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iDeal ChIP-seq kit for Transcription Factors

Cat. No. **C01010055** (24 rxns)
C01010170 (100 rxns)

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Introduction

Association between proteins and DNA is a major mechanism in many vital cellular functions such as gene transcription and epigenetic silencing. 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.

The different steps of the ChIP assay are cell fixation (cross-linking), chromatin shearing, immunoprecipitation, reverse cross-linking followed by DNA purification and analysis of the immunoprecipitated DNA.

In ChIP, living cells are first fixed with a reversible cross-linking agent to stabilize protein-DNA interactions. The most widely used reagent to fix cells is formaldehyde which generates covalent bonds between amino or imino groups of proteins and nucleic acids. Formaldehyde treatment crosslinks both DNA-protein as well as protein-protein complexes.

Following cross-linking, chromatin needs to be sheared very efficiently into homogeneous small fragments that can subsequently be used in immunoprecipitation (IP). The Bioruptor® shearing devices from Diagenode provide you with high quality sheared chromatin ready-to-ChIP. Currently, the Bioruptor® is the most widely used and the most cited chromatin fragmentation system as evidenced by more than 1000 publications. Shearing may also be accomplished with shearing kits from Diagenode which enable an easy and highly reproducible shearing process for any cell type. After fragmentation, the sheared chromatin is precipitated with a specific antibody (AB) directed against the protein of interest. The chromatin-AB complex is isolated using magnetic beads. Finally, the precipitated DNA fragments are released from the AB, and analyzed. Enrichment of specific sequences in the precipitated (IP'd) DNA indicates that these sequences were associated with the protein of interest in vivo. Analyzis of specific regions can be performed by quantitative polymerase chain reaction (qPCR). In recent years, ChIP combined with high-throughput Next-Generation sequencing (ChIP-seq) has become the gold standard for whole-genome mapping of protein-DNA interactions.

Although ChIP-seq is a powerful tool, the procedure requires tedious optimization of several reaction conditions that might lead to considerable time and lab expenditures. To reduce these tedious steps, Diagenode provides kits with optimized reagents and protocols for ChIP which enable successful ChIP-seq. The new iDeal ChIP-seq kit protocol has been thoroughly optimized by Diagenode for ChIP followed by high-throughput sequencing on Illumina® HiSeq.

Diagenode also offers a wide range of additional tools and products for ChIP analysis including the Bioruptor® for chromatin shearing and nucleic acid fragmentation, the IP-Star® Compact for automated ChIP and DNA methylation analysis, magnetic racks for 0.2 or 1.5 ml tubes which enable you to work with magnetic beads under optimal conditions (4°C), and ChIP and ChIP-seq grade antibodies against many epigenetic targets. In addition, Diagenode provides individual reagents, such as magnetic beads, negative IP controls (mouse and rabbit IgG), protease inhibitors and deacetylase inhibitors (sodium butyrate). Diagenode also offers several qPCR primer pairs for the analysis of IP'd DNA. Peptides can additionally be purchased for use in blocking experiments (negative ChIP controls).

All our products have been extensively validated in ChIP on various targets. The combination of all our quality-controlled kits, reagents and equipment is a perfect starting point that will lead to your success.

Kit method overview & time table

Table 1 : iDeal ChIP protocol overview

Step		Time needed	Day
1	Cell collection and DNA-protein cross-linking (for cultured cells)	1 to 2 hours	1
2	Cell lysis and chromatin shearing (for cultured cells)	1 to 2 hours	1
3	Magnetic immunoprecipitation	overnight	1-2
4	Elution, decross-linking and DNA purification	5 hours	2
5	Quantitative PCR and data analysis prior to Library preparation and Next-Generation Sequencing	2 to 3 hours	3

Kit materials

The iDeal ChIP-seq kit for Transcription Factors x100 contains reagents to perform 17 different chromatin preparations, 100 Chromatin Immunoprecipitations and DNA Purificationfrom cell collection to IP'd DNA. The iDeal ChIP-seq kit for Transcription Factors x24 contains reagents to perform 4 different chromatin preparations, 24 Chromatin Immunoprecipitations and DNA Purificationfrom cell collection to IP'd DNA. The kit content is described in Table 2. Upon receipt, store the components at the temperatures indicated in Table 2.

Table 2: Components supplied with the iDeal ChIP-seq kit

Description	Quantity (x24)	Quantity (x100)	Storage
Protease inhibitor cocktail	95 µl	385 µl	-20°C
5% BSA (DNA free)	940 µl	3.5 ml	-20°C
Rabbit IgG	10 µg (1 µg/µl)	40 µg (1 µg/µl)	-20°C
ChIP-seq grade CTCF antibody	10 µg (2.5 µg/µl)	40 µg (2.5 µg/µl)	-20°C
ChIP-seq grade H19 imprinting control region primer pair	100 µl	500 µl	-20°C
ChIP-seq grade Myoglobin exon 2 primer pair	100 µl	500 µl	-20°C
Carrier	80 µl	320 µl	-20°C
Glycine	10 ml	40 ml	4°C
Shearing buffer iS1b	7.3 ml	31 ml	4°C
DiaMag Protein A-coated magnetic beads	870 µl	3.3 ml	4°C - DO NOT FREEZE
Wash buffer iW1	10 ml	42 ml	4°C
Wash buffer iW2	10 ml	42 ml	4°C
Wash buffer iW3	10 ml	42 ml	4°C
Wash buffer iW4	10 ml	42 ml	4°C
ChIP-seq grade water	30 ml	120 ml	4°C
Elution buffer iE2	175 µl	720 µl	4°C
Fixation buffer	6,1 ml	26 ml	4°C
Wash buffer 1 w/o iso-propanol	2 ml	8 ml	4°C
Wash buffer 2 w/o iso-propanol	2 ml	8 ml	4°C
Buffer C	2 ml	8 ml	4°C
IPure Beads v2	385 µl	1.6 ml	4°C - DO NOT FREEZE
Elution buffer iE1	4 ml	16 ml	4°C
5x ChIP buffer iC1b	7.5 ml	32 ml	4°C
Lysis buffer iL1b	110 ml	470 ml	4°C
Lysis buffer iL2	66 ml	280 ml	4°C

⚠ Store DiaMag Protein A-coated magnetic beads and IPure Magnetic beads at 4°C. Do NOT freeze magnetic beads because they may become damaged. Keep the beads in liquid suspension during storage as drying will result in reduced performance.

