor is equal to log2(50)=5.64 and the formula to calculate the recovery will be as follows: %recovery = 2^[(Ct(input)-5.64 Ct(sample)]x100%.*

5.6. If an expected enrichment over positive loci with a low background is observed, proceed to the library preparation using an appropriate protocol (not supplied with iDeal TF kit).

Library preparation and Sequencing recommendations

The iDeal ChIP-seq for TF protocol has been validated for ChIP-seq on an Illumina®HiSeq Next-Gen sequencer. However, other sequencing systems such as the Illumina®MiSeq or the Life Technologies SOLiD™ systems can also be used.

For library preparation compatible with Illumina® sequencers, we highly recommend using Diagenode kits: MicroPlex Library Preparation Kit (Cat. No. C05010012, C05010001); or TAG kit for ChIPmentation (Cat. No. C01011030).

?

ASK THE EXPERTS

Please, do not hesitate to contact our customer support team if you have any questions about the design of your ChIP-seq experiment or the bioinformatics data analysis.

Contact for Europe, Asia, Oceania and Africa:
custsupport@diagenode.com
Contact for North and South America:
custsupport.na@diagenode.com

ChIP-seq data analysis recommendations from our bioinformaticians

ChIP-seq data analysis workflow

In the following chapter we will guide you through the basics of ChIP-seq data analysis. We will also provide some examples of software tools suitable for each step. We cite numerous analysis tools, including free and commercial softwares.

1. (Optional step) Trimming: use trimming to get rid of low quality bases and artefacts in the readset, such as adapter contaminations
   - **a.** Cutadapt
   - **b.** Trim Galore!
   - **c.** Trimmomatic
2. Alignment: in this step you will map the reads against a known reference sequence
   - **a.** ELAND
   - **b.** Tmap
   - **c.** BWA
   - **d.** Bowtie2
3. (Optional step) Quality control: you can check the general quality of the sequencing and the alignment
   - **a.** FastQC
   - **b.** Picard Tools
4. Peak calling: during peak calling the software will detect sites of enrichment along the genome
   - **a.** MACS2
   - **b.** SICER

d. ZINBA
e. PeakRanger
f. Pyicoteo
g. MUSIC
h. SPP
i. hiddenDomains

After above described basic analysis, the peaks can be analyzed further to get answers to our biological questions. There are countless ways and tools for further analyses, the project goals determine which ones we should pick. Just as in the case of basic analysis, we recommend to thoroughly study the manual of the chosen software tool to understand its purpose and its function. Below we will list a few common analysis types to further process the peaks, along with example tools again.

10. Visualization: the peaks, the reads, and other data (e.g. gene positions) can be displayed in a suitable genome browser

a. IGV
b. IGB
c. USCS Genome Browser

11. Descriptive statistics: the peaks can be described in various useful ways, like how many reads fall into them, what is their number, mean size and significance etc.; these figures are also very useful when you compare datasets

a. Peak callers usually provide per peak and/or summary statistics after peak detection
b. HOMER
c. GREAT
d. BEDTools

12. Motif search: especially in the case of TF data peaks frequently occur at specific motifs; therefore identifying these motifs and checking enrichments over them is essential for TF data analysis

a. HOMER
b. MEME Suit

c. ZINBA
d. PeakRanger
e. Pyicoteo
f. MUSIC
g. SPP
h. hiddenDomains

After above described basic analysis, the peaks can be analyzed further to get answers to our biological questions. There are countless ways and tools for further analyses, the project goals determine which ones we should pick. Just as in the case of basic analysis, we recommend to thoroughly study the manual of the chosen software tool to understand its purpose and its function. Below we will list a few common analysis types to further process the peaks, along with example tools again.

