TECHNOTE

ACTIVE MOTIF®
Enabling Epigenetics Research

PIXUL® Sonicator and Agilent Electrophoresis Systems for Chromosome Configuration Analysis

Advantageous combination of PIXUL® Multi-Sample Sonicator from Active Motif with Automated Electrophoresis systems from Agilent Technologies to study chromosome configuration using Hi-C

Introduction

Knowledge of epigenetics helps to advance therapeutic innovation and personalized medicine as a regulator of health, development, and disease. An important contributor to epigenetic regulations is the effect of long-range chromosomal interactions between distant genomic loci that regulate 3-dimensional (3D) configuration of chromatin, gene expression, and the participation of associated transcription factors. Long-range chromosomal interactions are dynamic, and changes can occur during developmental stages, disease conditions, drug treatment, and other physiological events, thereby impacting epigenetic regulations. These long-range interactions need to be studied because of their tremendous biological significance. The Hi-C assay is an efficient method for unbiased detection of chromosomal interactions and their 3D conformation. The data from Hi-C analysis provides significant insights into understanding gene regulatory networks and predicting transcription factor activity.

Like most epigenetic methods, the Hi-C workflow requires significant time, labor, and expenditure. As this assay has become more well-known, new tools have been developed to address workflow difficulties. Two major challenges to a successful Hi-C workflow are to obtain optimum DNA fragmentation within a precise range, and to obtain good quality of the final Hi-C library. The first challenge arises from inconsistencies in existing methods of DNA fragmentation, often due to inefficient methods, incompatibility with high-throughput platforms and high costs. Inconsistency in sizes of DNA fragments is a major contributor to the second challenge, obtaining a high-quality final Hi-C library. A poor-quality library leads to experimental failure or sub-optimal results and lost resources.

The first challenge of consistent DNA fragmentation is successfully addressed by the PIXUL® Multi-Sample Sonicator from Active Motif, which offers consistent, user-friendly, fast, and high-throughput sonication of 96-samples using an inexpensive microplate. Successful fragmentation of the samples should be confirmed by the Agilent automated electrophoresis systems at this step to ensure samples are within an optimal size range to further proceed with the Hi-C workflow.

The second challenge of obtaining high quality NGS (next-generation sequencing) libraries is aided by again using the Agilent automated electrophoresis systems for quality control, allowing for the identification of libraries that are high quality for sequencing or that may need further optimization. The Agilent systems perform accurate sizing and quantification of up to 96 samples at a time using only 1-2 µL per sample in a single run, while still maintaining a streamlined user-friendly workflow. By providing reliable DNA fragmentation and quality control, the combination of these technologies in the Hi-C workflow alleviates major challenges and contributes to overall experimental success.

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

Experimental Setup for PIXUL and Agilent Automated Electrophoresis Systems

Active Motif uses the Hi-C portfolio from Arima Genomics. The Hi-C workflow performed at Active Motif is schematically represented in Figure 2. First, samples are collected in the form of cells or tissue. Next, chromatin in the samples is crosslinked and fragmented, and the ends are repaired and biotinylated. The DNA is then ligated at proximal regions of genomic interactions as shown in step 5 of Figure 2. Ligated DNA is subjected to sonication where 100 µL of sample is loaded into each well of a 96-well round bottom plate (Corning 3799) which is sealed and loaded onto PIXUL® where the following sonication program is set – Pulse (N): 50, Pulse Rate Frequency: 1 kHz, Burst Rate: 20 Hz, Process Time 40 minutes. The program can be optimized as needed, with recommendations available upon request.

After sonication in step 6 of Figure 2, the 96-well plate is removed from PIXUL®, and centrifuged for ~10 seconds at 500 rpm for collecting all content into the wells. Next, the size of the fragmented DNA is confirmed by a quality control (step 7, Figure 2) using one of the Agilent automated electrophoresis systems, either the TapeStation or the Fragment Analyzer, and the associated kits. Both systems have multiple reagents and kits for nucleic acid analysis covering expansive

