Chromatrap

Chromatrap® ChIP-seq from native and cross-linked chromatin

Summary

This short application note provides a detailed genome wide comparison of native and cross-linked chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) using the patented solid state Chromatrap® spin column technology.

Introduction

ChIP is used to investigate protein DNA interactions and can be categorised into two methods, Native (N-ChIP) and cross linked (X-ChIP). N-ChIP uses native chromatin prepared by enzymatic digestion and can be used to investigate histone modifications and transcription factors tightly bound to DNA. X-ChIP however uses chemicals to cross link the DNA and protein components of chromatin and is suitable for both histone modifications and low abundant, or more loosely bound, transcription factor protein targets. Both of these assays can be performed at gene/locus specific sites through qPCR as well as genome wide analysis through downstream sequencing (N or X-ChIP-seq).

Chromatrap® supply high quality N-ChIP-seq (Cat no. 500237 & 500238) and X-ChIP-seq kits (Cat no. 500189 & 500190). This application note compares the enrichment of the histone H3K4me3 modification signal across the whole genome, from chromatin prepared by both methods (native vs cross link). H3K4me3 was chosen for this study to illustrate the sensitivity and efficiency of the Chromatrap® spin column technology for both native and cross-linked ChIP-seq. The histone mark H3K4me3 is associated with 'open' transcription start sites and gene activation. It is present in euchromatin throughout the genome and is an abundant epigenomic target.

Method

Chromatin preparation

Chromatin was prepared from the human endometrial epithelial cancer cell line Hec50 (Holinka et al., 1996) for N-ChIP and X-ChIP as per the Chromatrap® protocols. Briefly, for N-ChIP 1 x 10^6 cells were grown to 80% confluency, scraped in ice cold PBS and collected via centrifugation. Cells were lysed in Hypotonic Buffer, before the separated nuclei were subjected to micrococcal nuclease digestion to produce chromatin fragments between 100-500 bp in length. Samples were then dialysed to remove unwanted impurities and processed for ChIP. For X-ChIP 1 x 10^6 cells were grown to 80% confluency and fixed for 10 minutes in 1% formaldehyde. The fixation reaction was quenched with glycine and cells were collected in ice-cold PBS. Cells were spun down and the supernatant discarded before re-suspension and lysis in Hypotonic Buffer. The released nuclei were collected by centrifugation, lysed and the chromatin sheared to obtain 100 – 500 bp length fragments.

Chromatin IP

For immunoprecipitation, slurries were prepared at a 5:2 chromatin: antibody ratio (5 µg chromatin : 2 µg of H3K4me3 (Cat no. 70010) antibody. Slurries were incubated at 4°C for 1hr before immunoprecipitation with the Chromatrap® spin columns according to the Native or X-linked protocols respectively. Inputs containing 5 µg chromatin were prepared in parallel; these samples were used for analyses and not subjected to ChIP enrichment. Following elution, the X-ChIP samples were reverse cross-linked for 2 hours followed by proteinase K digestion. N-ChIP samples were immediately subjected to proteinase K digestion.

All samples were purified using Chromatrap® DNA purification columns prior to library preparation.

Library preparation

Libraries were prepared using the NEBNext® Ultra™ (NEB) library preparation kit and quantified using the KAPA Biosystems (Roche) quantification kit for NGS. Samples were processed on an Agilent Bioanalyzer (Agilent Technologies), to confirm sample size distributions between 200-300 bp (figure 1).

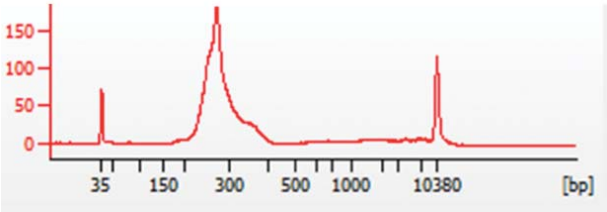


Figure 1 – *Representative Bioanalyzer trace showing chromatin sample size distribution between 200 and 300bp.*

For more information and help with designing your ChIP-seq experiment please refer to our **Top 10 tips for library preparation** brochure.

Chromatrap® ChIP-seq analysis

Paired end (2x75 bp reads) high throughput sequencing was performed using the Illumina® MiSeq, and the quality of the sequencing data analysed by FASTQC. Sequences were mapped to the human reference genome (hg19) using the ENCODE UCSC genome browser database and aligned using Bowtie2. Peak enrichment data were analysed by MACS2 using Chromatrap® ChIP-seq data analysis software, with input for both N and X-linked chromatin samples as background/baseline. Integrative genomics viewer online software (IgV1.4) was used to visualise H3K4me3 enrichment peaks for both the N-ChIP and X-ChIP samples.

