APPLICATION NOTE

From sample prep to sonication: A ChIP-Seq analysis workflow from various tissues using the Omni Bead Ruptor Elite and PIXUL™ Multi-Sample Sonicator.

Revvity, Inc.
Active Motif, Inc.

Summary

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, consistency in data and consistency in experiments is more important than ever. High quality tissue homogenization and consistent shearing is pivotal to generate data.

Herein, we demonstrate a robust workflow for ChIP-Seq sample preparation and analysis from clinically relevant tissue types using the Omni Bead Ruptor Elite bead mill homogenizer to dissociate tissues and the PIXUL™ Multi-Sample Sonicator for ChIP-Seq sample preparation.

Materials and methods

Equipment

OMNI equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat No. 19-042E)
- 2 mL Reinforced Tubes with Screw Caps & Silicone O-Rings (Cat No. 19-648)
- 5 mm Stainless Steel Beads Bulk, 325 g (Cat No. 27-7005)

For research use only. Not for use in diagnostic procedures.

Omni Bead Ruptor Elite bead mill homogenizer

Omni Bead Ruptor Elite bead mill homogenizer

Active motif equipment & reagents

- PIXUL™ Multi-Sample Sonicator (Cat No. 53130)
- AbFlex® Histone H3K9ac Antibody (Cat. No. 91103)
- Next Gen DNA Library Kit (Cat No. 53216, 53264)

PIXUL™ Multiple Sample Sonicator

Procedure

Fixation of primary tissues

Primary tissues were harvested 24 hours post-mortem and sent in preservation media to the research lab. Immediately upon arrival, tissues were washed in ice-cold 1X phosphate-buffered saline (PBS) (Gibco, Cat No. 20012050), diced with a razor blade in PBS + 4 % formaldehyde, transferred to a conical tube and fixed for 15 minutes at room temperature with overhead rotation. The reaction was quenched with 1/20th volume 2.5 M 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.

Sample preparation

Small pieces of previously fixed tissue were moved to a 2 mL reinforced tube (Cat No. 19-648) containing three 5 mm steel beads (Cat No. 27-7005) and 1 mL of Cell Shearing Buffer containing reagents as recommended by the ChIP protocol. Kidney, peripheral blood mononuclear cells (PBMCs), liver, and brain were homogenized using the Omni Bead Ruptor Elite bead mill homogenizer (Cat No. 19-042E) at 4 m/s for 20 seconds, while lung, heart, ovary, uterus and adipose were homogenized at 4 m/s for 2 cycles of 20 seconds with a 2-minute dwell.

Following homogenization, those samples prepared in Cell Shearing Buffer containing recommended reagents were centrifuged at 15,000 x g for 3 mins to pellet material before resuspension in 1 mL Cell Shearing Buffer with added reagents.

PIXUL™ Multi-Sample Sonication

After homogenization on the Omni Bead Ruptor Elite bead mill homogenizer, 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 (Active Motif, Cat No. 53130) 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 centrifuged at 15,000 x g 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 a non-sonicated control after input DNA prep on either Fragment Analyzer or TapeStation.

ChIP-Seq

After fragmentation, the samples were subjected to ChIP using 10 µg of sonicated chromatin per reaction and 4 µg of an AbFlex® Histone H3K9ac (Active Motif, Cat. No. 91103) antibody, which marks active chromatin. Less than 10 µg of sonicated chromatin may be required depending on antibody. Other Active Motif AbFlex® antibodies used in this experiment but not shown here are H3K27ac (Active Motif, Cat. No. 91193), H3K36me3 (Active Motif, Cat. No. 91265), and CTCF (Active Motif, Cat. No. 91285). The Low Cell ChIP-Seq Kit (Active Motif, Cat. No. 53084) was part of the protocol. Some other available options for consideration are PIXUL™ Chromatin Shearing Kit (Active Motif, Cat. No. 53132), PIXUL™ Chromatin Input Preparation Kit (Active Motif, Cat. No. 53134), etc.

Protein A/G agarose beads were prepared depending on the antibody. Chromatin was pre-cleared. Immunoprecipitation was performed on end-to-end rotor at 4 °C overnight followed by washing, elution, reversal of cross-links, and DNA purification.

For ChIP-Seq, library preparation was conducted 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. 20 M reads for each of the samples.

Results

Figure 1: PIXUL™ Multi-Sample Sonicator for preparation of DNA from Brain Tissue. Lane 1 shows the non-sonicated 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.

