SPOTLIGHT feature

Proteomics, Genomics & Microarrays

Chromatrap® Enzymatic Shearing Kit Isolates High Quality Chromatin for Excellent Target Enrichment

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Isolation of good quality, suitably fragmented chromatin is the most important prerequisite for successful Chromatin immunoprecipitation. Chromatin can be sheared enzymatically or by mechanical methods such as sonication.

Enzymatic shearing can be advantageous where expensive sonication equipment is not available or where native chromatin is to be examined.

Illustrated here is the effectiveness of the Chromatrap® Enzymatic Shearing kit in the fragmentation and isolation of high quality ChIP grade chromatin.

Chromatin immunoprecipitation (ChIP) is an effective tool in elucidating the protein/DNA interactions, thus enabling a greater understanding of the mechanisms of gene regulation. A critical step in the ChIP process is the preparation of high quality chromatin fragments between 100-500bp. These are generated either by mechanical shearing or restriction enzyme digestion.

A simple and cost effective alternative to mechanical shearing, enzymatic digestion does not require any expensive equipment. Added to this, the shearing process is milder and preferable to sonication in certain circumstances, for example, for ChIP using native chromatin *(see Table 1)*.

To demonstrate the utility of the Chromatrap® Enzymatic Shearing kit in the preparation of high quality ChIP-grade chromatin, ChIP was used to enrich high abundant H3 and low abundant ERα signal at the GAPDH and GREB1 gene loci respectively. Excellent quality chromatin, sheared to optimal fragment sizes was obtained using the Chromatrap® Enzymatic Shearing kit and excellent target gene enrichment was observed with high signal to noise ratio. The Chromatrap® Enzymatic Shearing kit therefore provides an excellent alternative to sonication for quick and simple preparation of high quality chromatin for use in ChIP.

Table 1. Comparison of shearing methods for chromatin preparation.

	Advantages	Disadvantages
Sonication	Random fragmentation. Suitable for difficult to lyse cell types.	Potential antigenic epitope damage through emulsification or overheating. Requires expensive equipment. Cannot be used for Native chromatin preparation (non cross-linked).
Enzymatic	Milder treatment, less damaging to epitopes of interest. Does not require any expensive equipment. Suitable for native chromatin preparation.	Restriction enzymes may exhibit some sequence bias during fragmentation. Not suitable for some difficult to lyse sample types.

Method

Chromatin Preparation

Chromatin was prepared from two human endometrial cell lines, the oestrogen responsive Ishikawa (Terakawa et al., 1987) and, the ERα negative Hec50 (Holinka et al.,1996). 1 million Hec50 and 500,000 Ishikawa cells were processed for chromatin isolation using the Chromatrap® Enzymatic shearing kit as per the standard protocol. Briefly, cells were grown to ~80% confluency before cross-linking in 1% formaldehyde, quenching with glycine and collection in ice-cold PBS. Cells were spun down and the supernatant discarded before re-suspension and cell membrane lysis in Hypotonic Buffer. The released nuclei were then collected by centrifugation and resuspended in Digestion Buffer for in-nucleus digestion of the chromatin.

A 10µl aliquot of the nuclei suspension was removed and lysed to measure the approximate concentration using a nanodrop spectrophotometer. Based on this concentration the appropriate volume of Shearing Cocktail was added to each stock nuclei suspension at a ratio of 1U/5µg chromatin and incubated at 37°C for 5 min. The reaction was stopped with Enzymatic Stop Solution and placing the suspensions on ice. Nuclei were again collected by centrifugation and lysed by addition of Lysis Buffer. Finally, suspensions were centrifuged to pellet cell debris and the supernatant, containing sheared chromatin stock, transferred to a fresh microcentrifuge tube. A 25µl aliquot of chromatin stock was removed, reverse cross-linked and proteinase K digested to measure the stock concentration and assess the chromatin quality using agarose gel electrophoresis *(see Figure 1).*

