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A Hologic Company

APPLICATION NOTE

From minuscule amounts to magnificent results: reliable ChIP-seq data from 10,000 cells with the True MicroChIP™ and the MicroPlex Library Preparation™ kits

Abstract

Diagenode has developed groundbreaking solutions for epigenetic studies on very low sample amounts. In this application note, we introduce our unique True MicroChIP Kit and the MicroPlex Library Preparation™ Kit, which enable the reliable detection of protein-DNA interactions by ChIP-PCR or ChIP-seq starting from inputs as low as 10,000 cells.

Introduction

As Next-Generation Sequencing (NGS) technologies have become widespread and more accessible, the primary method for genome-wide mapping of protein-DNA interactions is now chromatin immunoprecipitation followed by NGS detection (ChIP-seq), which enables the discovery of transcription factor binding sites or patterns of histone modifications. ChIP-seq is advantageous in providing high-throughput data of the whole genome that can be used for quantitative and qualitative analysis of the protein-DNA interactions (measured through the enrichment of bound DNA fragments), but it is not without its disadvantages. A major limitation is the requirement for high amounts of starting material, usually millions of cells. For scientists who work with limited sample amounts, such as ancient samples or difficult cell types, ChIP-seq has not been a viable analysis method. Diagenode has addressed this limitation with novel technology and kits optimized specifically for ChIP-seq from extremely low amount of starting materials.

Diagenode has a complete suite of ChIP and ChIP-seq solutions including extensively validated ChIP-seq grade antibodies, cell lysis and chromatin shearing devices (Bioruptor®), kits for high and low cell numbers, automated epigenetics sample prep, and kits specialized for ChIP-seq. For ChIP-seq with limited sample amounts, the solutions of choice are the True MicroChIP kit used in conjunction with the MicroPlex Library Preparation™ kit and our Premium grade antibodies, enabling ChIP-seq with just a few picograms of input. The MicroPlex kit is an exceptionally fast, user-friendly, and automatable solution with multiplexing capability optimized for ChIP-seq libraries from picogram inputs of chromatin. The library preparation requires only three simple steps, with no tube transfers or intermediate purifications required. Additionally, the True MicroChIP kit has been validated on the Illumina® sequencing platforms, Diagenode's IP-Star® Compact Automated Workstation, and with our Premium ChIP-seq grade antibodies, the most extensively validated antibodies on the market, selected specifically for ChIP-seq applications.

This application note demonstrates a successful example of using the True MicroChIP Kit followed by library preparation with the MicroPlex kit on as low as 10,000 cells. The True MicroChIP Kit contains everything you need for ChIP: buffers for lysis, washing, elution, precipitants, magnetic beads, and controls (antibody, IgG, PCR primers). Similarly the MicroPlex kit provides reagents necessary to perform a successful amplification and library preparation: buffers and enzymes for DNA synthesis and amplification, plus reagents for indexing. The overview of the simple and efficient protocols can be seen in Figure 1.