5. Visualization: the peaks, the reads, and other data (e.g. gene positions) can be displayed in a suitable genome browser
   a. IGV
   b. IGB
   c. USCS Genome Browser
6. Descriptive statistics: the peaks can be described in various useful ways, like how many reads fall into them, what is their number, mean size and significance etc.; these figures are also very useful when you compare datasets
   a. Peak callers usually provide per peak and/or summary statistics after peak detection
   b. HOMER
   c. GREAT
   d. BEDTools
7. Motif search: especially in the case of TF data peaks frequently occur at specific motifs; therefore identifying these motifs and checking enrichments over them is essential for TF data analysis
   a. HOMER
   b. MEME Suit

8. Annotation, Gene Ontology, Pathway analysis: after annotation/GO/Pathway analysis you will get a clear picture about which genomic features or pathways your peaks are associated to, providing an important information about disease mechanisms, the role of DNA binding proteins, or treatment effects

    a. HOMER
    b. GREAT
    c. BEDTools
    d. ReactomePA

9. Comparative analysis: this type of analysis is the obvious choice when you have several datasets from comparable conditions (e.g. treated and untreated cells) or when you want to check the performance of your ChIP-seq by comparing it to a reference; there are many different ways to compare peaks, including checking the overlaps, the correlation of enrichment sizes and performing statistical tests on the peaksets

