

A quick and quantitative chromatin immunoprecipitation assay for small cell samples

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TABLE OF CONTENT

1. Abstract
2. Introduction
3. Materials and reagents
 - 3.1. Antibodies
 - 3.2. Reagents
 - 3.3. Cells
 - 3.4. Materials
4. The Q²ChIP procedure
 - 4.1. Preparation of antibody-bead complexes
 - 4.2. DNA-protein cross-linking
 - 4.3. Cell lysis and chromatin fragmentation
 - 4.4. Immunoprecipitation and washes
 - 4.5. DNA elution, cross-link reversal and proteinase K digestion
 - 4.6. DNA isolation
 - 4.7. Real time PCR analysis and expression of the Q²ChIP results
 - 4.8. Freezing of samples
5. Notes
6. Perspective
7. Acknowledgement
8. References

1. ABSTRACT

Chromatin immunoprecipitation (ChIP) is to date a technique of choice for studying protein-DNA interactions. ChIP has been used for mapping the location of modified histones on DNA, often in relation to transcription or differentiation. A drawback of current ChIP protocols, however, is the requirement for large cell numbers, which limits the applicability of ChIP to rare cell samples. The procedure is also tedious and time consuming. We systematically evaluated and modified critical steps in the ChIP procedure to develop a quick and quantitative ChIP assay, referred to as Q²ChIP, suitable for up to 1,000 histone immunoprecipitations or 100 transcription factor immunoprecipitations from as few as 100,000 cells under cross-linking conditions. Analysis of the precipitated DNA by real time PCR enables quantification of the relative amount of a specific histone modification or transcription factor associated with a specific locus. This communication describes all steps of the Q²ChIP procedure as it is carried out in our laboratory.

2. INTRODUCTION

Gene expression is largely controlled by epigenetic modifications, which are defined as alterations of DNA or chromatin that do not affect DNA sequence. One such modification is cytosine methylation in CpG dinucleotides, a heritable mark that favors genomic integrity and is essential for long-term gene silencing (1). The second class of epigenetic alterations encompasses post-translational modifications of the amino-terminal tail of histones (2), among which those of core histones H3 and H4 have been best characterized (3,4). These modifications modulate interactions between histones and DNA and thereby affect binding of transcriptional regulators to target sequences. Histone modifications include combinatorial phosphorylation, ubiquitination, sumoylation, acetylation and methylation on specific residues. In particular, how acetylation and methylation of lysine (K, in single letter amino acid code) residues modulate transcription is the subject of intense investigations. Trimethylation of H3 lysine 9 (H3K9m3), H3K27m3 and H4K20m3, together

Quick and quantitative chromatin immunoprecipitation

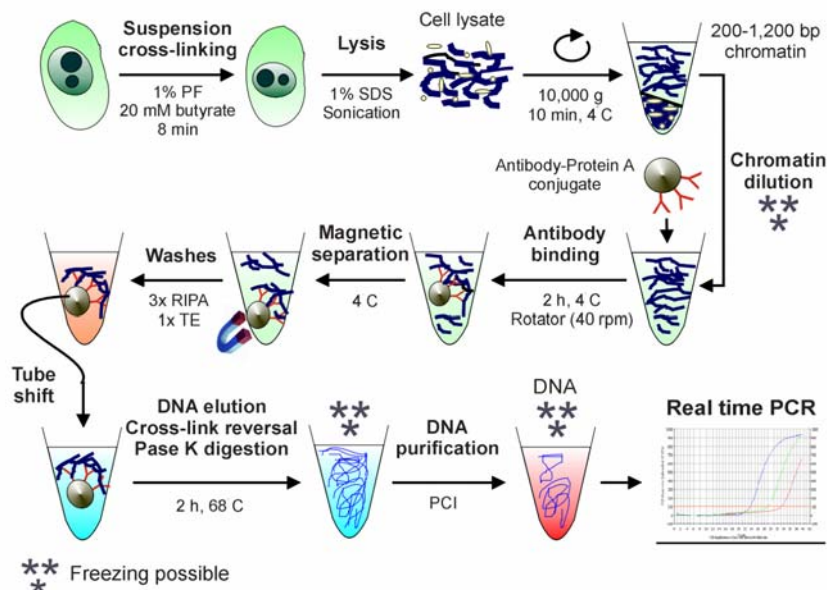


Figure 1. Summary diagram of the Q²ChIP assay. Steps where freezing of the samples is suitable without loss of efficiency are indicated. Reproduced with permission (12).

with deacetylation of H3K9, is associated with transcriptionally silent loci. In contrast, di- and tri-methylation of H3K4, H3K36 and H3K79, together with H3K9 acetylation (H3K9ac), marks a gene for transcription (2,5). Chromatin immunoprecipitation (ChIP) assays using antibodies directed against modified histones have enabled analysis of histone modifications in the context of embryo development (6-8), differentiation (9-12), aging (13) and cancer (14,15).