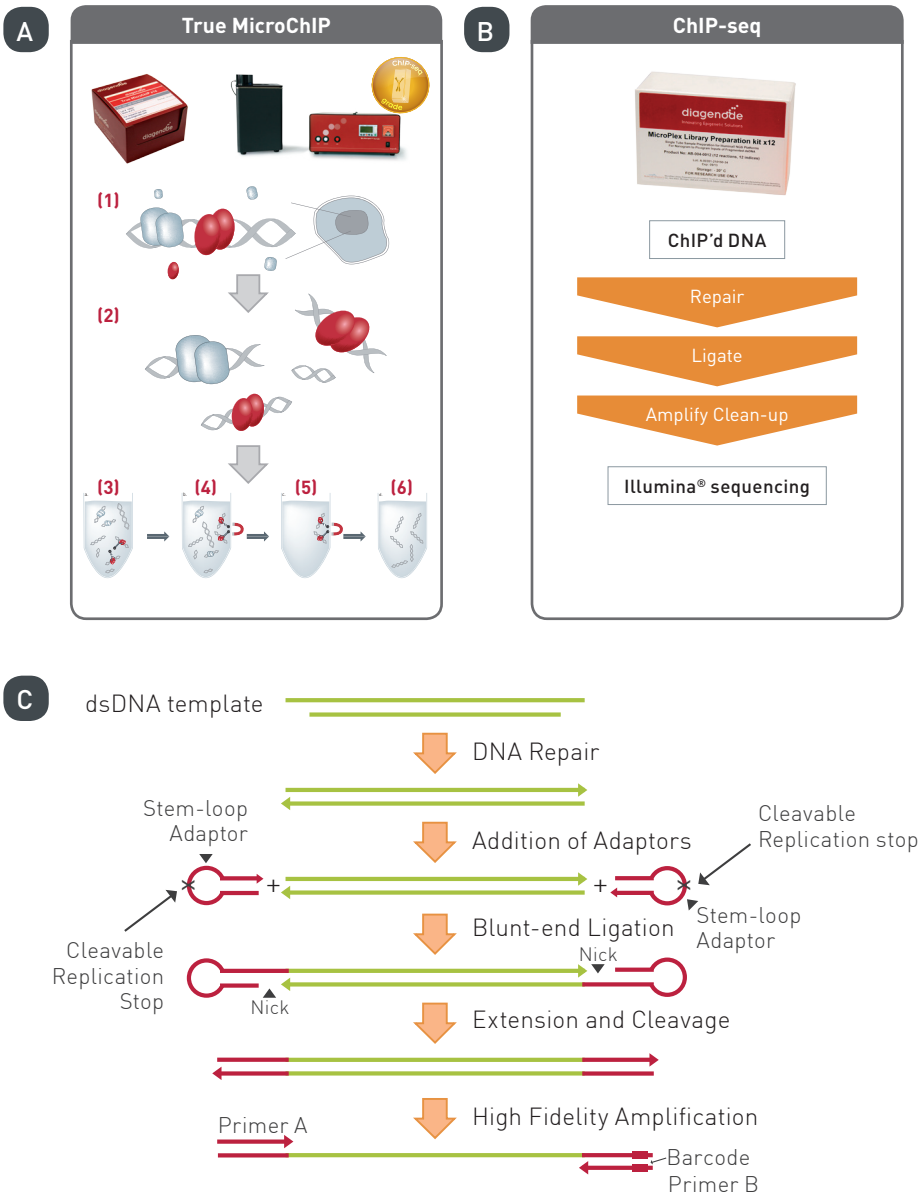

Fig.1A: Schematic of the process from sample prep to ChIP-seq with the True MicroChIP kit.

True MicroChIP procedure: 1. Cell fixation and DNA-protein cross-linking, 2. Cell lysis and chromatin shearing using the Bioruptor®, 3. Binding of the antibodies to the chromatin, addition of beads, 4. Magnetic immunoprecipitation, 5. Washes of immune complexes, 6. DNA purification and recovery of ChIP'd DNA; ChIP-sequencing procedure: 1. End-repair, 2. Adapter ligation, 3. Library amplification, 4. Library purification and sequencing on Illumina® platform.

Fig.1B: Schematic of the library preparation with the MicroPlex kit.

Both procedures can be performed on the IP-Star® Compact Automated Workstation.

Materials and methods

For the ChIP experiments, 10,000 HeLa-S3 cells were harvested in addition to a higher cell number (~100,000 cells) for comparison). Using the True MicroChIP Kit, the cells were fixed with formaldehyde and lysed. For chromatin shearing, 5 x 5 cycles of 30 seconds on/30 seconds off on the high power setting were done on the Bioruptor® Plus. Shearing efficiency was analyzed on 1.5% agarose gel.

The protein-DNA complexes were captured with Diagenode's ChIP-seq grade H3K4me3 antibody. Magnetic beads were used for easy isolation and elution. During washes with optimized buffers, DiaMag magnetic racks were used to reduce background. After elution, ChIP'd DNA was de-crosslinked and purified with phenol/chloroform/isoamyl alcohol solution. Two carriers were added during the ethanol precipitation in order to increase the DNA recovery.

