Reliable ChIP-seq results with the Diagenode True MicroChIP Kit and MicroPlex Library Preparation Kit on only 10,000 cells

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Abstract

Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) has become the gold standard for whole-genome mapping of protein-DNA interactions. However, conventional ChIP protocols require abundant amounts of starting material (at least hundreds of thousands of cells per immunoprecipitation) limiting the application for the ChIP technology to few cell samples.

The Diagenode True MicroChIP Kit has been developed with both an optimized protocol and reagents to enable successful ChIP on 10,000 cells. Moreover, the True MicroChIP kit protocol has been thoroughly optimized by Diagenode for ChIP followed by high-throughput sequencing on an Illumina® sequencer. In addition, to enable sequencing on the low amounts of DNA recovered after ChIP on 10,000 cells, Diagenode has developed a library preparation protocol for limited quantities of DNA. The MicroPlex Library Preparation Kit requires only picogram amounts of ChIP'd DNA inputs for library preparation compatible with the Illumina® platforms.

In this poster, we demontrated the successful use of the Diagenode True MicroChIP Kit in combination with the MicroPlex Library Preparation Kit for ChIP-seq on the Illumina® platform.

Introduction

The True MicroChIP Kit has been developed specifically for ChIP with limited cell numbers by optimizing several parameters; such as:

- Chromatin shearing
- Antibody concentration
- Use of different carriers
- Stringency of washes

The MicroPlex Library Preparation Kit is specifically designed for library preparation. It requires only 3 steps with a single purification. The kit requires only picogram amounts of DNA as starting material, allowing the sequencing of the low DNA amounts recovered after ChIP on low cell numbers.

Methods

ChIP-seq workflow

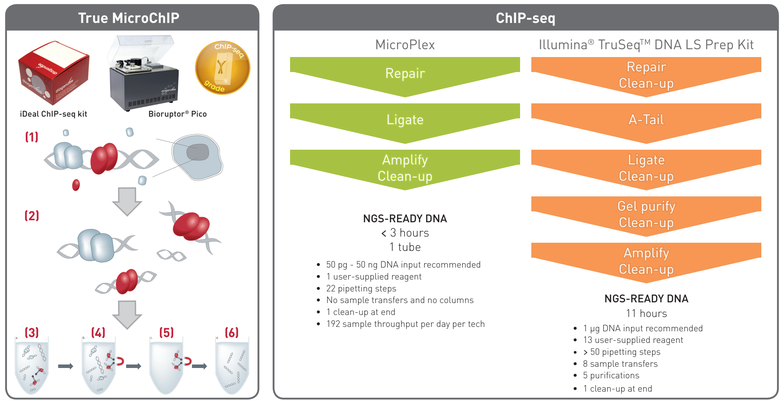
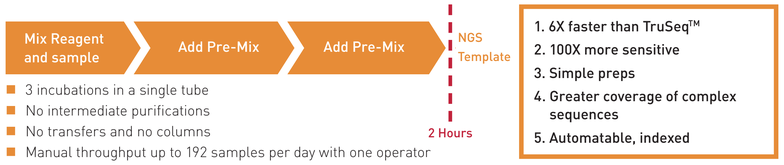


Figure 1

True MicroChIP procedure: 1. Cell fixation and DNA-protein cross-linking 2. Cell lysis and chromatin shearing using the Bioruptor® Pico 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: Comparison of the library preparation procedure using the MicroPlex kit and the TruSeq™ kit from Illumina®. The MicroPlex kit uses a 3-step protocol with a single purification at the end of the procedure. The Illumina® workflow for library preparation is composed of 5 steps with several purification procedures.

MicroPlex library preparation kit protocol overview



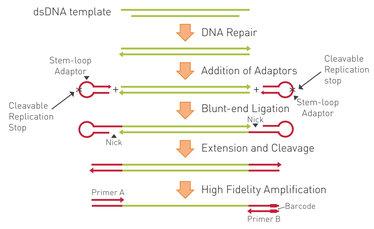


Figure 2. MicroPlex library preparation workflow.

50 pg to 50 ng of fragmented ds DNA are converted into sequencing-ready libraries for Illumina® NGS platforms using a fast, simple and sensitive 3-step protocol. After amplification, libraries are purified with AMPure XP beads. The purified libraries are ready for sequencing on an Illumina® platform. Indexing reagents included in the kit allow the multiplexing of 12 samples in a single sequencing lane.

Results

1. Efficient and easy chromatin shearing using Bioruptor® Pico and True MicroChIP kit Shearing Buffer

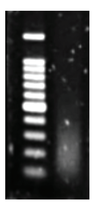


Figure 3. HeLa cells were fixed with 1% formaldehyde (for 10 minutes at RT). Cell lysis was performed using the Lysis Buffer of the Diagenode True MicroChIP kit. Samples corresponding to 10 000 cells were sheared during 5 rounds of 5 cycles of 30 seconds "ON" / 30 seconds "OFF" with the Bioruptor® Pico. Samples were vortexed before and after performing 5 sonication cycles, followed by a short centrifugation at 4°C. 10 µl of DNA (equivalent to 60,000 cells) were analysed on a 1.5% agarose gel.

