Rapid and easy method for Chromatin IP (ChIP) analysis of metastatic cells

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Abstract

We present a novel, rapid, plate-based method for chromatin immunoprecipitation (ChIP), validated against epigenetic targets in the human colon metastatic cancer cell line SW480. Fixation, lysis of cells and isolation of immunoprecipitated DNA fragments can be completed to allow downstream analysis in 6-8 hours. The method balances speed and sensitivity, and the key step lies in washing optimization, for which data will be presented. Finally, a series of applications in cancer cells, controlled with and without sodium butyrate treatment, are presented, which target chromatin analysis using multiple antibodies specific for histone modifications. The data demonstrates the utility of this method for functional genomic analysis of cancer cells.

Introduction

Even though most ChIP protocols require multiple labor-intensive steps and three days to complete, ChIP is an established technique used for studying protein/DNA chromatin interactions. Sigma's Imprint™ ChIP kit provides a one-day plate-based alternative to the longer traditional methods. The kit's plate-based format reduces or eliminates several of the labor-intensive manipulations, allowing for more samples to be processed simultaneously. In addition to a vast reduction in protocol time and increasing the number of samples, this method also requires fewer input cells. Combining these properties makes this method more appropriate for cell investigational and antibody screens.

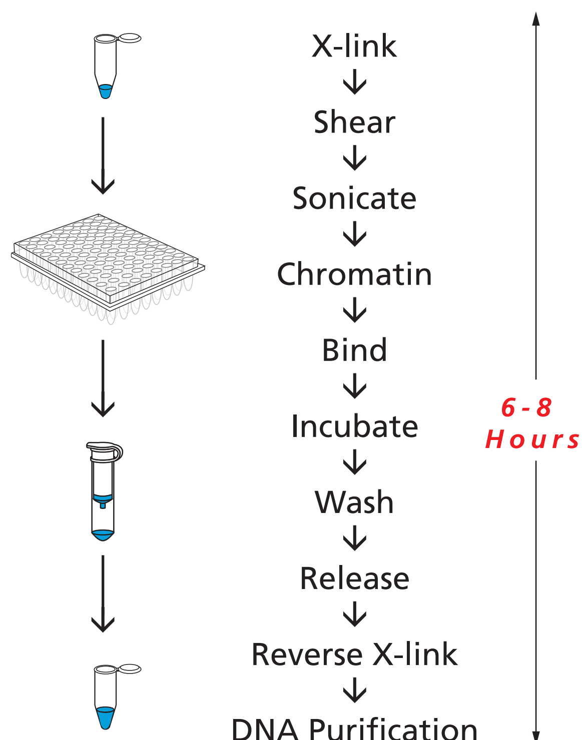
We have validated our rapid, one-day ChIP kit by investigating chromatin modifications on targets of the polycomb repressor complex II (PRC), in response to the histone deacetylase (HDAC) inhibitor – sodium butyrate. A recent ChIP study using another HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), revealed marked reduction of PRC binding and consequent H3K27 trimethylation, and accumulation of acetylated H3 at a PRC target gene promoter. The H3K27me3 mark is laid down by the PRC (Yu et al., Cancer Cell 2007).

In this study, using our rapid ChIP kit, we have demonstrated that sodium butyrate treatment of SW480 cells dramatically increases the accumulation of acetylated H3K9 and reduces H3K27 trimethylation on several established PRC targets (Kirmizis et al., Genes and Development 2004, Yu et al., Cancer Cell 2007)

Materials and Methods

SW480 cells obtained from ATCC (Cat. No. CCL-228, Manassas, VA) were cultured according to ATCC recommendations. Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (St. Louis, MO). Sodium butyrate (Cat. No. B5887) treatments were applied at 5 mM for 20-24 hours. SW480 cells were cross-linked and chromatin sonicated (Diagenode Bioruptor) per technical bulletin of Sigma's Imprint™ Chromatin Immunoprecipitation Kit (Cat. No. CHP1). Antibodies used for ChIP studies were α-IgG mouse (Cat. No. M8695) and α-RNA Polymerase II (RNAP, Cat. No. R1530) provided in CHP1 kit, α-H3K9ac (Cat. No. H9286) and α-H3K27me3 (Millipore, Cat. No. 07-449). Cells were processed as described in the CHP1 protocol with optional wash and 30 µl final elution. The *Staphylococcus aureus* (Staph. A) ChIP protocol was followed as described: **http://genomecenter.ucdavis.edu/expression_analysis (Updated 7/2/07).** Quantitative PCR was setup with 1.5-2 µl of ChIP DNA elution in a 25 µl SYBR Green (Cat. No. S4438) reaction, for a minimum of 35 cycles of 60 °C, 72 °C, 82 °C and 95 °C all for 20 seconds. Primers specific for GAPDH were used as a control. Primers for control and PRC target genes were used as described: GAPDH and MYT1 (Yu et al., Cancer Cell 2007), metastatic targets KRT17 and SOCS2 (Yu et al., Cancer Research 2007).

