Chromatin immunoprecipitation (ChIP) is a powerful technique used to study protein-DNA interactions. It involves the cross-linking of proteins to DNA, followed by isolation of the DNA bound to the proteins, and analysis of the bound DNA by sequencing. This method 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 cell type to few cells.

3 incubations in a single tube

ChIP-sequencing procedure:

1. Efficient and easy chromatin shearing using Bioruptor
2. True MicroChIP kit compatible with Automated System IP-Star® Compact
3. True MicroChIP kit on only 10,000 cells
4. High similarity, and even the 30 pg sample fulfils the Encode criteria (min. 80% of the top 40% of the peaks should overlap)

Conclusions

Methods

ChIP-seq workflow


MicroPlex Library Preparation Kit

The MicroPlex Library Preparation Kit is specifically designed for library preparation. It requires only several parameters; such as:

- Efficient and easy chromatin shearing using Bioruptor
- Proven and reliable ChIP results with the True MicroChIP Kit using Diagenode’s Premium
- True MicroChIP Kit: Efficient and easy chromatin shearing using Bioruptor® Pico
- True MicroChIP kit compatible with Automated System IP-Star® Compact

Figure 2. MicroPlex library preparation workflow. 30.50 pg of fragmented dsDNA was converted into sequencing-ready libraries for Illumina® NGS platforms using a fast, single 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. In this poster, we demonstrated the successful use of the Diagenode True MicroChIP Kit in combination with the MicroPlex Library Preparation Kit for ChIP on the Illumina® platform.

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 sonicated during 4 rounds of 5 cycles of 30 seconds, ‘ON’ /30 seconds ‘OFF’ with the Bioruptor® Pico. Samples were sonicated 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.

Figure 4. ChIP efficiency on 10,000 cells. ChIP assays were performed on 10,000 Hela cells with several Diagenode antibodies: H3K4me3 (0.25 μg/reaction), H3K27ac (0.5 μg/reaction), H3K9me3 (0.5 μg/reaction) and H3K27me3 (0.5 μg/reaction). Identical quantity of IgG was used as a control. Figure 5 shows the recovery, expressed as a percentage of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

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 8° Star Compact). 0.25 μg of IgG was used as a control. The qPCR was performed with primer to the TSH2B promoter and GAPDH 15S and the negative (no Myc-yield) seen 2 and 5′ΔΔCt. Figure 6 shows the recovery, expressed as a percentage of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

1. Efficient and easy chromatin shearing using Bioruptor® Pico and True MicroChIP kit Shearing Buffer
2. Proven and reliable ChIP results with the True MicroChIP kit using Diagenode’s Premium
3. True MicroChIP kit compatible with Automated System IP-Star® Compact

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 pg/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.

- The 36 bp tags were mapped to the human genome with the BLASTN aligner.
- During the subsequent peak calling by IDRER, the occurrences from low cell numbers could be identified with much more confidence as from millions of cells.

- 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:

- 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:

- Efficient library preparation on picogram amount of DNA without pre-amplification
- Multiplexing capacity of up to 2 samples using standard Illumina® index tags
- Compatible with all Illumina® sequencing platforms