

Chromatrap®: A fast, Reliable High-Throughput ChIP-seq Assay for Genome-Wide Protein-DNA Analysis

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Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a technique for genome-wide profiling of DNA-binding proteins and histone modifications. Allied with next-generation sequencing (NGS) technology, Chromatrap® provides a simple and easy to use ChIP format. Compatible with high and low cell numbers, validated on both transcription factor (TF) and epigenetic mark identification in primary and secondary cell lines.

The availability of Chromatrap® ChIP-seq in a 96-well plate allows for high-throughput analysis and, for the first time, provides an unrivalled tool for studying complex, co-ordinated gene regulation and epigenetic mechanisms on a global scale. This application note demonstrates the utility of the Chromatrap® ChIP-seq kit to elucidate the genome-wide binding patterns of nuclear receptor binding events in human adenocarcinoma cells; highlighting the fast multiplex capability alongside massively parallel NGS. **Chromatrap® ChIP-seq assays now enable unbiased, high resolution, low noise amplification for genome-wide understanding of enriched protein-DNA regulatory networks.**

Introduction

Mapping the chromosomal locations of TFs, nucleosomes, histone modifications, chromatin remodelling enzymes, chaperones, and polymerases is a key task in modern biology. The standard methodology for mapping these regulatory protein/DNA interactions is ChIP followed by massively parallel sequencing (ChIP-seq; summarised in Figure 1).

In this application note we present a high quality, reproducible format for ChIP-seq that enables confident analysis of data sets and integrative analysis. When coupled with the ability to conduct multiple target/sample analysis, Chromatrap® ChIP-seq 96 (high-throughput format) enables simultaneous, challenging TF complexes and epigenetic profiles to be deciphered. In this format, Chromatrap® provides a platform for genome-wide, multitarget screening in multiple cell types.

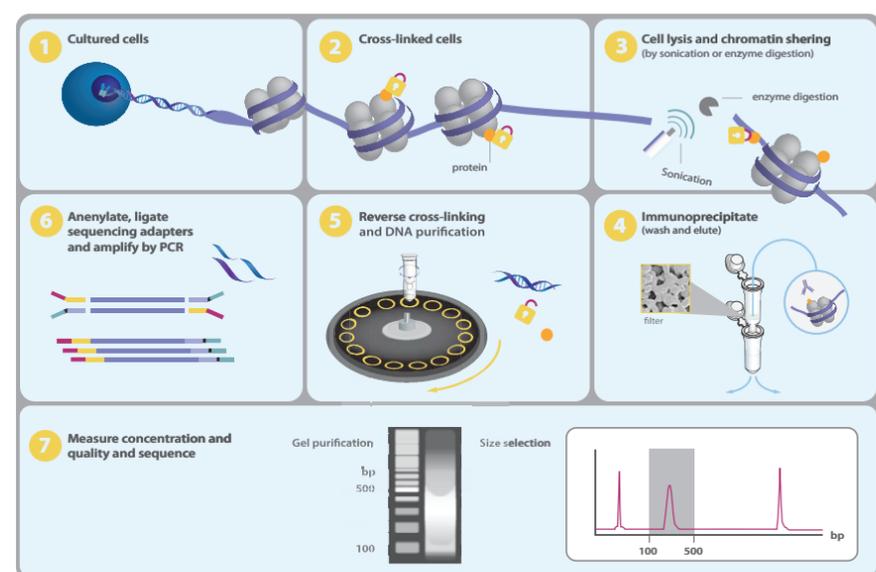


Figure 1. Overview of ChIP-seq process

To demonstrate the ability of Chromatrap® ChIP-seq to selectively and sensitively enrich DNA for NGS, the activity of oestrogen receptor (ER) was monitored in a cell line model. Here we demonstrate both ligand-dependent and - independent binding of ER in cells derived from human endometrial epithelial adenocarcinoma tumours. We also investigated the association of the epigenetic marks H3K4me3 and H3K27me3, these are associated with open and closed chromatin, respectively, in relation to ER binding.

