Mechanical DNA Fragmentation with the Q800R2 Sonicator

The Qsonica Q800R2 Sonicator offers an alternative method for mechanical DNA fragmentation for Illumina TruSeq® Library Preparation Kits.

Introduction

Several Illumina next-generation sequencing (NGS) kits require mechanical DNA fragmentation, including the TruSeq Nano DNA, the TruSeq DNA PCR-Free, and the TruSeq Exome Library Preparation Kits. Currently, the only instrument validated by Illumina for DNA fragmentation is the Covaris Ultrasonicator. For laboratories without access to Covaris instruments, or labs without the resources to acquire one, this may present a significant challenge. To address these challenges, Illumina tested DNA fragmentation with the Q800R2 Sonicator from Qsonica, LLC (Figure 1). As with the Covaris Ultrasonicator, the Q800R2 Sonicator uses high intensity sound waves to fragment DNA samples to a range of fragment sizes.

This technical note presents the experimental methods and results of a study designed to test and demonstrate the utility of the Q800R2 Sonicator for DNA fragmentation in Illumina library prep protocols. Settings for generating specific fragment sizes were tested and identified, fragmentation reproducibility was assessed, and the resulting libraries were sequenced to evaluate data quality. The results demonstrate that the Q800R2 Sonicator delivers excellent fragment size control and reproducibility, and produces libraries that generate high-quality sequencing data.†

Materials and Methods

Sample Sonication and Library Preparation

Four DNA sample replicates (QS1–QS4) were prepared from a human lymphocyte cell line (Coriell Institute for Medical Research, NA12878). To obtain 350 bp average insert sizes, DNA samples were sonicated in separate, generic, polypropylene tubes on the Q800R2 Sonicator (Qsonica, Model No. Q800R2) with identical instrument settings (Table 1). The white Qsonica tube holder was used (Qsonica, Part No. 4256), although several options are available for a variety of sample type and throughput needs (Table 4). Samples were quality checked for fragment size and fragment uniformity on the Fragment Analyzer (Advanced Analytical) using the High Sensitivity NGS Fragment Analysis Kit (Advanced Analytical Cat No. DNF-474). Four

Figure 1: The Q800R2 Sonicator—The Q800R2 Sonicator (Qsonica LLC) demonstrates excellent DNA fragment size control and reproducibility for Illumina library preparation kits.

libraries were prepared with the TruSeq Nano DNA Library Preparation Kit (Illumina, Cat No. FC-121-4001) and analyzed again with the Fragment Analyzer.

Sequencing and Preliminary Data Analysis

Libraries were sequenced on a HiSeq® 2500 System, in rapid run mode, as a paired-end 2 × 76 bp run, using HiSeq Rapid SBS Kit v2 sequencing reagents (Illumina, Cat No. FC-402-4023). Preliminary data analysis, including calculation of Q30 quality scores, was performed with BaseSpace® Sequence Hub, the cloud-based Illumina genomics computing environment.

Table 1: Q800R2 Sonication Settings for Library Replicates QS1–QS4

Parameters	Settings[a]
Target Fragment Size	350 bp
Sample Volume	52.5 µl
Total DNA Input	105 ng
DNA Concentration	2 ng/µl
Pulse (on/off)	15 sec/15 sec
Amplitude	20%
Temperature	4°C
Total Run Time	22 min.

a. Settings and run times should be optimized for the specific applications and buffers used.

†While the Qsonica method is a successful, demonstrated protocol for Illumina library sonication, it is not an Illumina supported protocol. For support-related questions, contact Qsonica at www.sonicator.com/contact.shtml.