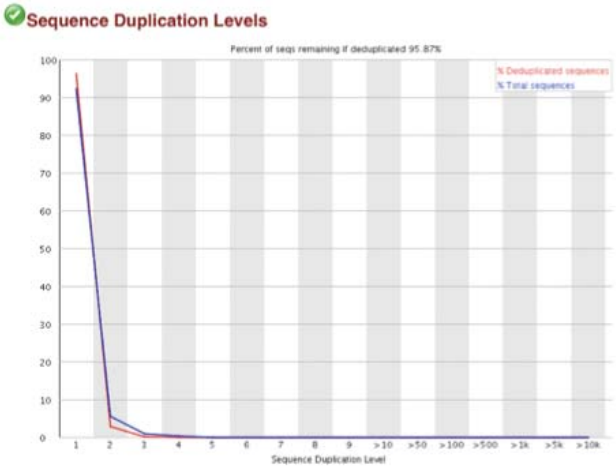
Results

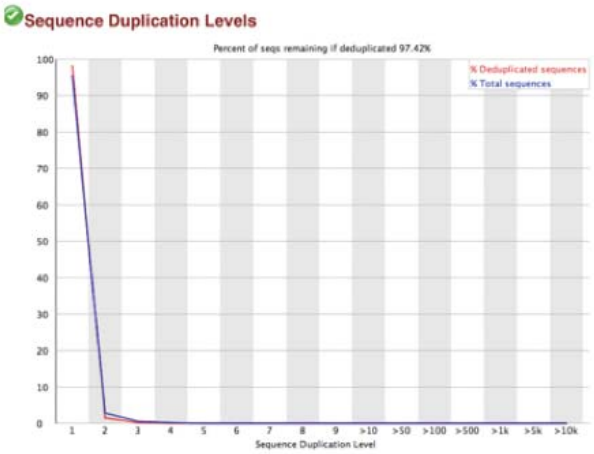
High quality sequence scores (FASTQC software) were obtained from both N and X-linked ChIP. Duplication rates were below 5% and Q scores greater than 30 (Q30 scores correlate to a 1 in 1000 probability of a base being called incorrectly) were observed for all samples. The FastQC scores and subsequent duplication levels obtained for H3K4me3 for both X-ChIP and N-ChIP are shown in figure 2 A-C, respectively.

Sequence quality and duplication

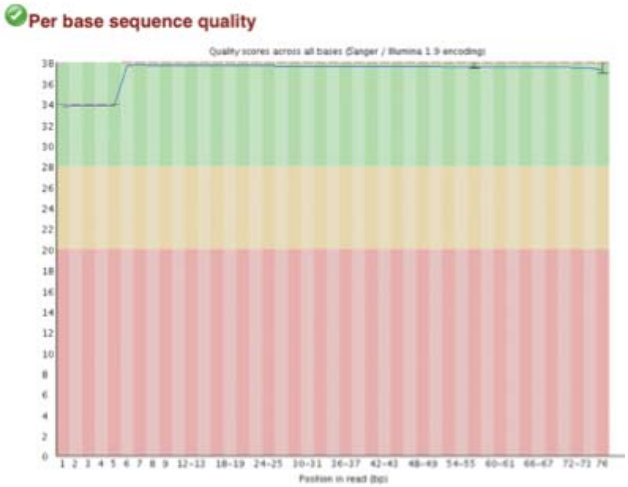
Sample	Q30	Duplication rates
N-ChIP H3K4me3	>30	4.13%
X-ChIP H3K4me3	>30	2.58%

B. Sequence duplication levels for N-ChIP and X-ChIP respectively





C. Q30 scores for N-ChIP and X-ChIP samples respectively



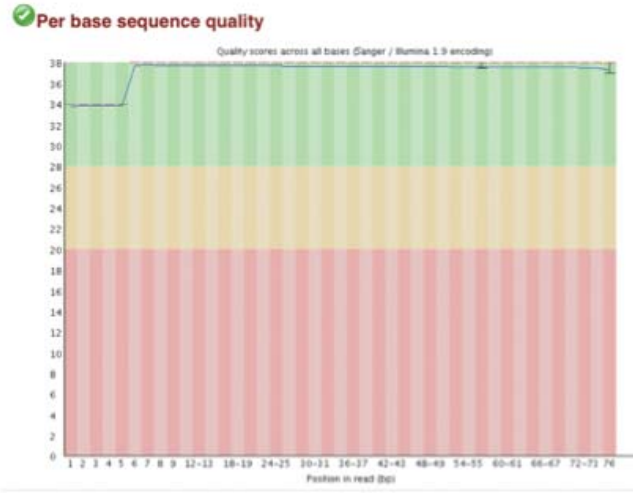


Figure 2 – *FastQC sequence quality and duplication for both N-ChIP and X-ChIP sequenced samples.*

Native and X-linked sequenced samples were analysed for peak enrichment using Chromatrap® ChIP-seq Data Analysis software. For N-ChIP samples 65000 peaks with strong enrichment were observed; peaks showed 3->30 fold enrichment over the input. For X-ChIP we obtained 39000 peaks with strong enrichment. A greater number of peaks would be expected from N-ChIP due to the potential of formaldehyde cross linking to mask protein epitopes leaving them less accessible to the antibody'.

