TECHNOTE

Use of PIXUL® Multi-Sample Sonicator with Agilent Automated Electrophoresis for ChIP-Seq

Targeted high-throughput epigenetics analysis using ChIP-Seq is facilitated by combining PIXUL® Multi-Sample Sonicator from Active Motif with the Agilent Automated Electrophoresis Portfolio

Introduction

Epigenetics answers significant questions regarding personalized medicine and a variety of diseases. Several challenges to epigenetics research include low sample quantity and sample preparation. Traditional epigenetic methodologies generally require large amounts of sample, which can be difficult to obtain from patients, and workflows involve several sample transfer steps resulting in sample loss. Additionally, consistent, quick, efficient, robust, and cost-effective preparation of high-throughput samples and their downstream analysis continue to pose significant challenges. To address some of the challenges associated with sample preparation, the PIXUL® Multi-Sample Sonicator from Active Motif can be combined with the Agilent automated electrophoresis systems, like the Fragment Analyzer and the TapeStation (Figure 1). The PIXUL provides rapid and consistent sample fragmentation through sonication, while the Agilent systems provide accurate sizing and quality metrics of the fragments as crucial aspects of next-generation sequencing workflows.

In a single run, PIXUL offers the flexibility to process 1 to 96 samples in a 96-well plate where 12 columns of that plate can be individually programmed. Its setup and run times are quick, and output is consistent. Both the Fragment Analyzer and TapeStation systems can be used for reliable evaluation of the sonicated material and the final NGS library, providing accurate sizing which is a crucial quality control step. If the analysis

indicates that the size distribution of the sonicated fragments needs to be altered, then the flexibility of the PIXUL makes the optimization process quick and convenient to identify best sonication parameters for a specific assay. The versatility of the PIXUL and the automated electrophoresis systems makes them adaptable to a variety of workflows across multiple omics realms.

In epigenetics, chromatin immunoprecipitation and sequencing (ChIP-Seq) is a popular method which provides genome-wide locations of interaction between a specific protein or histone modification and chromatin/DNA. ChIP-Seq is widely used in various fields of biological and biomedical research. Being an important, laborious, and expensive method, ChIP-Seq needs crucial quality control in its workflow. Hence, this Technical Note focuses on the combination of PIXUL with Agilent's Fragment Analyzer and TapeStation for crucial quality control in ChIP-Seq library preparation.

ChIP-Seq Workflow

ChIP-Seq maps genome-wide localization of a protein or histone modification of interest. Hence, crosslinking is performed for immobilization of chromatin-interacting proteins on DNA. Since the actual length of chromatin/DNA strands are not conducive to the ChIP-Seq workflow, they need to be fragmented down to compatible sizes. Hence, crosslinked samples undergo sonication to generate fragments around 300 base pairs, ideal for ChIP-Seq.

Figure 1: From left – PIXUL®, Agilent 4200 TapeStation system, and Agilent 5300 Fragment Analyzer system

Next, the fragments undergo immunoprecipitation (IP) using an antibody against the protein of interest which has been initially crosslinked to chromatin/DNA. An aliquot of fragmented chromatin is not subject to IP and serves as an input control. The DNA which is obtained by IP and the input DNA are purified, amplified, and sequenced. The sequencing results from the immunoprecipitated DNA and input control are compared to determine the success of ChIP-Seq and to interpret the results. An outline of the ChIP-Seq workflow is presented in Figure 2.

Two crucial steps in ChIP-Seq are 1) DNA sonication for generating consistent fragment sizes, and 2) antibody specificity. While several solutions regarding antibody improvement are commercially available, the critical criterion of generating consistent fragment sizes is not well-explored. Inconsistent and improper sonication causes

