A Duet of NGS Sample Preparation Techniques

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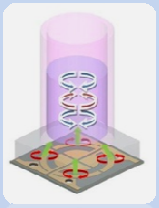
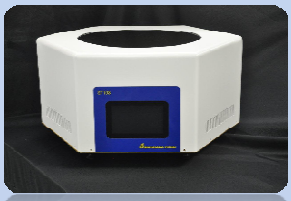
1. Introduction

Rapid, accurate DNA fragmentation is a critical step in Next-Generation Sequencing (NGS) and the ability to rapidly shear and measure the quality of this step is a workflow bottleneck in the library preparation process. A cost and time effective solution to address both the shearing and subsequent quantity/quality assessment is the use of this duet of newly released products: Bulk Lateral Ultrasonic (BLU)™ Energy for DNA shearing and a fluorescence-based capillary electrophoresis instrument both for accurate sizing and quantifying nucleic acids. Both technologies avoid the excessive use of expensive consumables and offer greater flexibility, overcoming shortcomings of most current techniques. This powerful, elegant duet provides considerably higher throughput and reduced labor to meet the needs of emerging demand for NGS.

2. High-Throughput Sample Prep



◄ **Figure 1.** 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.



► **Figure 2.** The *Microsonics ST*™ High-Throughput Sample Prep System.

Microsonic Systems' BLU energy (**Figure 1**) generates extremely high shear pressures – in excess of 3,000 psi – without cavitation, which enables a broad spectrum of control in fragment sizes using standard sample tubes.

The *Microsonics ST*™ High-Throughput Sample Prep System (**Figure 2**) is the first instrument utilizing BLU energy for NGS sample preparation. With the *Microsonics ST*™ system, a user would simply start with sample DNA in the Thermo Matrix tube, place the tube in the shearing device, select the target DNA fragment size, walk away and return with pinpoint accuracy on the requested number of base pairs.

3. Automated Fragment Sizing & Quantifying

The *Fragment Analyzer*™ Automated CE System (**Figure 3**) is a fluorescence-based capillary electrophoresis instrument for both sizing and quantifying nucleic acids. By using a sensitive intercalating dye coupled with a powerful LED light source, the system obviates the need for fluorescent labeled primers and can be used to separate dsDNA fragments and RNA. The *Fragment Analyzer*™ Automated CE System is the most flexible system in the market today, with the greatest sensitivity, the highest separation resolution over a wide size range, the widest dynamic range and the highest available sample throughput.

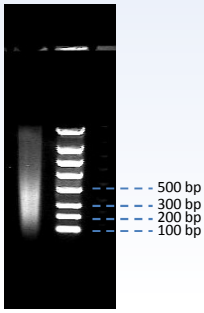
Figure 3. The *Fragment Analyzer*™ Automated CE System.

4. Chromatin Shearing Results

We placed chromatin samples extracted from lymphoblastoid cell line GM12878 ($2x10^7$; supplied by Dr. Richard Myers, HudsonAlpha Institute for Biotechnology) in 50 µL RIPA buffer in Matrix 2D-barcoded storage tubes (Thermo Scientific cat# 3711), 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 using FlashGel® Cassette gel electrophoresis system (Lonza cat# 57023; **Figure 4**) and *Fragment Analyzer*™ NGS kit (cat# DNF 481-0500; data not shown) to confirm the shearing results.

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

Figure 4. Chromatin shearing results. Agarose gel image showing chromatin extracted from GM12878 cell line sheared to 400-bp fragments using the *Microsonics ST* system.



5. DNA Shearing Results

We diluted Lambda phage DNA (SigmaAldrich cat# D3779) 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 250 bp to 2Kb. The shearing process was carried out at room temperature using the *Microsonics ST*™ system. We used the *Fragment Analyzer*™ system to validate the DNA shearing results, as shown in **Figure 5** and **Figure 6**.

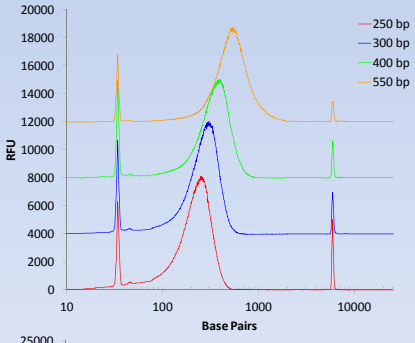


Figure 5. Lambda phage DNA samples sheared to 250, 300, 400 and 550 bp fragments using the *Microsonics ST*™ system. We used the *Fragment Analyzer*™ system and its NGS kit (cat# DNF 481-0500) to size the fragments, and then compared the four target fragment sizes with a composite chart.

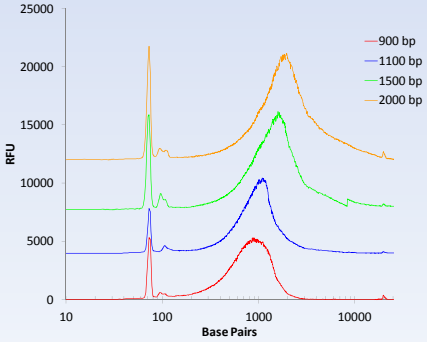


Figure 6. Lambda phage DNA samples sheared to 900, 1100, 1500 and 2000 bp fragments using the *Microsonics ST*™ system. We used the *Fragment Analyzer*™ system and its 75-20,000bp kit to size the fragments, and then compared the four target fragment sizes with a composite chart. We observed no biased affinity for high base pairs.

6. Conclusion

- The *Microsonics ST*™ system offers a wide range of fragment sizes, from 250 bp to 2 Kb and works with off-the-shelf Matrix storage tubes and requires only 50 µL of sample; protocols for higher fragment sizes will be available soon.
- The *Microsonics ST*™ system produces fragments with high consistency, and is easily scalable to meet future higher throughput sample preparation requirements.
- The *Fragment Analyzer*™ system has a wide fragment sizing range and no biased affinity for higher base pairs.
- The *Fragment Analyzer*™ system requires minimal sample preparation and no priming, which saves hands-on time significantly.
- Together, the *Microsonics ST*™ system and the *Fragment Analyzer*™ system provide a high-throughput NGS sample preparation solution.

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