Low resolution genome wide IGV visualised peak enrichment showed comparable H3K4me3 peaks for both Native and X-linked chromatin samples. The peak maps shown in Figure 3A and 3B highlight similar levels of H3K4me3 enrichment across the genome for both N-ChIP and X-ChIP respectively. This demonstrates that Chromatrap® immunoprecipitation is comparable and reproducible between N-ChIP and X-ChIP-seq on a genome wide scale.

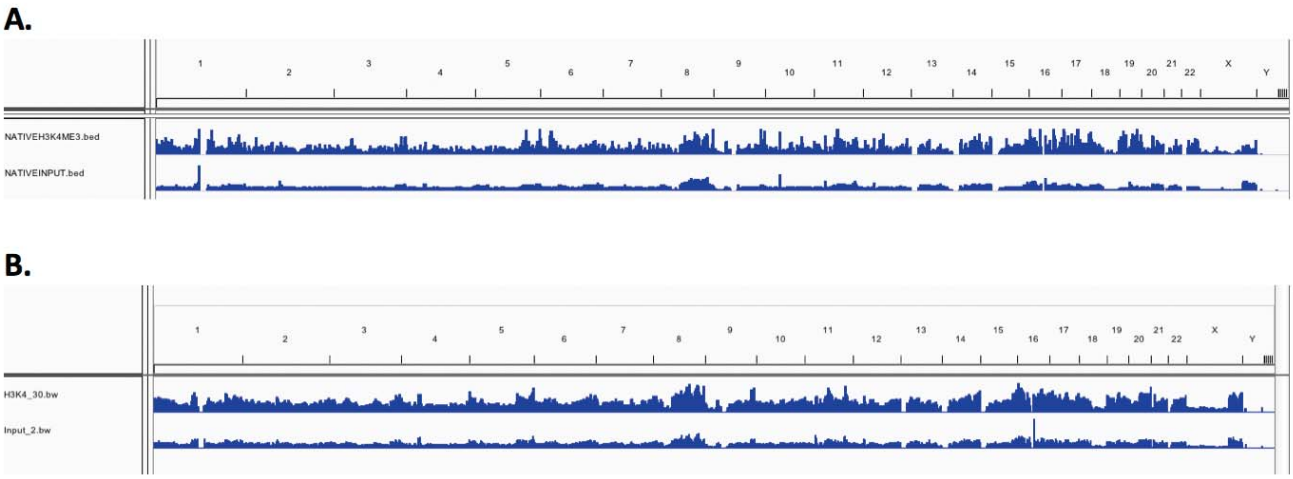


Figure 3 – *Enrichment peak maps of H3K4me3 across the whole genome (A) N-ChIP (B) X-ChIP*

Uniquely Identified Genes

In order to determine the similarities in the H3K4me3 genome wide landscape, the identified peaks were mapped to their closest genes loci (+/-5000 bp distance to the TSS in order to determine which peaks relate to each gene), and the uniquely identified genes compared between both N and X-linked ChIP samples. 20315 uniquely mapped genes were shown to be associated with H3K4me3 in the N-ChIP samples, while 19508 unique gene loci were associated with H3K4me3 in the X-linked ChIP samples, highlighting a 90% resemblance between samples (Figure 4).

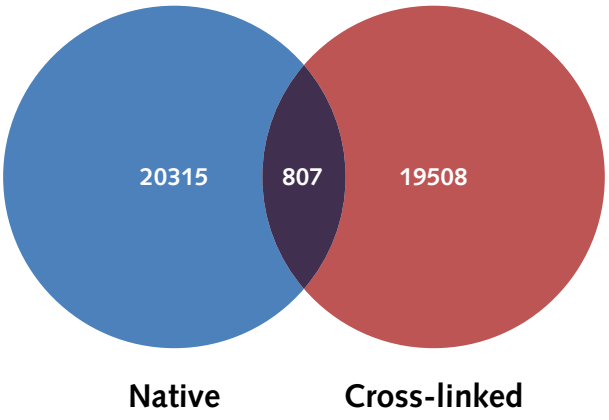


Figure 4 –*H3K4me3 uniquely identified genes*

Further validation of the homology between N-ChIP and X-ChIP-seq is observed when analysing specific gene loci. The ubiquitously expressed DNA binding protein ZNF566 showed comparable H3k4me3 peak enrichment above background in both N and X-linked ChIP samples, as shown in Figure 5.