Table 3 : Modules available separately

Description	Reference	Quantity
Chromatin Shearing Optimization Kit - Low SDS (for TFs)	C01020013	25 rxns
ChIP Cross-link Gold	C01019027	600 µl
IPure kit v2 x24	C03010014	24 rxns
IPure kit v2 x100	C03010015	100 rxns

Required materials not provided

Reagents

- Gloves to wear at all steps
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS) buffer
- 1 M Sodium butyrate (NaBu) (Cat. No. C12020010) **(optional)**
- 100% isopropanol
- RNase/DNase-free 1.5 ml tubes
- qPCR SYBR® Green Mastermix
- Reagents for library preparation, cluster generation (Illumina®) or ePCR (Ion Torrent™ PGM™) and sequencing
- Quant-IT dsDNA HS assay kit (Invitrogen)
- Diagenode ChIP cross-link Gold (Cat N° C01019027) (Optional)

For library preparation, we highly recommend

- High Resolution Library Preparation kit (Cat. No. C05010023)
- MicroPlex Library Preparation™ kit v2
  (Cat. No. C05010012, 12 reactions, 12 indices) (Cat. No. C05010013, 48 reactions, 12 indices)

Equipment

- Optional: ChIPettor™ System for Transcription Factors (Cat. No. C01010163)
- Diagenode DiaMag1.5 magnetic rack (Cat. No. B04000003) and Diagende DiaMag02 magnetic rack (Cat. No. B04000001)
- Diagenode Bioruptor® sonication device (Cat. No. Standard: B01010001, Plus: B01020001, and Pico: B01060001)
- Diagenode 1.5 ml TPX Microtubes (optimized for chromatin shearing with Bioruptor® Standard or Plus) (Cat. No. C30010010) or 1.5 ml Bioruptor® Microtubes with Caps (Cat No C30010016) optimized for chromatin shearing with Bioruptor® Pico
- Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
- Cell counter
- DiaMag Rotator (Rotating wheel) (Cat. No. B05000001)
- Vortex
- Thermomixer
- Qubit system (Invitrogen)
- qPCR cycler
- For tissues:
  - Petri dishes

Remarks before starting

1. Cell number

This protocol has been optimized for ChIP on 4,000,000 cells in 350 µl ChIP reaction. For using lower amounts of cells, simply dilute the chromation in shearing buffer before adding it to the IP reaction. For higher cell numbers you can increase the cell concentration in the shearing buffer, although this may require an additional optimization of the shearing conditions. Therefore, we recommend performing separate ChIP's and pool the IP'd DNA before purification.

2. Cell fixation

Formaldehyde is the most commonly used cross-linking reagent. However, formaldehyde is usually not effective to cross-link proteins that are not directly bound to the DNA. For example, chromatin interactions with inducible transcription factors or with cofactors that interact with DNA through protein-protein interactions are not well preserve with formaldehyde. When studying this kind of factors, we recommend the use of the Diagenode **ChIP cross-link Gold (Cat N° C01019027)**. This reagent is to use in combination with formaldehyde. The protocol involves a sequential fixation. A first protein-protein fixation by the ChIP cross-link Gold followed by protein-DNA fixation by formaldehyde.

3. Shearing optimization and sheared chromatin analysis

Before starting the ChIP, the chromatin should be sheared into fragments of 100 to 600 bp. Our kits and protocols are optimized for chromatin shearing using the Bioruptor® (Standard, Plus and Pico). The maximum volume for shearing with the Bioruptor® is 300 µl per 1.5 ml Microtube (depending on the specific type). We recommend using **TPX tubes (C30010010) for Bioruptor® Standard and Plus** as shearing has been shown to be more efficient and reproducible using these tubes. **For Bioruptor® Pico we recommend using 1.5 ml Microtubes with Caps (C30010016)**. The shearing conditions mentioned in the protocol are adequate for a variety of cell types. However, given that cell types are different, we recommend optimizing sonication conditions for each cell type before processing large quantities of cells or samples. It is important to perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation. A protocol to assess the shearing efficiency can be found in the "Additional Protocols" section.

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 lead to lower reproducibility. Do not freeze the beads.

The amount of beads needed per IP depends on the amount of antibody used for the IP. The protocol below uses 30 µl of beads. The binding capacity of this amount is approximately 10 µg of antibody. With most of Diagenode's high quality ChIP-seq grade antibodies the recommended amount to use is 1 to 2 µg per IP reaction. Therefore, you can reduce the amount of beads accordingly.

5. Negative and positive IP controls (IgG and control Ab)

The kit contains a negative (IgG) and a positive (CTCF) control antibody. We recommend including one IgG negative IP 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 (H19 imprinting control region and Myoglobin exon 2, respectively).

6. Quantification

Determine the concentration of the IP'd DNA after the ChIP with a highly sensitive method such as the 'Quant-IT dsDNA HS assay kit' on the Qubit system from Invitrogen. 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 expected DNA yield will be dependent on different factors such as the cell type, the quality of the antibody used and the antibody target. The expected DNA yield obtained with the positive control CTCF antibody on 4,000,000 HeLa cells is approximately 20 ng.

7. Quantitative PCR

Before sequencing the samples, we recommend analysing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. The kit contains a positive and negative control primer pair which can be used for the CTCF positive control antibody in SYBR® Green qPCR assay using the protocol described in the manual. Use your own method of choice for analysing the appropriate control targets for your antibodies of interest.

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 them in triplicate is recommended to be able to identify potential outliers.

7. Quantitative PCR data interpretation

The efficiency of chromatin immunoprecipitation of particular genomic loci can be expressed as the recovery of that locus calculated as the percentage of the input (the relative amount of immunoprecipitated DNA compared to input DNA). If the amount used for the input was 1% of the amount used for ChIP, the recovery can be calculated as follows:

% recovery = $2^{(Ct_{input} - Ct_{sample})}$

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. This equation assumes that the PCR is 100% efficient (amplification efficiency = 2). For accurate results the real amplification efficiency, if known, should be used.

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%.

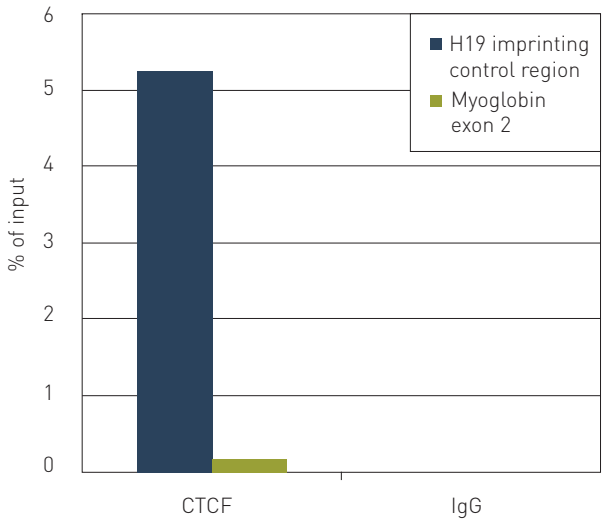


Figure 1: ChIP was performed on human HeLa cells using the control antibodies from the iDeal ChIP-seq kit for Transcription Factors. Sheared chromatin from 4 million cells, 0.5 µl of the positive control antibody and 1 µl of the negative IgG control were used per IP. Quantitative PCR was performed with the positive control H19 imprinting control region and the negative control Myoglobin exon 2 primer sets from the kit. The recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis)

Short protocol for experienced users

STEP 1. Cell collection and DNA-protein cross-linking

Note:
When studying inducible transcription factors or cofactors, it is recommended to perform the fixation using the ChIP cross-link Gold (C01019027) in addition to the formaldehyde fixation

1. Dilute formaldehyde in **Fixation buffer** to a final concentration of 11%.
2. Add **1/10 volume of diluted formaldehyde** directly to the cell culture medium.
3. Incubate at room temperature for 10 to 20 minutes with gentle shaking.
4. Stop the fixation by adding **1/10 volume of glycine**.

