Novel Epigenetics Technology for High-Throughput Processing of Limited Samples to Study Cancer Using Cavitation-based Pixelated Ultrasound and Tagmentation-indexing ChIP-Seq

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ACTIVE MOTIF®
Enabling Epigenetics Research

Overview

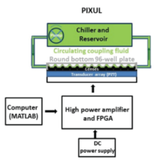
Epigenomic profiling methods are powerful tools for discovery and clinical research. An indispensable method used in epigenetics research to understand gene regulation is chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq). However, implementation into translational medicine has been slow due to challenges in complex workflows and sample availability. ChIP-seq's success relies on several factors including large amounts of sample quantity, consistent chromatin fragmentation, antibody specificity, etc., which can be time-consuming, and impractical if the sample source is limited.

To address the limitations, we present a new technology with Tagmented, Indexed and Pooled ChIP-Seq (TIP-ChIP), a novel epigenetics assay developed to achieve high-throughput, multi-mark ChIP-seq. TIP-ChIP allows for low-input, multi-target epigenomic profiling through unique barcoding of crosslinked samples using Tn5 tagmentation, followed by pooling and splitting of all samples into multiple immunoprecipitations. An overview of the workflow is shown on the right. The experimental readouts are the genome-wide occupancy maps of proteins or histone modifications of interest, similar to ChIP-seq but with several added advantages.

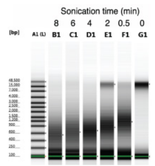
The workflow also features another cutting-edge technology utilizing pixelated ultrasound. Although the technology is generally used for DNA and chromatin fragmentation, but in this specific workflow, the technology is used to obtain nuclear extract after tagmentation. It is important to note that the pixelated ultrasound technology is a rapid and consistent method for sample preparation. Apart from ChIP-seq and DNA fragmentation, the pixelated ultrasound technology is implemented in DNA and RNA methylation analysis, RNA-seq, protein extraction for LC-MS/MS, and it can process a variety of samples including tissues and FFPE.

Overall, we demonstrate two novel technologies which enable epigenetics research by increasing throughput while reducing per-sample input. Our workflow further reduces labor, time, and costs, while maintaining consistency and minimizing sample-to-sample variation that can arise from individual processing. Optimization of this technology to its fullest potential will greatly benefit discovery-based research as well as translational medicine.

TIP-ChIP - Active Motif Technology



- 1-96 samples
- Versatile programming for 12 columns of 96-well plate
- Applications
  - ChIP-seq
  - Genomics
  - RNA-seq
  - LC-MS/MS
  - Methylation seq for DNA & RNA



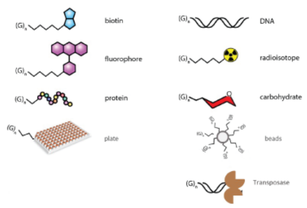
PIXUL

A 96-well plate ultra sonicator capable of generating 96 ChIP-ready samples in less than 30 minutes. This sonicator allows for uniform sonication of chromatin using time to achieve desired fragment length.

CDRs cloned from NGS of reverse transcribed RNA

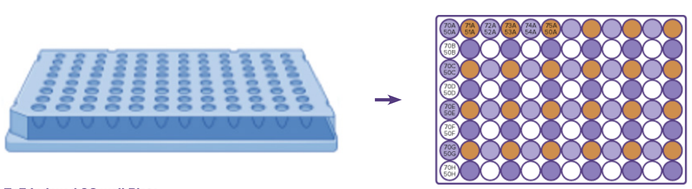
Standard frameworks and constant region backbone

Sortase Recognition Tag
Avidin Tag
6X-**H**is Tag



AbFlex™ Recombinant Antibodies

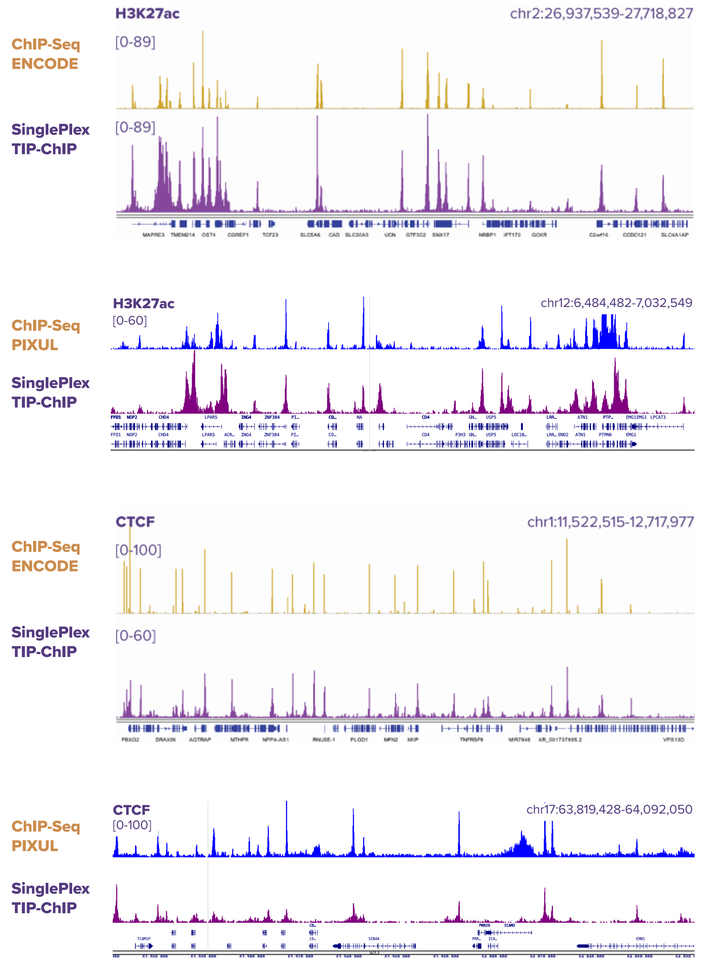
Antibodies that have been engineered to produce a highly specific and reproducible consistency. Each AbFlex® antibody contains distinguishable tags, allowing flexible labeling and purification options.



