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Best Workflow Practices for ChIP-seq Analysis with Small Samples

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Abstract:

Combined chromatin immunoprecipitation and next-generation sequencing (ChIP-seq) has become the gold standard to investigate genome-wide epigenetic profiles. However, ChIP from a limited amount of cells has been a challenge. Diagenode and Advanced Analytical Technologies, Inc. (AATI) provide the first complete and robust workflow solution for successful ChIP-seq from small numbers of cells.

Diagenode's Bioruptor® Pico, True MicroChIP Kit and the MicroPlex Library Preparation™ Kit efficiently prepare chromatin from very low input amounts as these combined solutions:

- Allow reproducible shearing from low inputs of chromatin using the Bioruptor® Pico
- Assure quality with a validated ChIP protocol for histone marks down to 10,000 cells.
- Provide accurate sequencing library preparation from just picogram inputs

Quality control analyses are expedited with the Fragment Analyzer™ Automated CE System from AATI; a fast, high resolution parallel capillary electrophoresis (CE) instrument that provides accurate sizing and quantification down to 1,000 cells. The routine quality control checkpoints, after shearing of DNA and after library preparation, can be completed with limited number of cells on the Fragment Analyzer using the DNF-474 High Sensitivity NGS Fragment Analysis Kit, replacing high concentration dependent agarose gel electrophoresis. In this context, the Fragment Analyzer:

- Conserves the ChIP'ed sample for sequencing with superior quantification sensitivity
- Accurately sizes the sheared DNA smears through 6,000 bp with only 1,000 cells
- Automatically quantifies library samples
- Improves overall ChIP-seq quality control analysis

Introduction:

With advances in next-generation sequencing (NGS), the primary method for genome-wide mapping of protein-DNA interactions is chromatin immunoprecipitation with NGS detection (ChIP-seq) (Figure 1). ChIP-seq allows for the detection and characterization of transcription factor binding sites or patterns of histone modifications for the entire genome.

Traditionally, ChIP-seq required high amounts of starting material, usually millions of cells, making rare

or difficult cell types unsuitable for analysis. Diagenode has developed groundbreaking solutions for epigenetic studies on small sample sizes with the True MicroChIP Kit and the MicroPlex Library Preparation™ Kit enabling reliable analysis of protein-DNA interactions by ChIP-PCR or ChIP-seq with inputs as low as 10,000 cells(1).

Conventionally, ChIP-seq quality control checkpoints required agarose gel electrophoresis with a minimum input of 50,000 cells for reliable quality assessment. The Fragment Analyzer™ Automated CE System from

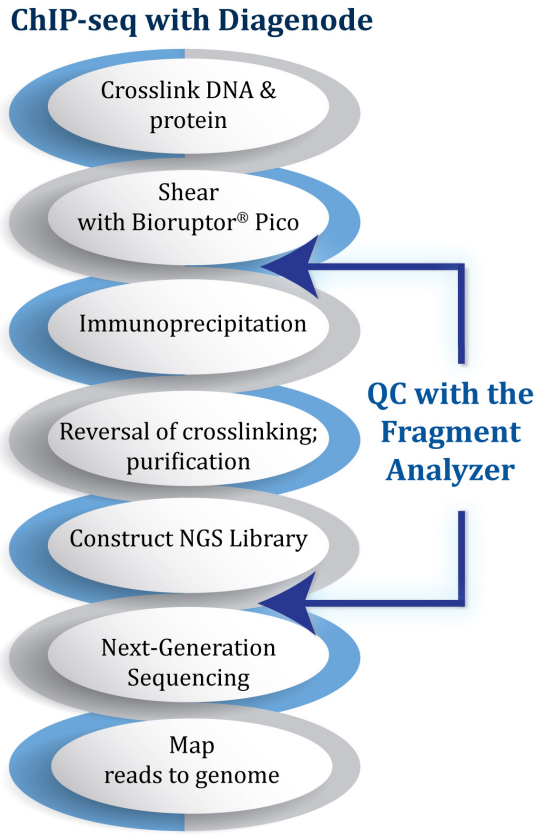

Figure 1- ChIP-seq workflow overview. Fragment Analyzer replaces agarose gel electrophoresis at critical quality control checkpoints.

