diagenode

A Hologic Company

iDeal ChIP-FFPE Kit

Cat. No. C01010190

USER GUIDE

Version 1 10_2022

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 remodelling and transcription regulator recruitment to specific genomic sites.

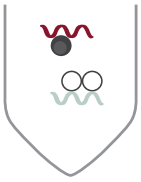
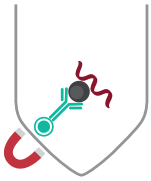
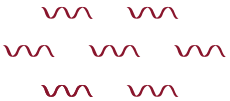
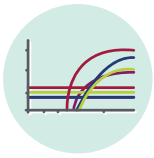
Formalin-fixed paraffin embedded (FFPE) samples are a gold standard for storage of pathology samples for morphological analysis. The number of archival FFPE samples is in the millions, providing an invaluable repository of information for analysis. While it is common to perform ChIP from cells and fresh or frozen tissues, FFPE samples are challenging due to extensive crosslinking.

The iDeal ChIP-FFPE Kit provides a robust ChIP protocol suitable for investigation of proteins associated with chromatin. The protocol involves deparaffinization, followed by chromatin extraction and shearing by sonication. 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 DiaPure columns. The eluted DNA is used for qPCR analysis.

The iDeal ChIP-FFPE Kit samples offers unique benefits:

- Fast and user-friendly deparaffinization workflow
- Easy-to-follow, user-friendly protocol
- Increased yield of chromatin
- Magnetic beads make ChIP easy, fast and more reproducible
- Eluted DNA suitable for qPCR analysis or other down-steam applications

Kit method overview & time table

			Time needed	Day
STEP 1		Deparaffinization	40 minutes	1
STEP 2		Chromatin extraction and shearing	3 - 4 hours	1
STEP 3		Magnetic immunoprecipitation	Overnight +2h	1-2
STEP 4		Elution, decross-linking and DNA purification	5 hours	2
STEP 5	 qPCR	Quantitative PCR and data analysis	2 to 3 hours	2

LEGEND

- Protein of interest
- Other protein
- DNA
- Antibody
- Magnetic bead
- Magnet

Kit materials

The iDeal ChIP-FFPE kit contains enough reagents to perform 10 individual chromatin preparation from FFPE sections, up to 24 immunoprecipitations and 50 DNA purifications (from 10 input, 24 IPs and 10 shearing assessment samples), as described in Table 1.

Table 1. Number of reactions included in the iDeal ChIP-FFPE Kit.

Kit reference	Number of chromatin preparations	Number of ChIP reactions	Number of DNA purifications
C01010190	10	24	50

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-FFPE Kit

	Qty	Storage
Lysis Buffer iL1b	6 ml	4°C
Lysis Buffer iL2	6 ml	4°C
Shearing Buffer iS1b	6 ml	4°C
ChIP-seq grade water	26.6 ml	4°C
DiaMag protein A-coated magnetic beads	480 µl	4°C
5x ChIP Buffer iC1b	6.8 ml	4°C
Wash Buffer iW1	8.4 ml	4°C
Wash Buffer iW2	8.4 ml	4°C
Wash Buffer iW3	8.4 ml	4°C
Wash Buffer iW4	8.4 ml	4°C
Elution Buffer iE1	7,8 ml	4°C
Elution Buffer iE2	320 µl	4°C
Protease inhibitor cocktail	80 µl	-20°C
5% BSA (DNA free)	144 µl	-20°C
MicroChIP DiaPure columns	50	RT

	Qty	Storage
ChIP DNA Binding Buffer	50 ml	RT
DNA Wash Buffer *	6 ml	RT
DNA Elution Buffer	10 ml	RT

** Ethanol must be added to DNA Wash Buffer before the first use*

Required materials not provided

Materials and Reagents

- Gloves to wear at all steps
- Tweezers
- RNase/DNase-free 1.5 ml tubes
- Heptane, molecular grade
- Methanol, molecular grade
- Ethanol, molecular grade
- RNAse cocktail (e.g. Ambion AM 2286A)
- ChIP-grade antibodies – www.diagenode.com
- qPCR SYBR® Green Mastermix
- Fluorescence-based assay for DNA concentration measurement, e.g. the Qubit High Sensitivity assay (Life Technologies #Q32851)