STEP 2. Cell lysis and chromatin shearing

For adherent cells (~25 million cells):

5a. Remove the medium and wash the cells once with **20 ml of PBS**. Keep everything at 4°C from now on.

6a. Add **5 ml of cold lysis buffer iL1b** to the plate and collect the cells by scraping.

7a. Rinse the flask with an additional **20 ml of buffer iL1b**.

8a. Incubate at 4°C for 20 minutes.

For suspension cells (~25 million cells):

5b. Collect the cells by centrifugation at 1,600 rpm and 4°C for 5 minutes.

6b. Wash the cells once with **20 ml of PBS**. Keep everything at 4°C from now on.

7b. Resuspend the cells in **25 ml of cold lysis buffer iL1b**.

8b. Incubate at 4°C for 20 minutes.

9. Discard the supernatant and resuspend the cells in **15 ml lysis buffer iL2**. Incubate at 4°C for 10 minutes with gentle mixing.

10. Centrifuge at 1,600 rpm and 4°C for 5 minutes.

11. Add **1/200 volume protease inhibitor cocktail** (PIC) to shearing buffer iS1b.

12. Discard the supernatant and resuspend the cells in **shearing buffer iS1b + PIC** to a final concentration of 15 million cells/ml. Resuspend by pipetting up and down.

13. Shear chromatin by sonication using the Bioruptor®. An initial time course experiment is recommended.

- For Bioruptor® Standard or Plus use High power setting for 10-30 cycles (30 seconds ON, 30 seconds OFF). Stop the system after each run of 10 cycles, vortex and spin down sample.
- For Bioruptor® Pico, sonicate samples for 8-10 cycles (30 seconds ON, 30 seconds OFF). Vortexing is not required between runs.

14. Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin.

STEP 3. Magnetic immunoprecipitation

The immunoprecipitation step can also be performed using the semi-automated **ChIPettor™ System.** If doing so, please refer to the corresponding protocol delivered with this product. Alternatively, the protocol can also be downloaded from the website www.diagenode.com

15. Prepare **4 ml of 1x ChIP buffer iC1b**. Add **80 µl of 5% BSA**.
16. Take the required amount of **magnetic beads (30 µl/ChIP)** and was them 3 times with **1 ml 1x ChIP buffer iC1b + BSA**.
17. Resuspend the beads in the original volume of **1x ChIP buffer iC1b + BSA**.
18. Prepare the following ChIP reaction mix:
    6 µl of 5% BSA
    1.8 µl of 200x PIC
    20 µl of 5x ChIP buffer iC1b
    42.2 µl H2O
    30 µl beads
19. Add the required amount of **antibody**.
20. Incubate for 2-4 hours (or overnight) at 4°C on a rotating wheel.
21. Add **250 µl sheared chromatin**. Keep **2.5 µl chromatin** aside to serve as input.
22. Incubate overnight at 4°C on a rotating wheel.
23. The next day, briefly spin the tubes, place them in the ice-cold magnetic rack and discard the supernatant.
24. Add **350 µl ice-cold Wash buffer iW1** and incubate for 5 min at 4°C on a rotating wheel. Discard the wash buffer using the **Diagenode magnetic rack**.
25. Repeat step 24 and 25 once with **Wash buffer iW2, iW3 and iW4**, respectively.

STEP 4. Elution, decross-linking and DNA purification

26. After removing the last wash buffer, add 100 µl of **Elution buffer iE1** to the beads and incubate for 30 min on a rotating wheel at room temperature. Resuspend the beads pellet and transfer it into a new 200 µl tube
27. Briefly spin the tubes and place them into the Diagenode magnetic rack. Transfer the supernatant to a new tube and add 4 µl of **Elution buffer iE2**. Also 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.
28. Purify the DNA using the IPure kit.
29. Add 2 µl of **carrier** to each IP and input sample.
30. Add 100 µl of 100% isopropanol to each IP and input sample.
    ATTENTION: 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.
31. Resuspend the **IPure beads v2** and transfer 10 µl to each IP and input sample.
32. Vortex well and incubate 10 minutes at room temperature on a rotating wheel (40 rpm).
33. Prepare the **Wash buffer 1** containing 50% isopropanol:

Wash buffer 1		
	24 rxns	100 rxns
Wash buffer 1 w/o isopropanol	2 ml	8 ml
Isopropanol (100%)	2 ml	8 ml
Total volume	4 ml	16 ml

35. Briefly spin the tubes, place into the **DiaMag0.2 - magnetic rack**, wait 1 min and discard the buffer. Add 100 µl **Wash buffer 1 in each tube**. Close the tubes, incubate for 30 seconds at room temperature on a rotating wheel (40 rpm).

36. Prepare the **Wash buffer 2** containing 50% isopropanol as follows:

Wash buffer 2		
	24 rxns	100 rxns
Wash buffer 2 w/o isopropanol	2 ml	8 ml
Isopropanol (100%)	2 ml	8 ml
Total volume	4 ml	16 ml

38. Briefly spin the tubes, place into the **DiaMag0.2 - magnetic rack**, wait 1 min and discard the buffer. Keep the captured beads and add 100 µl **Wash buffer 2** per tube. Close the tubes, resuspend the beads and incubate for 5 min at room temperature on a rotating wheel (40 rpm).

39. Briefly spin the tubes and place them into the DiaMag0.2 or DiaMag1.5, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, 25 µl buffer C (alternatively, a higher volume can be used for the elution if necessary). Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 15 minutes at room temperature on a rotating wheel (40 rpm). Resuspend the pelleted beads using the pipet and make sure that you drop them on the bottom of the tube

STEP 5. Quantitative PCR analysis

40. Spin the tubes and place them into the **DiaMag0.2 - magnetic rack**, wait 1 min and transfer the supernatants into a new labelled 1.5 ml tube and discard the beads.

41. 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 mix
    4 µl of water
    5 µl IP'd or input DNA

42. Use the following PCR program: 3 to 10 min denaturation step at 95°C (please check carefully supplier's recommendations about Taq polymerase activation time), followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72 °C. These conditions may require optimization depending on the type of Master Mix or qPCR system used.

Detailed protocol

STEP 1. Cell collection and DNA-protein cross-linking

1. Dilute formaldehyde in **Fixation buffer** to a final concentration of 11%, e.g. add **5 ml of a 37% formaldehyde** solution to **11.8 ml Fixation buffer**. For a T175 culture flask you will need ~2 ml of diluted formaldehyde.