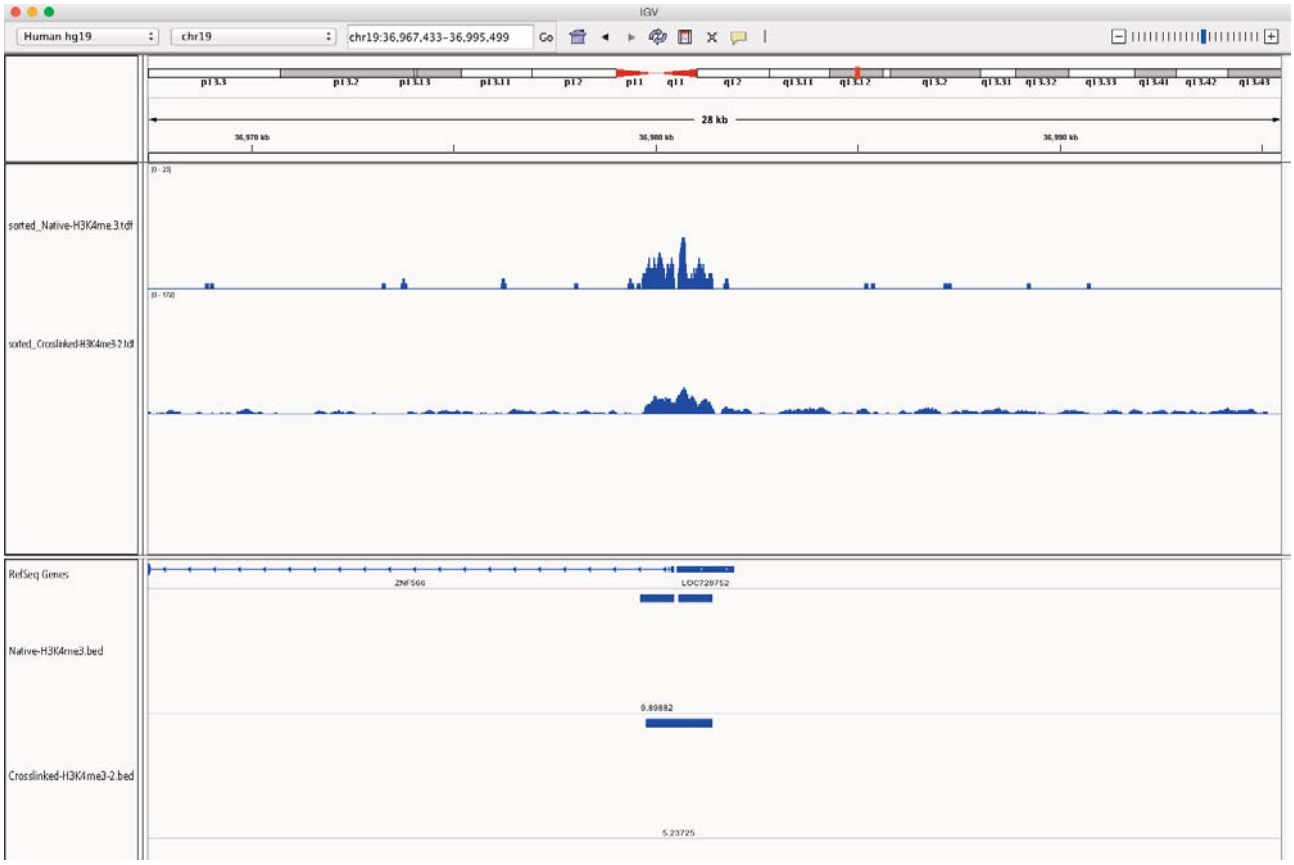


Figure 5 – *H3K4me3 enrichment onto ZNF566 for both N and X-linked Chromatrap ChIP-seq*

Conclusion

In this application note we demonstrate strong and comparable enrichment of H3K4me3 from both Native and Cross-linked chromatin. The study highlights that both Native and Cross-linked chromatin preparation methods are compatible with Chromatrap® extraction and capture technology. Providing excellent datae these methods ensure superior H3K4me3 enrichment followed by high throughput sequencing. High quality FASTQC metrics and significant genome wide peak enrichment levels are obtained using both assay technologies. The wide range of ChIP assays available from Chromatrap® provides users with abundant flexibility and choice for different methods of chromatin preparation and enrichment for downstream next generation sequencing.

Chromatrap

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