Optional supplies

- IgG control (Rabbit IgG, Diagenode Cat. No. C15410206 or mouse IgG, Diagenode Cat. No. C15400001)
- Pierce™ Spin Cups - Cellulose Acetate Filter Catalog number: 69702

Equipment

- Microtome and blade
- Equipment for tissue disruption and homogenization:
  - Dounce homogenizer with a loose pestle (1 ml)
  - or TissueLyser (Qiagen) with 2 ml tubes and stainless steel beads, 5 mm (Qiagen, Cat. No.69989)
- Bioruptor® sonication device and the associated microtubes:
  - Bioruptor® Plus (Diagenode, Cat. No. B01020001) and 1.5 ml TPX Microtubes (Cat. No. C30010010)
  - Bioruptor® Pico (Diagenode, Cat. No. B01060001) and 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
- Refrigerated centrifuge for 1.5 ml tubes

- DiaMag Rotator (Rotating wheel) (Diagenode, Cat. No. B05000001)
- Magnetic rack for 1.5 ml tubes
- Vortex
- Thermomixer
- Qubit® Fluorometer (ThermoFisher Scientific)
- qPCR cycler
- Fragment Analyzer (Advanced Analytical, High Sensitivity NGS Fragment Analysis Kit (DNF-473) or BioAnalyzer (Agilent, High sensitivity kit and reagents) for chromatin shearing assessment

Remarks before starting

1. Starting amount, chromatin yield and number of IPs

The protocol describes the preparation of chromatin from up to 6 FFPE sections (up to 10 µm in thickness). The total volume of the sheared chromatin per one chromatin preparation is about 600 µl. The exact number of IP that can be performed from this chromatin pool depends on the chromatin yield which is estimated by DNA quantification. The minimum DNA amount recommended per one IP is about 300 ng. However, a higher starting amount is recommended when possible. Please use a required volume up to 500 µl of sheared chromatin (containing minimum 300 ng of DNA) per IP. Take into account that 10% of a chromatin volume used per IP should be kept as input. Additionally, an aliquot of 50 µl is required for the shearing assessment.

Example:

- The volume of the chromatin pool is 500 µl
- DNA concentration is 8 ng/µl
- Total chromatin yield (500 µl x 8 ng/µl) is 4 µg
- 150 µl of chromatin containing 1.2 µg of DNA will be used per IP
- 3 IPs can be run from this chromatin preparation
- 15 µl of chromatin from the pool (10% out of 150 µl) saved as input
- 50 µl of sheared chromatin for shearing assessment

Please keep in mind that the chromatin yield varies depending on samples (tissue type, fixation, storage). Therefore, the exact amount of sections giving a sufficient chromatin yield should be optimized by the user.

2. Deparaffinization

Tissue sections must be first deparaffinized prior to chromatin extraction. The described protocol uses heptane as a fast and less toxic alternative to a conventional xylene-based approach. Deparaffinized sections **do not**

require additional re-hydratation removing laborious washes with serial dilutions of ethanol. Please note that conventional deparaffinisation described in a separate section "Deparaffinization and rehydrataion using xylene" is compatible with the further protocol.

3. Antigen retrieval

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

4. Magnetic beads

This kit includes DiaMag Protein A-coated magnetic beads. 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. 20 µl of DiaMag Protein A-coated magnetic beads can bind up to 6.5 µg of antibody.

If the antibody of interest 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 (Cat. No. C03010021).

5. ChIP grade antibodies

The quality of antibodies used in ChIP 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

6. 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. We recommend including one input (10% of chromatin used per one IP) for each chromatin preparation from an individual FFPE sample.

