Chromatrap

Chromatrap® Enzymatic Shearing Kit isolates high quality chromatin for excellent target enrichment

Summary

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 in this short application is the effectiveness of the Chromatrap® Enzymatic Shearing Kit in the fragmentation and isolation of high quality ChIP grade chromatin. We also highlight comparable data for enzymatic versus sonication using Chromatrap® ChIP kits.

1.0 Introduction

Chromatin immunoprecipitation (ChIP) is an effective tool in elucidating 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. See table 1 for advantages and disadvantages of the two different methods of chromatin preparation.

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 Histone 3 (H3) signal at the Glyceraldehyde-3-phosphate *(GAPDH)* gene loci. Chromatin was also prepared using Chromatrap® Spin Column Sonication Kit to compare the quality of chromatin between the different shearing methods. Excellent quality chromatin, sheared to optimal fragment sizes was obtained using both the

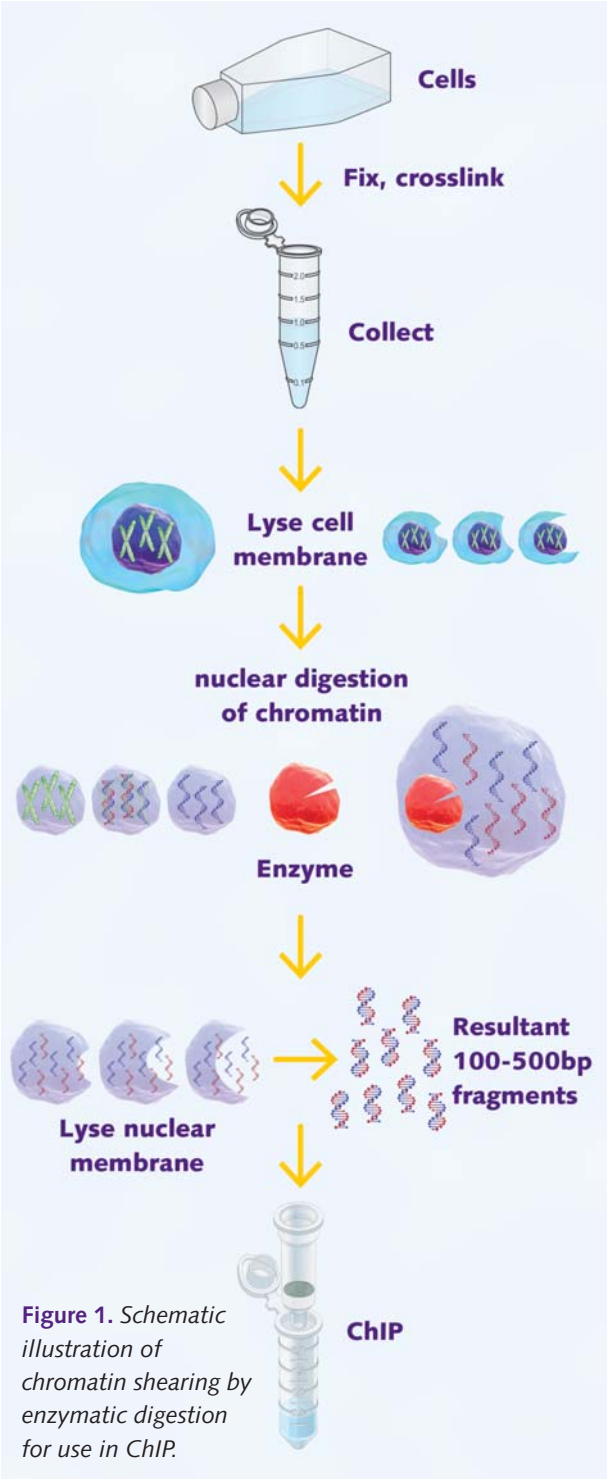

Figure 1. *Schematic illustration of chromatin shearing by enzymatic digestion for use in ChIP.*

Chromatrap® Enzymatic Shearing Kit and the Chromatrap® Spin Column Sonication Kit. Excellent target gene enrichment with high signal to noise ratio was observed with both chromatin preparations. The Chromatrap® Enzymatic Shearing Kit can provide an excellent alternative to sonication for quick and simple preparation of high quality chromatin for use in ChIP without the need for expensive sonication equipment.

	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.

