Imprint® Ultra Chromatin IP Kit: for Successful ChIP-Seq with a Low Abundance Transcription Factor

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Abstract

Performance of Sigma's Imprint® Ultra Chromatin IP Kit (CHP2) was tested by conducting immunoprecipitation of chromatin complexes (ChIP) containing a rare transcription factor (TF), EZH2, followed by qPCR for target and nontarget DNA sequences (ChIP-qPCR) and by deep sequencing (ChIP-Seq). With Imprint® Ultra ChIP-qPCR, target sequence was enriched 45-fold and non-target enrichment was negligible. The kit's modified Staph-Seq binding matrix allowed 23% more mappable Illumina sequence reads than untreated Staph A. We conclude that the Imprint® Ultra ChIP kit is highly suited to ChIP-Seq studies of rare TFs.

Introduction

The Imprint® Ultra Chromatin IP Kit is Sigma's second generation chromatin immunoprecipitation kit, developed for maximum sensitivity and optimum next-generation sequencing results. This kit uses modified Staphylococcus aureus (Staph A) to immunoprecipitate and purify chromatin complexes. Sequencing quality Staph A (Staph-Seq) is achieved with a proprietary DNA-blocking treatment to prevent Staph A DNA from contributing to ChIP-Seq. Staph-Seq is ideally suited for studying recruitment of low abundance TF in genome wide location analysis experiments such as ChIP-chip and ChIP-Seq. The high density of protein A on the cell wall of Staph A serves as an excellent matrix to pull-down rare TF-associated chromatin complexes. This high capacity coupled with a very stringent wash buffer improves the sensitivity and specificity of the Imprint® Ultra ChIP kit, resulting in high yields of pure DNA with extremely low backgrounds. This development will now allow researchers to explore the genome-wide binding sites of low abundance TF's as well as novel histone modifications.

The Imprint® Ultra ChIP protocol was adapted from and validated in consultation with the laboratory of Dr. Peggy Farnham, UC Davis. It is optimized for ChIP reactions with chromatin from 10^7 cells (up to ≈ 50 µg DNA), and can also be scaled up (or several preparations pooled) to accommodate 10^8 cells for genome-wide binding studies in ChIP-chip and ChIP-Seq applications. Our Staph-Seq DNA-blocking treatment virtually eliminates Staph A DNA contamination of Illumina-libraries prior to massively parallel next-generation sequencing (Figs. 1-2), without altering performance (Fig. 3). Successful performance of the Imprint® Ultra ChIP kit has been demonstrated in a ChIP-Seq experiment performed in collaboration with the Farnham lab (Palhan et al., manuscript in preparation). ChIP was performed using 200 million DU145 cells and antibody to EZH2 (a low abundance transcription factor and catalytic component of Polycomb Repressor Complex) using either untreated (C) or Staph-Seq (T) cells (Fig. 3). Use of Staph-Seq resulted in correct mapping of 23% more sequence reads, or 5 million additional unique sequences, compared with untreated Staph A. (Fig. 5). Thus we have developed a unique procedure to block the Staph A DNA while retaining functionality of Staph-Seq.

In this poster, we demonstrate the use of the Imprint® Ultra ChIP kit in ChIP-qPCR experiments for rare and high abundance TF's (Fig. 7), as well as ChIP-Seq analysis with a rare TF, EZH2 (Figs. 4-5). In addition, we show that the kit outperforms competing products in EZH2 ChIP-qPCR (Fig. 6).

CHP2 is Designed for ChIP-Seq.

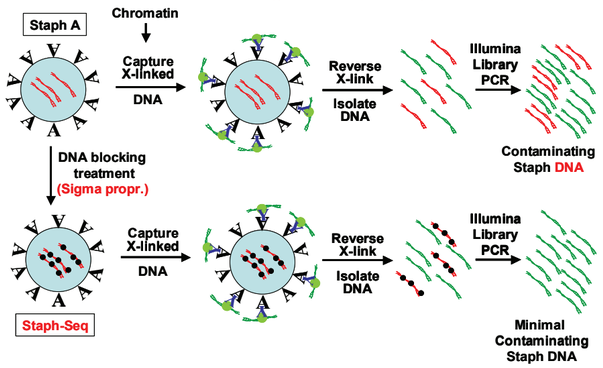


Figure 1: Use of untreated (top panel) Staph A (blue circle) in a ChIP-Seq assay results in Staph A DNA sequences (red) contaminating the genomic ChIP'ed DNA sequences of interest (green). Both the genomic DNA and the Staph A DNA are amplified in the Illumina library. With Staph-Seq (lower panel), however, Staph A DNA is blocked and is not amplified during library preparation.