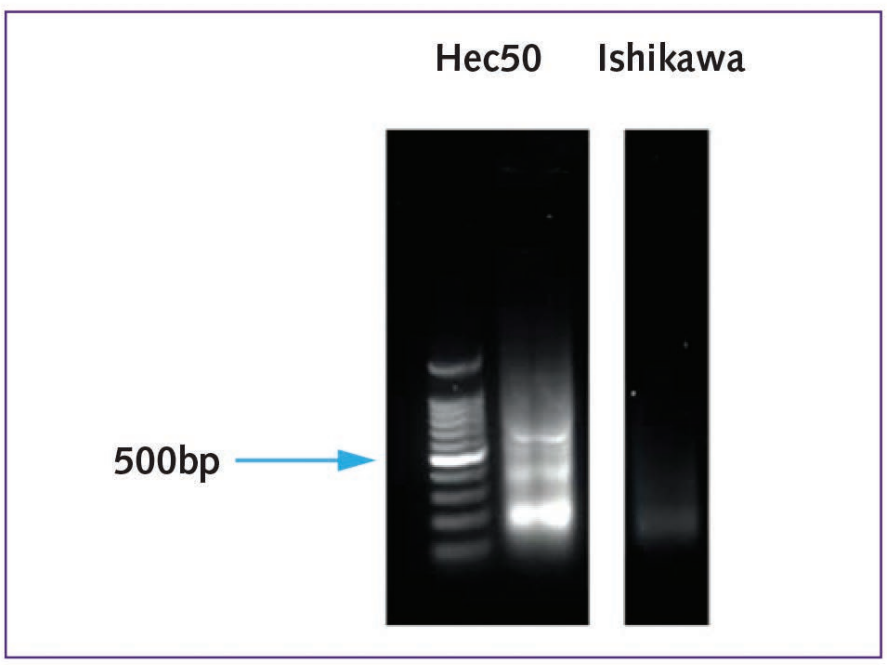

Figure 1. Agarose gel electrophoresis of 400ng/µl Hec50 and 100ng/µl Ishikawa chromatin prepared using the Chromatrap® Enzymatic Shearing Kit. The typical ladderlike banding pattern shows fragments of 200bp, 400bp and 600bp – ideal for ChIP using Chromatrap® ChIP columns or high throughput plates.

Immunoprecipitation Using Chromatrap® Spin Columns

Antibody and gene targets

The common epigenetic mark, core histone H3, and the transcription factor ERα were selected as antibody targets for the study. H3, one of four histones comprising the protein component of chromatin, is ubiquitous within chromosomes and therefore serves as an abundant antibody target for ChIP. The glyceraldehydes-3-phosphate (GAPDH) locus provides an abundant gene target and is actively expressed in all cell types (Barber et al., 2005).

The steroid hormone oestrogen (E2 or 17β-oestradiol) regulates diverse processes in many mammalian tissues, via several mechanisms, including the ligand activated nuclear transcription factor oestrogen receptor alpha (ERα) (Prossnitz et al., 2008). Oestrogen receptor activation of transcription occurs through interaction of a conserved DNA-binding domain with palindromic sequences in DNA known as oestrogen response elements (EREs) (Mader et al., 1993). The GREB1 promoter region contains three such consensus EREs and recruitment of ERα to this region has been demonstrated (Deschênes et al., 2007). Therefore an antibody directed against ERα was used to demonstrate transcription factor ChIP on chromatin from these endometrial cancer lines with the gene target GREB1.

Immunoprecipitation

Chromatin stocks were standardised by preparation of 50ng/µl working stocks in sterile distilled water. Slurries were subsequently prepared with 1µg total chromatin and 2µg relevant antibody. ChIP was carried out using a Chromatrap® Pro-A spin column kit as per the standard protocol. Inputs were prepared in parallel containing 1µg chromatin from each cell line in a total volume of 20µl, these samples were used for analyses and not subjected to ChIP enrichment. Following elution, samples were reverse cross-linked, alongside the inputs, and proteinase K digested to release DNA suitable for qPCR.