Advanced Analytical Technologies, Inc. (AATI) offers quick assessment of chromatin quality, size and quantification with as little as 1,000 cells with the DNF-474 High Sensitivity NGS Fragment Kit (HS NGS Fragment Kit) (2,3).

The low sample input requirement makes it the perfect replacement for agarose gel electrophoresis. Diagenode's ChIP solutions in combination with Fragment Analyzer from AATI offer a complete solution to the ChIP-seq high cell input dilemma.

Method Overview:

Low Input Chromatin Shearing and Sizing

The first critical step of a successful ChIP experiment is the preparation of sheared chromatin with a suitable fragment size range which is representative of the biological scenario of interest. The Bioruptor® Pico from Diagenode uses ultrasound ACT (Adaptive Cavitation Technology) in a temperature-controlled environment to efficiently shear chromatin, providing high quality chromatin with preserved epitopes for ChIP-seq. Quality control checks prior to immunoprecipitation of the protein of interest are conventionally done with agarose gel electrophoresis, which requires a minimum of 50,000 cells for reliable visualization. This becomes a problem when working with a limited population of cells.

Chromatin from HeLa cells were crosslinked and sheared from 100 bp to 600 bp fragments using the Bioruptor® Pico for small volume samples. After reversal of cross-linking and purification, samples were separated using the Fragment Analyzer with the HS NGS Fragment Kit (Figure 2A&B) or agarose gel electrophoresis (Figure 2C).

The Fragment Analyzer offers highly accurate and sensitive analysis of chromatin shearing with as little as 1,000 cells. *PROSize*® Data Analysis Software automatically provided a smear size and concentration while also offering a feature to overlay sample profiles for easy visualization of the targeted DNA (Figure 2A). The electropherogram overlay displayed that all the smears had a similar shape regardless of the initial cell count. This confirms consistent chromatin shearing among samples from different cell counts with the Bioruptor® Pico from Diagenode. Chromatin shearing by agarose gel electrophoresis was best visualized with a sample from 50,000 cells.

With confident analysis over a broad dynamic concentration range, the Fragment Analyzer can be used in place of agarose gel electrophoresis not only for small 1,000 cell sample size, but also for larger 50,000 cell sample sizes. Sheared chromatin quality control checks with the Fragment Analyzer offers analysis over a wide range of sample sizes, while reporting distribution profiles and accurate sizing results important for successful chromatin immunoprecipitation.

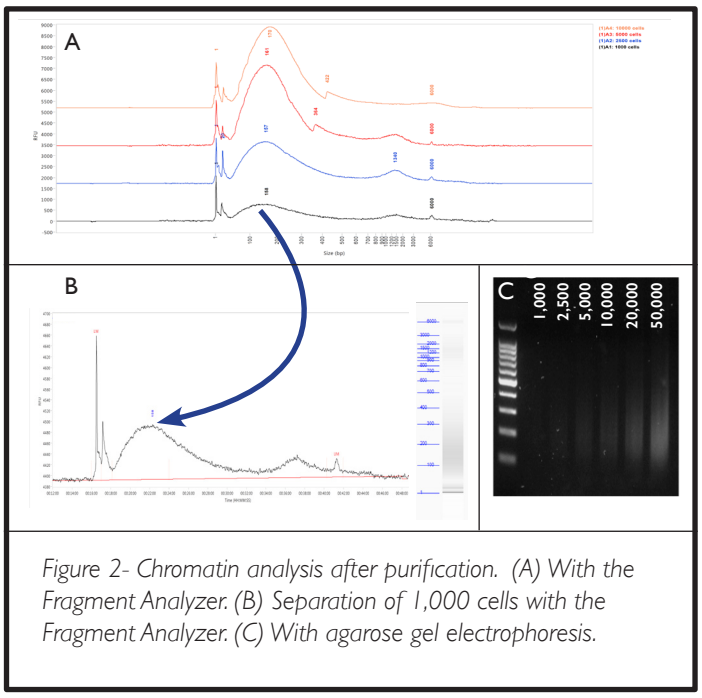

Figure 2- Chromatin analysis after purification. (A) With the Fragment Analyzer. (B) Separation of 1,000 cells with the Fragment Analyzer. (C) With agarose gel electrophoresis.

