Chromatrap

Chromatrap® Native ChIP

Chromatrap® is pleased to announce the release of the latest addition to its epigenetic catalogue, a Native Chromatin Immunoprecipitation (N-ChIP) Kit (Catalogue no. 500237 & 500238). This kit focuses on extracting chromatin without the need for chemical fixation, a benefit for those wanting to work with chromatin in its native state. With native chromatin, the proteins and histones associated with DNA are naturally linked and traditional methods of shearing can easily disrupt the DNA-protein complexes. The DNA is biologically sheared using Chromatrap®'s Shearing Cocktail to prevent this disruption. Native ChIP is mainly used for the study of histone modifications and certain abundant transcription factors that are likely to be bound to DNA.

Traditional cross-linked (X-ChIP) involves chemical fixation of the cells prior to chromatin extraction, preserving DNA-protein complexes. This enables researchers to investigate a wider range of epigenetic modifications, however chemical fixation can present challenges that can be overcome with N-ChIP.

Introduction

When performing ChIP, there are two main options on how to prepare your chromatin: through cross-linking the chromatin with a chemical agent such as formaldehyde (X-ChIP), or by non-cross linking the chromatin, known as native ChIP (N-ChIP). Chromatrap is fully flexible, dependent on the researcher and scientific questions being asked. Outlined in table 1 are the advantages and disadvantages of each method.

Native ChIP		Cross-linked ChIP	
ADVANTAGES	DISADVANTAGES	ADVANTAGES	DISADVANTAGES
Suitable for investigating histone marks and abundant targets.	Slightly longer due to overnight dialysis.	ChIP carried out in 5 hours!	Chemical fixation required.
No chemical fixation of cells; cells remain in a more natural 'native' state.	Can only shear chromatin enzymatically.	Can shear chromatin by either sonication or enzymatically.	Cannot capture chromatin from cells in their 'native' state.
In some cases there is increased affinity of antibody binding to antigens on native chromatin as it is more accessible.	Some low abundant transcription factors will not be detected.	Can investigate a wide range of histone marks and transcription factors.	Cross-linked chromatin can occasionally mask epitopes of some antibodies, affecting antibody/ chromatin binding.

