TECHNOTE:

Sonicating Limited Tissue Samples with PIXUL™ Multi-Sample Sonicator for ChIP-Seq Analysis

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PIXUL & CHIP-SEQ WITH LIMITED AMOUNTS OF TISSUE SAMPLES

Introduction

Epigenetics has a huge potential in personalized medicine as it becomes evident that it plays a role in many diseases. However, preparing samples in a clinical setting can be challenging. With this TechNote we describe how to use the **PIXUL™ Multi-Sample Sonicator** to process tissue samples for analysis using ChIP-Seq.

Challenges of Sample Processing from Large Cohort Studies and Clinical Samples

Preparation of clinical samples for analysis can be challenging for several reasons. Limited amounts of tissue are available with clinical samples, which leads to a lack of sufficient material to answer relevant questions in this setting. Due to the nature of clinical samples, chromatin preparation is complicated.

Furthermore, traditional methods of tissue homogenization have limitations such as sample loss and low amounts of cells being available, which in turn yields a low amount of starting material that can make some experiments impossible. With such limitations from small amounts of starting material and difficult-to-process samples, reproducibility in data and consistency in experiments is more important than ever. Consistent shearing is necessary to generate reproducible data.

Solution: PIXUL™ Multi-Sample Sonicator

In 2020, Active Motif launched the PIXUL™ Multi-Sample Sonicator, which delivers consistent, high throughput shearing of cells, tissue or purified biological samples with multiple parameters in one run and on standard 96-well round bottom plates. Each column in the 96-well plate is controlled by its own pair of transducers. As a result it is possible to run up to 12 different optimization protocols at one time, each column can run its own parameters. With that, **PIXUL** enables the sonication of clinically relevant numbers of samples simultaneously, alleviating bottlenecks from traditional sonication methodology.

Methodology and Data

So far 10 different human tissues have been tested on the **PIXUL**: kidney, liver, uterus, colon, adipose tissue, ovary, PBMCs, heart, brain, and lung, providing a near to complete snapshot of relevant tissue samples.

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We found that human tissues could be sonicated on **PIXUL** in 72 minutes under a standard set of parameters. We also tested 7 mouse tissues and found that those can be sheared optimally in 36 minutes, indicating some difference in optimal programs depending on species and tissue type. With the ability of PIXUL to run 12 different programs simultaneously, optimization of these programs per sample type is easy.

Fixation of Primary Tissues

Primary tissues were harvested 24 hours post-mortem and sent in preservation media to Active Motif. Immediately upon arrival, tissues were washed 1x in ice cold PBS, diced with a razor blade in PBS +4% formaldehyde, moved to a conical tube and fixed for 15 minutes at room temperature with overhead rotation. The reaction was quenched with 1/20th volume 2.5M glycine for 5 minutes at room temperature with overhead rotation. Following fixation, samples were centrifuged to remove the media before being snap-frozen in liquid nitrogen for long term storage at -80°C.

Bead Ruptor Homogenization

Small pieces of previously fixed tissue were moved to a 2 mL Omni Bead Ruptor tube containing three 5 mm steel beads and 1 mL of either Cell Shearing Buffer (+PIC/PMSF) or Cell Shearing Buffer without N-laurylsarcosine (+PIC/PMSF). Kidney, PBMCs, liver, and brain were homogenized at 4 m/s for 20 sec. Lung, heart, ovary, uterus, adipose were homogenized 2 for cycles of 4m/s for 20 sec. with ~2 mins between cycles.

After homogenization, samples homogenized in Cell Shearing Buffer without N-lauryl sarcosine were centrifuged at full speed for 3 mins to pellet material before resuspension in 1 mL Cell Shearing Buffer (+PIC/PMSF).

PIXUL™ Multi-Sample Sonication

After Bead Ruptor homogenization, 100 µL/well of homogenized suspension were moved into 1 column (8 wells) of a 96-well round-bottom plate and sonicated on the PIXUL™ Multi-Sample Sonicator at a Pulse [N] of 50, PRF [kHz] 1.00, Burst Rate [Hz] 20.00, and Process Time [min] 72. After sonication, the plate was spun at full speed for 1 min at 4°C and the samples were pooled and aliquoted into Eppendorf tubes for long term storage at -80°C. Sonicated material was compared with unsonicated control after input DNA prep on either Fragment Analyzer or TapeStation.

