High-Throughput Ultrasonic DNA Shearing for NGS Sample Preparation

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Introduction

As Next-Gen sequencing throughput continues to accelerate, a serious sample preparation bottleneck is emerging, calling for a higher throughput DNA shearing technique. The solution must not compromise the quality of the sheared fragments and should not add to the existing cost of sample/library prep kits. Microsonic Systems Inc. (Microsonics) has developed a unique, new ultrasonic technology "Bulk Lateral Ultrasonic (BLU)™ energy" and created a core building block the Microprocessor for Life Sciences™, to commercialize this exciting new technology. Together, these form the basis for a multi-channel DNA shearing device – the *Microsonics ST*™ High-Throughput Sample Prep System.

BLU Energy – Technology Overview

Microsonic Systems' BLU energy is a new form of ultrasonic energy that produces a broad beam of energy using a Micro-Electrical-Mechanical Systems (MEMS) transducer. It enables a very high power output with a very small form factor. As shown in **Figure 1**, BLU energy creates regions of lateral ultrasonic thrust in alternating counter-rotating vortices. These vortices in turn generate extremely high shear pressures – in excess of 3,000 psi – without cavitation. The ability to generate strong ultrasonic forces without cavitation enables a broad spectrum of control – from gentle non-destructive mixing of proteins and cells to very strong shearing pressures.

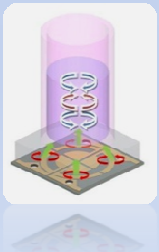

Figure 1. How does it work? Regions of bulk lateral ultrasonic thrust in alternating counter-rotating vortices create controllable shear forces in a sample tube, enabling powerful DNA shearing for a wide range of fragment sizes.

Materials and Method

To evaluate the effectiveness of BLU energy for DNA shearing, we used Lambda phage DNA (SigmaAldrich cat# D3779) and human DNA samples (supplied by Dr. Richard Myers, HudsonAlpha Institute for Biotechnology). We diluted the genomic DNA samples to 50 µL in Matrix 2D-barcoded storage tubes (Thermo Scientific cat# 3711) at various concentrations, and subjected them to optimized shearing protocols for specific target fragment lengths ranging from 300 bp to 1.5 Kb. The shearing process was carried out at room temperature using the *Microsonics ST* system powered by the BLU energy (**Figure 2**). We used the Agilent Bioanalyzer 2100 with DNA High Sensitivity Kit (cat# 5067-4626) and DNA 12000 Kit (cat# 5067-1508) to validate the DNA shearing results.

Figure 2. Lambda DNA sample, as indicated by the blue arrow in the figure, in an off-the-shelf Matrix 2D-barcoded storage tube is positioned over a BLU energy form factor to be sheared to target fragment length.

To test chromatin shearing with BLU energy, we placed chromatin samples extracted from GM12878 ($2x10^7$; supplied by Dr. Richard Myers, HudsonAlpha Institute for Biotechnology) , a lymphoblastoid cell line from the blood of a female donor, in 50 µL RIPA buffer in the Matrix tubes, and processed the sample tubes in the *Microsonics ST* system. To confirm that we have successfully sheared chromatin, we reversibly cross-linked post-shearing chromatin samples by incubating the samples at 65°C overnight. We then purified the samples with Qiagen PCR Purification Kit (cat# 28106), and analyzed the samples with a FlashGel® Cassette gel electrophoresis system (Lonza cat# 57023) to confirm the shearing results.

DNA Shearing

I. Dial a DNA Fragment

Using the *Microsonics ST* system, a user would simply start with sample DNA in the Matrix tube, place the tube in the shearing device, select the target DNA fragment size, and walk away. As shown in **Figure 3**, six Lambda DNA samples were sheared to target sizes from 300 bp to 1.5 Kb by specifying the "Dial a DNA Fragment" settings. We are developing shearing conditions for 3 Kb and 5 Kb fragments.

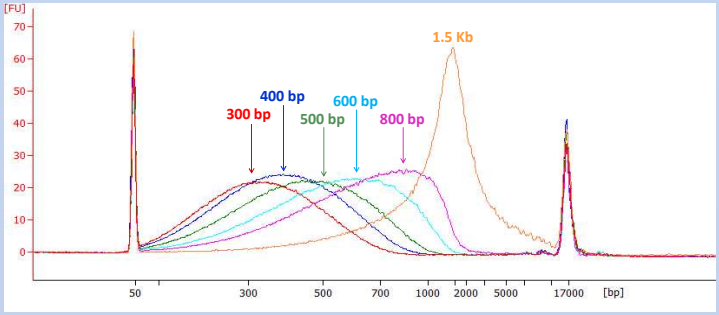

Figure 3. Bioanalyzer results of Lambda DNA sheared to target sizes of 300 bp, 400 bp, 500 bp, 600 bp, 800 bp and 1.5 Kb by specifying the "Dial a DNA Fragment" settings.

II. DNA Sample Concentration Independence

Eliminating the sample concentration normalization step further simplifies the sample preparation process. To check the DNA sample concentration dependency, we diluted human DNA samples to 20 ng/µL, 50 ng/µL and 80 ng/µL and then sheared to 400 bp. In **Figure 4**, all three samples share the same bell curve pattern, which shows that the difference in sample concentration does not affect sheared fragment length.

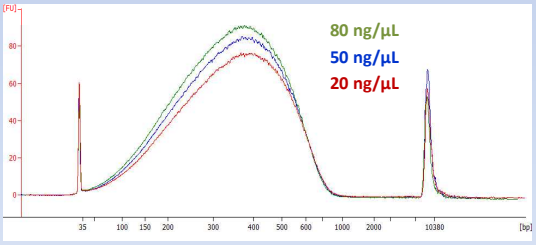

Figure 4. Bioanalyzer results of three Human DNA samples, at 20, 50 and 80 ng/µL, sheared to target size of 400 bp.

Chromatin Shearing

Chromatin Immunoprecipitation (ChIP) is a technique used to investigate the relationship of proteins with specific genomic regions *in vivo*, and ChIP-sequencing (ChIP-seq) has been used to study the interaction pattern of any protein with DNA in high resolution.

Properly shearing chromatin to the desired fragment sizes is important for the precision of the ChIP-seq assay, and here we show that the BLU energy is an effective ultrasonic shearing technique for chromatin (**Figure 5**) and a high-throughput alternative to existing methods.

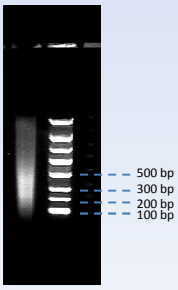

Figure 5. Agarose gel image showing chromatin extracted from GM12878 cell line sheared to 400-bp fragments using the *Microsonics ST* system.

Conclusion

- BLU energy is a new form of non-contact, ultrasonic technology for DNA and chromatin shearing, which works with off-the-shelf Matrix storage tubes and requires only 50 µL of sample.
- Powered by the BLU energy, the *Microsonics ST* system offers a wide range of fragment sizes, from 300 bp to 1.5 Kb; protocols for higher fragment sizes will be available soon.
- There is no need to dilute or condense samples to a specific starting concentration when using the *Microsonics ST* system for sample shearing.
- The *Microsonics ST* system produces fragments with high consistency, and is easily scalable to meet future higher throughput sample preparation requirements.

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