

# Optimization of Covaris Settings for Shearing Bacterial Genomic DNA by Focused Ultrasonication and Analysis Using Agilent 2200 TapeStation

## Application Note

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### Abstract

Shearing of bacterial gDNA within a specific size range prior to sequencing library construction is a critical step in Next Generation Sequencing workflows. The quality control of the sheared bacterial gDNA is required in large multiplexed formats for large volume workflows, such as those used in the 100K Pathogen Genome Sequencing Project. Using the Covaris E220 instrument, the power and treatment time were varied to determine the effect on the optimal fragment size (150–350 bp) in the resulting sheared gDNA of four bacterial pathogens: *Salmonella enterica* subsp. *enterica* serovar Saint Paul strain Sp3 and serovar *Typhimurium* strain LT2, *Klebsiella* sp. and *Vibrio* spp. DNA fragment quantification and sizing were measured using an Agilent 2200 TapeStation system, and Agilent High Sensitivity D1000 ScreenTape assay. The 2200 TapeStation system was suitable to determine size distribution after fragmentation of gDNA in a 96-well plate format, a format suitable for high-throughput workflow and compatible with shearing technologies that use a 96-well plate multiplexed format. This approach enabled the measurement of gDNA and sheared DNA using a single technology.



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## Introduction

Large scale sequencing projects, such as the 100K Foodborne Pathogen Genome Project (<http://100kgenome.vetmed.ucdavis.edu/>), require high-throughput procedures from DNA extraction to library construction and sequencing. This effort to sequence the genomes of 100,000 microbes important to food security is supported by a consortium of government, academic, and industrial partners, including Agilent Technologies, Inc. This global effort aims at creating a publicly available database of genomics information on foodborne pathogens to develop precise and robust new molecular-based tests to detect foodborne pathogens in the food chain, from farm to kitchen table.

In the workflow for genome sequencing, especially for the 100K sequencing project, multiplexed methods for DNA quantification, purity, and size qualification are important in streamlining robust quality control methods in the pipeline (Figure 1). A crucial step in the automated sequencing library construction pipeline for automated sequencing is shearing (or fragmenting) the genomic DNA (gDNA) to an optimal size range, based on sequencing platform selected [1,2].

A variety of methods are available to fragment gDNA (nebulization, sonication, and enzymatic) [3]. Mechanical shearing by focused ultrasonication (Adaptive Focused Acoustic technology commercialized by Covaris Inc.) is an established fragmentation method commonly used for many sequencing technologies [4]. It reproducibly produces fragments from gDNA within specific sizes without GC bias or thermal damage. Agilent Technologies integrated the Covaris DNA shearing technology in their Agilent SureSelect Target Enrichment Systems for human sequencing [5,6]. The optimization of the focused ultrasonication key parameters (peak incident power and treatment time) is essential to obtain sheared DNA fragments of the targeted size range for pathogenic bacteria.

The first step in the QC process in library construction is to determine the sheared DNA size distribution and quantity (Figure 1). The Agilent 2100 Bioanalyzer is the gold standard instrument to determine size distribution after fragmentation. The major next-generation sequencing providers recommend using the 2100 Bioanalyzer at this critical step in the library construction workflow. Up to 12 samples could be analyzed per chip (per run) in up to 45 minutes. The 2100 Bioanalyzer is the perfect solution when few samples are required to be analyzed.

### Next-generation sequencing pipeline

NGS Workflow	Workflow need	100K Application notes
DNA extraction	<input checked="" type="checkbox"/> Consistent cell lysis to yield HMW gDNA	Lysis reagents (5991-3722EN)
QC: DNA quality and quantity	<input checked="" type="checkbox"/> HTP method to measure HMW gDNA	Agilent 2200 TapeStation (5991-4003EN)
Shear DNA	<input checked="" type="checkbox"/> Consistent and inexpensive method	
QC: fragmentation	<input checked="" type="checkbox"/> HTP method to measure fragmented gDNA	→ This Application Note ←
Library construction	<input checked="" type="checkbox"/> Automated protocols	Long read - PacBio (5991-4482EN) Short read - Kapa (5991-4296EN)
Library normalization and pooling	<input checked="" type="checkbox"/> Consistent ratios of each library	Needs automation
Submit for sequencing	Rapid sequencing < 12 hours	Available on some platforms

Figure 1. 100K Foodborne Pathogen Genome Project Workflow. This figure details the NGS workflow used in our laboratory, and the application notes that were created for each step of the workflow. HTP: high-throughput; gDNA: genomic DNA; HMW: high molecular weight.

A method to determine size distribution after fragmentation of gDNA in a multiplexed format (that is, 96-well plate) is required for high-throughput workflows. The Agilent 2200 TapeStation system for DNA quantitation and sizing allows the use of the 96-well plate sample format for the entire workflow, with the fast analysis times and constant cost per sample required for a library construction pipeline in a genomic sequencing project (Figure 1) [6,7,8].

DNA analyses were done using the 2200 TapeStation system with the ready-to-use Genomic DNA ScreenTape [9] and the Agilent High Sensitivity D1000 ScreenTape [10]. Genomic DNA ScreenTape assay was used to monitor the efficiency of the focused ultrasonication in shearing gDNA and High Sensitivity D1000 ScreenTape for size distribution of the fragments. The 2200 TapeStation instrument automatically loads the prepared samples from the 96-well plate onto the High Sensitivity D1000 or Genomic DNA ScreenTape. Electrophoresis and imaging of an electropherogram or a gel image, as well as analysis, were all automated within the instrument.