Table 1. *Comparison of shearing methods for chromatin preparation.*

2.0 Method

Chromatin preparation

Chromatin was prepared from the human endometrial cell line Hec50 (Holinka et al., 1996). 1 million Hec50 cells were processed for chromatin isolation using the Chromatrap® Enzymatic shearing Kit and the Chromatrap® Spin Column Sonication 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. For Enzymatic shearing the released nuclei were then collected by centrifugation and re-suspended 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 nuclei stock 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 suspensions placed 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).

For chromatin prepared by sonication, after cell membrane lysis the nuclei were released using the appropriate volume of Lysis Buffer and the isolated nuclear fraction was separated by centrifugation. The chromatin was sheared using a water bath sonicator with 30 second bursts with 30 second intervals at a power setting of 3 for 15 minutes to achieve optimal fragment lengths of 100-500bp (see Figure 2).

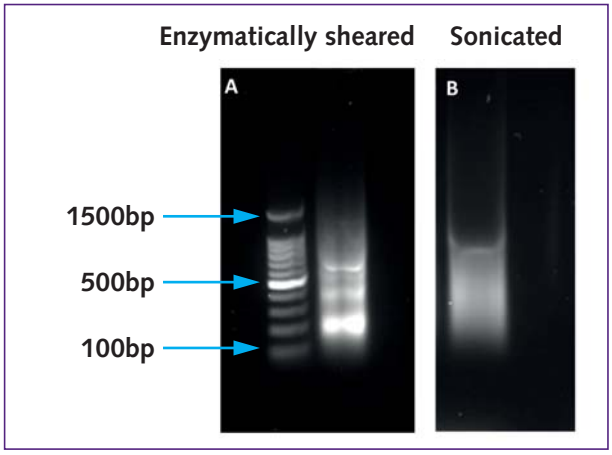

Figure 2. Agarose gel electrophoresis of Hec50 chromatin prepared using the Chromatrap® Enzymatic Shearing Kit and Chromatrap® Spin Column Sonication Kit. *The typical ladder-like banding pattern observed after enzymatic digestion, demonstrate fragments of 200bp, 400bp and 600bp – ideal for ChIP using Chromatrap® ChIP columns or high throughput microplates. (1A). Uniform chromatin fragment lengths between 100 and 500bp visualised with sonicated chromatin (1B).*

Immunoprecipitation using Chromatrap® spin columns

Antibody and gene targets – The common epigenetic mark, core H3 was selected as an antibody target 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).

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 *GAPDH* gene loci using antibody directed against H3. 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.

3.0 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 and 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 4.87% for *GAPDH* from Hec50 chromatin. This is comparable to Hec50 chromatin prepared by sonication which demonstrates a real signal of 4.56% of H3 at the GAPDH loci. Excellent reproducibility is clearly demonstrated indicating low variability between samples, independent of starting cell number (see Figure 3).

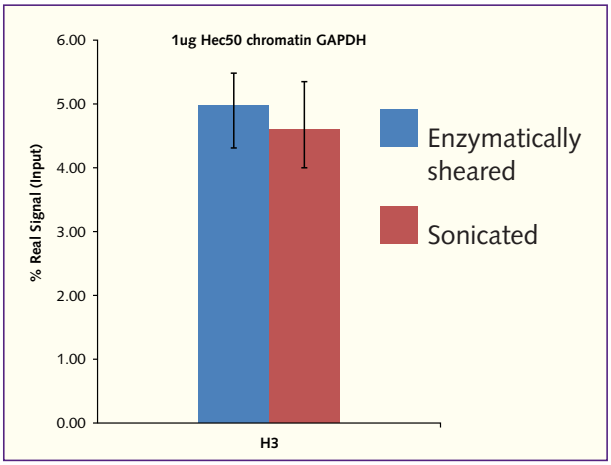

Figure 3. H3 signal enrichment at the GAPDH promoter. *Using chromatin prepared using the Chromatrap® Enzymatic Shearing Kit and Chromatrap® Spin Column Sonication Kit, strong amplified signal was obtained at the GAPDH gene promoter following H3 IP. Excellent signal to noise ratio in both Hec50 chromatin preparations.*

4.0 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 anti-histone H3 antibody for both sonicatated and enzymatically digested chromatin. With its quick and simple protocol this makes the Chromatrap® Enzymatic Shearing Kit the perfect cost effective alternative to sonication.

5.0 References

Albitar, L., Pickett, G., Morgan, M., Davies, S., Leslie, K. (2007). Models representing type I and type II human endometrial cancers: Ishikawa H and Hec50co cells. *Gynecol. Oncol.* 106: 52-64.

Barber, R. D., Harmer, D. W., Coleman, R. A., Clark, B. J. (2005). GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiological genomics* **21**, 389-95.

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