    a. HOMER
    b. BEDTools
    c. DiffBind

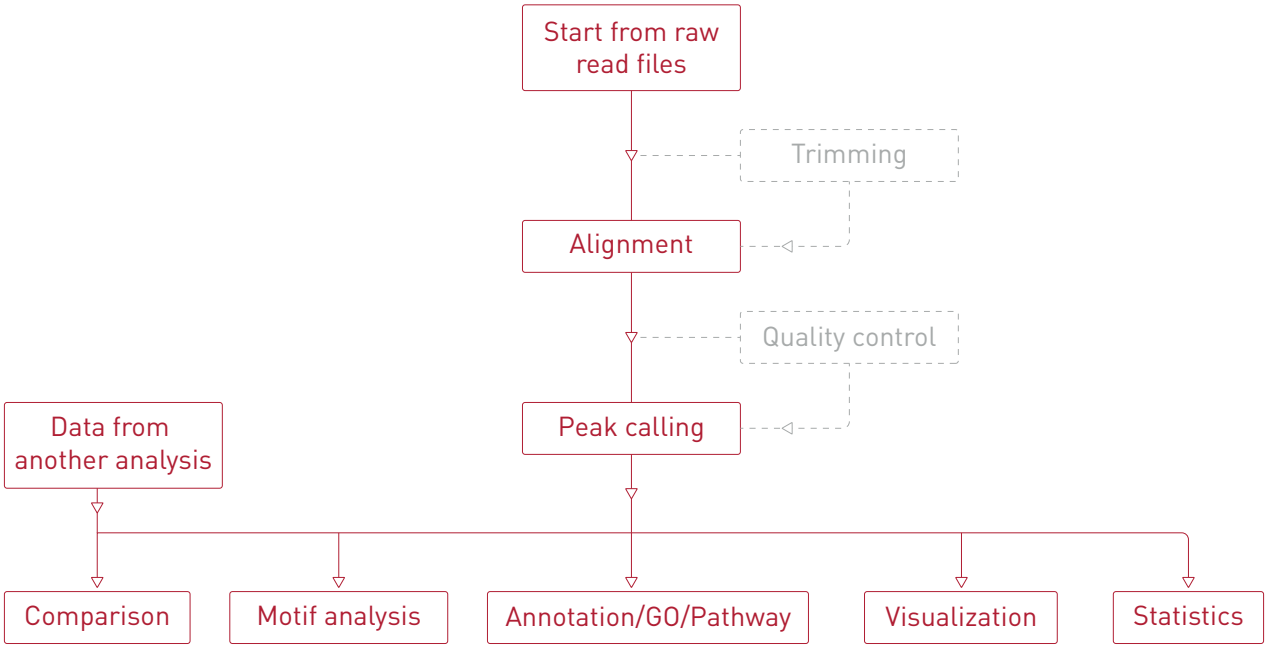


Figure 1. ChIP-seq data analysis workflow

Example of results

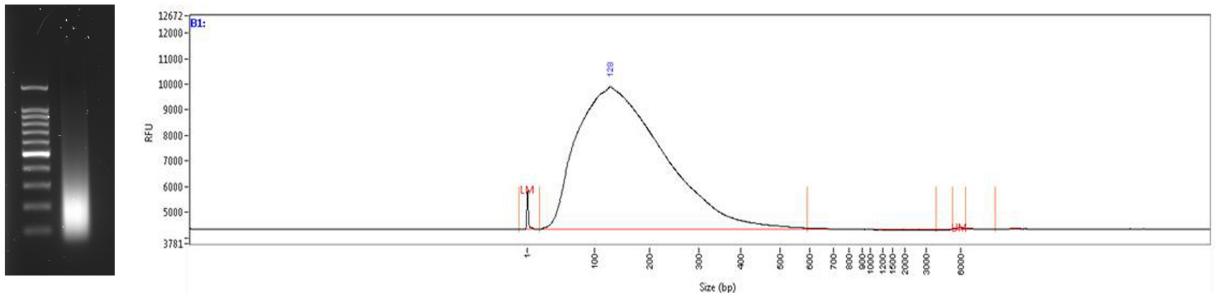


Figure 2. Successful chromatin shearing using the Bioruptor® Pico and the iDeal-ChIP-seq for TF.

HeLa cells were fixed with formaldehyde and chromatin was prepared accordingly to the iDeal-ChIP-seq for TF protocol. Samples were sonicated for 8 cycles of 30" ON/30" OFF using the Bioruptor® Pico. A 100 bp ladder was loaded as size standard. Panel A: Fragment size assessment by an agarose gel electrophoresis. Panel B: Fragment size assessment using a Fragment Analyzer (Advanced Analytical).

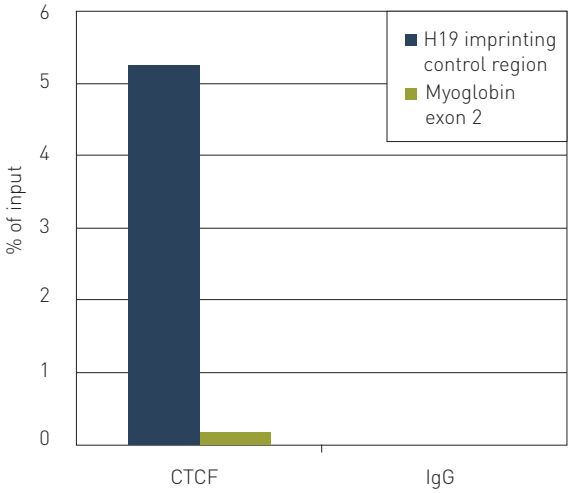


Figure 3. Chromatin immunoprecipitation analysis using control CTCF antibody

ChIP was performed on human HeLa cells using the control antibodies from the iDeal ChIP-seq kit for Transcription Factors. Sheared chromatin from 4 million cells, 1 µg of the positive control antibody and 1 µg of the negative IgG control were used per IP. Quantitative PCR was performed with

the positive control H19 imprinting control region and the negative control Myoglobin exon 2 primer sets from the kit. The figure shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

For the positive control antibody (CTCF) the recovery of the positive control target (H19 imprinting control region) is expected to be approximately 5% although this will depend on the cell type used. The recovery of the negative control target (Myoglobin exon 2 locus) should be below 0.5% as shown in Figure 3.