Analysis

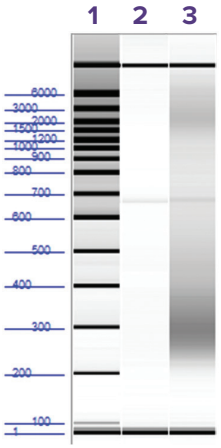

Fig. 1: **PIXUL Multi-Sample Sonicator** for Preparation of DNA from Brain Tissue.

Fragmented chromatin from 10 different human tissue samples was analyzed on a Fragment Analyzer and compared to unsonicated control.

Lane 1, DNA ladder.
Lane 2, unsonicated control sample.
Lane 3, sonicated brain tissue.

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As an example we show human brain below. Lane 1 shows the unsonicated control, lane 2 shows the same sample sonicated for 72 minutes on PIXUL™ Multi-Sample Sonicator. The sonicated sample shows an enrichment of fragments around 300 base pairs, which is the ideal size for ChIP-Seq analysis.

Protocol Details

Bead Ruptor Homogenization:

Small pieces of previously fixed tissue were moved to a 2 mL Omni Bead Ruptor tube containing three 5 mm steel beads and 1 mL of Cell Shearing buffer (+PIC/PMSF) or Cell Shearing Buffer without N-lauryl sarcosine (+PIC/PMSF). Kidney, PBMCs, liver, and brain were homogenized at 4 m/s for 20 seconds Lung, heart, ovary, uterus, and adipose were homogenized 2 for cycles of 4m/s for 20 seconds with ~2 minutes between cycles.

After homogenization, samples homogenized in Cell Shear without N-lauryl sarcosine were centrifuged at full speed for 3 mins to pellet material and resuspended in 1 mL of Cell Shearing buffer (+PIC/PMSF).

PIXUL Sonication:

After Bead Ruptor homogenization, 100 µL/well of homogenized suspension was moved into 1 column (8 wells) of a 96-well U-bottom plate and sonicated on PIXUL at a pulse of 50, PRF 1, burst of 20 for 72 mins (36 mins tested for liver and kidney only).

After sonication, the plate was spun at full speed for 1 minute at 4°C and the material was pooled and aliquoted into Eppendorf tubes before long term storage at -80°C. Sonicated material was checked for sizing versus unsonicated control after input DNA prep on either a fragment analyzer or TapeStation.

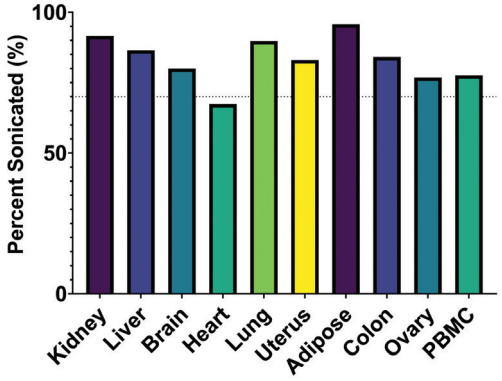

Figure 2: Overview of human tissues sonicated with **PIXUL™ Multi-Sample Sonicator**. All but one sample surpassed our internal QC metric of 70% sonicated fragments.

Kidney and adipose tissue nearly sonicate to 100%. Some tissues, such as heart, are more challenging, but this sample also approached a 70% sonication level which is considered acceptable for downstream ChIP-Seq analysis.

ChIP-Seq

After fragmentation, the samples were subjected to ChIP-Seq analysis using 10 µg of sonicated chromatin per reaction and 4 µg of an AbFlex ® Histone H3K9ac (Cat. No. 91103) antibody, which marks active chromatin.