NOTE:
When studying inducible transcription factors or cofactors, it is recommended to perform the fixation using the ChIP cross-link Gold (C01019027) in addition to the formaldehyde fixation

2. Add **1/10 volume of the diluted formaldehyde** directly to the cell culture medium.
3. Incubate the cells for 10 to 20 minutes at room temperature with gentle shaking. The fixation time can depend on your target of interest.
4. Add **1/10 volume of Glycine** to the cell culture medium to stop the fixation. Incubate for 5 minutes at room temperature with gentle shaking

NOTE:
The fixed cells can be stored at -80°C for up to 4 months. However, we strongly recommend using freshly fixed cells for preparation of sheared chromatin prior to ChIP for ChIP-sequencing.

STEP 2. Cell lysis and chromatin shearing

For adherent cells:

5a. Remove the medium and wash the cells once with **20 ml of PBS**. Keep everything at 4°C from now on.

6a. Add **5 ml of cold Lysis buffer iL1b** to the plate and collect the cells by scraping.

7a. Add an additional volume of **Lysis buffer iL1b** to rinse the flask and add this to the collected cells. The total volume of Lysis buffer iL1b should be about 10 ml per 10^7 cells (e.g. for a T175 culture flask (~25 milllion cells), rinse with an additional **20 ml of buffer iL1b**.

8a. Incubate at 4°C for 20 minutes.

For suspension cells:

5b. Pellet the cells by centrifugation at 1,600 rpm and 4°C for 5 minutes. Discard the cell culture medium.

6b. Wash the cells once with PBS. Resuspend the cells in **20 ml of PBS**, centrifuge at 1,600 rpm and 4°C for 5 minutes and discard the supernatant. Keep everything at 4°C from now on.

7b. Add **1 ml ice-cold lysis buffer iL1b** to the cell pellet and resuspend the cells by pipetting up and down several times. Add an **additional amount of buffer iL1b** to obtain a total volume of about 10 ml per 10^7 cells (e.g. for a T175 culture flask (~25 milllion cells), add an additional 24 ml of buffer iL1b.

8b. Incubate at 4°C for 20 minutes.

9. Pellet the cells by centrifugation at 1,600 rpm for 5 minutes and 4°C and discard the supernatant.

10. Add **1 ml ice-cold Lysis buffer iL2** to the cell pellet and resuspend the cells by pipetting up and down several times. Add an additional amount of buffer iL2 and incubate for 10 minutes at 4°C with gentle mixing. For 25 million cells the total amount of iL2 should be 15 ml.

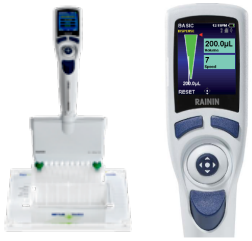
11. Pellet the cells again by centrifugation for 5 minutes at 1,600 rpm (500 x g) and 4°C and discard supernatant.

12. Add **200x protease inhibitor cocktail to Shearing buffer iS1b**. Prepare 1 ml of complete shearing buffer per tube of 15 million cells. Keep on ice

13. Add **1 ml of complete Shearing buffer iS1b** to 15 Million cells. Resuspend the cells by pipetting up and down several times. The final cell concentration should be 1.5 Million cells per 100 µl buffer iS1b. Split into aliquots of 100 to 300 µl and transfer the cell suspension to 1.5 ml TPX microtubes (Diagenode cat. No. M-50001) when using the Bioruptor Standard or Plus or to 1.5 ml Bioruptor® Microtubes with Caps (Cat No C30010016) optimized for chromatin shearing with the Bioruptor® Pico.

14. Shear the chromatin by sonication using the Bioruptor®. When using the Bioruptor Standard or Plus, shear for 1 to 3 runs of 10 cycles [30 seconds "ON", 30 seconds "OFF"] each at high power setting. Briefly vortex and spin between each run. Shear for 8-10 cycles [30 seconds "ON", 30 seconds "OFF"] when using the Bioruptor Pico. These shearing conditions will work excellent for many cell types. However, depending on the cell type and Bioruptor® system used, optimisation may be required.

STEP 3. Magnetic immunoprecipitation



The immunoprecipitation step can also be performed using the semi-automated **ChIPettor™ System.** If doing so, please refer to the corresponding protocol delivered with this product. Alternatively, the protocol can also be downloaded from the website www.diagenode.com

15. Centrifuge at 13,000 rpm (16,000 x g) for 10 minutes and collect the supernatant which contains the sheared chromatin. Use the chromatin immediately in immunoprecipitation or store it at -80°C for up to 2 months. If desired, the chromatin shearing effciciency can be analysed at this step (see the protocol in Additional protocols.)

This protocol has been optimised for ~4 Million cells per ChIP, although it is possible to reduce or increase the amount of cells. For using lower amounts of cells, simply dilute the chromation in shearing buffer before adding it to the IP reaction. For higher cell numbers you can increase the cell concentration in the shearing buffer, although this may require an additional optimization of the shearing conditions. Therefore we recommend performing separate ChIP's and pool the samples before purification of the DNA

16. Determine the total nr. of IP's in the experiment. Please note that we recommend to include one negative control in each experiment (IP with the IgG negative control. Take the required amount of **DiaMag Protein A-coated magnetic beads** (30 µl/IP). Prepare **4 ml of 1x ChIP buffer iC1b** by adding **3.2 ml ChIP-seq grade water** to **0.8 ml 5x ChIP buffer iC1b**. Add **80 µl of 5% BSA**. Keep the diluted ChIP buffer iC1 on ice.

17. Wash the beads 3 times with **1 ml of ice-cold 1x ChIP buffer iC1b**. To wash the beads add 1x ChIP buffer iC1, resuspend the beads by pipetting up and down several times and incubate at 4°C with gentle shaking for 5'. Spin the tubes and place them in the **1.5 ml Diagenode magnetic rack** (Cat. No. B04000003). Wait for one minute to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 2 times. Alternatively, you can centrifuge the tubes for 5 minutes at 1,300 rpm, discard the supernatant and keep the bead pellet.

18. After the last wash, resuspend the beads in the original volume **1x ChIP Buffer iC1b**.

19. Take the required number of tube and add **30 µl of the washed beads** to each tube.

20. Prepare the ChIP reaction mix according to Table 3. If required, NaBu (20 mM final concentration) or other inhibitors can also be added. Add 70 µl of ChIP reaction mix to the tubes containing the beads. In table 3, x is the amount of antibody needed. If you will use different amounts of antibody (e.g. when performing a titration curve), we recommend to add the water separately.

Table 3: ChIP reaction mix

N° of IP's	5% BSA (µl)	200x Protease inhibitor cocktail (µl)	5x buffer iC1b (µl)	ChIP-seq grade water (µl)	Antibody (µl)
1	6	1.8	20	42.2-x	x
2	12	3.3	40	84.4-x	x
4	24	7.2	80	168.8-x	x
6	36	10.8	120	253.2-x	x
8	48	14.4	160	337.6-x	x

21. Add the antibody to the reaction mix. Use **1 µl of the IgG negative control antibody** for the negative control IP. If a positive control IP is included in the experiment, use **0.5 µl of the CTCF positive control antibody**.