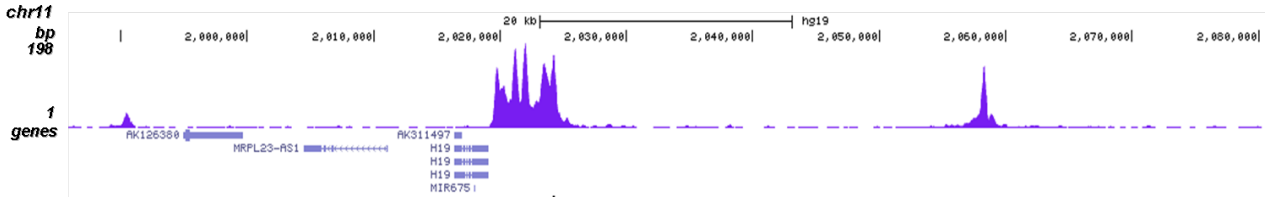


Figure 4. ChIP-seq results obtained with the Diagenode antibody directed against CTCF

ChIP was performed on sheared chromatin from 4,000,000 HeLa cells using the iDeal ChIP-seq kit for Transcription Factors and 1 µg of the CTCF positive control antibody. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 4 shows the peak distribution in a genomic region surrounding the H19 imprinting control region.

Protocol for chromatin shearing analysis

General remarks

We recommend using an agarose gel analysis or the Fragment Analyzer (Advanced Analytical) for the size 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. RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing.

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 using **IPure** beads (30 minutes)
- Fragment size assessment (agarose gel or Fragment Analyzer) (1 h)

RNAse treatment

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

6. Add **4 µl of Elution buffer iE2**, mix thoroughly.

7. Incubate samples at 65°C for 4 hours (or overnight).

DNA purification

> ***NOTE**: The protocol below describes DNA purification using the IPure magnetic beads included in the kit. Other methods of DNA purification (columns-based DNA clean-up, e.g. DiaPure columns from Diagenode or a phenol–chloroform extraction followed by ethanol precipitation) can be used also.*
>
> ***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.*

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 v3** by vortexing and add **20 µl** to the sample.

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

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

15. Spin the tubes again and place them on the magnetic rack. 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 rotator.

16. Spin the tubes and place them into the magnetic rack, wait 1 minute and transfer the supernatants into a new 1.5 ml tube. Discard the beads.

Fragment size assessment

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)).

FAQs

What is the amount of DNA I can precipitate after IP?

The amount of DNA you precipitate largely depends on the antibody and cell type used. With the CTCF control antibody the amount is ~20 ng from 4 million HeLa cells. A typical yield for a transcription factor will be in the range of 1-5 ng.

Can I use iDeal ChIP-seq kit for proteins other than transcriptional factors?

The iDeal ChIP-seq kit for transcription factors is intended for all non-histone proteins, including transcription factors, histone modifying enzymes and other chromatin associated proteins.

Is the included control CTCF antibody compatible with mouse?

The included control CTCF antibody has been raised in rabbit against human CTCF, but it is compatible with mouse and rat.

Are the included controls primers compatible with mouse?

The included control primers are specific for human DNA sequences and will not perform on DNA from other species.

How much antibody is needed per IP?

The amount of antibody needed depends on different factors of which the antibody itself is the most important. Most of Diagenode ChIP-grade antibodies have been tested at different concentrations to determine the optimal amount and the suggested amount of antibody is given in the data sheet. Please note, however, that this can be assay dependent and might need to be optimized for each experimental setting. If the antibodies come from other companies, please refer to the corresponding technical data sheet. If no required amount of antibody is given, we suggest performing a titration experiment. For ChIP-seq it is important to select the amount of antibody which gives the lowest background signal.

What is the binding capacity of DiaMag Protein A-coated magnetic beads?

30 µl of DiaMag Protein A-coated magnetic beads can bind 10 µg of antibody.

What is the specificity of protein A-coated magnetic beads?

Ideal TF ChIP kit contains DiaMag Protein A-coated magnetic beads which allow an efficient capture of rabbit, guinea pig, dog and pig polyclonal and monoclonal Abs, mouse IgG2a, IgG2b and IgA and human IgG1, IgG2 and IgG4. If the antibody of interests belongs to a different class of immunoglobulins (mouse IgG1 and IgG3, rat or goat polyclonal Abs, and human IgG3), DiaMag Protein G-coated magnetic beads should be used instead of protein A coated beads. These beads are available separately (C03010021).