When coupled with the ability to conduct multiple target/sample analysis, Chromatrap® ChIP-seq in its highthroughput format enables simultaneous, challenging TF complexes and epigenetic profiles to be deciphered. In this format, Chromatrap® now provides a platform for genome-wide, multi-target screening across multiple cell types.

Methodology

1 x 10⁷ human endometrial epithelial adenocarcinoma cells were cultured in phenol red-free medium with 10% charcoal-stripped FBS for 48 hours, followed by treatment with 10 nM 17 β -estradiol (E2) for 4 hours. Chromatin was prepared according to the **Chromatrap® ChIP-seq spin column protocol** (chromatrap.com/protocols). Prior to sequencing it is important to validate the immunoprecipitation at a specific genomic locus; Chromatrap® provides positive antibody controls and primers for this purpose. In this experiment, validation of the immunoprecipitation enrichment for H3K4me3 and ER antibodies was conducted by qPCR for the GAPDH and XBP1 locus respectively (Figure 2).

Subsequently, immunoprecipitations were performed for sequencing using 30 μ g of chromatin and 5 μ g of antibody (ER, H3K4me3 or H3K27me3). An equivalent quantity of IgG was also used in a separate IP and used as a background control during data analysis. After immunoprecipitation, chromatin was reverse crosslinked and purified using the illustra GFX PCR DNA and Gel Band Purification Kit and eluted in 50 μ l Tris-HCl elution buffer. ChIP DNA was validated using the Agilent Bioanalyzer to verify sample size distribution and concentration (Figure 3).

Samples were quantified using the Qubit 2.0 fluorometer and library preparation was performed according to the Illumina® TruSeq ChIP Sample Prep Kit protocol (IP-102-1012). Illumina® recommends an initial starting quantity of 5-10 ng ChIP DNA. For this study, 25 ng of DNA was used to increase library complexity and minimise the potential for PCR duplications.

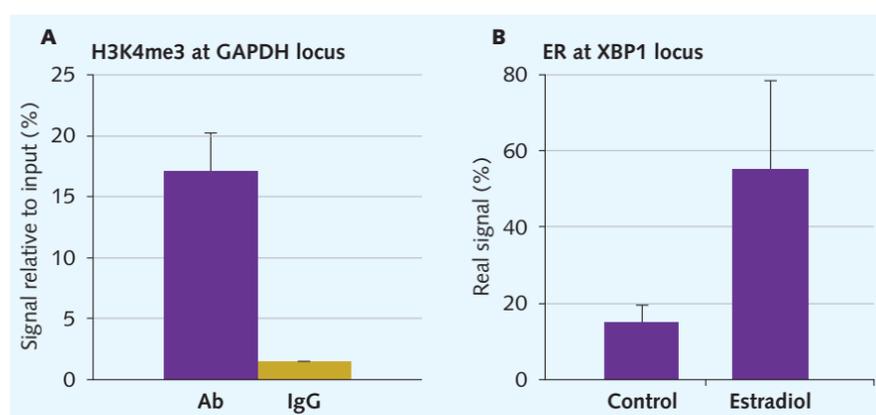


Figure 2. Chromatin IP validation. The enrichment of H3K4me3 associated with GAPDH (A) and ER associated with XBP1 (B) was validated prior to library preparation with a small-scale 2 μ g IP reaction.

Chromatrap® ChIP-seq data analysis – Computational mapping of the sequenced DNA identifies the genomic locations of DNA-binding enzymes, modified histones, chaperones, nucleosomes, and TFs, illuminating the role of these protein-DNA interactions in gene expression and other cellular processes (Mardis et al, 2007). Sequences were mapped to the human reference genome (USCS human genome assembly hg19) and were aligned using Bowtie 2. Data was analysed using MACS or SICER for ER or H3K4me3

Genes belonging to each treatment group, are compiled into a database that contains information about genomic loci, a description of the gene's function, the start and end position of the gene, and whether the peak occurs upstream, downstream or within the gene itself (Figure 7). Many of the genes associated with the E2 group belong to cell proliferation and metabolic pathways.