Interactions between proteins and DNA are essential for many vital cellular functions such as genomic stability, DNA replication and repair, chromosome segregation, transcription and epigenetic silencing of gene expression. ChIP has become a technique of choice in the study of protein-DNA interactions and for unraveling transcriptional regulatory circuits within the cell (16-18). ChIP has been used for mapping the location of post-translational histone modifications, transcription factors, chromatin modifiers and other non-histone chromosome-associated proteins. In a typical ChIP assay, DNA and proteins are reversibly cross-linked to maintain the association of proteins with their target DNA sequences. However, when analyzing histone modifications associated with DNA sequences, cross-linking may be omitted and the assay is then referred to as native ChIP (8,19). Regardless of the approach, chromatin is sheared, usually by sonication, to fragments of 200-1,000 base pairs and cleared for cellular debris by sedimentation. The supernatant is used for immunoprecipitation of a specific protein-DNA complex using antibodies coupled to beads. Immunoprecipitated complexes are washed under stringent conditions, the precipitated chromatin is eluted, cross-link is reversed, proteins are digested and DNA is purified. DNA sequences associated with the precipitated material can be identified by polymerase chain reaction (PCR),

molecular cloning and sequencing, ChIP-display (20) or by hybridization to genome-wide or tiling arrays (ChIP-chip) (16-18,21,22). Several reviews have also described parameters and variations of ChIP assays (23,24) and the various analytical tools which can be combined with ChIP to address the landscape of DNA-protein interactions (21,25,26).

ChIP is a versatile tool; however, a drawback of current protocols is the requirement for large cell numbers ($5\text{-}50 \times 10^6$ per immunoprecipitation), which limits the applicability of ChIP to rare or limiting cell samples. Current ChIP procedures are also lengthy and require extensive sample handling, favoring a loss of material and creating room for technical errors. We describe here in detail a quick and quantitative (Q²)ChIP assay suitable for up to 1,000 histone immunoprecipitations or up to 100 transcription factor immunoprecipitations, from as few as 100,000 cells (12). The procedure involves alterations of conventional ChIP protocols such as that of Spencer and colleagues (27). Analysis of input equivalent and precipitated DNA by real time PCR enables the quantification of the amount of a histone modification or transcription factor associated with a specific locus as fold precipitation relative to input. The Q²ChIP procedure is illustrated in Figure 1.

3. MATERIALS AND REAGENTS

3.1. Antibodies

We recommend using ChIP-grade antibodies when available. We have used antibodies against specific modifications of histone H3, such as, from Upstate Biotechnology (www.upstate.com), anti-H3K9ac (No. 06-942), anti-H3K9m2 (No. 07-441), anti-H3K9m3 (No. 07-442) and anti-H3K27m3 (No. 05-851), and from Abcam

Quick and quantitative chromatin immunoprecipitation

(www.abcam.com), anti-H3K4m2 (Ab 7766) and anti-H3K4m3 (Ab 8580). We have also successfully used rabbit polyclonal antibodies to ChIP the pluripotency-associated transcription factor Oct4 (antibody H-65, Santa Cruz Biotechnology; www.scbt.com). Other companies (e.g., Diagenode SA; www.diagenode.com) also sell ChIP-grade antibodies to modified histones and transcriptional regulators also well suited for Q²ChIP.

3.2. Reagents

Reagents other than antibodies, including RPMI 1640 culture medium, fetal calf serum, formaldehyde and the histone deacetylase inhibitor sodium butyrate are from Sigma-Aldrich (www.sigmaaldrich.com) unless indicated otherwise. Protein A-coated paramagnetic beads (Dynabeads® Protein A) are from Dynal Biotech (cat. No.100-02D; www.invitrogen.com).