22. Incubate the tubes for 2-4 hours at 4°C under constant rotation at 40 rpm on a rotating wheel.
23. Briefly spin the tubes and add **250 µl of sheared chromatin**. Put 2.5 µl of the sheared chromatin aside to be used as an input the next day. Incubate the tubes overnight at 4°C under constant rotation at 40 rpm on a rotating wheel.
24. The next morning, after the overnight incubation, briefly spin the tubes and place them in the magnetic rack. Wait for one minute and remove the supernatant. Wash the beads with **Wash buffer iW1**. To wash the beads, add **350 µl of iW1**, gently shake the tubes to resuspend the beads and incubate for 5 minutes on a rotating wheel at 4°C.
25. Repeat the wash as described above once with **Wash buffer iW2, iW3 and iW4**, respectively.

STEP 4. Elution, decross-linking and DNA purification

26. After removing the last wash buffer, add 100 µl of **Elution buffer iE1** to the beads and incubate for 30 min on a rotating wheel at room temperature. Resuspend the beads pellet and transfer it into a new 200 µl tube
27. Briefly spin the tubes and place them into the Diagenode magnetic rack. Transfer the supernatant to a new tube and add 4 µl of **Elution buffer iE2**. Also 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.
28. Pool samples if necessary.

NOTE:
Up to 2 samples can be easily pooled. If more than 2 samples need to be pooled, process each sample purification individually, pool final eluates at the end of the IPure purification and concentrate (e.g. using Microcon®)

29. Add 2 µl of **carrier** to each IP and input sample.
30. Add 100 µl of 100% isopropanol to each IP and input sample.

    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.
31. Resuspend the provided **IPure beads v2** and transfer **10 µl** to each IP and input sample.
    - Keep **IPure beads v2** in liquid suspension during storage at 4°C and at all handling steps, as drying will result in reduced performance.
    - The final volume is now 116 µl per IPure reaction.
32. Incubate IP and input samples for 10 minutes at room temperature on a rotating wheel (40 rpm).
33. Prepare the **Wash buffer 1** containing 50% isopropanol:

Wash buffer 1		
	24 rxns	100 rxns
Wash buffer 1 w/o isopropanol	2 ml	8 ml
Isopropanol (100%)	2 ml	8 ml
Total volume	4 ml	16 ml

34. - Never leave the bottle open to avoid evaporation.
35. Briefly spin the tubes, place in the **DiaMag02**, wait 1 min and discard the buffer. Add 100 µl **Wash buffer 1 per tube**. Close the tubes and vortex well until the beads pellet is completely broken. In order to avoid the beads pellet to be too difficult to break down, do not let the beads for too long on the magnet, incubate for 30 seconds at room temperature on a rotating wheel (40 rpm).
    - Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning into the **Diagenode Magnetic Rack**. Prepare the **Wash buffer 2** containing 50% isopropanol as follows:

Wash buffer 2		
	24 rxns	100 rxns
Wash buffer 2 w/o isopropanol	2 ml	8 ml
Isopropanol (100%)	2 ml	8 ml
Total volume	4 ml	16 ml

- Never leave the bottle open to avoid evaporation.

36. Wash the IP and input samples with the Wash buffer 2 as follows. Briefly spin the tubes, place into the **DiaMag1.5**, wait 1 min and discard the buffer. Add 100 µl **Wash buffer 2 per tube**. Close the tubes and vortex well until the beads pellet is completely broken. In order to avoid the beads pellet to be too difficult to break down, do not let the beads for too long on the magnet, incubate for 30 seconds at room temperature on a rotating wheel (40 rpm).

- Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning in the DiaMag rack.

NOTE:
This **Elution buffer (buffer C)** is suitable for direct qPCR analysis, whole genome amplification, chip hybridization and Next-Generation sequencing.

37.

a. Briefly spin the tubes and place them into the **DiaMag02**, wait 1 min and discard the buffer. Spin the tubes again and place them on the DiaMag02 magnetic rack. Discard the remaing Wash buffer 2 if necessary. Resuspend the beads pellet with 25 µl of buffer C. Incubate at room temperature for 15 minutes on a rotating wheel (40 rpm).

b. Spin the tubes and place them into the **DiaMag1.5**, wait 1 min and transfer the supernatants into a new labelled 1.5 ml tube and discard the beads

c. Place the DNA on ice and proceed to any desired downstream applications, or store it at -20°C or -80°C until further use.

40. Take 5 µl (10%) of IP'd DNA and determine the concentration with 'Quant-IT dsDNA HS assay kit' using the Qubit system or a similar method.

41. Store the DNA at -20°C until you are ready to analyze it with qPCR or by high throughput sequencing.

STEP 5. Quantitative PCR analysis

Before sequencing the samples, we recommend analysing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. The kit contains a positive (H19 imprinting control region) and negative (Myoglobin Exon 2) control primer pair which can be used for the positive control antibody provided in the kit (CTCF ChIP-seq grade antibody) in SYBR® Green qPCR assay using the protocol described below. Use your own method of choice for analysing the appropriate control targets for your antibodies of interest.

42. 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 mix
- 4 µl of water
- 5 µl IP'd or input DNA

43. Use the following PCR program: 3 to 10 min denaturation step at 95°C (please check carefully supplier's recommendations about Taq polymerase activation time), followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72 °C. These conditions may require optimization depending on the type of Master Mix or qPCR system used.

ChIP-sequencing

The iDeal protocol has been optimized 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.

?

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:
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ChIP-seq data analysis recommendations

To find the captured regions of the genome after sequencing you must perform a) a reference alignment followed by b) a peak calling, then c) further data analysis (annotation, visualization etc.) to help you find what you are looking for. There are abundant software tools for each task that use different approaches to the same problem; choose your preferred one considering your dataset and scientific goals. The workflows for different sequencers basically differ only in the alignment step, since every sequencer has its own characteristic read set (short or long, fixed or variable length, nucleotide or colour code etc.).

a) The built-in aligners with default settings worked very well for our ChIP-seq experiments (e.g. **ELAND** for Illumina®®, **TMAP** for PGM). If you cannot access them, open source tools are also available; we have positive experience with **BWA**: http://bio-bwa.sourceforge.net. If you use a multipurpose aligner, do not forget to use settings appropriate to your dataset; please consult with the manual of your software.

b) The purpose of the peak calling is to find the enriched regions in the alignment. Take extreme care when you choose and set up your peak caller, since the outcome can vary widely depending on the used software and its settings. We advise you to read the comparative literature and the software manuals to fully understand

how a certain program works. One of the key features of your data is the expected length of the enrichment regions. Transcription factors tend to produce short and sharp peaks, while histone marks create broad islands of enrichment. A remarkable tool for sharp peak detection is **MACS**, while **SICER** is dedicated to histone marks, and tools like **ZINBA** can be used for both with decent outcomes. **MACS 2** is reported to be better suited for histone marks than previous versions.