Sigma Imprint ChIP Protocol



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Results

One Day ChIP Enrichment

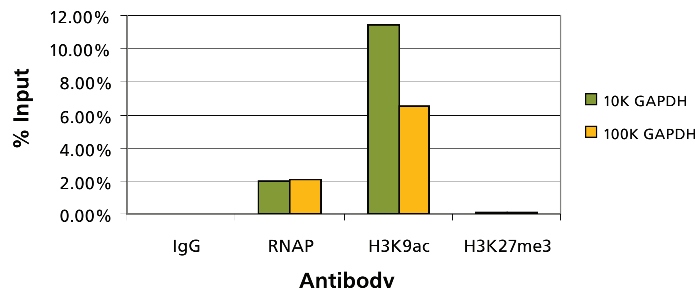

Figure 1: One day is sufficient to acquire ChIP enriched DNA using Sigma's Imprint ChIP protocol. ChIP with antibodies against mouse IgG, H3K9ac, RNAP and H3K27me3 was completed on 10,000 and 100,000 SW480 cells per reaction. A fraction (1%) of the sonicated chromatin was set aside as "input" DNA before the antibody affinity manipulations. DNA was purified following cross-link reversal. The resulting enriched DNA was probed for GAPDH using specific primers by qPCR. Percent input was calculated by 100 x $2^{(Ct_{\text{adjusted Input}} - Ct_{\text{Enriched}})}$. Input DNA Ct was adjusted from 1% to 100% equivalent by subtracting 6.644 Cts or Log_2 100.

Enrichment was observed with both RNAP and H3K9ac using either 10,000 or 100,000 cells per ChIP. Mouse IgG represents non-specific background after IP. No significant enrichment was observed for the H3K27 trimethyl (polycomb repressive mark) on the ubiquitously transcribed GAPDH gene promoter, thus demonstrating the specificity of our ChIP assay.

Excellent ChIP enrichment with only 10,000 cells input

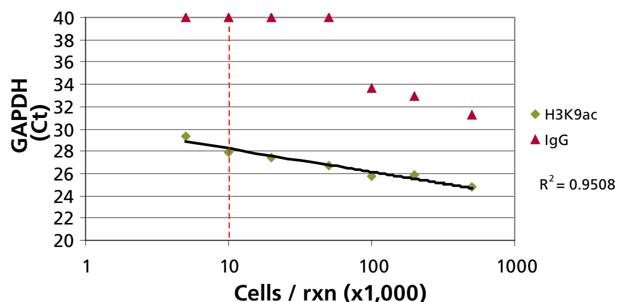

Figure 2: The chromatin from varying numbers of SW480 cells was crosslinked, isolated and sheared as described in the technical bulletin of the Imprint ChIP kit. The resulting chromatin was immunoprecipitated using either α-H3K9ac antibody or purified IgG, washed, crosslinks hydrolyzed and DNA purified, then probed by qPCR using primers specific for GAPDH (shown) or SOCS2 (not shown).

Nonspecific binding in the IgG (noise) IP samples was noticeable above 100,000 cells; although DNA enrichment was still observed. This experiment, mirrored using SOCS2 primers, shows that the enrichment was linear from 10,000 - 500,000 cells per ChIP.

Imprint ChIP kit captures the trend of butyrate-mediated changes

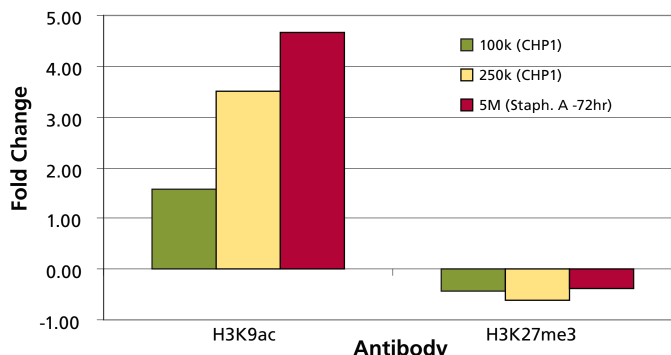

Figure 3: Imprint ChIP kit protocol captures the trend of butyrate – mediated changes in chromatin modifications on the SOCS2 promoter. Similar trends were observed with CHP1 kit that uses fewer cells and a faster (1-day) ChIP protocol, and Staph. A-based ChIP protocol, that uses 5 million cells and takes 72 hours. The Imprint ChIP samples were processed as described **(Fig. 2)** while the Staph. A protocol followed was as mentioned in Methods.