3.3. Cells

Q²ChIP can be performed with chromatin from any cultured cell type and, potentially, from cells dissociated from tissue samples. The protocol presented here has been carried out with undifferentiated and differentiated human embryonal carcinoma NCCIT cells (American Type Culture Collection; www.atcc.org) (12), as well as with 293T embryonic kidney epithelial cells and mesenchymal stem cells from human adipose tissue. NCCIT cells have been established from a mediastinal germ cell tumor and can differentiate into derivatives of endoderm, mesoderm and ectoderm germ layers (28). NCCIT cells are cultured in RPMI 1640 supplemented with 10% fetal calf serum and passaged 1:8 every 3 to 4 days.

3.4. Materials

Standard molecular biology laboratory equipment and supplies are required for Q²ChIP. Notably, 0.5-ml centrifuge tubes (Axygen cat. No. 321-05-051, www.axgen.com) are used for chromatin preparation, and 200-μl PCR tubes, in strip format (Axygen, cat. No. 321-10-051) are used for immunoprecipitation and washes. Antibody-chromatin incubations and washes are carried out on a rotator of the type Stuart SB3 (Science Lab; www.sciencelab.com) placed at 4°C. Sonication of cells for chromatin fragmentation is done on ice with a Labsonic-M sonicator from Sartorius (www.sartorius.com) fitted with a 3-mm diameter probe. Where specified in the protocol, sample mixing is performed on a Thermomixer (Eppendorf; www.eppendorf.com). For quantitative PCR analysis of precipitated DNA, we use a MyiQ Real-time PCR Detection System with IQ SYBR® Green (BioRad; www.biorad.com).

4. THE Q²ChIP PROCEDURE

4.1. Preparation of antibody-bead complexes

1. Prepare a working solution of RIPA buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate, 140 mM NaCl).
2. Prepare a slurry of Protein A-coated paramagnetic beads (Dynabeads® Protein A): for 20 ChIPs, take out 220 μl of well-suspended bead stock solution, remove the

buffer after magnetic capture, remove from the magnet and add 500 μl RIPA buffer. Mix by vortexing and remove the buffer. Repeat the washing procedure once. Resuspend the beads in 210 μl RIPA buffer.

3. Aliquot 90 μl RIPA buffer into 200-μl PCR tubes, with one tube for each ChIP reaction.
4. Add 10 μl of pre-washed Protein A-bead slurry and 2.4 μg of a specific antibody. Incubate at 40 rpm on a Stuart SB3 rotator for 2 h at 4°C. If necessary, prolong incubation on the rotator until chromatin samples are ready for immunoprecipitation (see Section 4.4). Note that for this and subsequent steps, the PCR tubes used are in the form of 8-tube strips for easier handling than individual tubes (Note 1).

4.2. DNA-protein cross-linking

1. Immediately before harvesting the cells, add 20 mM sodium butyrate from a 1 M stock to the culture medium and mix gently. Note that butyrate is added to all solutions thereafter unless otherwise stated (Note 2).
2. Discard the medium to remove dead cells (if cells are growing adherent), and add room temperature phosphate buffered saline (PBS) containing 20 mM butyrate (e.g. 10 ml of PBS/butyrate solution to a 175 cm² flask).
3. Harvest cells by trypsinization or as per standard protocol. Trypsin or other harvesting solution should contain 20 mM butyrate.
4. Count cells and resuspend 10⁵ cells in 500 μl PBS containing 20 mM butyrate, at room temperature, in a 0.5-ml tube.
5. Add formaldehyde to 1% (v/v), mix by gentle vortexing and allow cross-linking to take place for 8 min at room temperature (Note 3).
6. Stop the cross-linking reaction by adding glycine to a final concentration of 125 mM and incubate for 5 min at room temperature.

4.3. Cell lysis and chromatin fragmentation

This section describes the fragmentation of chromatin to produce fragments of size suitable for ChIP and PCR analysis of the immunoprecipitated material (200-1,000 base pairs). After chromatin fragmentation, it is necessary to assess fragmentation by agarose gel electrophoresis following DNA purification. This step may be omitted once sonication results are consistent and conditions have been well established for a particular cell type. Carry out all subsequent steps on ice unless otherwise stated.