The availability of the mentioned softwares:

- **MACS**: http://liulab.dfci.harvard.edu/MACS
- **MACS 2**: https://github.com/taoliu/MACS/tree/master/MACS2
- **SICER**: http://home.gwu.edu/~wpeng/Software.htm
- **ZINBA**: http://code.google.com/p/zinba

We are extensively using **MACS 1.4.1** for our experiments. While it is a prominent tool for shorter peaks, sometimes it has difficulties with broader regions, therefore we recommend you to set a wider local peak background and lower the pvalue cutoff if necessary for histone marks. In some cases turning off the local lambda calculation provides a better coverage of broad enrichment islands, though this can result in more false positive peaks detected. Please refer to the MACS manual (http://liulab.dfci.harvard.edu/MACS/README.html) if you are not sure how to tweak the parameters.

c) Having your peaks you can start decrypting the epigenetic code.

The visual inspection is a common first step, especially if the aim of your experiment was to see if certain genes have certain histone modifications/transcription factors attached, or you want to check some positive/negative control sites for enrichment. Choose the appropriate viewer software according to the output format of your peak caller and your preferences.

Annotation is always very useful, since you can identify biological features that are relevant to your peaks, or check if you have the peaks at the expected loci, like H3K4me3 enrichments in the promoter regions of active genes. You can expand the annotation with a gene ontology/pathway analysis of the peak associated genes, thus discovering how your transcription factor/histone modification is involved in the cell's or the whole organism's life.

Motif search is almost an obligatory analysis for the sequence specific transcription factors, but you may find common motifs among histone modification sites as well, so you can check for example if you indeed have promoter specific motifs in your theoretically promoter specific enrichments.

A lot of programs, including peak callers themselves output descriptive statistics of the peaks, measuring for example their enrichment ratios, significances, width, heights, reads in peaks. This characterization helps you better understand your data, which is essential for most applications; a typical example is the comparison of performance of different sample preparation protocols or different sequencer setups.

The final recommended analysis type is the comparative analysis. We encourage scientists to use replicates in their experiments; removing peaks that are not common could effectively reduce false positives. You can also use a validated reference set of peaks for comparisons, but that is rarely available. Additionally, if you have other biologically relevant data from your samples, it is wise to compare and integrate them. For example, an RNA-seq dataset is a great source of validation for histone marks that are supposed to regulate gene expression.

Recommended free tools for the peak analysis:

- **IGV** (visualization): http://www.broadinstitute.org/igv
- **UCSC Genome Browser** (visualization): http://genome.ucsc.edu
- **HOMER** (motif search, annotation, gene ontology, comparison, statistics): http://biowhat.ucsd.edu/homer
- **PinkThing** (annotation, conservation, comparison, gene ontology, statistics): http://pinkthing.cmbi.ru.nl
- **GREAT** (annotation, statistics): http://great.stanford.edu

When analysing ChIP-seq, please always keep an eye on sequencing quality and the performance of the software tools used for analysis. For example with a low quality sequencing with a lot of read errors you will have a hard time finding the peaks you are looking for, despite your excellent IP'd DNA. To control the quality use the **vendor supplied software** and metrics, like the ones available in the Illumina®® pipeline for GA II. Open source tools can

also be used, e.g. the **FastQC** by Babraham Institute: http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc.

Throughout this chapter we recommended some free tools, because they are accessible for everyone and we have tested most of them. Please note that there are commercial softwares for the same purposes as well, most of them capable of performing several tasks, or even a complete ChIP-seq workflow. Here are a few examples that we know of (but we have not tested them):

- **CLC Genomics Workbench**: http://clcbio.com
- **Partek Genomics Suite**: http://www.partek.com/partekgs
- **NextGENe**: http://www.softgenetics.com/NextGENe.html
- **Avadis NGS**: http://www.avadis-ngs.com
- **Geneious**: http://www.geneious.com/web/geneious/geneious-pro
- **GenoMiner**: http://www.astridbio.com/genominer.html
- **GenoMatix**: http://www.genomatix.de

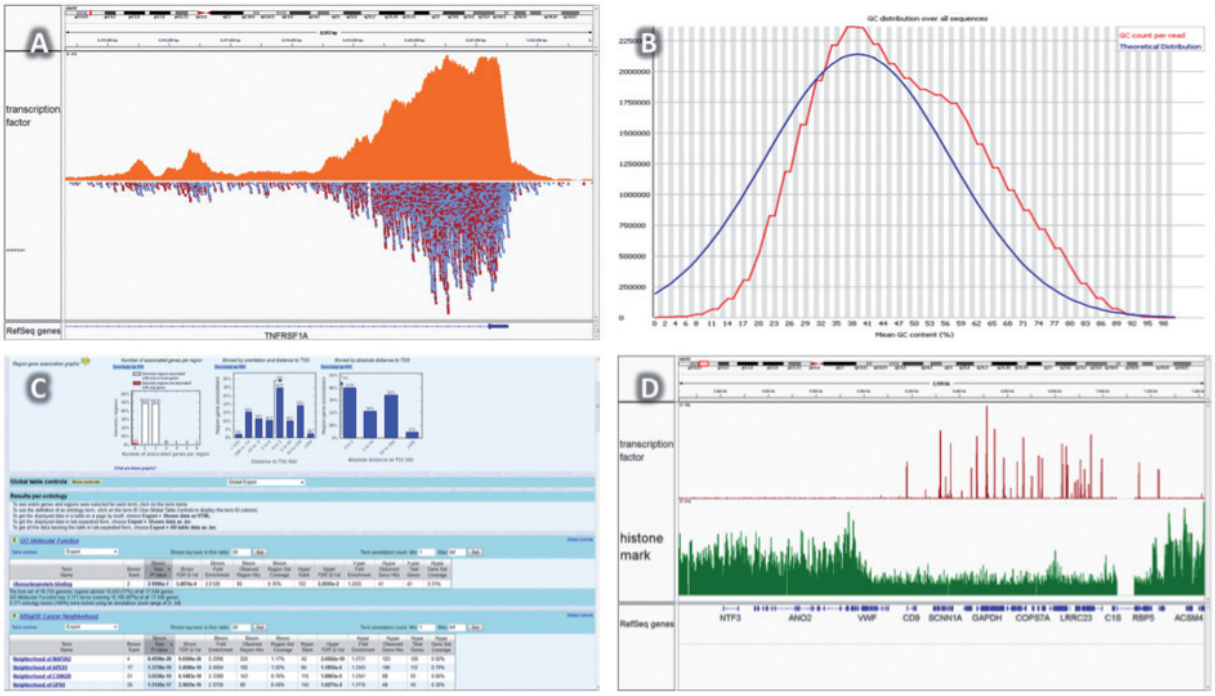

Figure 5
Various stages of bioinformatics data analysis

Representative images made during bioinformatics analysis of ChIP-seq data.