Each chromatin/antibody combination was carried out in triplicate to demonstrate the reproducibility of the enrichment using chromatin prepared using the kit.

qPCR

A significant advantage of the Chromatrap® spin column kit is the suitability of the buffer system for proceeding directly to downstream processing without the need for DNA clean up. qPCR was used to analyse precipitation of the GREB1 and GAPDH gene loci using antibodies directed against ERα and H3 respectively. In addition, precipitation of these loci using non-specific IgG was determined. The percentage of real signal was calculated as a proportion of the input chromatin, normalised using the signal generated by non-specific binding of unspecific IgG. Error bars represent the standard error of the mean of the triplicate ChIPs.

Results and Discussion

Sonicated chromatin from primary cells and cancer cell lines has been reproducibly enriched for high and low abundant targets using Chromatrap® spin columns and Chromatrap® 96HT. To demonstrate the application of the Chromatrap® Enzymatic Shearing kit as an alternative for the preparation of high quality ChIP-grade chromatin, chromatin from two endometrial cell lines was extracted and immunoprecipitated using Chromatrap® Pro-A spin columns.

Immunoprecipitation using 1µg chromatin (equivalent to approximately 160,000 cells) resulted in a 12-fold increase in specific H3 enrichment at the GAPDH promoter, compared with the non-specific IgG; Illustrating the high signal to noise ratio resulting from chromatin extraction using the Chromatrap® Enzymatic Shearing kit. High real signal was obtained for H3, precipitated at this locus with 5.63% and 4.87% for GAPDH from Ishikawa and Hec50 chromatin respectively. Excellent reproducibility is clearly demonstrated indicating low variability between samples, independent of starting cell number *(see Figure 2)*.

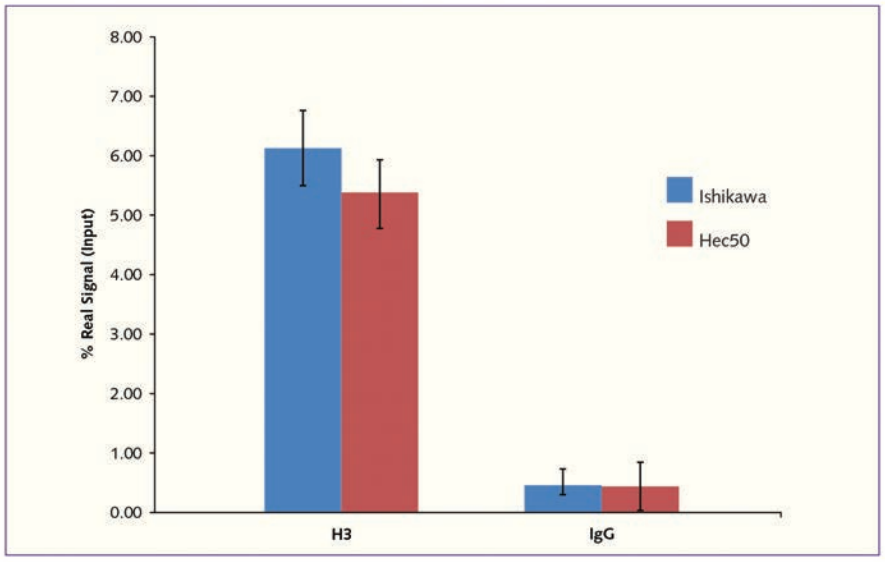

Figure 2. H3 signal enrichment at the GAPDH promoter. Using chromatin prepared using the Chromatrap® Enzymatic Shearing Kit, strong amplified signal was obtained at the GAPDH gene promoter following H3 core histone IP. Low non-specific IgG background demonstrates excellent signal to noise ratio in both Ishikawa and Hec50 chromatin.