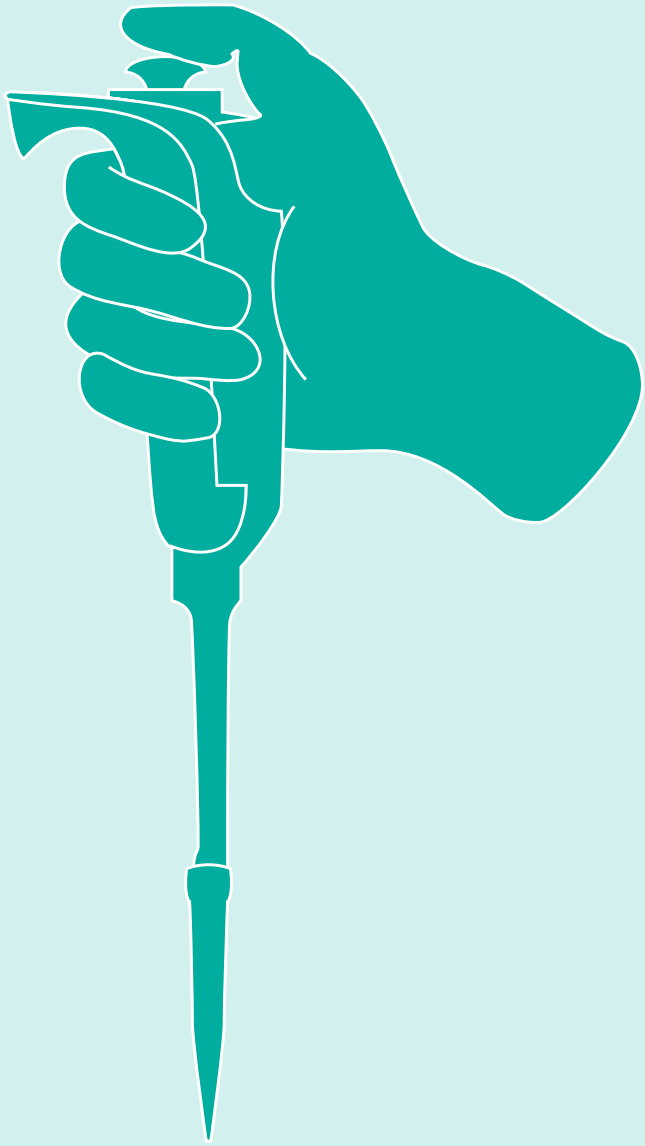
7. Negative control

IgG could be used as a negative control in parallel with specific antibody at the same concentration as the specific antibody to monitor the background and specificity of the IP. While it is recommended including one negative IgG control in each series of ChIP reactions, it is not always possible due to a low chromatin yield from FFPE samples. The kit **does not** contain a negative IgG control. It should be purchased separately (Rabbit IgG - Diagenode, Cat. No. C15410206 or mouse IgG – Diagenode, Cat. No. C15400001-100).

8. Quantitative PCR analysis

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. Each specific antibody will require specific control primers designed by the user. For each primer pair, run the input DNA alongside the immunoprecipitated samples. PCR reactions should be performed at least in duplicate although performing in triplicate is recommended to be able to identify potential outliers.

PROTOCOL



MANUAL PROCESSING

STEP 1

Deparaffinization

1 *Day 1* *40 minutes*

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

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

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

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

1.4 Add **50 µl** of **100% methanol** and vortex for 30 seconds. Centrifuge at 16,000 x g for 2 minutes at room temperature.

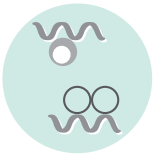
1.5 Remove the supernatant carefully and discard.

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

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

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

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



STEP 2

Chromatin extraction and shearing

Day 1

3 to 4 hours

2.1 Resuspend the sample in **0.6 ml** of **ice-cold Lysis Buffer iL1b**.

2.2 Incubate at 4°C for 10 minutes with gentle mixing on a DiaMag Rotator. Pellet the sample by centrifugation at 10.000 x g for 5 minutes at 4°C, carefully discard the supernatant and keep the pellet.