Reduction of Staph A DNA in Staph-Seq ChIPs

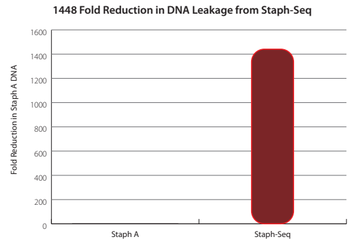


Figure 2: Staph A DNA contamination in ChIPs prepared with Staph-Seq versus untreated Staph A control cells was quantitated by qPCR with primers targeting Staph A DNA sequence for rRNA. Treatment led to a 10.5 Ct, or 1448-fold, reduction in Staph A DNA in Staph-Seq.

Validation of Staph-Seq ChIPs: 1) Post-Library Preparation

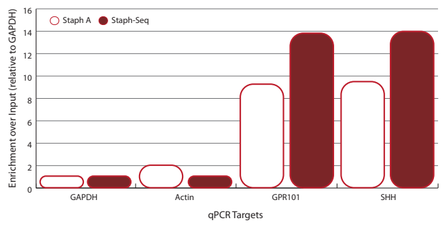


Figure 3: Specific targeted recruitment of EZH2 was demonstrated on the SHH and GPR101 gene promoters, but not on non-target Actin and GAPDH promoters, in scaled-up ChIPs with untreated Staph A (C) and Staph-Seq (T) cells after library preparation before Illumina-sequencing.

2) .. & Illumina Sequencing

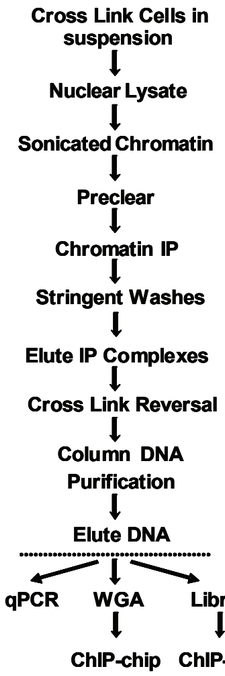
Figure 4: Sequencing results from Staph-Seq EZH2 libraries validates EZH2 recruitment observed in the Illumina libraries before sequencing (Fig. 3 above). Integrated Genome Browser snapshot of the chromosome 7 region surrounding the SHH gene shows a strong peak directly above the qPCR amplicon (shown in red).

EZH2 ChIP-Seq Results Summary

	Control "Staph A"	Treated "Staph-Seq"
Total Reads	45,368,364	34,942,887
Mapped Reads (HG19)	29,878,289 (65.9%)	31,074,058 (88.9%)
Unique	17,171,199	22,200,188

Figure 5: Summary of ChIP-Seq results with Staph-Seq vs Staph A cells. EZH2 ChIPs were performed with Staph A and Staph-Seq and cross-linked chromatin from 200 million DU145 cells. Illumina sequencing was performed by the UC Davis Genome Center. **(http://genomecenter.ucdavis.edu/dna_technologies).** 36 cycles were run on the Illumina Genome Analyzer II. Bioinformatics analysis was done using "SOLE Search" program **(http://havoc.genomecenter.ucdavis.edu/cgibin/chipseq.cgi).** Compared to regular Staph A cells, DNA-blocked Staph-Seq cells gave a 23% gain in mappability of reads when blasted against the human genome (HG19 build).