Tn5 Indexed 96-well Plate

A 96-well plate with 96 unique indexed Tn5 Transposase enables high-throughput tagmentation reactions to be performed simultaneously. In addition, this 96-well plate is automation-friendly, allowing researchers to simplify workflows for different applications.

Singleplex TIP-ChIP Yields Comparable Results with ChIP-Seq

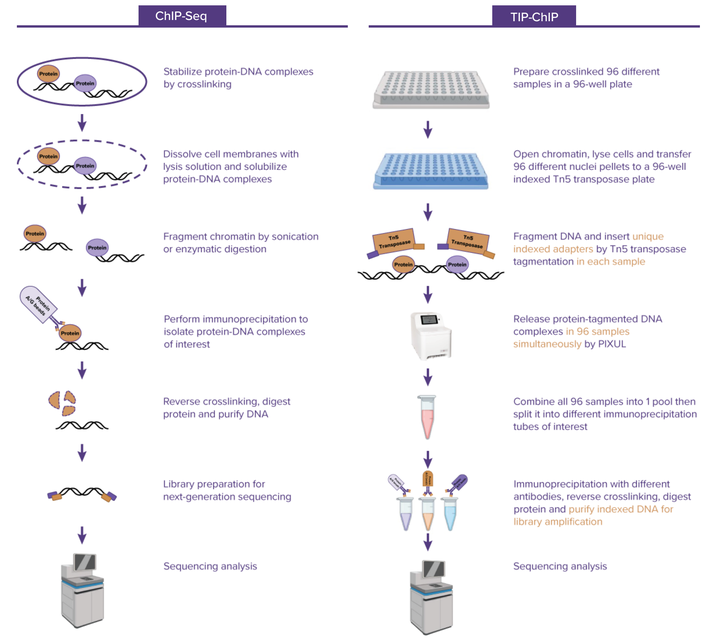


Singleplex TIP-ChIP in Multiple Marks Accomplishes Similar Results with ChIP-Seq

Genome tracks for K562 cells with a histone mark (H3K27ac), and a transcription factor (CTCF)

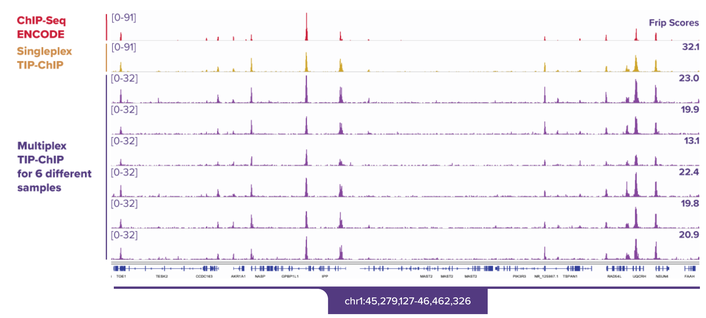
ChIP-Seq ENCODE (gold), singleplex TIP-ChIP (purple), and PIXUL-ChIP-Seq (blue)

ChIP-Seq and TIP-ChIP Comparison for 96 Samples



	Advantages of TIP-ChIP	ChIP-Seq	TIP-ChIP
Number of cell/nuclei input	Lowering input requirement	1 - 10 million/sample	50,000 - 100,000/sample
Number of targets	Targeting multiple targets per sample	One antibody/sample	Up to 4 different antibodies/sample
Cost of service per sample	Reducing cost significantly	$2500 - $3000	$1800 - $2000
Time to process 96 samples	Shortening hands-on time	1.5 - 2 months	1 - 2 weeks
Input types	Compatible with different input types	Tissue, cell, and nuclei	Tissue, cell, and nuclei

Sample Multiplexing of H3K27ac with TIP-ChIP



TIP-ChIP Performs Promisingly for a Pool of 6 Different Samples with H3K27ac

Genome tracks for K562 cells with H3K27ac: ChIP-Seq ENCODE (red) and singleplex TIP-ChIP (gold) compared with 6 different samples (purple) multiplexed by TIP-ChIP, which were demultiplexed successfully by unique indices.

Future Directions

- Testing and validating method with additional histone marks and transcription factors (BRD4, YAP1, Estrogen receptor, etc.)
- Limit of detection study for cell/nuclei input
- Method optimization for compatibility with multi-channel pipettes and automated liquid handling instrumentation
- Development of spike-in control for normalization

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PIXUL has been developed in collaboration with Matchstick Technologies

Check out Active Motif resources at
activemotif.com/resources