***NOTE**: Care should be taken not to disturb and lose the pellet after centrifugation.*

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

2.4 Pellet the sample again by centrifugation at 10.000 x g for 5 minutes at 4°C and discard the supernatant.

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

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

- Dounce homogenizer: transfer the sample to the homogenizer and disaggregate it using a tight fitting pestle to get a homogeneous suspension

- Alternatively, TissueLyser can be used. Transfer the sample to a 2 ml tube containing 2 stainless steel beads, diameter 5 mm. Place the tube in the Tissue Lyser Adaptor precooled at 4°C. Operate the system for 5 minutes at 25 Hz.

2.7 Transfer the sample into 1.5 ml tube and incubate for 1h30 at 65°C with shaking on Thermomixer for antigen retrieval.

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

2.8 Transfer the sample 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)

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

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

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

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

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

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

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

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

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

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

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

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

***NOTE**: This step can be omitted when optimal sonication settings for given sample type 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 (de-cross-linking).*

2.18 The remaining chromatin is ready for immunoprecipitation with an antibody of interest. Define the volume of sheared chromatin (up to 500 µl) containing at least 300 ng of DNA to be run per IP. Determine

the number of IP from one chromatin preparation. Include negative IgG control if possible. Keep aside the chromatin corresponding to 10% of a total volume used per IP and keep it at 4°C to be used as an INPUT starting from step 4.2.

2.19 Proceed to the Step 3 Magnetic immunoprecipitation or store sheared chromatin at -80°C for up to 2 months.

STEP 3

Magnetic immunoprecipitation

1-2 *Day 1-2* *Overnight + 2 hours*

3.1 Take the required volume of sheared chromatin and prepare the ChIP reaction mix as described below:

Component	Quantity per IP
Sheared chromatin	X µl
Protease inhibitor cocktail	1.8 µl
BSA	6 µl
ChIP-grade antibody or negative IgG	Y µl
1 x ChIP Buffer iC1b*	Adjust the total volume to 500 µl

X - volume of sheared chromatin containing at least 300 ng of DNA per one IP

Y - The amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody.

** - Dilute the required volume of 5x ChIP Buffer iC1b with ChIP-grade water to obtain 1x iC1b buffer.*

3.2 Incubate the tubes overnight at 4°C under constant rotation on the DiaMag Rotator.

3.3 Next day, take a required amount of **DiaMag Protein A-coated magnetic beads**. 20 µl of beads are needed per IP. Scale the amount accordingly to the number of IP to proceed.

3.4 Prepare 1 ml of 1x ChIP Buffer iC1b by mixing **200 µl of 5x ChIP Buffer iC1b and 800 µl ChIP-grade water**. Wash the beads 3 times with 0.3

ml of ice-cold 1X Buffer iC1b. To wash the beads, add **0.3 ml** of **ice-cold 1x Buffer iC1b** directly to the beads suspension, resuspend the beads by pipetting up and down several times. 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.

3.5 After the last wash, resuspend the beads in **1x Buffer iC1b** adding the original volume of beads (this means 20 µl per IP).

3.6 Add **20 µl of the washed beads** to each tube containing ChIP reaction and incubate for 2 hours at 4°C under constant rotation on the DiaMag Rotator.

3.7 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** and resuspend by pipetting up and down several times.

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

STEP 4

Elution, de-crosslinking and DNA purification

2 *Day 2* *5 hours*

***NOTE**: Before the first use of the DNA Wash Buffer, 24 ml of ethanol must be added to 6 ml of the Buffer. Never leave the bottle open during storage to avoid evaporation.*

4.1 After removing the last wash buffer, add **200 µl** of **Elution Buffer iE1** to the beads, resuspend the beads pellet and incubate for 15 minutes at 65°C with shaking on Thermomixer.

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 **8 µl of Elution Buffer iE2**. At the same time add the **Buffer iE1** to the **INPUT** sample to reach the final volume **200 µl** and add **8 µl of Buffer iE2**. Incubate for 4 hours or overnight at 65°C with shaking on Thermomixer.