CHP2 Workflow



Competitor Comparison

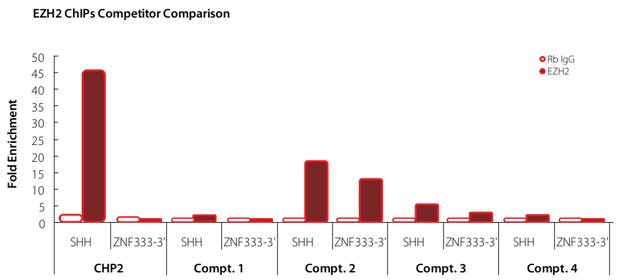


Figure 6: ChIP was performed with 2 µL of EZH2 Ab (Diagenode, pAb39) and 1 µL of Rabbit IgG (Sigma, I5006) and cross-linked chromatin from 2 million DU145 cells. 2 µL ChIP'ed DNA (out of 30 µL eluate) was analyzed by qPCR using primers for the sonic hedgehog (SHH) gene promoter, a target of the EZH2 containing Polycomb Repression Complex, and a non-target region 3' to the ZNF333 gene (ZNF333-3'). Target sequence was enriched 45-fold and non-target was negligible for Imprint® Ultra, whereas target enrichment was < 20-fold and non-target was > 50% of target for competitor 3, the closest competitor.

H3K27me3 & EZH2 ChIPs

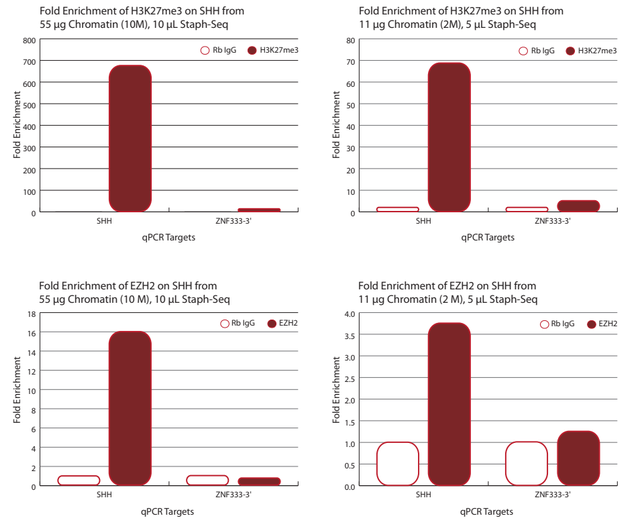


Figure 7: ChIPs were performed with 2 µL of H3K27me3 Ab (Diagenode-pAb069, top panel), or 2 µL of EZH2 Ab (Diagenode-pAb039, bottom panel) and 1 µL of Rabbit IgG (Sigma, I5006) with chromatin from DU145 cells as indicated (10 million cells on left panel and 2 million on right panel). 2 µL ChIP'ed DNA (out of 30 µL eluate) was analyzed by qPCR using primers for the sonic hedgehog (SHH) gene promoter, a target of the EZH2 containing polycomb repression complex and a non-target ZNF333 gene (ZNF333-3').

Conclusions

- The Imprint® Ultra ChIP kit is highly sensitive, allowing ChIP-Seq studies of rare TFs
- A proprietary treatment during the manufacture of Staph-Seq blocks amplification of Staph A DNA without affecting performance – Contamination in ChIP'ed DNA reduced by > 3 logs (Fig. 2) but no loss in target sequence detection by qPCR (Fig. 3). We demonstrate a 23% increase in mappable reads (5 million additional unique reads) with Illumina sequencing of DNA ChIP'ed with Staph-Seq compared to regular Staph A cells (Figs. 4-5).
- Performance of Imprint® Ultra is superior to competing kits – Imprint® Ultra was the only kit tested that gave unequivocal results in ChIP-qPCR for EZH2, a rare TF. Target sequence was enriched 45-fold and non-target enrichment was negligible, versus target enriched < 20-fold and non-target > 50% of target for the closest competitor (Fig. 6).

To Order:

Product	Cat. #
Imprint® Ultra ChIP kit with controls	CHP2-24 Rxn
Imprint® Ultra ChIP kit without controls	CHP2NC-48 Rxn
Imprint® Chromatin Optimization kit	CHROP-15 Rxn