A: The reads are accumulating around the binding site to form a peak like structure in the coverage graph. Peak callers are used to detect these peaks.

B: A quality control software (like FastQC) anlyses numerous parameters that can help us assess the goodness of sequencing. Here we can monitor the GC content distribution.

C: Descriptive statistics and annotation output by GREAT.

D: Transcription factors tend to produce sharp peaks (upper red band), while broad enrichments are characteristic of many histone modifications (lower green band).

Aditional protocols

Sheared chromatin analysis

Reagents not supplied with the iDeal ChIP-seq kit

- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- 100% Ethanol
- 70% Ethanol
- DNA precipitant (Cat. No. C03030002)
- DNA co-precipitant (Cat. No. C03030001)

1. Take an aliquot of 50 µl of sheared chromatin and spin the chromatin at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.
2. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1µl of cocktail in 150 µl of ChIP-seq grade water).
3. Add 2 µl of diluted RNase cocktail.
4. Incubate 1h at 37°C.
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 4h (or overnight).
8. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the sample at RT for 10 min on a rotating wheel.
9. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
10. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.
11. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
12. Precipitate the DNA by adding 10 µl DNA precipitant, 5 µl of co-precipitant, and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
13. Centrifuge for 25 min at 13,000rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.
14. Centrifuge for 10 min at 13,000 rpm at 4°C. Carefully remove the supernatant, leave tubes open for 30 min at RT to evaporate the remaining ethanol.
15. Re-suspended the pellet in 20 µl of TE buffer.
16. Run samples (20 µl of DNA + 4 µl of 6x loading dye) in a 1.5% agarose gel.

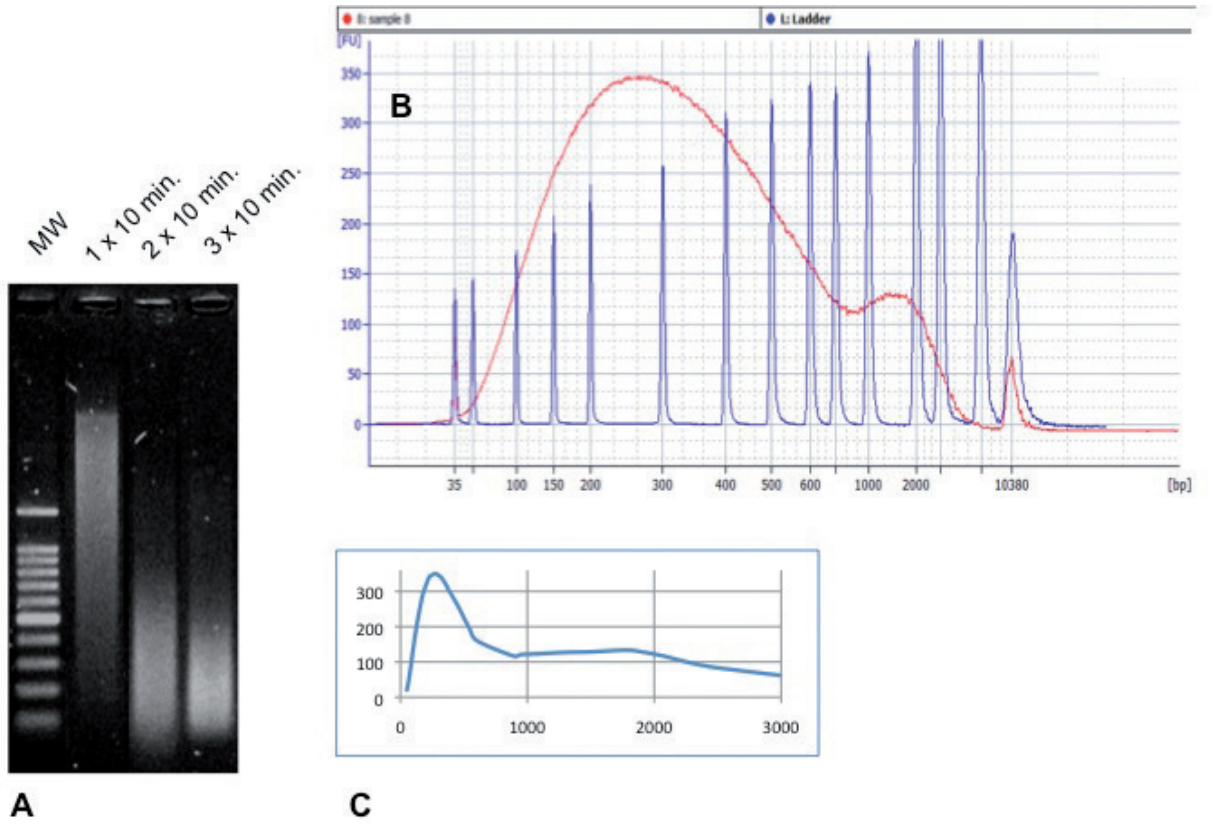

Figure 6

Superior chromatin shearing results with the Bioruptor® Plus using buffers and protocol of the Diagenode iDeal ChIP-seq kit

Hela cells were fixed with 1% formaldehyde (for 8 min at RT). Nucleus isolation of five million fresh or frozen (stored at -80°C) cells are performed using buffers of the Diagenode iDeal ChIP-seq kit (Cat. No. C01010050) and are then resuspended in 200µl of **Shearing Buffer iS1** prior to chromatin shearing.

Samples are sheared during 1, 2 or 3 rounds of 10 cycles of 30 sec ON / 30 sec OFF with the Bioruptor® Plus combined with the Bioruptor® Water cooler at HIGH power setting (position H). For optimal results, samples are vortexed before and after performing 10 sonication cycles, followed by a short centrifugation at 4°C. All samples were treated with RNase (see "**Additional Protocols**").

Panel A: 10 µl of DNA (equivalent to 300 ng) are analyzed on a 1.5% agarose gel.

Panel B and C: Sample 3 (3x 10 min) was analyzed on Bioanalyzer 2100 using DNA High Sensitivity chip. The default log scaled Bioanalyzer output can be seen in Panel A, while Panel C represents their linear transformation for better vizualisation. Out of range fragments were not shown in this graph.

In this example, the optimal shearing condition corresponds to 3 rounds of 10 cycles (30 sec ON / 30 sec OFF).