Fold change was calculated by the equation

$$[2^{(Ct_{\text{Enriched control}} - Ct_{\text{Input control}} - Ct_{\text{Enriched butyrate}} + Ct_{\text{Input butyrate}})}] - 1.$$

Following butyrate treatment, with increasing number of cells per ChIP a corresponding accumulation of H3K9 actylation was observed, whereas the reduction in H3K27 trimethylation was independent of either the number of cells per ChIP or the protocol followed. This work suggests that targets requiring high sensitivity detection may be best immunoprecipitated using the extended (72-hour) Staph. A-based ChIP protocol, which can accommodate a higher number of cells per reaction. The fold change in GAPDH promoter levels for H3K27me3 were insignificant (data not shown) demonstrating the specificity of butyrate-mediated inhibition of a PRC metastatic target (SOCS2).

Results

Butyrate reverses PRC-mediated repression on metastatic targets.

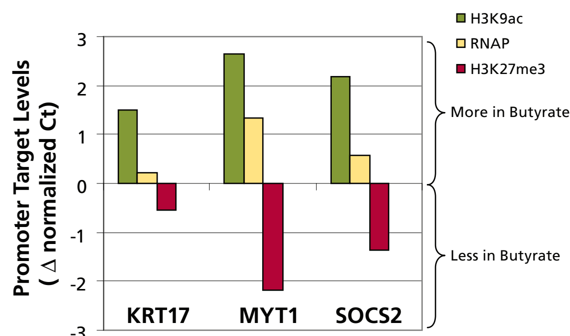

Figure 4: Imprint ChIP kit captures alterations in chromatin modifications and RNA Polymerase (RNAP) recruitment on several PRC targets post sodium butyrate treatment of metastatic SW480 cells. Antibodies against H3K9ac, RNAP and H3K27me3 were used with 250,000 cells in the previously described CHP1 method. Delta normalized Cts were calculated by

$$(Ct_{Enriched} - Ct_{Input})_{control} - (Ct_{Enriched} - Ct_{Input})_{butyrate}$$

A positive value indicates more DNA target was present in the sodium butyrate treated sample when compared to the control. The "input" values for all the targets tested were comparable before and after butyrate treatment.

Consistent with previous HDAC inhibitor treatment results; the butyrate treatment resulted in an increase in H3K9ac and concurrent increase of RNAP recruitment on PRC targets KRT17, MYT1, and SOCS2. In addition to the probable transcription increase, based on increased RNAP recruitment, a decrease in H3K27 trimethylation was also observed on the same PRC targets. These results indicate that the HDAC inhibitor, sodium butyrate, inhibits the PRC responsible for the trimethylation of H3K27. Combined with previous results (Yu J., et al., Cancer Cell, 2007) this study extends the application scope of HDAC inhibitors to include inhibiting PRC-mediated gene repression on metastatic targets (KRT17, SOCS2).

Applications

The speed and sensitivity of the Imprint ChIP kit opens experimental possibilities, especially for screening assays. Applications include:

1. Screening antibodies to determine ChIP compatibility;
2. Testing various gene activation conditions, e.g., compounds, small molecules, agonists/antagonists, cellular treatments, UV, IR, heat shock, inhibitors, etc.;
3. Measuring the effect of several siRNA's/miRNA's against transcription regulators, DNA-binding activators/repressors, co-activators or subsequent chromatin modifiers;
4. Surveying multiple cell lines to determine the ideal candidate for further studies of a transcription regulator when endogenous expression levels of the transcription factor are unknown;
5. Optimizing cross-linking agents {formaldehyde, dimethyl 3, 3′-dithiobispropiomimidate (DTBP), dimethyladipimadate (DMA), disuccinimidyl suberate (DSS), dithiobis [succinimidyl propinoate] (DSP), and ethylene glycobis [succinimidyl succinate] (EGS)} either alone or in combination to enrich for co-activators/co-repressors that do not bind DNA directly.

Conclusions

- The one-day Imprint ChIP procedure demonstrates butyrate-mediated repression of PRC metastatic target genes.
- Imprint ChIP protocol works with a small (10,000 cells) input per reaction.
- Imprint mirrors the established Staph. A. ChIP results; both may be used to see the trend effect of butyrate on metastatic targets.
- ChIP protocols requiring 5 million cells and 72 hours are not required to see butyrate effects on the target genes tested.
- Butyrate treatment reduced PRC methylation (H3K27me3) function on promoters of metastatic target genes (KRT17, SOCS2).
- Repression of metastatic target genes by PRC may be reversed by the HDAC inhibitor, sodium butyrate, suggesting therapeutic potential.

References

Yu J. et al., Cancer Cell 12:419-431, 2007. "Integrative genomics analysis reveals silencing of β-adrenergic signaling by polycomb in prostate cancer."

Kirmizis A., et al., Genes and Development 10:1592-1605, 2004. "Silencing of human polycomb target genes is associated with methylation of histone H3Lys27."

Yu J., et al., Cancer Res. 67:10657-10663, 2007. "A polycomb repression signature in metastatic prostrate cancer predicts cancer outcome."

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