***NOTE**: If desired, include a chromatin sample for shearing assessment (from step 2.17). Perform de-crosslinking 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 Proceed to DNA purification using MicroChIP DiaPure columns. Add **1 ml of ChIP DNA Binding Buffer** to each sample and mix briefly.

4.4 Transfer **600 µl** of a mixture to the provided spin column in a collection tube and centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through.

4.5 Transfer the remaining **600 µl** of a mixture to the same spin column in a collection tube and centrifuge at ≥10,000 x g for 30 seconds. Discard the flow-through.

4.6 Add **200 µl** of **DNA Wash Buffer** to the columns. Centrifuge at ≥10,000 x g for 30 seconds. Repeat wash step.

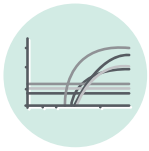
4.7 Add **50 µl** of **DNA Elution buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥10,000 x g for 30 seconds to elute DNA. DNA is now ready to use for qPCR analysis, quantification and library preparation.

***NOTE**: A smaller elution volume down to 6 µl can be used in order to get a higher DNA concentration when required.*

4.8 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.

***NOTE**: IP'd DNA yield from FFPE samples is usually low and samples should not be diluted prior to qPCR. If a sequencing experiment is planned, keep at least 20 µl of each sample for DNA quantification and library preparation.*

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



STEP 5

Quantitative PCR analysis

2 *Day 2* *2 to 3 hours*

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

5.1 Take an aliquot of immunoprecipitated DNA and a corresponding INPUT.

5.2 Prepare the qPCR mix as follows (20 µl reaction volume):

- 10 µl of a 2x SYBR® Green qPCR master mix
- 1 µl of primer pair (user provided)
- 4 µl of water
- 5 µl of 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:

$$\%recovery = 2^{((Ct_{input} - 3.32) - Ct_{sample})} * 100\%$$

- 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
- 3.32 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 10% of INPUT was used as suggested in the protocol (e.g.25 µl INPUT vs 250 µl of chromatin per IP). If the amount of INPUT used is different from 10%, an introduction of a compensatory factor in the formula is required to correct the input dilution (X) as follows:*

$$\%recovery = 2^{[(Ct_{input} - log2(X\%)) - Ct_{sample}]} * 100\%$$

Where: log2(X) accounts for the INPUT dilution

Example of results

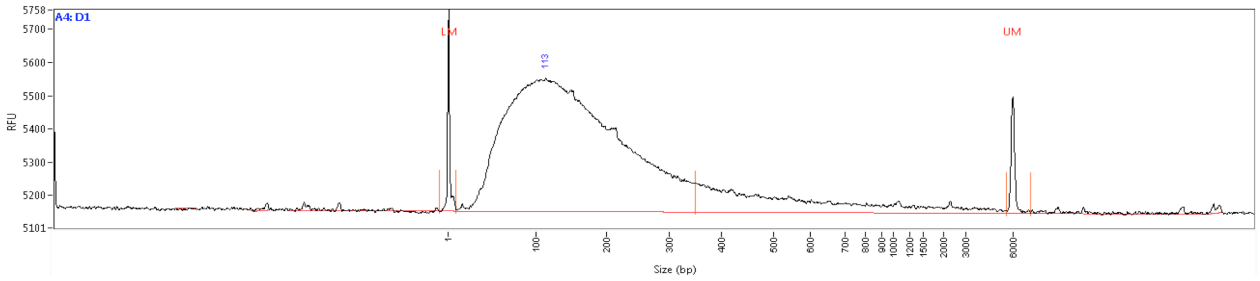


Figure 1. Succesful chromatin shearing using the Bioruptor Pico and iDeal ChIP-FFPE Kit.

Sections of FFPE Mouse liver (3 year storage) were proceeded accordingly to the iDeal ChIP-FFPE protocol. The recovered sheared DNA was analysed using Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit (DNF-473). The Fragment Analyzer trace shows the fragment size distribution between 100-400 bp ideal for ChIP experiment.