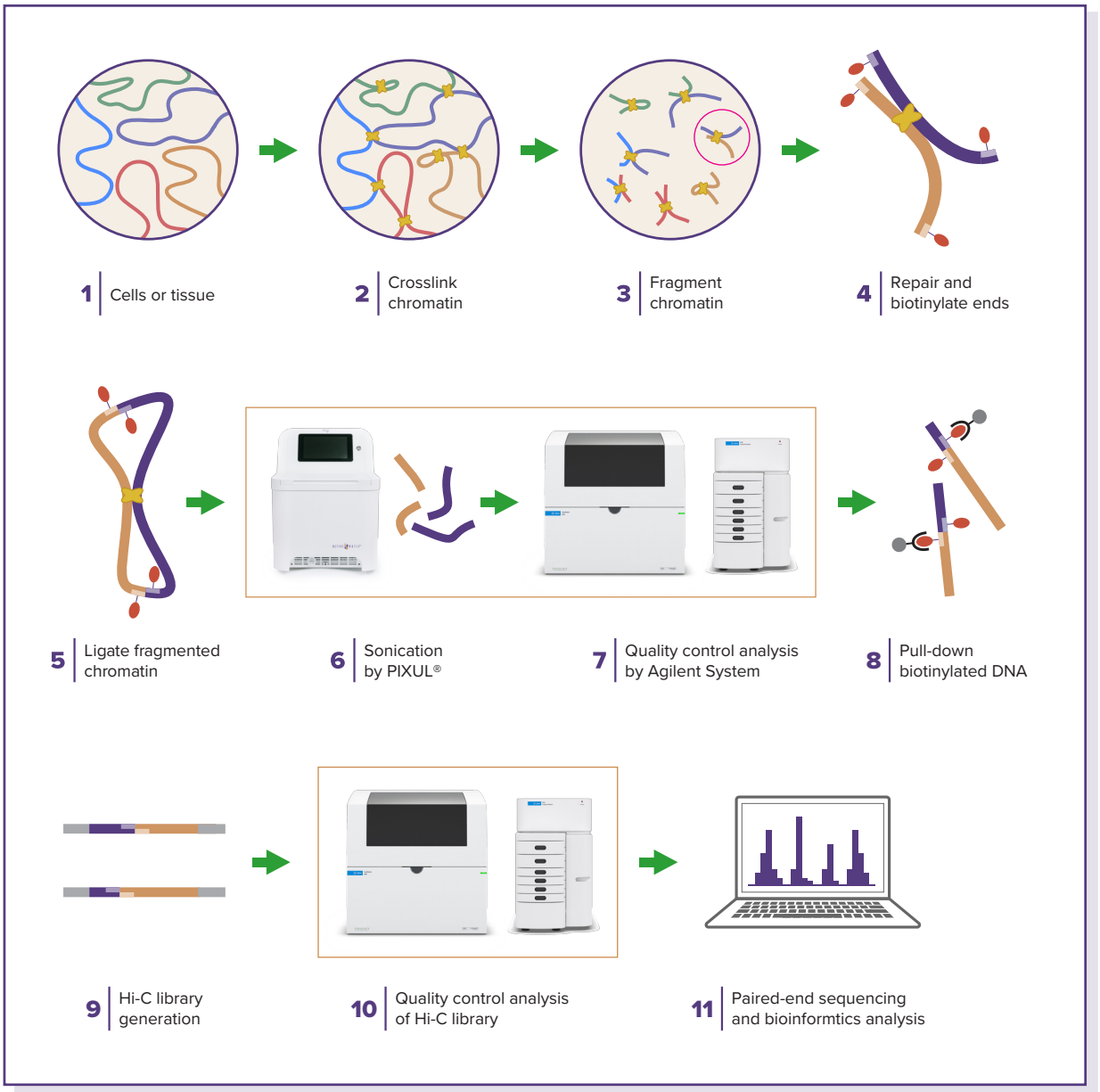


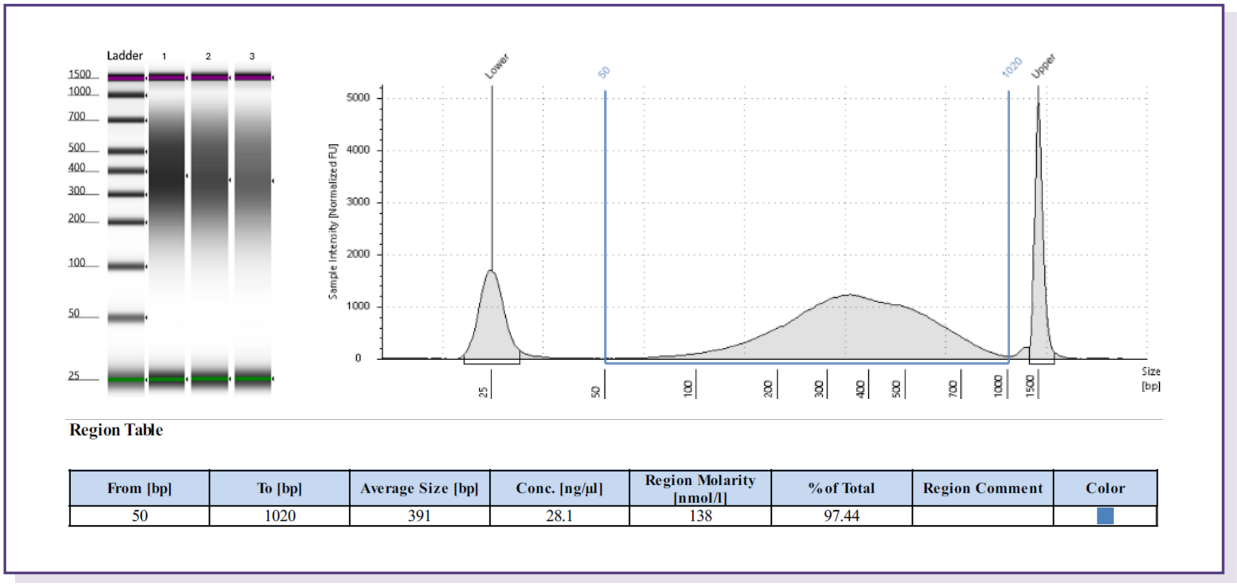
Figure 2. Active Motif Hi-C service workflow. Sonication by the PIXUL® is performed in step 6. The Agilent automated electrophoresis systems, either the TapeStation or the Fragment Analyzer, are used for quality control of the fragmented sample and of the final Hi-C library, as shown in steps 7 and 10, respectively.

sizing and concentration ranges. The sample quality is considered acceptable when the output appears as the smear shown in Figure 3 with the largest portion of the smear around 400 base pairs. The experiment will then proceed to immunoprecipitation and Hi-C library preparation (steps 8 and 9, Figure 2).

The final Hi-C libraries undergo quality control using the Agilent TapeStation or Fragment Analyzer (step 10, Figure 2) for fast and accurate analysis of sample size distribution, quantification, and purity. This quality control check is crucial to determining whether the libraries can proceed to sequencing and bioinformatics analysis (step 11, Figure 2).

Results

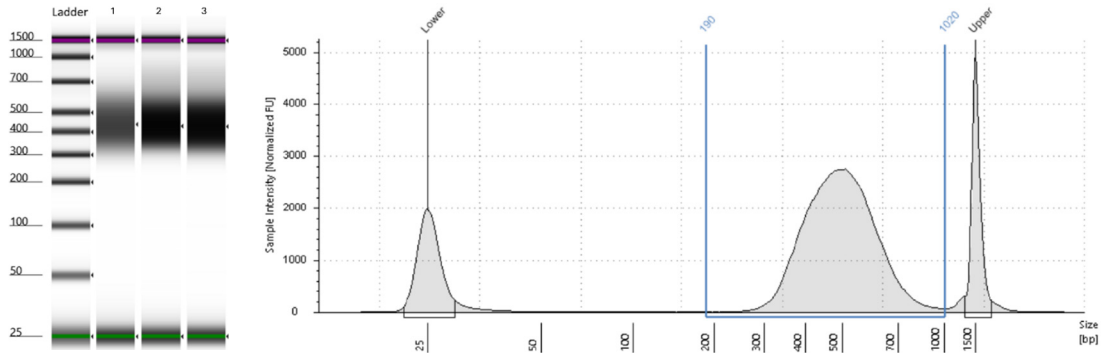
Prior to immunoprecipitation, DNA fragments sonicated by PIXUL® are analyzed using Agilent automated electrophoresis systems for accurate quantification and sizing assessment as a quality control step. Figure 3 represents an example of the analysis of PIXUL®-sonicated samples using the TapeStation. The 3 lanes of the electronic gel in Figure 3 show the sizes of DNA fragments from 3 samples sonicated by PIXUL®. TapeStation data can be presented as either a digital gel image, or as individual electropherograms. Additionally, a data table shows information such as the concentration and average size of each sample. The example shown in Figure 3 demonstrates the fragmented pattern expected for PIXUL sonicated samples in the Hi-C workflow. Accurate analysis of the samples by TapeStation confirms that the samples have sufficiently met Active Motif's DNA fragment size and consistency standards as shown in Figure 3 where the largest portion of the smear appears around 400 base pairs. Samples which pass the crucial quality control steps using the Agilent systems proceed to immunoprecipitation, followed by library preparation.