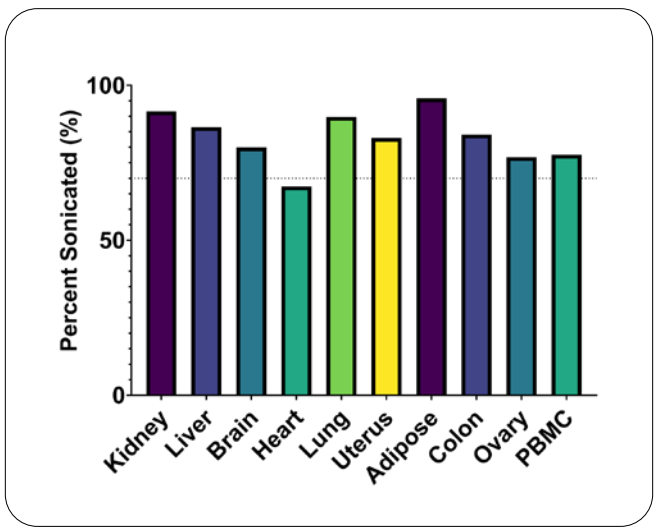

Figure 2: Overview of human tissues sonicated with PIXUL™ Multi-Sample Sonicator. All but one sample surpassed our internal QC metric of 70 % sonicated fragments. Kidney and adipose tissue nearly sonicate to 100 %. Some tissues, such as heart, are more challenging, but this sample also approached a 70 % sonication level which is considered acceptable for downstream ChIP-Seq analysis.

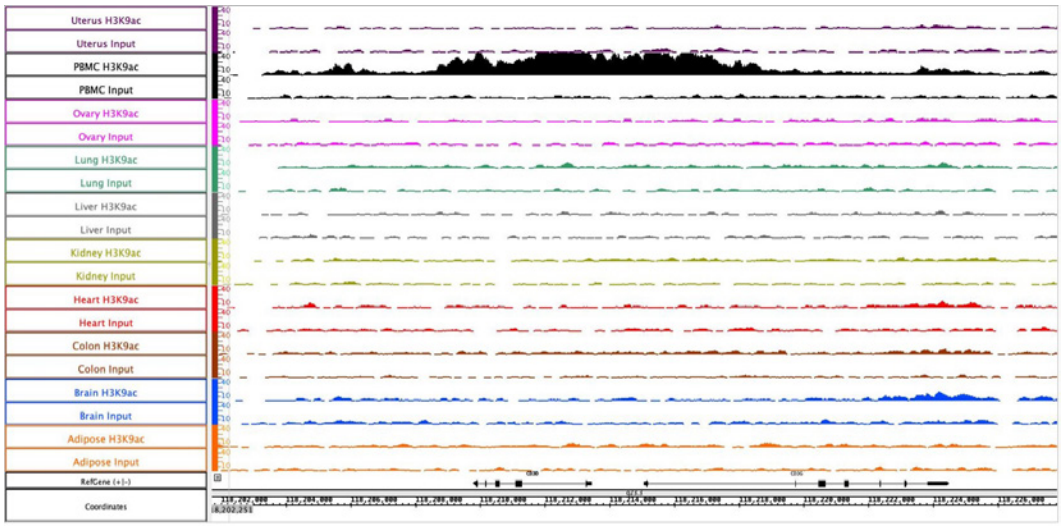

Figure 3: Genome browser tracks of ChIP-Seq results for all tissue types at the CD3 locus which specifically marks T-cells. This is a sub-unit of the CD3 receptor complex where strong H3K9 acetylation is only observed throughout the entire gene body in the PBMC population, as PBMCs play a leading role 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.

Conclusion

Herein, we demonstrate the suitability of the ChIP-Seq workflow using the Omni Bead Ruptor Elite bead mill homogenizer for upstream tissue dissociation and PIXUL™ Multi-Sample Sonicator for preparation of relevant sample types and amounts. The Omni Bead Ruptor Elite bead mill homogenizer streamlines sample preparation allowing for quick tissue dissociation, releasing analytes of interest for downstream analysis. Alongside the PIXUL™ Multi-Sample Sonicator, it is possible to sonicate chromatin under a standard set of parameters and in a high-throughput manner, yielding material that is appropriately sheared to the correct size and suitable for downstream ChIP-Seq analysis.

The 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.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

202206/OM