1. Wash the cross-linked cells twice by sedimentation (300 g for 10 min at 4°C) and resuspension in 0.5 ml PBS/butyrate (for 10⁵ cells). Sediment the cells as above, assess pellet volume and discard the supernatant.
2. Lyse cells by thorough resuspension of the pellet in 120 μl lysis buffer (50 mM Tris-HCl, pH 8, 10 mM EDTA, 1% SDS, broad-range protease inhibitor cocktail, 1 mM PMSF and 20 mM butyrate). Use

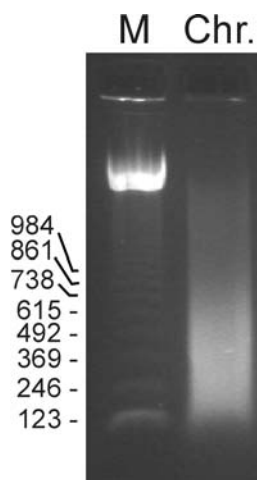


Figure 2. Assessment of chromatin fragmentation by sonication. Chromatin (Chr.) was prepared by sonication as described in Section 4.3, DNA was purified by phenol-chloroform isoamylalcohol as described in Section 4.6 and resolved in a 1.2% agarose gel stained with ethidium bromide. M, 123-base pair ladder. Size markers are shown on the right (in base pair).

room temperature lysis buffer to avoid SDS precipitation.

3. Suspend the cells by pipetting and incubate for 5 min on ice.
4. Sonicate each sample for 5 x 30 sec on ice. Allow a 30 sec pause on ice between each pulsing session. We use a Labsonic-M sonicator from Sartorius (www.sartorius.com) fitted with a 3-mm probe, at 30% power, cycle 0.5 (i.e., 0.5 sec pulse, 0.5 sec pause). With 293T and NCCIT cells, this sonication regime produces chromatin fragments of ~500 base pairs (range, 200-1,000 base pairs; Figure 2) (Note 4).
5. After sonication, centrifuge the lysate at 12,000 g for 10 min at 4°C. Use a swing-out rotor. Collect the supernatant into a clean tube placed on ice and take care to avoid aspiration of the pelleted complexes. Do not collect the top lipid layer.
6. Chromatin fragmentation can be assessed in a duplicate sample (which may be prepared in advance) dedicated to gel electrophoresis. To this end, carry out crosslink reversal and proteinase K digestion (see Section 4.5, step 2 onwards, as for input chromatin) and purify DNA by phenol-chloroform isoamylalcohol extraction (see Section 4.6). We recommend also carrying out an RNase digestion of the extracted sample.
7. Analyze the DNA sample by electrophoresis in a 1.2% agarose gel stained with ethidium bromide as per standard procedure. We recommend using a 123-base pair DNA ladder to evaluate size of the fragments produced (Figure 2).

4.4. Immunoprecipitation and washes

1. Dilute chromatin 10-fold or more (see below) in RIPA buffer containing protease inhibitor cocktail, 1 mM PMSF and 20 mM butyrate, to reduce SDS concentration to ~0.1%. Note that the chromatin

preparation can be diluted as much as 1,000- fold (and frozen in aliquots) to allow 1,000 immunoprecipitations from the sample.

2. Briefly spin PCR tubes containing antibody-coated Protein A beads (see Section 4.1) to bring down liquid caught in the lid. Place tubes in an ice-cold magnetic rack, wait for 1 min and discard the supernatant. Remove tubes from magnetic field.
3. Transfer 100 µl of diluted chromatin to each 0.2-ml PCR tube, and incubate on a rotator at 40 rpm for at least 2 h at 4°C. This step can be carried out over night if more convenient.
4. Remove another 100 µl of diluted chromatin from which DNA will be isolated (see Section 4.6) and used as input chromatin reference for real-time PCR (see Section 4.7). Keep this sample on ice until Section 4.5.
5. Wash immune complexes (i.e., the ChIP material) three times in fresh 100 µl RIPA buffer. Always do a brief spin of the tubes to bring down liquid caught in the lid prior to positioning in the magnetic rack. Bring the beads in suspension by gentle manual agitation prior to each wash. Each wash lasts for 4 min on a rotator at 40 rpm at 4°C. Discard the buffer between each wash.
6. Wash antibody-bead complexes once in 100 µl of Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0, 10 mM EDTA). Incubate for 4 min on a rotator at 40 rpm at 4°C. Do not place tubes in the magnetic rack after washing.
7. Transfer the suspension of beads carrying ChIP material into a clean 0.2-ml tube, magnetically capture the beads and remove the TE buffer (Note 5).