Troubleshooting guide

Critical steps	Troubles, solutions and comments	
Cross-linking	Cross-linking is too weak.	Make sure you perform the fixation step for the correct period of time, at the right temperature and with the correct formaldehyde concentration. e.g: incubate for 10-20 minutes at room temperature with 1 % formaldehyde final concentration (weight/ volume). Also, use high quality, fresh formaldehyde.
	Cross-linking is too strong.	
	Proteins have unique ways of interacting with the DNA. Some proteins are not directly bound to the DNA but interact with other DNA-associated proteins.	Very short or very long cross-linking time can lead to DNA loss and/or elevated background, therefore the optimal cross-linking time should be found empirically as maximal specificity and efficiency of ChIP.
	Both cross-linking time and formaldehyde concentration are critical.	Cross-linking can affect both efficiency of chromatin shearing and efficiency of specific antigen immunoprecipitation. Shorter cross-linking times (5 to 10 minutes) and/ or lower formaldehyde concentrations (<1%, weight/ volume) may improve shearing efficiency while, for some proteins especially those that do not directly bind DNA, this might reduce the efficiency of cross-linking and thus the yield of precipitated chromatin.
	The optimal duration of cross-linking varies between cell type and protein of interest.	It is possible to optimize the fixation step by testing different incubation times: such as 10, 20 and 30 minutes. Do not cross-link for longer than 30 minutes as cross-links of more than 30 minutes can not be efficiently sheared.
	Efficient fixation of a protein to chromatin in vivo is a crucial step for ChIP. The extent of cross-linking is probably the most important parameter.	Two major problems concerning the subsequent immunoprecipitation step should be taken into account: 1/ an excess of cross-linking can result in the loss of material or reduced antigen availability in chromatin, or both. 2/ the relative sensitivity of the antigen epitopes to formaldehyde. It is essential to perform the cross-linking step with care.
	It is essential to quench the formaldehyde.	Use glycine to stop the fixation: quench formaldehyde with 125 mM glycine for 5 minutes at room temperature (add 1/10 volume of 1.25M glycine). Alternatively, wash the fixed cells properly and make sure you get rid of ALL the formaldehyde.
Cell lysis	Temperature is critical.	Perform cell lysis at 4°C (cold room) or on ice. Keep the samples ice-cold at all times during the cell lysis and use ice-cold buffers see STEP 3.
	Protein degradation during lysis can occur.	Add the protease inhibitors to the lysis buffer immediately before use.
Cell type	Kit protocol validation.	HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells) and Kerationocytes have been used to validate the Magnetic ChIP protocol.
Chromatin shearing	Optimal shearing conditions are important for ChIP efficiency.	Shearing conditions for each cell type must be optimized from cell collection, fixation to shearing method (settings of the sonicator apparatus).
	Critical points for shearing optimization.	1) Not to start with a too high concentration of cells (15 x 10e6 cells/ml or less is ok) 2) Keep samples cold (4°C)
	Shear the samples of chromatin using the Bioruptor® Pico from Diagenode.	Maintain temperature of the samples close to 0°C. The chromatin shearing needs to be optimized for each cell type. A troubleshooting guide for Bioruptor-chromatin shearing is available at Diagenode.
Sheared chromatin analysis	Purify the DNA from the sheared chromatin as described in the kit protocol to analyse the shearing.	Extract total DNA from an aliquot of sheared chromatin and run on 1% agarose gel (stain with EtBr). In order to analyse the sheared chromatin on gel, take DNA purified from the sheared chromatin input -prepared at STEP 3 . Some unsheared chromatin can be analysed on gel as well (purify it as done with the input sample (see "6. Additional protocols" section). Chromatin eqvivalent to 100,000 cells, one million cells or more can for sure be visualized on a gel.
	Do not load too much DNA on a gel.	Loading of large quantities of DNA on agarose gel can lead to poor quality pictures, which do not reflect the real DNA fragmentation. The DNA amount to load depends on the size of the well and on the gel size.
	Agarose concentration.	Do not use more than 1-1.5% agarose gel and run slowly (Volt/cm and time depend on the gel size).
	Running buffer concentration.	1x TAE or TBE is preferred to 0.5x TAE, which can lead to smears on agarose gel.
Sheared chromatin amounts	How much sheared chromatin do I need to prepare?	Most of the sheared chromatin is to be used in the ChIP experiment, but remember that some of the sheared chromatin is needed as control as it corresponds to the input sample for the ChIP experiment and it can also be checked on agarose gel.

Antibody binding beads	Beads are in suspension.	The provided beads are coated with protein A. Resuspend into a uniform suspension before each use.
	Bead storage.	Store at 4°C. Do not freeze.
	Antibody binding capacity.	~ 10 µg / 30 µl
Protease inhibitors	Storage.	Some inhibitors are unstable in solution. The provided P.I. mix should be kept frozen at -20°C, and thawed before use.
Negative ChIP control(s)	Use non-immune IgG in the IP incubation mix.	Use the non-immune IgG fraction from the same species the antibodies were produced in.
	Do not add antibody to the IP.	Incubation with beads, which were not coated with antibodies antibodies could also be used as a negative ChIP control.
	Use a specifically blocked antibody in parallel.	Use one antibody in ChIP and, the same antibody that is blocked with specific peptide. To specifically block an antibody: pre-incubate the antibody with saturating amounts of its epitope specific peptide for about 30 minutes at room temperature before use in the IP incubation mix. Use the blocked antibody as a negative control in parallel with the unblocked antibody.

Antibody in IP	How many negative controls are necessary?	If multiple antibodies - of the same species - are to be used with the same chromatin preparation then a single negative ChIP control is sufficient for all of the antibodies used.
	Why is my antibody not working in ChIP?	Antibody-antigen recognition can be significantly affected by the cross-linking step resulting in loss of epitope accessibility and/or recognition.
	Which antibody should I use in ChIP?	Use ChIP-grade antibodies. If not available, it is recommended to use several antibodies directed against different epitopes of the same protein. Verify that the antibodies can work directly in IP on fresh cell extracts. Also, when testing new antibodies, include known ChIP-grade antibodies as positive control for your ChIP assay.
	How do I choose an antibody for ChIP?	Be aware of the possible cross-reactivity of antibodies. Verify by Western blot analysis the antibody specificity. Antigen affinity purification can be used to increase titer and specificity of polyclonal antibodies.
	Are my antibodies going to bind the protein A or protein G?	There is a significant difference in affinity of different types of immunoglobulins to protein A or G. Thererfore, in function of the antibody used for your ChIP, it is recommended to choose either protein A or protein G coated beads.

Species	Immunglobulli Isotype	Protein A	Protein G
Human	IgG1	+++	+++
	IgG2	+++	+++
	IgG3	-	+++
	IgG4	+++	+++
	IgGM	Use anti Human IgM	
	IgGF	-	+
	IgGA	-	+
Mouse	IgG1	+	+++
	IgG2a	+++	+++
	IgG2b	++	++
	IgG3	+	+
	IgGM	Use anti Mouse IgM	
Rat	IgG1	-	+
	IgG2a	-	+++
	IgG2b	-	++
	IgG2c	+	++
Chicken	All Isotypes	-	++
Cow	All Isotypes	++	+++
Goat	All Isotypes	-	++
Guinea Pig	All Isotypes	+++	++
Hamster	All Isotypes	+	++
Horse	All Isotypes	++	+++
Pig	All Isotypes	+	++
Rabbit	All Isotypes	+++	++
Sheep	All Isotypes	-	++

Freezing	Avoid multiple freeze/thawing.	Snap freeze and thaw on ice (e.g.: fixed cell pellets and sheared chromatin)

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