ChIP-Seq to either fail or to provide incorrect and misleading information. Issues with this crucial step in the ChIP-Seq workflow are easily resolved with the PIXUL sonicator from Active Motif, which consistently fragments chromatin/DNA down to sizes that are ideal for ChIP-Seq. Following sonication, the Agilent automated electrophoresis systems enable precise analysis of fragment sizes, a pivotal quality control step that helps to decide the course of the workflow. If the fragments are of the appropriate size, the sample proceeds through library preparation as shown in Figure 2. The Agilent automated electrophoresis systems can be utilized a second time in the ChIP-Seq workflow for quality control analysis of the final library prior to sequencing. The combination of the PIXUL and the automated electrophoresis instruments helps ensure the generation of consistent fragment sizes and successful ChIP-Seq results.
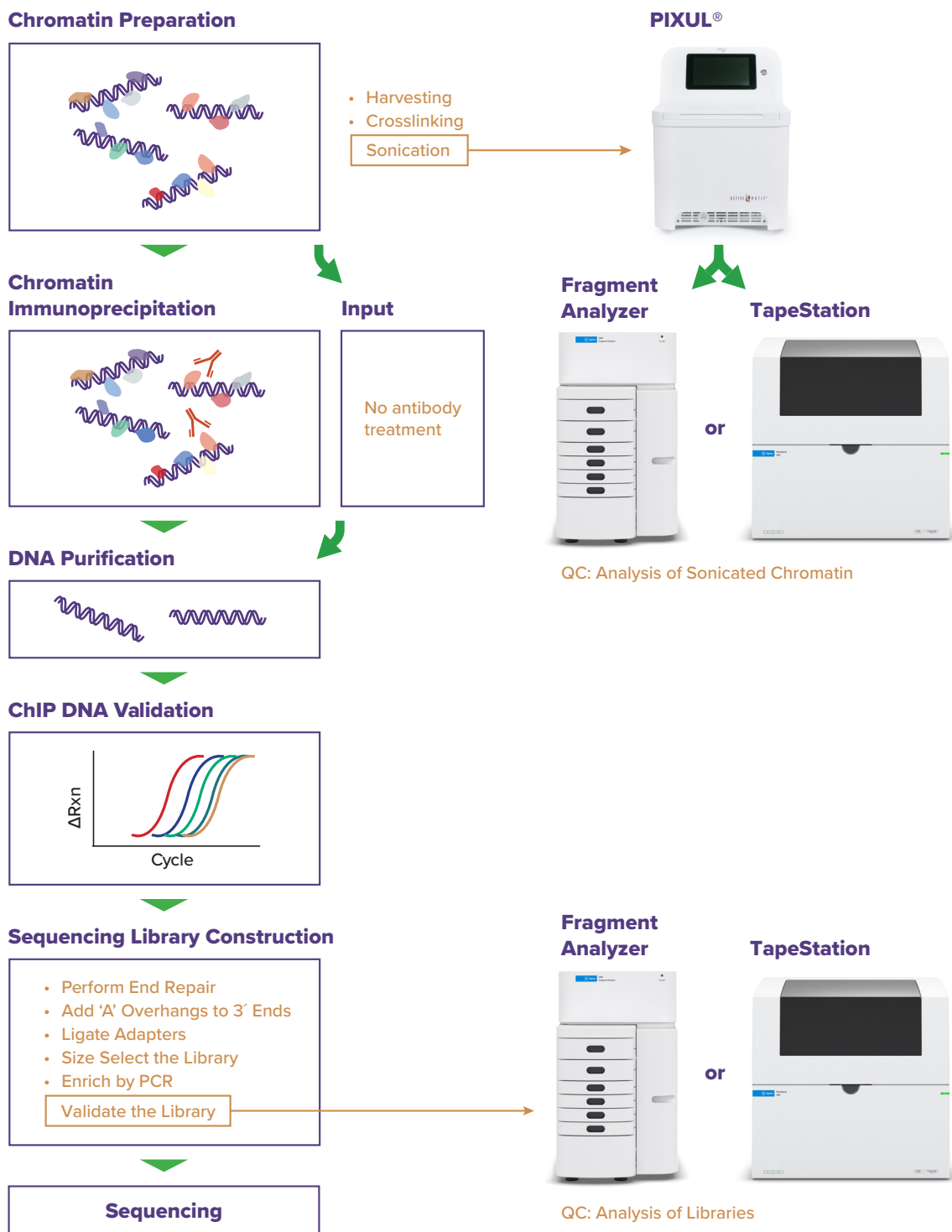
Use of PIXUL® Multi-Sample Sonicator with Agilent Automated Electrophoresis for ChIP-Seq

ACTIVE MOTIF

Chromatin Preparation

- Harvesting
- Crosslinking
- Sonication →

PIXUL®

Chromatin Immunoprecipitation

Input

No antibody treatment

Fragment Analyzer   **TapeStation**

 or

QC: Analysis of Sonicated Chromatin

DNA Purification

ChIP DNA Validation

ΔRxn

Cycle

Sequencing Library Construction

- Perform End Repair
- Add 'A' Overhangs to 3´ Ends
- Ligate Adapters
- Size Select the Library
- Enrich by PCR

Validate the Library →

Fragment Analyzer   **TapeStation**

 or

QC: Analysis of Libraries

Sequencing

Figure 2: ChIP-Seq workflow, indicating the steps where the PIXUL®, the Agilent 5300 Fragment Analyzer system, and the Agilent 4200 TapeStation system are used. PIXUL is used for sonication of chromatin into fragments whose sizes are compatible with ChIP-Seq. The Agilent equipment are used to quantify the sizes of the sonicated fragments and later for ChIP-Seq library quality control analysis.