4.5. DNA elution, cross-link reversal and proteinase K digestion

In contrast to procedures reported in conventional ChIP protocols, elution of the DNA from the immunoprecipitated DNA-protein complex, reversal of the DNA-protein cross-link, and digestion of the proteins are combined into a single 2-h step (Note 6). Also perform cross-link reversal and proteinase K treatment of the input chromatin sample (see Section 4.4, step 4).

1. Add 150 µl elution buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 20 mM sodium butyrate, 50 mM NaCl) containing 1% SDS, and 50 µg/ml proteinase K to the washed ChIP material
2. Put the input chromatin sample at room temperature and add 200 µl elution buffer and proteinase K to a final concentration of 50 µg/ml.
3. Incubate samples for 2 h at 68°C, 1,300 rpm, on an (Eppendorf) Thermomixer.
4. Remove from the Thermomixer, briefly spin, capture the beads, collect the supernatant and place it into a clean 1.5-ml tube at room temperature.
5. Add 150 µl of elution buffer/SDS/proteinase K to the ChIP material and re-elute for another 5 min at 68°C, 1,300 rpm, on the Thermomixer.
6. Remove from the Thermomixer, capture the beads, collect the supernatant and combine it with the first supernatant.

Quick and quantitative chromatin immunoprecipitation

7. One can now either move onto the next step, or alternatively, the pooled supernatants can be stored on ice until the next day or frozen at -20°C.

4.6. DNA isolation

1. Add 200 µl elution buffer to the eluted ChIP material.
2. Also add 200 µl elution buffer to the input chromatin sample.
3. Extract DNA once with an equal volume of phenol-chloroform isoamylalcohol and transfer 460 µl of the aqueous phase to a clean tube. Extract once with an equal volume of chloroform isoamylalcohol and transfer 400 µl of the aqueous phase to a clean tube. Centrifuge at 15,000 g for 5 min at room temperature in order to separate the phases. We recommend the use of filter tips when adding phenol-chloroform isoamylalcohol and chloroform isoamylalcohol to avoid dripping during transfer.
4. Precipitate the DNA with 1/10 volume of 3 M NaAc, pH 7.0, 10 µl of 0.25% acrylamide carrier and 2.5 volume of ice-cold 96% ethanol at -80°C.
5. Centrifuge at 20,000 g for 15 min at 4°C to pellet the precipitated DNA, and wash pellet twice in 1 ml of 70% -20°C ethanol. Centrifuge at 20,000 g for 10 min at 4°C between washes.
6. Dissolve the DNA in 100 µl TE buffer (pH 8.0) (30 µl when ChIPs are carried out with chromatin from 100 cell equivalents, i.e., from input chromatin diluted 1,000-fold).

4.7. Real time PCR analysis and expression of Q²ChIP results

The immunoprecipitated DNA is analyzed by real time PCR using your laboratory's standard operating procedure, starting from 5 µl template DNA per 25 µl reactions (Note 7). Starting quantities of DNA in the ChIP samples and the input sample are determined based on a standard curve. We present the data as a fold enrichment of precipitated DNA associated with a given histone modification relative to a 1/100 dilution of input chromatin (11,12). This, then, represents the percentage of precipitated material relative to the amount in the input sample.

Figure 3 illustrates Q²ChIP results of precipitation of acetylated H3K9, di- and trimethylated H3K9, di- and trimethylated H3K4 and trimethylated H3K27 from undifferentiated NCCIT cells, a human embryonal carcinoma cell line. Note that dilution of the chromatin prior to carrying out immunoprecipitations maintains the histone modification profiles on the *OCT4* proximal promoter and proximal enhancer, *NANOG* promoter, *PAX6* promoter and *GAPDH* promoter. Nevertheless, some background starts appearing after a dilution of input chromatin to 100 cell equivalents (i.e., a 1,000-fold dilution of input chromatin). Note also that dilution of input chromatin results in enhancement of the amount of immunoprecipitated material relative to background as a result of increased ratio of antibody-bead complexes to target chromatin. Thirty to eighty percent of a specific input chromatin target can be precipitated (Figure 3). Figure 4 illustrates a Q²ChIP analysis of binding of the

transcription factor Oct4 to the *NANOG* promoter in undifferentiated NCCIT cells. The Figure also shows that Oct4 dissociates from the *NANOG* promoter in retinoic acid-differentiated cells (Figure 4A). Specific Oct4 protein binding to the *NANOG* promoter can also be detected after a 10-fold or a 100-fold dilution of input chromatin from 100,000 cells (12) (Figure 4B).