The IP'd DNA was quantified on a Qubit system with the Quant-IT dsDNA HS assay kit of Invitrogen. Subsequently qPCR assays were performed with the provided primers for positive and negative control regions to evaluate the ChIP quality.

For sequencing library preparation, we used the MicroPlex kit -- 30 pg and 300 pg of input DNA immunoprecipitated from 10,000 cells and 100,000 respectively were used. After end repair of the double-stranded DNA templates, we ligated the cleavable stem-loop adaptors containing the sequencing primer sites, and after proper DNA extension we used them to amplify our sample with a proprietary high fidelity amplification method. After amplification the amount was quantified with the qPCR based Illumina® library quantification kit from Kapa Biosystems. The amplification was performed by following the MicroPlex manual recommendations. The libraries were purified with the Agencourt AMPure® XP bead-based purification system. After purification, the libraries were ready for direct sequencing with the Illumina® platform. No size selection was necessary.

The ChIP and the library preparation were done manually, but we performed additional validations on the IP-Star® Compact Automated Workstation to explore the capabilities of automating the kits.

The libraries were sequenced on an Illumina® HiSeq2000 with standard Illumina® reagents, 36 bp single end protocol. The cluster generation and sequencing were carried out according to the manufacturer's instructions.

The sequencing was followed by bioinformatics analyses, the primary analysis (cluster filtering, base calling, etc.) was done by the standard Illumina® pipeline. The filtered reads were aligned with ELAND to the human genome (version hg19/GRCh37.3). The peak calling was done with SICER with the recommended settings for H3K4me3, and the subsequent custom analyses were performed with Homer, Bedtools, and internally developed custom scripts.

Detailed information about the above procedures can be found in the respective manuals, downloadable from our website. Please see the "Further information" section below.

Results

The chromatin shearing of 10,000 cells was very efficient, represented by the smear in Figure 2. We used 30 pg of DNA extracted from 10,000 cells and 300 pg from the higher cell number experiment. The successful IP was confirmed by qPCR (Figure 3) which shows good enrichments in positive control regions and negligible signal in negative control regions. For comparison and proof of consistency, we provide other data obtained with H3K27me3, H3K9me3, and H3K27me3 antibodies, using 10,000 cells and the True MicroChIP Kit. The automated ChIP performed well, demonstrated by a series of ten IP reactions that were reproducible and highly comparable with the manual ChIP results (see Figure 4).