Can I use a monoclonal antibody in ChIP experiment?

ChIP can be performed using either monoclonal or polyclonal antibodies. In general, polyclonal antibody populations will recognize a number of different epitopes, in contrast to monoclonal antibodies, which recognize a single epitope. Because monoclonals recognize a single epitope on a target protein, they often provide a high level of specificity, low non-specific binding, and low background signals. The major disadvantage of a monoclonal antibody is its recognition of only one epitope, which can be masked by cross-linking, decreasing the efficiency of immunoprecipitation.

Why do I observe a different DNA size on agarose gel and Bioanalyzer for sheared chromatin?

We recommend using agarose gel or Fragment analyzer (Advanced Analytical) for an accurate size assessment of sheared chromatin.

Although Agilent BioAnalyzer 2100 is widely used for size assessment of DNA fragments before library preparation for NGS, this technology is less optimal for analysis of sheared chromatin. For example, some inconsistencies between agarose gel and BioAnalyzer 2100 profiles have been documented. It may be linked to a higher sensitivity of microfluidics chips to residual contaminants (ions, SDS, proteins, carrier used for DNA

precipitation, etc.), overloading and to conformation/spacial structure of DNA molecules which might be affected by fixation and not fully relieved by de-crosslinking.

Moreover, BioAnalyzer 2100 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.

Another key point is about quantitation of BioAnalyzer 2100 peaks. If each region is calculated using molarity which represent number of molecules in a particular range, a significantly higher level of molecules are found in low molecular weight region (fig. below).