4.8. Freezing of the samples

Q²ChIP is compatible with freezing of the samples at various stages of the procedure, without affecting the efficiency of the following steps. Stages compatible with freezing are illustrated in Figure 1. Freezing can safely occur after dilution of the chromatin prior to the immunoprecipitation itself, after the chromatin elution step/cross-link reversal/Proteinase K digestion, and after purification and solubilization of the precipitated DNA. Samples are frozen at -20°C. In addition, it is possible to freeze the cells to be examined, after formaldehyde cross-linking, as a dry pellet of cells washed in PBS containing 20 mM sodium butyrate. In this case, snap-freeze in liquid nitrogen and store at -80°C.

5. NOTES

1. Q²ChIP assays are performed in 100 µl of sonicated and cleared undiluted (from 100,000 cells) or diluted chromatin (12).

2. Histone acetylation is a labile modification which is degraded unless histone deacetylase inhibitors are used in the ChIP reaction buffers. In order to preserve as much histone acetylation as possible, we add sodium butyrate to cells immediately prior to fixation and supplement all solutions and buffers thereafter with 20 mM butyrate. Under these conditions, we find that ChIP efficiency is enhanced 3-fold compared to when butyrate is added from the lysis step onward. As expected, enhancement is specific for acetylated histones, because the amount of DNA precipitated using an antibody against H3K9m2 is unaltered. When transcription factors are immunoprecipitated, sodium butyrate can be omitted. For ChIP analysis of phosphorylated residues (e.g., phosphorylated serine 10 of histone H3), phosphatase inhibitors may be used.

3. Formaldehyde cross-linking stabilizes DNA-protein interactions. Cross-linking makes Q²ChIP versatile in that not only histones but also transcription factors and other DNA-bound proteins can be immunoprecipitated from a single chromatin preparation (see Figure 4). Adherent cells are usually fixed in culture dishes or flasks, making harvesting tedious and inefficient. To simplify this step and enhance cell recovery, we fix cells in suspension, whether they grow adherent or in suspension. Time of formaldehyde treatment may vary from minutes to hours depending on the nature of the target protein to be assessed. In general, 8-10 min treatment with 1% formaldehyde will result in the cross-linking to DNA of only nuclear components located within 2 Å of DNA (29).