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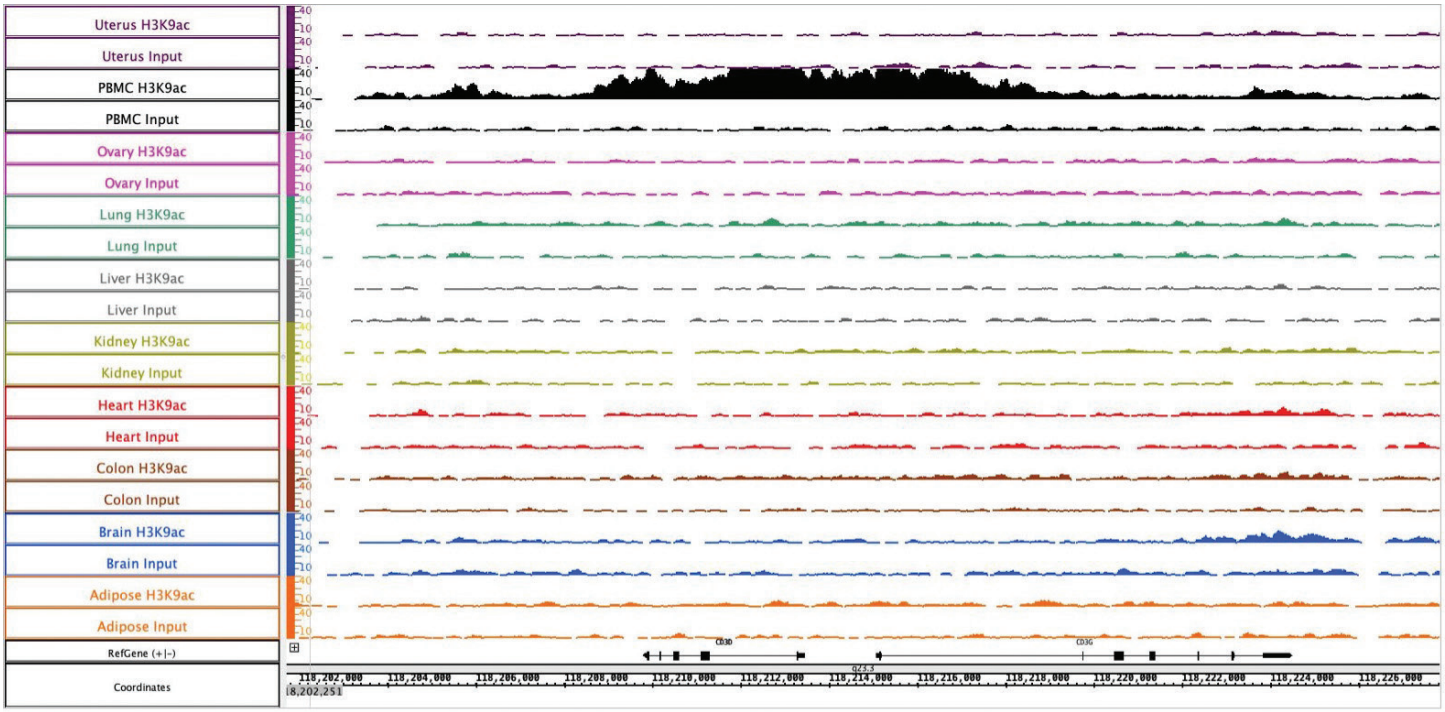

Library preparation was carried out using the Active Motif Next Gen DNA Library Kit (Cat. No. 53216, Cat. No. 53264). The sequencing was completed on the Illumina NextSeq500 and utilized approx. 20M reads for each of the samples.

Pictured above, genome browser tracks of ChIP-Seq results at the CD3 locus which specifically marks T-cells. This is a sub-unit of the CD3 receptor complex where strong H3K9 acetylation is only observed throughout the entire gene body in the PBMC population, as peripheral blood mononuclear cells play a central role in the immune response.

In contrast to that, no crosstalk is observed in any of the other tissues that were surveyed, indicating a strong tissue specificity from these experiments using the **PIXUL™ Multi-Sample Sonicator**.

Summary

Here, we demonstrate the suitability of the **PIXUL™ Multi-Sample Sonicator** for preparation of clinically relevant sample types and amounts. With the **PIXUL™ Multi-Sample Sonicator** it is possible to sonicate chromatin under a standard set of parameters, yielding material that is appropriately sheared to the correct size and suitable for downstream ChIP-Seq analysis.

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The main advantages of the **PIXUL** are:

- High throughput, tissue-specific results from PIXUL-sonicated chromatin to analyze tissue specific differences in their biology under a uniform, time-saving set of parameters. The resulting chromatin performs reliably in ChIP-Seq analysis.
- Epigenomic profiling via ChIP-Seq was performed in 10 human tissues and 7 mouse tissues for 3 histone modifications and 1 transcription factor by a single scientist in a few weeks (only the human tissues and 1 mark are shown in this Technote). This was previously impossible due to linear processing of single samples, inconsistent shearing and cumbersome optimization procedures of available sonicators.
- **PIXUL™ Multi-Sample Sonicator** presents a new and robust technology for consistent and fast chromatin shearing for clinical volumes of samples, enabling epigenomic studies and translational research.

CHECK OUT OUR QUICK GUIDE:

SHEARING CHROMATIN FROM FROZEN TISSUES WITH PIXUL™

Get recommendations for fragmenting chromatin with the PIXUL Multi-Sample Sonicator using the PIXUL Chromatin Shearing Kit (Cat. No. 53132) using both human and mouse tissues."

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