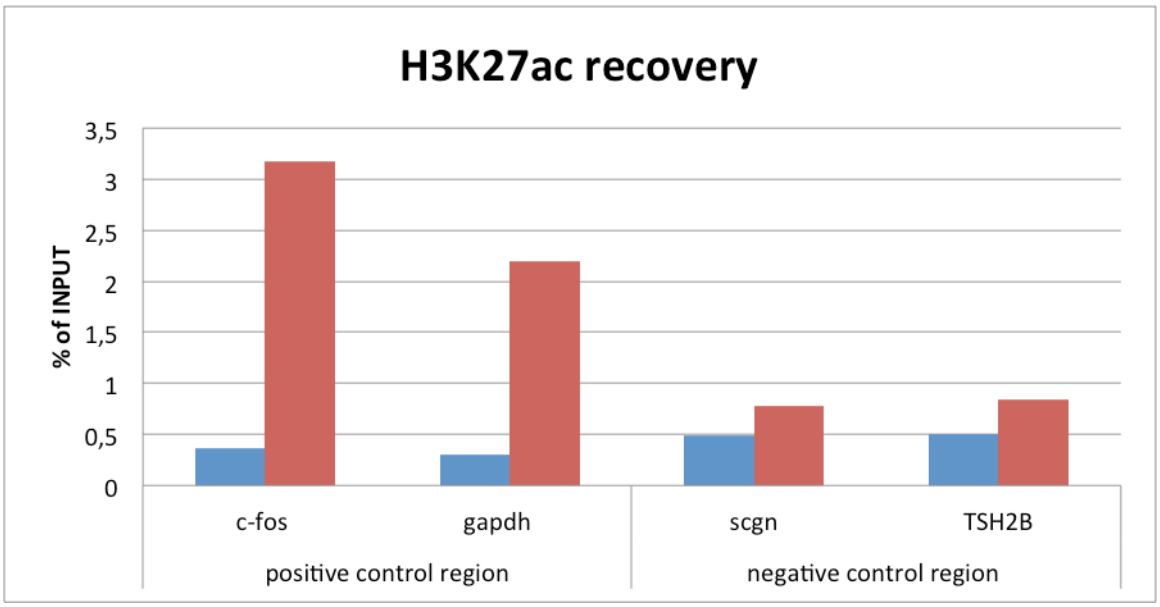


Figure 2. Chromatin immunoprecipitation analysis using H3K27ac antibody.

ChIP was performed on Mouse liver FFPE samples accordingly to the iDeal ChIP-FFPE protocol using 1 µg of H3K27ac antibody (Diagenode, Cat. no. C15410174)