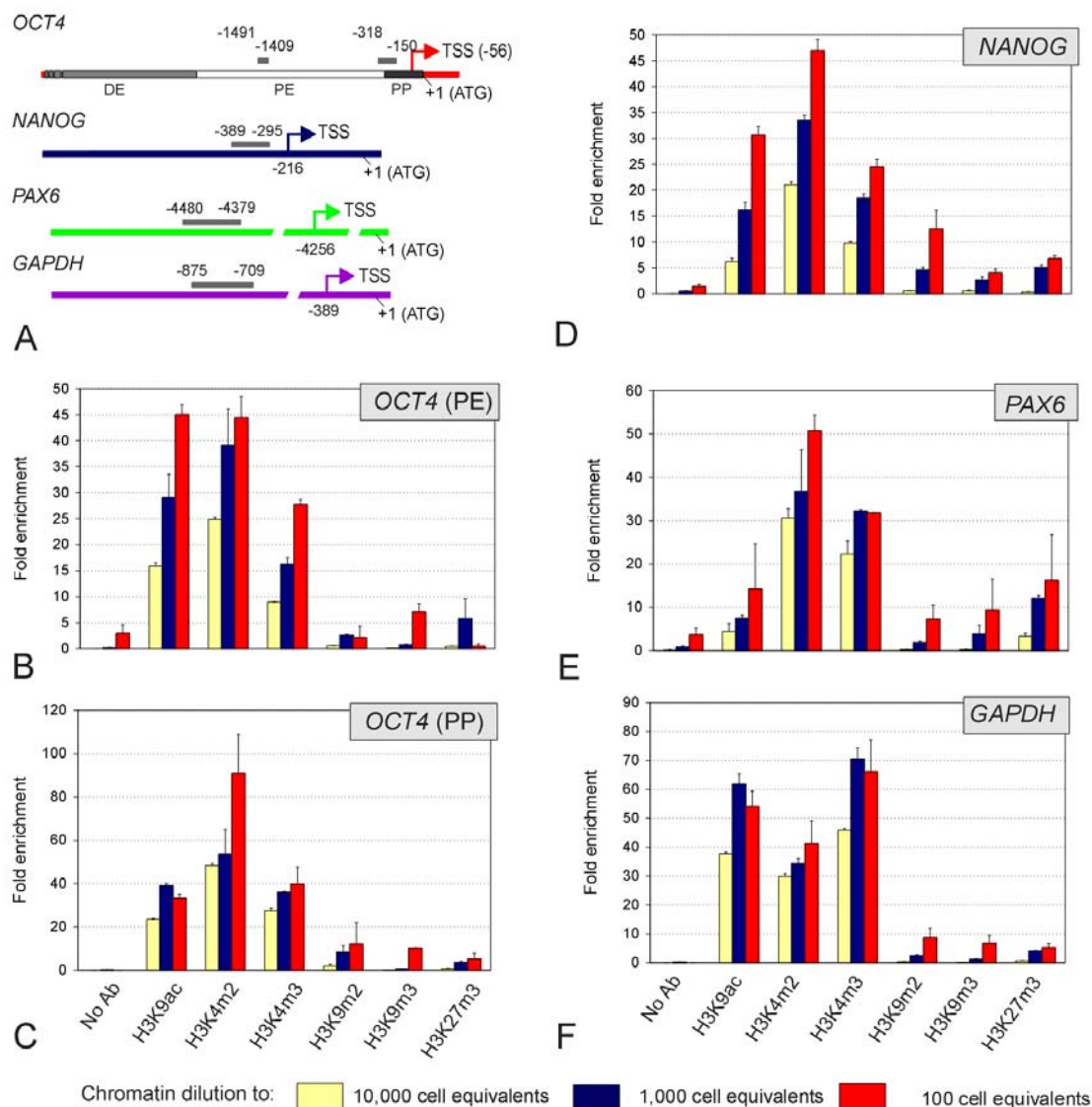


Figure 3. Q²ChIP analysis of histone H3 modifications on the *OCT4*, *NANOG*, *PAX6* and *GAPDH* regulatory regions in undifferentiated NCCIT cells. (A) Regions examined by Q²ChIP on the pluripotency-associated genes *OCT4* (two regions, one in the proximal promoter [PP], one in the proximal enhancer [PE]) and *NANOG*, on the developmentally regulated gene *PAX6* and on the housekeeping gene *GAPDH*. Chromatin was prepared from 100,000 NCCIT cells and diluted 10, 100 or 1,000-fold prior to immunoprecipitation of indicated histone modifications. Dilutions produced chromatin from the equivalent of 10,000, 1,000 or 100 cells. (B) *OCT4* PE, (C) *OCT4* PP, (D) *NANOG* promoter, (E) *PAX6* promoter and (F) *GAPDH* promoter. Note the maintenance of histone modification profiles across all loci examined irrespective of the amount of input chromatin. With chromatin from 100 cell equivalents, however, background becomes noticeable and errors between replicate ChIPs tend to increase (data from two independent Q²ChIPs). Reproduced with permission from (12).

4. Conditions for sonication need to be assessed for each cell type examined. Efficiency of sonication varies to some extent with changes in sample volume and tube size. We use 0.5 ml tubes for sonication to increase sample depth. The tip of the sonicator probe should be kept as deep as possible while not touching the tube wall, and no more than a few millimeters above the bottom of the tube. This is important for two reasons: 1) continuous contact between sonicator probe and tube wall will lead to reduced efficiency of chromatin shearing; 2) positioning the tip of

the probe too close to the sample surface will lead to foaming and inefficient sonication. If foaming is not eliminated by taking these precautions, try reducing sonicator output energy. Throughout the sonication procedure the sample tube is kept in an ice slurry to avoid excessive heating.

