High-Throughput DNA Shearing Using Bulk Lateral Ultrasonic (BLU)™ Energy

Smriti Sharma, Kapil Dev, Parvez Deshmukh, Babur Hadimioglu, Jean Shieh, Bruce Jamieson and Vibhu Vivek
Microsonic Systems Inc.

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. 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, making possible a multi-channel DNA shearing device that will shear multiple DNA samples simultaneously.

The Company is currently finalizing the design of its first high throughput DNA shearing instrument which will be sufficient to meet the needs of current and emerging Next-Gen sequencing throughput demands, while increasing the quality of the fragmented DNA.

BLU Energy – Technology Overview

Microsonic Systems' bulk lateral ultrasonic 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 (**Figure 1**). The company calls the transducer the "Microprocessor for Life Sciences" to emphasize its wide range of potential uses. The operation begins with an excitation signal that the MEMS transducer converts into ultrasonic waves that enter a sample as a broad beam of acoustic energy. As shown in **Figure 2**, the 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. The BLU energy transducer is a small form factor device that can generate ultrasonic forces powerful enough to shear DNA without cavitation. It is readily scalable to meet high-throughput sample preparation needs by utilizing parallel, simultaneous channels.

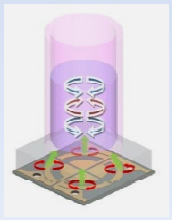

Figure 2. How does it work? Regions of bulk lateral ultrasonic thrust in alternating counter-rotating vortices create controllable shear forces in a sample tube, enabling powerful DNA shearing for a wide range of fragment sizes.

Materials and Method

To evaluate the effectiveness of the 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 a prototype device powered by the BLU energy (**Figure 3**). 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 3. 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.

DNA Shearing Results

I. Dial a DNA Fragment

Using the Microsonics' shearing device, a user will 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 4**, six Lambda DNA samples were sheared to target sizes from 300 bp to 1.5 Kb by specifying the "Dial a DNA Fragment" settings.

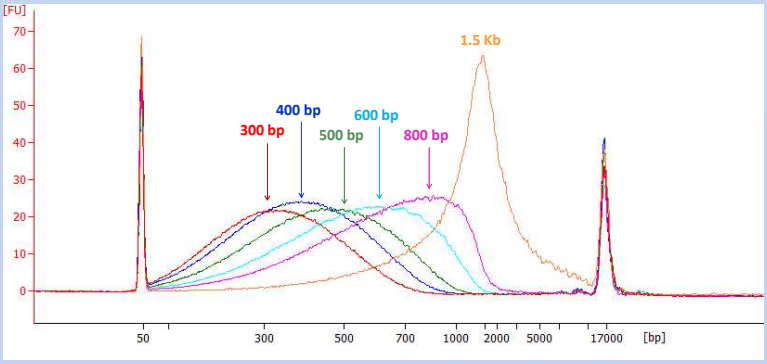

Figure 4. Bioanalyzer results of Lambda DNA sheared to target sizes of 300 bp, 400 bp, 500 bp, 600 bp, 800 bp and 1.5 Kb by specifying the "Dial a DNA Fragment" settings.

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 5**, all three samples share the same bell curve pattern, which shows that the difference in sample concentration does not affect sheared fragment length.

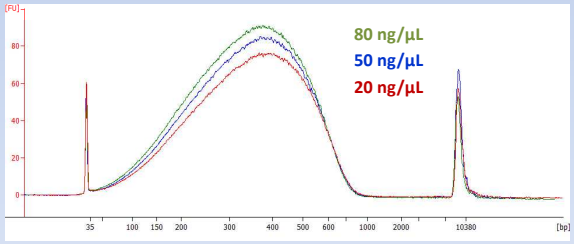

Figure 5. Bioanalyzer results of three Human DNA samples, at various starting sample concentrations, sheared to target size of 400 bp.

III. High Shearing Reproducibility

To confirm the shearing reproducibility, we sheared four human DNA samples under identical conditions. The Bioanalyzer data in **Figure 6** shows that the four curves overlapped with one another demonstrating high reproducibility.

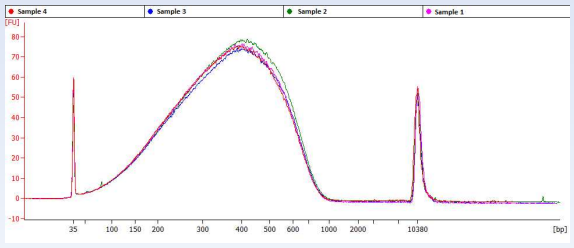

Figure 6. Bioanalyzer results of four human DNA samples (50 ng/µL) sheared using the same BLU energy shearing protocol.

Conclusion

In conclusion -

1. The BLU energy is a new form of non-contact, ultrasonic technology for DNA shearing, which works with off-the-shelf Matrix storage tubes and requires only 50 µL of DNA sample.
2. The BLU energy offers a wide range of genomic DNA fragment sizes, from 300 bp to 1.5 Kb.
3. There is no need to dilute or condense DNA samples to a specific starting concentration when using the BLU energy for DNA shearing.
4. The BLU energy produces DNA fragments with high consistency, and is easily scalable to meet future higher throughput sample preparation requirements.

MICROSONIC SYSTEMS™
76 Bonaventura Dr.
San Jose, CA 95134
www.microsonics.com