Table 1 – *Comparison of Chromatrap®'s native vs cross-linked ChIP kits*

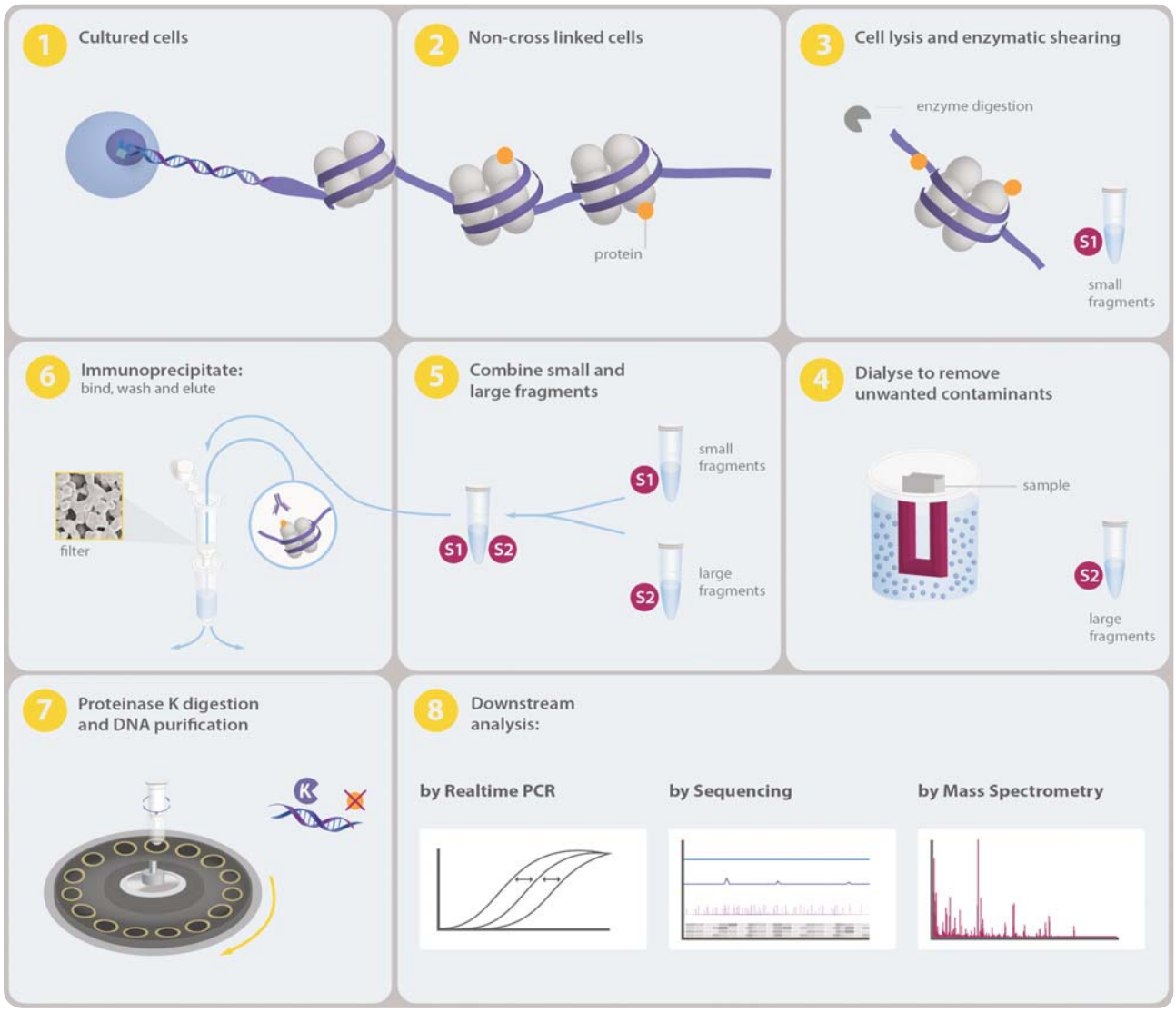

Figure 1 – *Overview of the Chromatrap® Native ChIP process*

In order to highlight the ability of Chromatrap®'s Native ChIP Kit (Catalogue no. 500237 & 500238) and standard ChIP-qPCR Spin Column Kit (Catalogue no. 500071 & 500117) to selectively enrich histone marks and low abundant transcription factors, two epigenetic marks: H3K27me3 and EZH2, were chosen for this study. EZH2 is an enzymatic subunit of the Polycomb Repressive Complex 2 (PRC2) associated with repressive transcription and is a methyltransferase that can control the methylation status of the histone mark H3K27me3 (Yoo & Henninghausen, 2012). Abnormal EZH2 expression and therefore H3K27me3 increased methylation is associated with various cancers (Yoo & Henninghausen, 2012). Together this transcription factor and histone modification are detectable at target gene locations in endometrial epithelial cancer cell lines (Hec50).

Method

Chromatin Preparation

For both N-ChIP and X-ChIP, chromatin was prepared from the human endometrial epithelial cancer cell line Hec50 (Holinka et al., 1996) as per each Chromatrap® protocol.

Experimental Design

Both N-ChIP and X-ChIP provide a sensitive and robust platform for a ChIP assay. Both methods have been shown to amplify signal for histone marks and low abundant transcription factors, highlighting the sensitivity of the Chromatrap® technology. Each antibody/chromatin combination was carried out in triplicate to demonstrate the reproducibility of Chromatrap®'s range of ChIP assay kits.

Antibody and Gene Targets

The epigenetic mark EZH2 forms part of the PRC complex, associated with repressive gene transcription. This methyltransferase enzyme is responsible for the addition of methyl groups to the histone methylation mark H3K27me3. This increase in methylation on lysine 27 of histone H3 aids with repressing transcription and switching genes off. When levels of this methylation increases in cancer it can lead to tumour suppressor genes being switched off. The Myelin Transcription Factor 1 (MYT1) gene is generally associated with heterochromatin and repressive transcription; it is a valid positive target for both of these antibodies. Glyceraldehyde-3-phosphate (GAPDH) is actively expressed in all cell types; it is an appropriate negative gene target for both of these epigenetic marks. In addition to the negative gene targets, non-specific IgG antibody from the same species as the test sample antibodies was included to demonstrate the low non-specific background binding obtained using the Chromatrap® technology.

Immunoprecipitation

N-ChIP

Each antibody and negative control IgG, was added at a 5:2 chromatin: antibody ratio (5µg chromatin: 2µg antibody). Inputs were prepared in parallel containing 5µg chromatin; these samples were used for analyses and not subjected to ChIP enrichment. Slurries were incubated at 4°C on an end-to-end rotator for 1hr. After washing to remove any unbound chromatin, any selectively enriched chromatin specifically targeted using the appropriate antibody, was eluted using the specially formulated Native ChIP Elution Buffer. Samples were then proteinase K digested and subjected to DNA purification prior to qPCR analysis.

X-ChIP

Each antibody and negative control IgG, was added at a 2:1 ratio to the chromatin. (2µg antibody: 1µg chromatin). Inputs were prepared in parallel containing 1µg chromatin; these samples were used for analyses and not subjected to ChIP enrichment. Slurries were loaded into the Chromatrap® columns and these were then immediately incubated at 4°C on a rocker for 1hr. ChIPs were then washed to remove any unbound chromatin, and eluted using ChIP Elution Buffer. The samples then underwent the reverse cross-linking process and Proteinase K digestion prior to qPCR analysis.

qPCR analysis

qPCR was used to analyse MYT1 and GAPDH specific DNA fragments associated with immunoprecipitated EZH2 and H3K27me3 antibodies. Each precipitation assay was completed using an equivalent loading of IgG antibody as a negative control. The percentage of the input chromatin was 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. Both primer sets were used at an optimum annealing temperature of 60°C and at a working concentration of 4mM.