5. A drawback of most ChIP procedures is non-specific chromatin background. Background becomes significant when reducing the amount of input material in

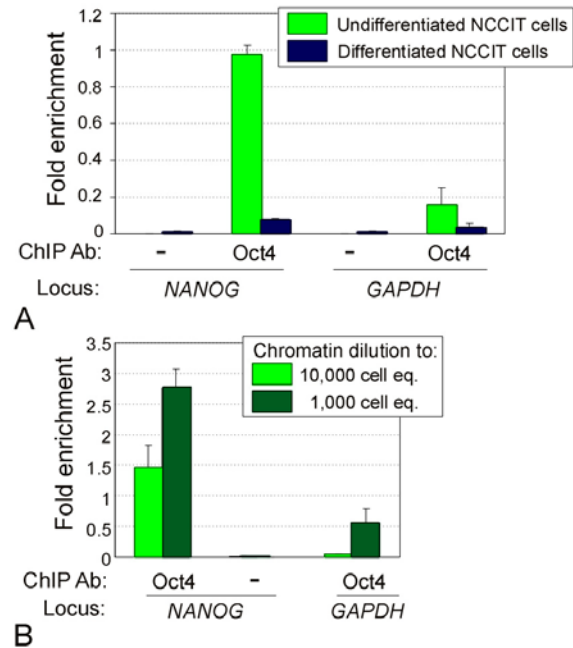


Figure 4. Q²ChIP analysis of Oct4 protein binding to the *NANOG* promoter in undifferentiated and differentiated EC cells. (A) Oct4 was immunoprecipitated by Q²ChIP from chromatin of 100,000 NCCIT cells (NCCIT) or NCCIT cells differentiated with retinoic acid, and Oct4 binding to the *NANOG* and *GAPDH* promoters was examined by real time PCR. Control ChIPs were done with beads only. (B) Chromatin from 100,000 NCCIT cells was diluted to 10,000 and 1,000 cell equivalent (cell eq.) and Oct4 binding to the *NANOG* and *GAPDH* promoters was assessed by Q²ChIP. Ab, antibody. Reproduced with permission from (12).

the ChIP assay and with a reduction of sample volumes, due to an increase in tube surface to reaction volume ratio. The ‘tube switch’ step while the ChIP material is in TE buffer has in our hands proven to be essential for enhancing specificity of the ChIP assay. Low background is essential when scaling down ChIP in order to maintain specificity.

6. We have shown that cross-link reversal, proteinase K digestion and DNA elution do not need to be carried out as separate steps (totaling ~9 h) but rather, can be combined into a single 2-h step for simpler sample handling and increased time efficiency (12). We have shown that a 2-h incubation step does not affect the efficiency of crosslink reversal (12).

7. As with any real time PCR assay, PCR efficiency of the primers used is critical. This should be emphasized in the context of Q²ChIP whereby the amount of template DNA available per PCR reaction is limited.

6. PERSPECTIVE

A native ChIP assay relying on the co-precipitation of *Drosophila* carrier chromatin (carrier ChIP,

or CChIP) in the sample of interest has recently been reported for cell numbers as low as 100 (8). CChIP has notably enabled the analysis of histone modifications in mouse blastocysts and in embryonic stem cells. Introduction of carrier chromatin in the Q²ChIP procedure may permit a further reduction in minimal cell numbers required for ChIP, a possibility that remains to be tested. In the context of CChIP however, it is imperative that the carrier chromatin be from a species sufficiently distant from that of the sample to be analyzed in order not to be amplified by PCR. Whether CChIP is also efficient with cross-linked samples remains to be demonstrated although this is anticipated. We are currently adapting the Q²ChIP procedure to enable the study of rare cell samples, or even embryos.

7. ACKNOWLEDGEMENT

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Abbreviations: ChIP: chromatin immunoprecipitation, H3K9ac: acetylated histone H3 lysine 9, H3K4m2: dimethylated H3K4, H3K4m3: trimethylated H3K4, H3K9m2: dimethylated H3K9, H3K9m3: trimethylated H3K9, H3K27m3: trimethylated H3K27, PBS: phosphate buffered saline, Q²ChIP: quick and quantitative chromatin immunoprecipitation, RT-PCR: reverse transcription polymerase chain reaction

Key Words: Chromatin Immunoprecipitation, Epigenetics, Gene Expression, Histone Acetylation, Histone Methylation

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