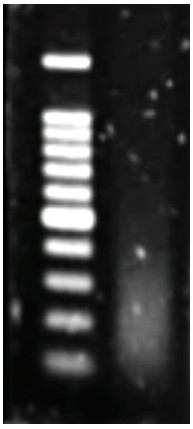

Figure 2:
The even smear without residue shows efficient sonication

IP-Star® Compact Automated Workstation

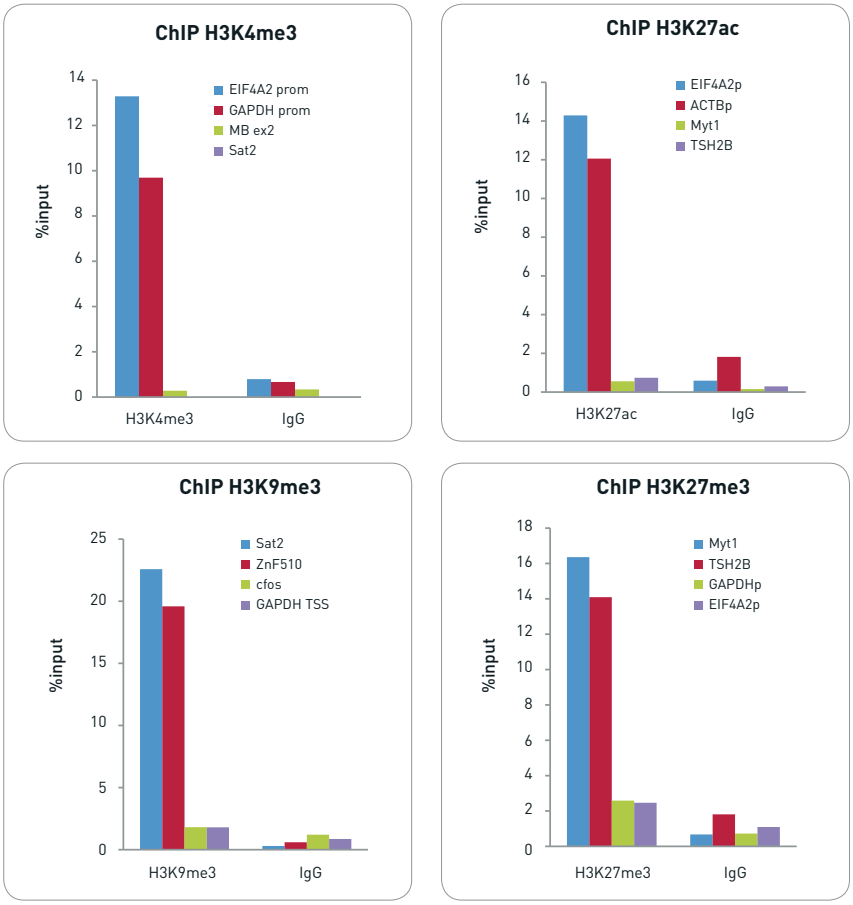

Figure 3: ChIP efficiency on 10,000 cells.

ChIP assays were performed with several Diagenode antibodies: H3K4me3 antibody (0.25 µg/reaction), H3K27ac (0.1µg/reaction), H3K9me3 (0.5 µg/reaction) and H3K27me3 (0.25 µg/reaction). Identical quantity of IgG was used as a control. The qPCR was performed with primers for two positive loci and two negative loci for each ChIP assay. Figure shows the recovery, expressed as a percentage of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

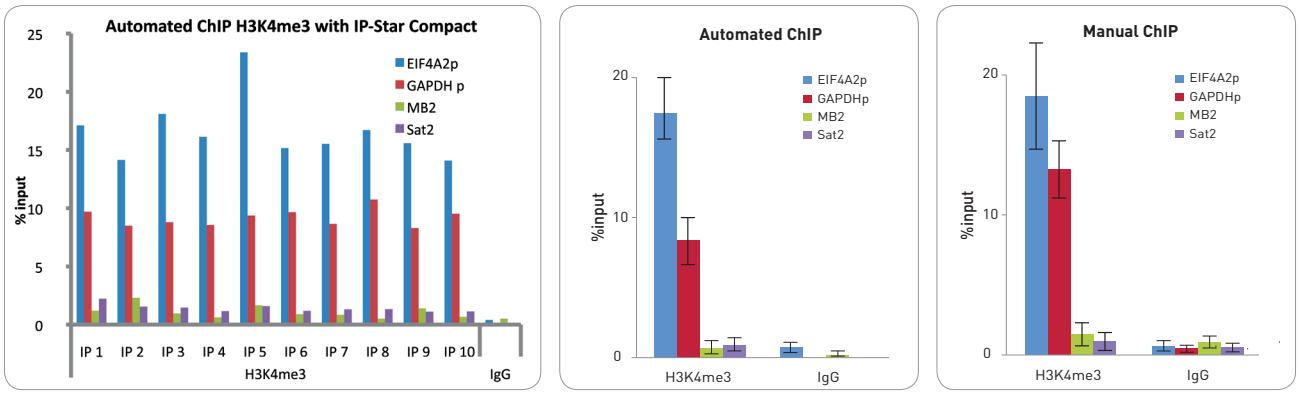

Figure 4: Automated ChIP assay on 10,000 cells.