Materials and Methods

To sonicate samples for ChIP-Seq using PIXUL, crosslinked chromatin is resuspended in lysis buffer and added in each well of a 96-well round bottom plate, which is then sealed carefully (Active Motif Catalog No. 53139 or Corning Catalog No. 3799). The sealed plate is loaded into PIXUL as per instructions in the PIXUL manual. The PIXUL touchscreen is operated for the next set of actions.

On the PIXUL touchscreen, Circulation is turned on to maintain low temperature during fragmentation, and to remove any bubbles which may appear underneath the external surface of the 96-well plate upon loading it into PIXUL. The touchscreen is then used to set the sonication program for 1 to 12 columns of the 96-well plate and to Start sonication. For DNA fragmentation the following simple parameters are used – Pulse (N): 50, Pulse Rate Frequency: 1 kHz, Burst Rate: 20 Hz, Process Time: optimized as needed. Information on PIXUL protocols for various applications can be found here. After sonication, the 96-well plate is removed from PIXUL and centrifuged for ~10 seconds to collect all fluids down to the bottom of wells.

The size of the sonicated DNA is assessed with the Fragment Analyzer using the qualitative dsDNA 930 Reagent kit (Agilent p/n DNF-930-K0500) according to the manufacturer's instructions. This is one of the most crucial quality control steps in the ChIP-Seq workflow. The fragmented DNA is then subjected to the subsequent steps of the ChIP-Seq workflow as shown in Figure 2. When the libraries are prepared, the Fragment Analyzer is used again for quality control analysis. Alternately, quality control can also be performed using the Agilent TapeStation (Figure 2).

Results

The size of fragmented DNA/chromatin undergoing immunoprecipitation is crucial to successful ChIP-Seq workflows. To test whether PIXUL sonication fragments the DNA into sizes ideal for ChIP-Seq, an accurate method of analysis is implemented using either the Fragment Analyzer (Figure 3) or TapeStation (Figure 4). This analysis helps researchers make the critical decisions about whether the fragments qualify for downstream processing, such as immunoprecipitation, library preparation, and sequencing.

Shown in this application note are examples of the quality control steps in the ChIP-Seq workflow where Agilent systems are implemented. As shown in Figure 2, the first QC step is analysis of the sonicated chromatin. Example of the results achieved at this QC step can be seen in Figures 3 and 4. Analysis with the Agilent automated electrophoresis systems presents the results as an electropherogram or a digital gel. In this example, unsonicated and PIXUL-sonicated chromatin which is purified from human brain samples are compared on the Fragment Analyzer (Figure 3). Compared to the sonicated DNA, the unsonicated sample

is characterized by the absence of lower molecular weight DNA in the gel lane. In contrast, the PIXUL sonicated sample yields fragments around 300 bp, the ideal size range for the ChIP-Seq workflow. Figure 4 shows a similar analysis of mouse brain using the TapeStation, which also provides accurate size analysis of sonicated DNA (Y) and very definitive contrast to the unsonicated control (N).

Once the size of the sonicated sample has been confirmed, the ChIP-Seq workflow continues through library preparation as depicted in Figure 2. The sonicated samples shown in Figures 3 and 4, were thus used for immunoprecipitation of the sonicated chromatin from human and mouse brain using an antibody against histone H3 lysine 9 acetylation (H3K9ac). ChIP-Seq libraries were prepared and analyzed on the Agilent automated electrophoresis systems. Representative examples of the input control and the ChIP-Seq libraries generated from the immunoprecipitated DNA are shown on the TapeStation (Figure 5).
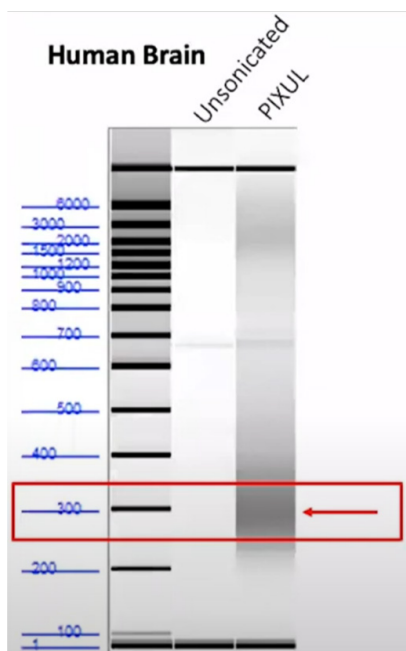