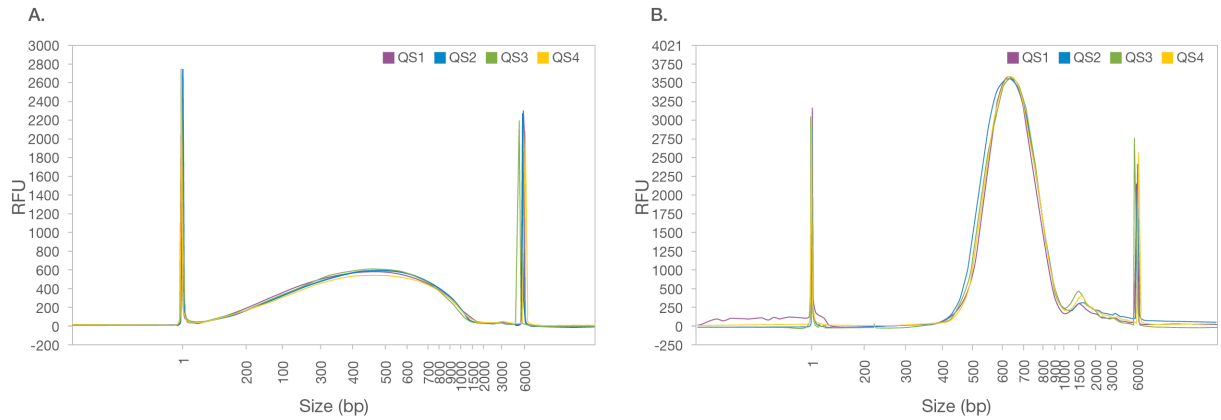

Figure 2: Q800R2 Fragmentation Reproducibility— A. DNA samples QS1–QS4 were sonicated by the Q800R2 Sonicator then assessed with the Fragment Analyzer. **B.** Libraries were prepared with the TruSeq Nano DNA Library Prep Kit and assessed with the Fragment Analyzer.

Results

Fragment Size Distribution and Fragmentation Reproducibility

To evaluate fragment size distribution and reproducibility of fragmentation, QS1–QS4 samples were assessed with the Fragment Analyzer after Q800R2 sonication, and again after TruSeq Nano library preparation (Table 2). The postsonication QS1–QS4 samples and the postlibrary prep samples demonstrated highly reproducible average fragment sizes (Figure 2). The Fragment Analyzer results (postsonication samples) demonstrated a broad size range, as is typically seen with Covaris sonication. The average fragment size for samples QS1–QS4 was ~420 bps. Subsequent steps in the library preparation process, such as bead-based clean-up steps, narrow the size range further to 350 bp.

Table 2: Average Insert Size and Library Size for Replicates QS1–QS4

	QS1	QS2	QS3	QS4
Avg Size After Sonication	421 bp	428 bp	426 bp	423 bp
Avg Size After Library Prep	553 bp	544 bp	564 bp	554 bp

Sequencing Data Quality

Sequencing data quality from each library was assessed by Q30 scores and percent alignment metrics. Libraries prepared with Q800R2 sonication produced reproducible, high-quality data sets, with all libraries showing ≥ 94% reads aligned and ≥ 94% of reads ≥ Q30 (Table 3).

Q30 score: A Q30 score indicates an error probability (1 in 1000) in base calling.

To learn more about NGS quality scores, read technical note *Understanding Illumina Quality Scores*.

Table 3: Sequencing Data Quality

Statistics[a]	QS1	QS2	QS3	QS4
Percent Aligned	94.9 %	94.7 %	94.6 %	95.1%
Percent ≥ Q30	94.5 %	94.4 %	94.4 %	94.8%
Median Fragment Length	365 bp	351 bp	364 bp	359 bp

a. Statistics shown are an average of Read 1 and Read 2 data.

Summary

The Q800R2 Sonicator offers a second method for DNA fragmentation in Illumina library prep kit protocols. The Q800R2 Sonicator demonstrates excellent reproducibility and generates libraries with high-quality sequencing data results. Although the TruSeq Nano DNA, TruSeq DNA PCR-Free, and TruSeq Exome Library Prep Kits currently use the Covaris Ultrasonicator for DNA fragmentation, the results of this study show that Q800R2 sonication offers a reliable alternative, adding greater flexibility to the Illumina library preparation workflow.

Table 4: Qsonica Sample Tube Holders

Description[a]	Sample Tube Size	Part Number
8 Tube Holder (white)	1.5 ml polystyrene (Evergreen)[b]	4256
12 Tube Holder (black)	0.5 ml thin walled PCR tubes	4255
18 Tube Holder (blue)	0.3 ml thin walled PCR tubes	4262
12 Tube Holder (grey)	0.5ml brandtech tubes	4263

a. In addition to the standard tube racks, Qsonica can design and manufacture custom sample tube racks to meet your specific process requirements. Visit www.sonicator.com/61-sample-tube-holders.html or contact a Qsonica representative for more information.
b. Evergreen Scientific: Sample tube Part No. 214-3721-010, Cap Part No. 300-2911-020 are recommended.

To learn more about the Q800R2 Sonicator, visit www.sonicator.com/19-q800r2-sonicator.html.

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