Results

Enrichment of H3K27me3 onto the positive gene locus MYT1 is comparable for both X-ChIP (Figure 2) and N-ChIP (Figure 4). Excellent signal to noise is shown for both H3K27me3 and the low abundant transcription factor EZH2 respectively by the low enrichment for the negative gene target GAPDH (Figure 2/4) and the non-specific IgG (figure 3).

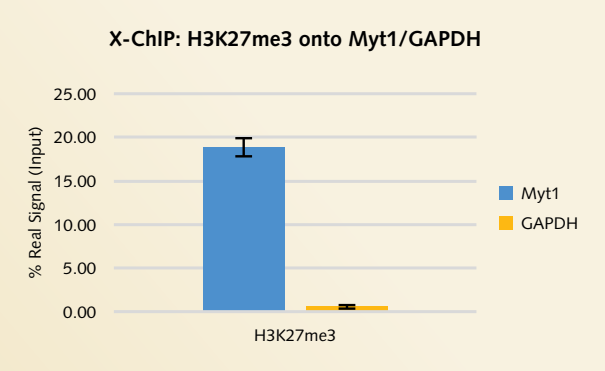

Figure 2 – X-ChIP H3K27me3 onto Myt1/GAPDH: *Low signal is produced by the negative gene target GAPDH when H3K27me3 is enriched using the qPCR Standard Chromatrap® Spin Column Kit.*

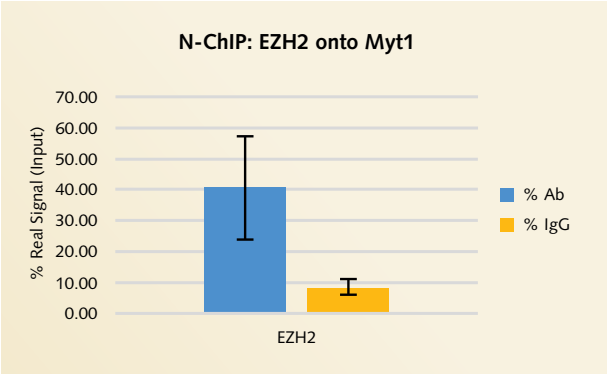

Figure 3 – N-ChIP EZH2 onto Myt1: *% Ab vs % IgG: Excellent signal to noise is demonstrated with the Chromatrap® Native ChIP Kit when specific antibody enrichment is compared to the enrichment of non-specific IgG.*

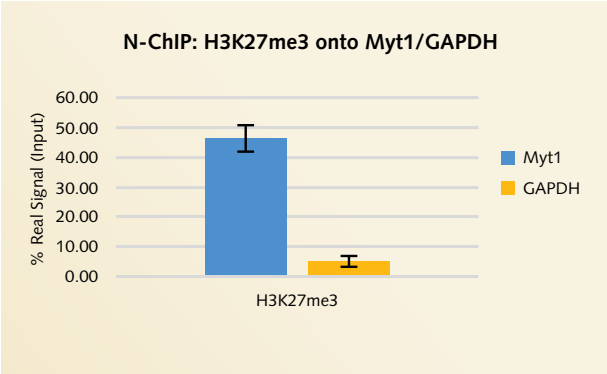

Figure 4 – N-ChIP H3K27me3 onto Myt1/GAPDH: *High levels of enrichment is produced using the Chromatrap® Native ChIP Kit onto the positive gene target MYT1. Low signal is produced for the negative gene target GAPDH, highlighting the highly specific enrichment of H3K27me3 onto the positive gene loci.*

Again with cross-linked ChIP, low background signal is seen with the Chromatrap® technology. Figure 4 highlights the low signal that is produced by enrichment of H3K27me3 onto the negative gene target (GAPDH) when compared to the high signal produced by the positive gene target (MYT1).

Conclusion

With the addition of the Native ChIP Kit (Catalogue no. 500237 & 500238) Chromatrap® have further expanded their product range to allow the scientific user a multitude of options when choosing how to prepare their chromatin. This application note highlights the difference between native and cross-linked ChIP, allowing the user to decide which method is appropriate for their research. Excellent positive enrichment has been demonstrated when compared to the negative gene target and non-specific IgG enrichment for both the Native ChIP Kit and the qPCR standard Chromatrap® Spin Column Kit. The Chromatrap® Native ChIP Kit allows the possibility of preparing chromatin without the need for harsh chemical fixation. With the Chromatrap® technology, native chromatin can be used for studying histone modifications and transcription factors, including the low abundant target EZH2 as described in this study. This has been achieved with a low concentration of chromatin demonstrating the excellent specificity and high sensitivity of the Chromatrap® Native ChIP Kit.

References

Yoo, K. H. & Henninghausen, L. (2012). EZH2 Methyltransferase and H3K27 Methylation in Breast Cancer. Internation Journal of Biological Sciences. 8(1): 59-65.

Chromatrap

European Chromatrap® Technical Support Team Porvair Sciences Ltd
Clywedog Road South Wrexham Industrial Estate Wrexham LL13 9XS UK Tel: +44 (0)1792 666222 Fax: +44 (0)1978 660007

www.chromatrap.com