Figure 3: PIXUL-sonicated and unsonicated chromatin purified from an input prep using human brain sample, analyzed on the Agilent 5300 Fragment Analyzer system.
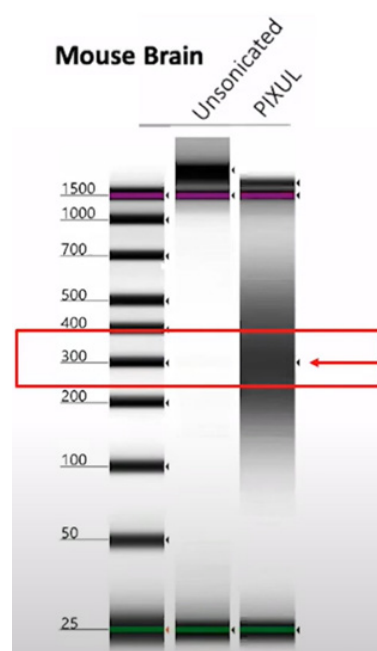

Figure 4: PIXUL-sonicated (Y) and unsonicated (N) chromatin purified from an input prep using mouse brain sample, analyzed on the Agilent 4200 TapeStation system.
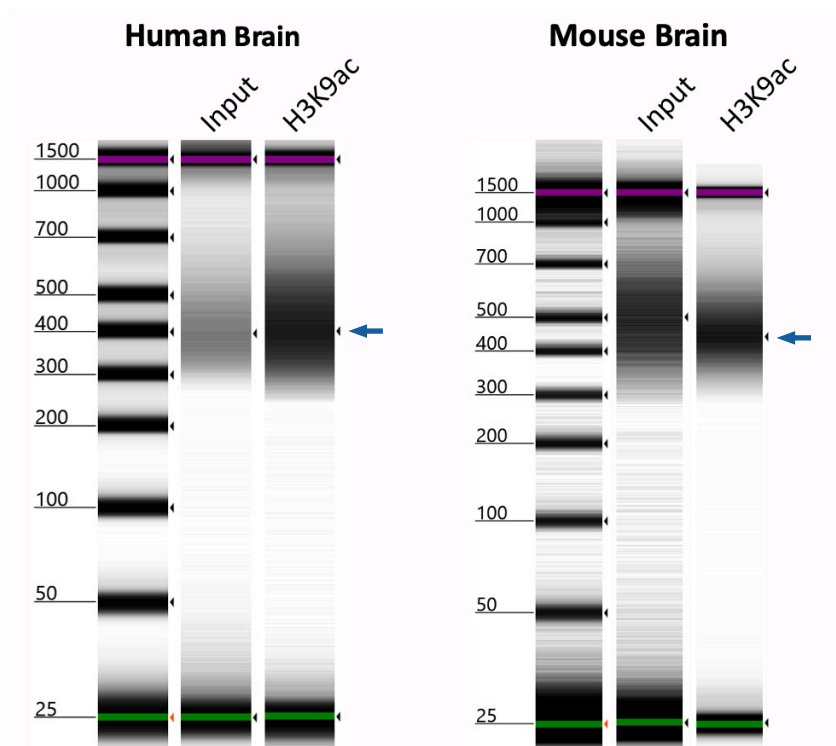
Figure 5: ChIP-Seq libraries of immunoprecipitated (IP) DNA and corresponding input DNA from human and mouse brain samples, analyzed on the Agilent 4200 TapeStation system. IP using antibody against histone H3 lysine 9 acetylation (H3K9ac). Sizes (blue arrows) are higher than Figures 3 and 4 because these fragments are ligated with sequencing adapters during library preparation.

Conclusion

The combination of PIXUL sonication and quality control analysis with the Agilent automated electrophoresis portfolio, *i.e.,* the Fragment Analyzer and TapeStation, throughout the ChIP-Seq workflow significantly contributes to its success. PIXUL provides consistent sizes of chromatin fragments which is a necessity for ChIP-Seq. The automated electrophoresis systems enable quality control at two key steps of size analysis in the workflow: the sheared DNA and the final library. Together, the use of these systems leads to high quality library preparation with very consistent size distribution, which are ideal for ChIP-Seq. Further details on the advantages to using the Agilent Fragment Analyzer can be found here. Likewise, the TapeStation has several advantages which are found here. Overall, the PIXUL and Agilent automated electrophoresis systems provide important solutions towards successful execution of complex, laborious, and expensive methods like ChIP-Seq.

Agilent PR7001-1524

by
Rwik Sen, Ph.D. and Dana Meents, M.S.