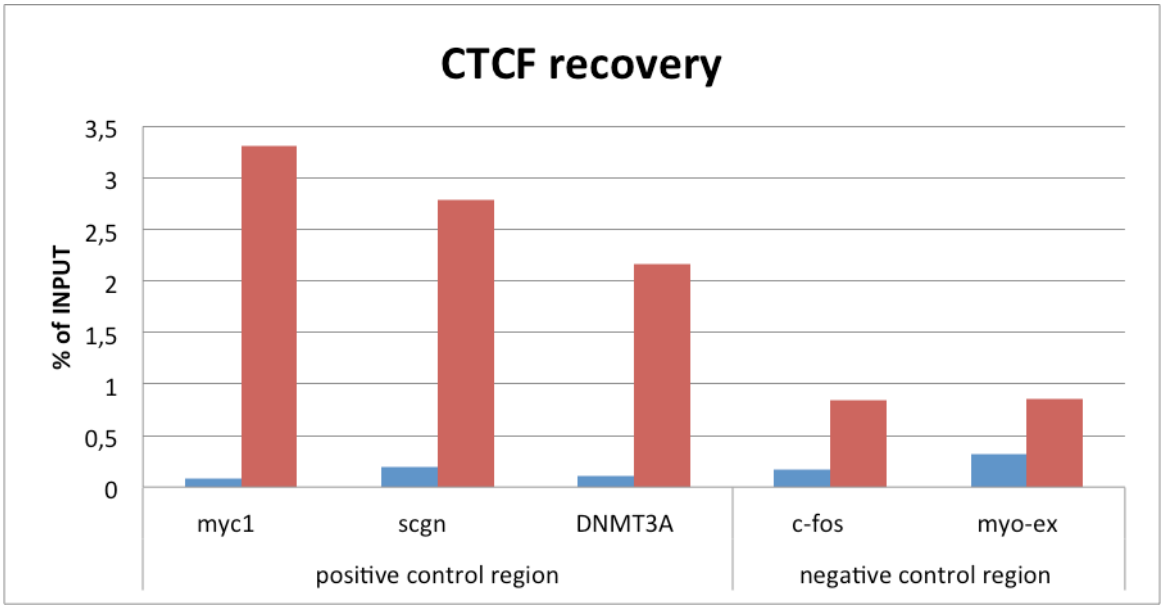
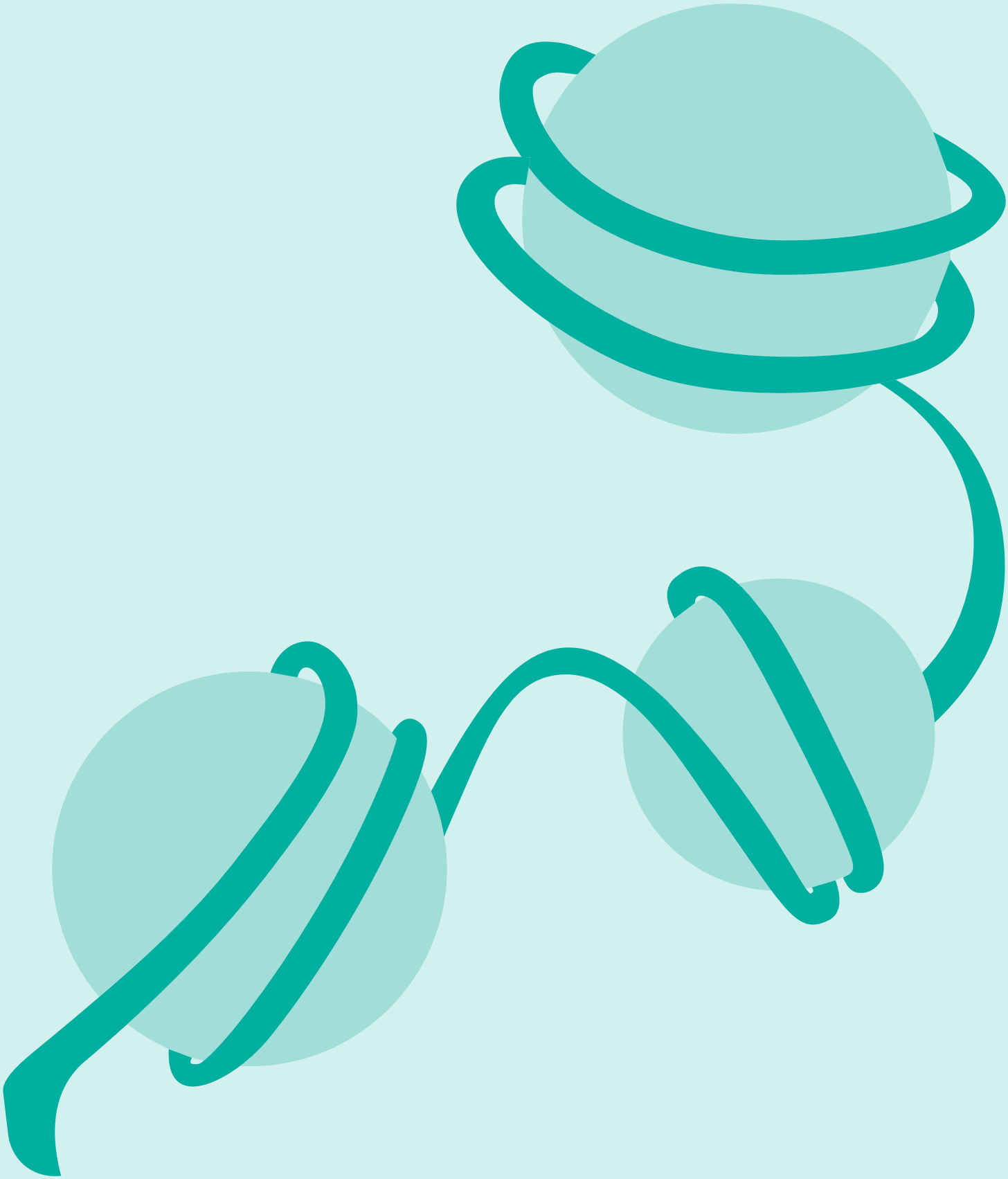