Nuclear Receptor Function and the Epigenetic Landscape

H3K4me3 is typically associated with the open chromatin structure of active genes; many of which are constitutively active due to their involvement in essential cellular processes. To demonstrate the utility of high throughput Chromatrap® ChIP-seq on multiple marks, the simultaneous enrichment of ER and histone methylation marks associated with open and closed chromatin structures was assayed.

We sought to identify the number of genes that are commonly associated between ER and H3K4me3 or H3K27me3 under basal conditions. Of the 376 genes associated with ER, approximately one third are also commonly found in association with H3K4me3 (Figure 8a). Interestingly, a larger proportion of genes were associated with H3K27me3, an epigenetic modification typically associated with silenced regions (Figure 8b). The number of peaks associated with histone modifications far exceeded those identified for ER.

Summary

New products and technologies are needed to transform genome wide ChIP-seq assays to high-throughput experiments that are capable of mapping multiple marks simultaneously. Chromatrap® ChIP-seq enables further

understanding of complex epigenetic and TF mechanisms which control gene regulation. Understanding these mechanisms in both normal physiology and disease is crucial for the design of therapeutic strategies. Furthermore, cohort and metastudies require the need for standardised sample preparation, IP, and analysis techniques, encouraging normalised archiving of large data sets for public use and metadata analysis.

Demonstrated here is the ability of Chromatrap® ChIP-seq to investigate genome-wide nuclear receptor binding in a complex epigenetic landscape in cell lines expressing multiple isoforms. Understanding the dynamic nature of TF binding helps to elucidate the complicated transcriptional regulation of genes involved in cancer progression. We were able to identify over 2000 precise genomic locations of direct ER binding and provide information on the association of histone modifications linked with active or silenced chromatin regions.

The Chromatrap® ChIP-seq kit provides the user with high quality DNA to perform multiple library preparations from a single IP, and provides a robust enrichment signal. With chromatin loadings of up to 30 µg, the Chromatrap® ChIP-seq kit provides a dynamic range for sample loading that allows users to load lower amounts of chromatin and minimises antibody requirements. The kit is fully compatible with major sequencing platforms including the Illumina® MiSeq and HiSeq instruments.

References

Mardis, E.R. ChIP-seq: welcome to the new frontier. Nature Methods (2007), 4:613-614.

Chen, Y. Systematic evaluation of factors influencing ChIP-seq fidelity. Nature Methods (2012), 9(6):609-14.



Figure 8. ER recruitment and the histone methylation landscape. The number of genes associated with H3K4me3 (left) and H3K27me3 (right) when compared with ER binding under basal conditions. Each diagram shows the number of unique and commonly shared genes between each group.



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New Thermal Cycler offers High-Class qPCR

Analytik Jena AG introduces the new qTOWER³ real-time PCR thermal cycler. The manufacturer of instruments in the field of biotechnology and molecular diagnostics made the announcement at the Biotechnica exhibition in Hannover.

The patented fibre optic shuttle system with unique light source powered by 4 high-performance LED's guarantees ideal excitation and detection of known fluorescent dyes up to dark red. In addition, the system's highly sensitive detection module can be equipped with up to 6 different colour modules. Colour modules are upgradeable, allowing the system to benefit from future Analytik Jena developments. And, enjoy peace of mind knowing that the qTOWER³'s optical components are backed by an industry-leading 10-year warranty.

Silver block technology is at the heart of the qTOWER³, offering outstanding homogeneity of only ±0.1°C over the entire 96 well block. Optionally, the linear gradient function is the optimal tool to easily adjust the instrument to different assays.

The qTOWER³ is available either as stand-alone device with integrated 10" tablet or as PC-based system. The software comes with a broad spectrum of optimised analysis algorithms including absolute and relative quantification, melt point analysis, delta-delta Ct method, PCR efficiency, allelic discrimination, endpoint and protein determination.



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