2. Proven and reliable ChIP results with the True MicroChIP Kit using Diagenode's Premium antibodies

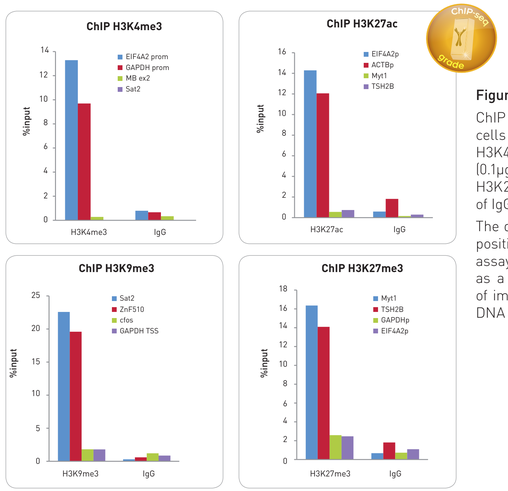


Figure 4. ChIP efficiency on 10,000 cells.

ChIP assays were performed on 10,000 Hela cells 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. Figures show the recovery, expressed as a percentage of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

3. True MicroChIP kit compatible with Automated System IP-Star® Compact

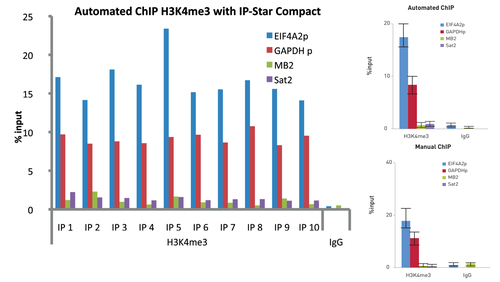


Figure 5. Automation of ChIP assay on 10,000 cells.

ChIP assays were performed on 10,000 Hela cells with the Diagenode antibody 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 exon 2 and Sat2. Figure shows the recovery, expressed as a percent of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

4. Library preparation on low amount of ChIP'd DNA with no pre-amplification step using the MicroPlex kit

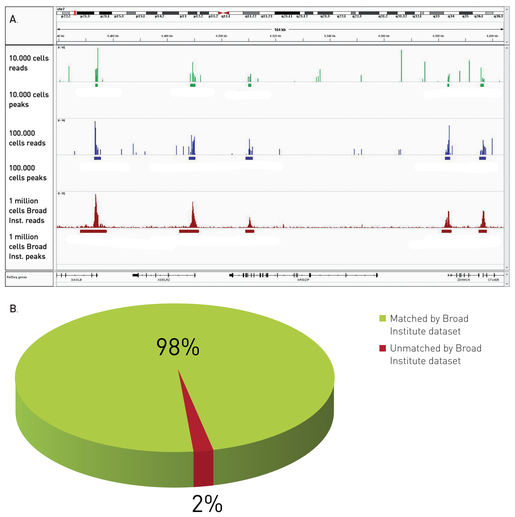


Figure 6. Library generation with the MicroPlex kit.

ChIP assays were performed on 10,000 and 100,000 HeLa cells with Diagenode H3K4me3 antibody (0.25 µg/reaction). Libraries were made with the MicroPlex Library Preparation Kit. The generated libraries were then analysed on an Illumina® HiSeq2000. Cluster generation and sequencing were performed according to the manufacturer's instructions.

A. The 36 bp tags were mapped to the human genome with the ELAND aligner. During the subsequent peak calling by SICER the enrichments from low cell numbers could be identified with as much confidence as from millions of cells.

B: The datasets were analyzed and compared with each other and to the reference data generated by the Broad Institute. We proved that our low cell samples are consistent and have very high similarity, and even the 30 pg sample fulfils the Encode criteria (min. 80% of the top 40% of the peaks should overlap.)

Conclusions

True MicroChIP Kit:

- The first and unique ChIP kit for very low starting cell numbers
- Optimized for an automated format
- Fully validated using ChIP-qPCR on multiple key epigenetic marks
- ChIP-seq validation using ChIP'd DNA from as low as 10,000 cells with the MicroPlex kit without pre-amplification

MicroPlex Library Preparation Kit:

- Fast and simplified protocol for NGS library preparation
- Efficient library preparation on picogram amount of DNA without pre-amplification
- Multiplexing capacity of up to 12 samples using standard Illumina® index tags
- Compatible with all Illumina® sequencing platforms

RUBICON GENOMICS *breaking the sample barrier*

MicroPlex Library Preparation kit x12 contains ThruPLEX technology developed and manufactured by Rubicon Genomics, Inc.,Ann Arbor, Michigan, USA and covered by US Patent 7,803,550; EP1924704; and US and international patents pending.

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