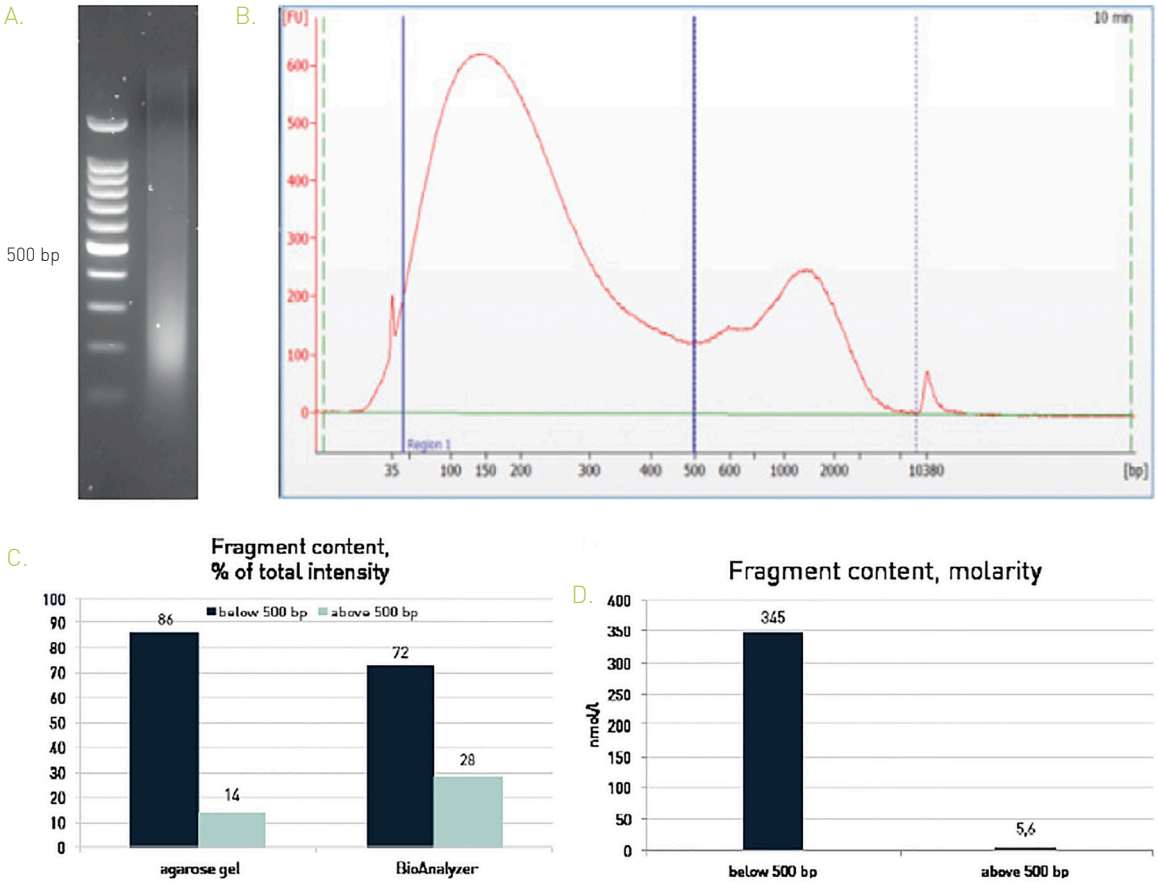


Figure 5. Size assessment of sheared chromatin using an agarose gel and the BioAnalyzer.

HeLa cells were fixed with formaldehyde and chromatin was prepared according to Diagenode's protocol. Samples were sonicated for 10 cycles of 30" ON/30" OFF with the Bioruptor® Pico using 1.5 ml Bioruptor® microtubes with caps (C30010016) and analyzed by agarose gel (panel

A) or by BioAnanlyzer, High sens Agilent DNA kit (panel B). Fragment content below and above 500 bp was calculated as percentage of total surface (panel C). Panel D shows fragment content calculated as molarity (BioAnalyzer trace only).

What is the composition of buffers included in the kit?

The composition of the buffers is proprietary.

Related products

Product	Cat. No.
ChIP Cross-link Gold	C01019027
Chromatin EasyShear Kit – Low SDS	C01020013
Chromatin EasyShear Kit - Ultra Low SDS	C01020010
MicroPlex Library Preparation Kit v2 (12 indices)	C05010012
MicroPlex Library Preparation Kit v2 (12 indices)	C05010013
MicroPlex Library Preparation Kit v2 (48 indices)	C05010014
iDeal ChIP-seq kit for Histones	C01010050
iDeal ChIP-seq kit for Histones	C01010051
iDeal ChIP-seq kit for Histones	C01010059
IPure kit v2	C03010014
IPure kit v2	C03010015
Bioruptor Pico®	B01080010

Validated antibodies – check out the complete list at www.diagenode.com

ChIP-seq grade antibody	Cat. No.
AML1-ETO polyclonal antibody	C15310197
CBFb polyclonal antibody	C15310002
CTCF polyclonal antibody	C15410210
E2F6 polyclonal antibody	C15410314
ER alpha monoclonal antibody	C15100066
ETO polyclonal antibody	C15310001
FOXA1 polyclonal antibody	C15410231
FOXM1 polyclonal antibody	C15410232
GR monoclonal antibody	C15200010
GTF2E2 polyclonal antibody	C15410264
NF-E2 polyclonal antibody	C15410240
NFKB p65 polyclonal antibody	C15310256

ChIP-seq grade antibody	Cat. No.
OCT4 polyclonal antibody	C15410305
p53 polyclonal antibody	C15410083
Pol II monoclonal antibody	C15200004
Pol II S2p monoclonal antibody	C15200005
Pol II S5p monoclonal antibody	C15200007
RARA polyclonal antibody	C15310155
TAL1 monoclonal antibody	C15200012
TARDBP polyclonal antibody	C15410266
TBP monoclonal antibody	C15200002
ZHX2 polyclonal antibody	C15410260
ZMYM3 monoclonal antibody	C15200016

Revision History

Version	Date of modification	Description of modifications
Version 5 04_2023	**April 2023**	- Replacement of the IPure beads v2 by IPure beads v3 - Removal of discontinued references

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