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	%of Total	Region Comment	Color
50	1020	391	28.1	138	97.44		

Figure 3. Agilent TapeStation analysis after PIXUL® sonication of 3 samples, prior to pull-down of biotinylated DNA. The electropherogram and region table correspond to the sample in lane 1 of digital gel. Blue lines on electropherogram indicate region analysis settings. Region table shows average size, concentration, and percent total of portion of sample within the user-specified region.



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
190	1020	509	31.7	100	95.89		

Figure 4. Agilent TapeStation analysis of Hi-C libraries. The electropherogram and region table correspond to the sample in lane 1 of the digital gel image.

The Agilent automated electrophoresis systems can then be utilized a second time in the Hi-C workflow for quality control of the final NGS library prior to sequencing. Figure 4 shows TapeStation analysis of final Hi-C libraries. The 3 lanes of the electronic gel in Figure 4 are the final Hi-C libraries generated from the same 3 samples represented in Figure 3. Hi-C libraries with size distributions and patterns similar to Figure 4, qualify as passing. Libraries which do not pass quality control should not proceed to sequencing and risk a failed experiment or incorrect interpretation of results, along with loss of precious resources invested in the workflow. The Agilent automated electrophoresis systems allow the user, in this case Active Motif, to make crucial decisions regarding the course of the experiment.

Figures 3 and 4 provide examples of the data assessment provided by TapeStation systems in the Hi-C workflow. Alternately, the Agilent Fragment Analyzer can also be used to examine fragmented DNA and Hi-C libraries using the ProSize data analysis software (data not shown).

Conclusion

Hi-C helps to detect epigenetic effectors like chromosomal conformation and 3-dimensional interactions between genomic loci which can be analyzed in healthy versus disease states, various stages of development, drug treatment, and other physiological events. As these scenarios are often studied within disease research and therapeutic development, Hi-C is a valuable assay within therapeutic and pharmaceutical industries.

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This application note demonstrates the importance and benefits of integrating the PIXUL® and Agilent automated electrophoresis systems into the Hi-C workflow. Sonication by PIXUL® provides consistent DNA fragmentation, which is necessary for downstream processing. The automated electrophoresis systems are integral to crucial quality control steps throughout, including confirming the size of the fragmented DNA sample and the quality of the Hi-C NGS library before sequencing. Both PIXUL® and Agilent systems are compatible with high-throughput workflows as they improve the simplicity and speed of the protocol with increasing accuracy and precision.

Additionally, the PIXUL and Agilent systems make optimization of the Hi-C workflow easier. This is because PIXUL® allows up to 12 different sonication programs to be assigned to the 12 columns of a 96-well microplate for simultaneous processing, making it possible to test for multiple sonication conditions in a single run. Afterwards, the high-throughput samples can be prepared in a simple manner and rapidly analyzed with the TapeStation. The Fragment Analyzer can also be used for precise analysis of high-throughput samples because it allows up to three 96-well plates to be loaded and analyzed without user intervention. Both Agilent systems require only 1-2 µL of sample, minimizing sample loss. Together, a combination of PIXUL® and Agilent automated electrophoresis systems significantly shorten sample optimization time and assures successful Hi-C sequencing.

Further details and advantages of TapeStation and Fragment Analyzer can be found here at agilent.com
TapeStation
Fragment Analyzer

For more information on the PIXUL® Multi-sample Sonicator visit activemotif.com/pixul
PIXUL®

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