ChIP assays were performed with the Diagenode antibody against H3K4me3 (0.25 µg/reaction) on the IP-Star® Compact. 0.25 µg of IgG was used as a control. The qPCR was performed with primers for the positive loci EIF4A2 promoter and GAPDH TSS and the negative loci Myoglobin exon2 and Sat2. The figure shows the recovery, expressed as a percent of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

The bioinformatics analyses after the sequencing show outstanding results from low amounts of starting material. The 30 pg dataset generates low background noise and highly reliable enrichments (peaks) which are confirmed by both the 300 pg dataset and the H3K4me3 dataset generated by the Broad Institute for the ENCODE project which was used as an external reference. The 300 pg dataset is also performed well and showed better results than the 30 pg dataset. One notable datapoint is the "Top40 overlap ratio", which refers to a standard method used in the ENCODE project in which two datasets are considered identical if at least 80% of the best 40% of the peaks ranked by significance score overlap. The 30 pg dataset fulfills these criteria for both the 300 pg dataset (considering all of its peaks, not just the best 40%) and the Broad data). The 300 pg dataset shows almost identical peaks to Broad with its 98% top40 overlap ratio. See Figure 5 for the matching peaks and comparative chart.

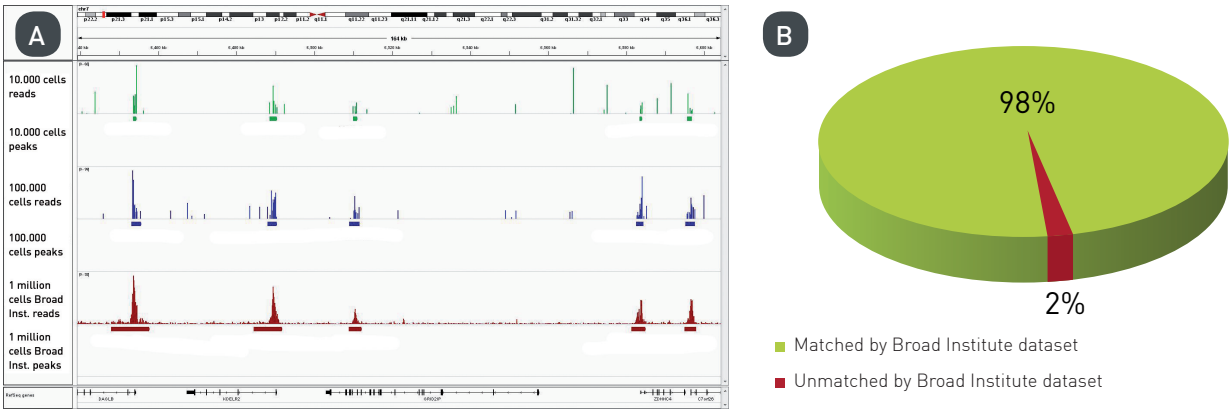

Figure 5A: Screenshot of the IGV Genome Browser comparing the peaks of different datasets.

The top line (green) is the 30 pg data, the middle one (blue) shows the 300 pg data, while the bottom line (red) is the Broad Institute data.

Figure 5B: Percentage of peaks from the 300 pg set confirmed by Broad Institute data

Conclusion

We demonstrated that with the True MicroChIP Kit and MicroPlex Library Preparation™ Kit, it is possible to get consistent, reproducible and reliable data from samples as low as 10,000 cells (30 pg).

- The True MicroChIP kit is the first kit on the market that allows successful ChIP-seq on such low amounts
- The two kits provide a unique solution to standardize ChIP-seq experiments with low starting amounts of cells, cell subpopulations, or biopsy samples
- The MicroPlex Library Preparation™ Kit was successful in the high fidelity amplification of low input amounts without introducing significant bias
- The two kits are optimal for automated ChIP and ChIP-seq applications and provide high quality results
- The True MicroChIP kit provides high quality ChIP-PCR results as shown by the results from several key epigenetic regions (both positive and negative controls)

Further information

For further details about applications, the kits, and other referenced products (sonicators, automated workstations, antibodies etc.), please contact a Diagenode sales representative in your area. You can also contact the Diagenode headquarters directly at custsupport@diagenode.com and techsupport@diagenode.com. Please also visit www.diagenode.com to find general information about our products, including manuals, applications, pricing, and more.