Reliable Chromatin Immunoprecipitation and Library Preparation from 10,000 Cells and Pre-Sequencing Quality Control Check

The True MicroChIP Kit allows performing a high efficiency ChIP on as few as 10,000 cells.

The successful immunoprecipitation obtained with H3K4me3, H3K27me3, H3K9me3, and H3K27me3 antibodies was confirmed by qPCR which shows good enrichments in positive control regions and negligible signal in negative control regions. In ChIP, checking for enrichment is crucial before proceeding with library preparation. Please see https://www.diagenode.com/img/documents/application-notes/AATI_Diagenode_qPCR.jpg to view the enrichment data from this assay.

NGS-ready libraries were constructed with 30 pg of immunoprecipitated DNA using the MicroPlex Library Preparation Kit. Thus, the True MicroChIP Kit used in conjunction with the MicroPlex Library Preparation™ Kit and premium grade antibodies enable ChIP-seq with just a few picograms of input.

Prior to sequencing, it is necessary to check the quality of the NGS library to ensure successful results. The Fragment Analyzer™ Automated CE System offers quick analysis of the integrity, molarity, and size of the DNA smear before sequencing. After library amplification, the samples were separated on the Fragment Analyzer to confirm distribution profile and sizing results (Figure 3). The Fragment Analyzer offers fast separation, automatic sizing, and quantification of low concentration samples, confirming the quality of a library for sequencing.

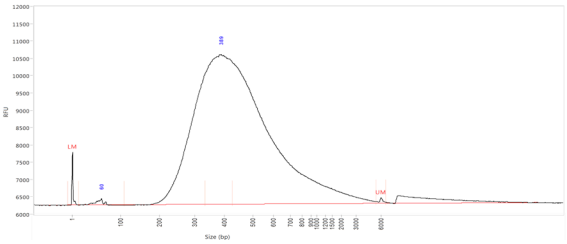

Figure 3- Quality control check of a ChIP-seq library on the Fragment Analyzer. High Efficiency ChIP performed on 10,000 cells.

Bioinformatics Analysis

Bioinformatics analysis after sequencing displayed excellent results from low amounts of starting material. Comparison of 30 pg of DNA extracted from 10,000 cells and 300 pg of DNA extracted from a higher cell number was performed.

The 30 pg dataset generated low background noise and highly reliable enrichments (peaks), which were confirmed by both the 300 pg dataset and the H3K4me3 dataset generated by the Broad Institute for the ENCODE project (Figure 4). The 30 pg dataset also fulfilled the criteria for the Top40 overlap ratio(1). Bioinformatics analysis confirms that Diagenode and AATI have provided a successful pathway for small input sample analysis with ChIP-seq.

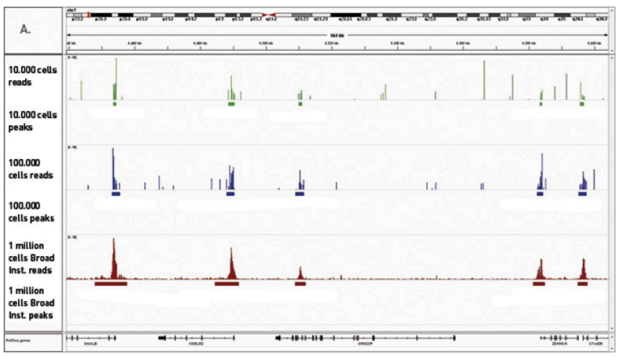

Figure 4- Screenshot of the IGV Genome Browser comparing the peaks of different datasets. The top line (green) is the 30 pg dataset, the middle line (blue) shows the 300 pg dataset, and the bottom line (red) is the Broad Institute data. Both the 30 pg and 300 pg fulfill the Top40 overlap ratio criteria.

Summary

Diagenode and AATI are the first to provide a reliable and comprehensive workflow for small sample analysis with Chip-seq. A combination of Diagenode's True MicroChIP Kit and the MicroPlex Library Preparation Kit and AATI's Fragment Analyzer™ Automated CE System with the HS NGS Fragment Kit enables reliable detection of protein-DNA interactions by ChIP-seq with inputs as low as 10,000 cells and quality control checks from as little as 1,000 cells, thus advancing epigenetics research.

References

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