Figure 3. Chromatin immunoprecipitation analysis using CTCF antibody.

ChIP was performed on Mouse liver FFPE samples accordingly to the iDeal ChIP-FFPE protocol using 1 µl of CTCF antibody (Diagenode, Cat. no. C15410210).



ADDITIONAL PROTOCOLS

Protocol for deparaffinization and rehydrataion using xylene

Required reagents (not included in the kit):

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

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

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

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

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

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

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

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

6. Incubate for 10 minutes at room temperature.

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

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

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

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

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

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

Protocol for chromatin shearing analysis

General remarks

We recommend using the Fragment Analyzer (Advanced Analytical) or BioAnalyzer Agilent) for the size assessment of FFPE-derived chromatin due to a high sensitivity of these systems. A conventional agarose gel analysis is not recommended due to a low sensitivity.

For accurate size determination of the chromatin fragments, reverse crosslinking, followed by DNA purification including RNase treatment, 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:

- Reverse cross-linking (4 hours or overnight)
- DNA purification using MicroChIP DiaPure columns with on columns-RNase treatment (30 minutes)
- Fragment size assessment (Fragment Analyzer or BioAnalyzer) (1 hour)

1. Take **50 µl** of **sheared chromatin** (step 2.17 in the protocol) and transfer to a 1.5 ml microtube.

2. Add **50 µl** of **Elution Buffer iE1** and **4 µl** of **Elution Buffer iE2**, mix thoroughly and incubate samples at 65°C for 4 hours or overnight with shaking on Thermomixer.

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

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

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

6. Add **1 µl of RNase cocktail** directly to the center of the spin column membrane and incubate for 15 minutes at room temperature.

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

8. Add **6 µl** of **DNA Elution Buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥10,000 x g for 30 seconds to elute the DNA.

9. Analyze the purified DNA using Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit (DNF-473) or BioAnalyzer (High sensitivity kit).

Related products

Product	Cat. No.
Rabbit IgG	C15410206
Mouse IgG	C15400001
DiaMag Rotator	B05000001
Bioruptor Plus	B01020001
Bioruptor Pico	B01060001

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
H2A.Z polyclonal antibody	C15410201
H2BK15ac polyclonal antibody	C15410220
H3K27ac polyclonal antibody	C15410196
H3K27me3 polyclonal antibody	C15410195
H3K36me3 polyclonal antibody	C15410192
H3K4me1 polyclonal antibody	C15410194
H3K4me3 polyclonal antibody	C15410003
H3K79me3 polyclonal antibody	C15410068

ChIP-seq grade antibody	Cat. No.
H3K9/14ac polyclonal antibody	C15410200
H3R17me2(asym)K18ac pAb	C15410171
H4K20ac monoclonal antibody	C15210008
H4K20me3 polyclonal antibody	C15410207
macroH2A.1/H2A.2 mAb	C15210003
NF-E2 polyclonal antibody	C15410240
NFKB p65 polyclonal antibody	C15310256
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 1 10_2022	**October 2022**	Removal of the DiaMag1.5 magnetic rack Replacement of the DiaFilters by centrifugation

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