Included here as a low abundant transcription factor target, in the absence of the activating ligand oestradiol, recruitment of ERα to the GREB1 promoter was selectively precipitated. ERα occupancy of GREB1 was observed in chromatin from both Ishikawa and Hec50 cells. Hormone receptor positive Ishikawa cells (Albitar et al., 2007) demonstrated 4-fold higher ERα signal (2% real signal) at this gene promoter compared to Hec50 (0.5%). This increased signal in Ishikawa cells indicates the selective and sensitive amplification of low abundant IP targets from enzymatically-sheared chromatin using the Chromatrap® Enzymatic Shearing Kit. Hec50 cells originate from poorly differentiated endometrial cancers and thus have been shown to express very little to no steroid hormone receptors (Kumar et al 1998).

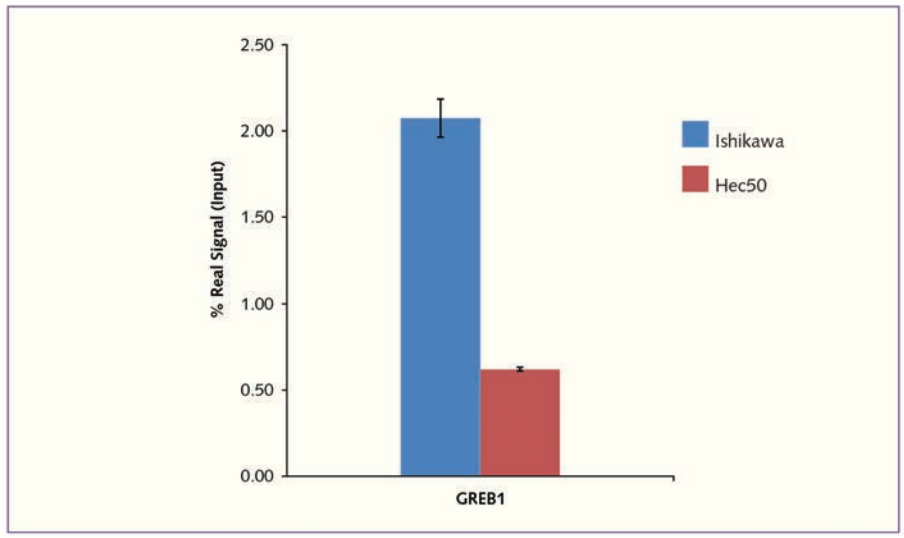

Figure 3. GREB1 promoter enrichment from Ishikawa and Hec50 chromatin. Low abundant signal amplification, targeting ERα occupancy at the GREB1 promoter was easily achieved in Ishikawa and Hec50 isolated chromatin sheared using the Chromatrap® Enzymatic Shearing Kit.

Conclusion

The Chromatrap® Enzymatic Shearing Kit provides an excellent methodology for the preparation of high quality, ideally fragmented chromatin for ChIP analysis. This short technical note has demonstrated excellent enrichment, independent of starting cell number, with comparable GAPDH enrichment from immunoprecipitation of 1µg chromatin with an antihistone H3 antibody. Sensitive enrichment demonstrating differences in ER occupation of the GREB1 locus in steroid hormone receptor positive and negative cell lines has also been clearly shown. With its quick and simple protocol this makes the Chromatrap® Enzymatic Shearing Kit the perfect cost effective alternative to sonication.

Chromatrap® enzymatic shearing kit supplies all the necessary reagents and buffers for up to 10 chromatin preparations. The success of a ChIP assay is highly dependent on the quality of chromatin prepared. Chromatrap® enzymatic shearing kit allows you to determine optimal shearing conditions for your chromatin preparations and can supply you with enough chromatin to perform up to 24 ChIPs if using standard Chromatrap® ChIP spin column kit or up to 96 IPs if using Chromatrap® ChIP 96 High throughput microplate.

References

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