This application note presents a study on the optimization of the focused ultrasonication parameters for shearing gDNA from foodborne bacterial pathogens. We are showing that the 2200 TapeStation system could be used to determine size distribution and shearing efficiency in the library construction pipeline in a way comparable to the 2100 Bioanalyzer system.

## Experimental

### Bacterial culture

*Vibrio* sp. was grown in Tryptic Soy Broth (TSB; Difco, Franklin Lakes, New Jersey). *Salmonella enterica* subsp. *enterica* serovar Saint Paul strain Sp3 and serovar *Typhimurium* strain LT2 as well as *Klebsiella* sp. were cultured in Luria Broth (LB; Difco, Franklin Lakes, New Jersey). Bacteria were cultured in their respective liquid media at 37 °C aerobically for 12–16 hours before sampling for DNA extraction. For this experiment, bacterial cell pellets for gDNA extraction were collected by centrifugation at 2,000 x g for 10 minutes.

### DNA extraction and quantification

DNA from bacterial cell pellets was extracted with a commercial DNA extraction kit followed by purification using a silica spin column [11,12]. DNA extracts were quantified and their purity assessed by measuring the UV absorbance at 260 and 280 nm using a NanoDrop 2000 UV-Vis spectrophotometer

(Thermo Fisher Scientific, Waltham, Massachusetts). The quantity of input DNA to shear with Covaris was determined, in all the extracts, with Qubit dsDNA HS Assay Kit (Q32854; Invitrogen, Carlsbad, California) on a Qubit 2.0 Fluorometer (Q32866; Invitrogen, Carlsbad, California). When possible, concentrations of bacterial gDNA in extracts were adjusted to 30 ng/μL.

### DNA fragmentation (shearing) by focused ultrasonication

Fragmentation of bacterial gDNA was performed by focused ultrasonication using a Covaris E220 instrument [13]. Fragmentation was performed in 96-well format according to manufacturer specifications [14]. Two parameters were modulated to optimize the yields of gDNA fragments in the size range of interest: peak incident power (W) and treatment time (seconds) [140 W at 120, 140, 160 seconds; 175 W at 120, 140, 160, 170, 175, 180 seconds] with duty cycle (10 %) and number of cycles per burst (200) remaining the same.

### Quantification, sizing, and integrity analysis using the Agilent Genomic DNA and High Sensitivity D1000 ScreenTape assays

The Agilent 2200 TapeStation Nucleic Acid System (G2965AA), Agilent High Sensitivity D1000 ScreenTape (5067-5584), Agilent High Sensitivity D1000 Reagents (5067-5585) as well as Agilent Genomic DNA ScreenTape (5067-5365), and Agilent Genomic DNA Reagents (5067-5366) were provided by Agilent Technologies (Waldbronn, Germany), and these were used in accordance to manufacturer instructions [9,10,15].

The samples for the High Sensitivity D1000 ScreenTape assay were prepared by mixing 2 μL of sheared gDNA sample with 2 μL of High Sensitivity D1000 Sample Buffer. A 2 μL amount of High Sensitivity D1000 Ladder was mixed with 2 μL of High Sensitivity D1000 Sample Buffer, and was placed in the first well of a 96-well plate, followed by the samples. The prepared plate was vortexed on high speed, covered with foil to prevent evaporation, centrifuged, and placed in the 2200 TapeStation instrument [10].

The samples for the Genomic DNA ScreenTape assay were prepared by mixing 1 μL of gDNA sample, or sheared gDNA sample, with 10 μL of Genomic DNA Sample buffer. A 3 μL amount of the prepared Genomic DNA Ladder was placed in the first well of a 96-well plate, followed by the samples. The prepared plate was vortexed on high speed, covered with foil, centrifuged, and placed in the 2200 TapeStation instrument [9].

Agilent 2200 TapeStation Analysis Software A.01.04 was used to analyze the data. Electropherograms from analysis of intact and sheared bacterial gDNA and standards were aligned and scaled to MW range. The Region view function in the software was used to integrate a specific region (150–350 bp) of the electropherograms for High Sensitivity D1000 ScreenTape data.

## Results and Discussion

Shearing bacterial gDNA is a critical step in NGS library construction (Figure 1). It is necessary to optimize parameters that control fragmentation or shearing of gDNA to maximize the quantity of DNA fragments in the right target size range (150–350 bp). Focused ultrasonication, a technology used by

Covaris instruments, is one of the most commonly used to fragment gDNA prior to library construction. Essentially, two parameters are to be optimized for a given type of sample: peak incident power and treatment time [16]. Also, we hypothesized that shearing efficiency and recovery of fragments in the right target size range could be influenced by the origin of the gDNA. This is why we decided to optimize the main shearing parameters using four different model bacteria: *Salmonella enterica* subsp. *enterica* serovar Saint Paul strain Sp3 and serovar *Typhimurium* strain LT2, *Klebsiella* sp. and *Vibrio* sp. We used the 2200 TapeStation system to determine size distribution after fragmentation of gDNA in a 96-well plate format. This format is suitable for a high-throughput workflow, and is perfectly compatible with Covaris technology that could work in 96-well plate format.

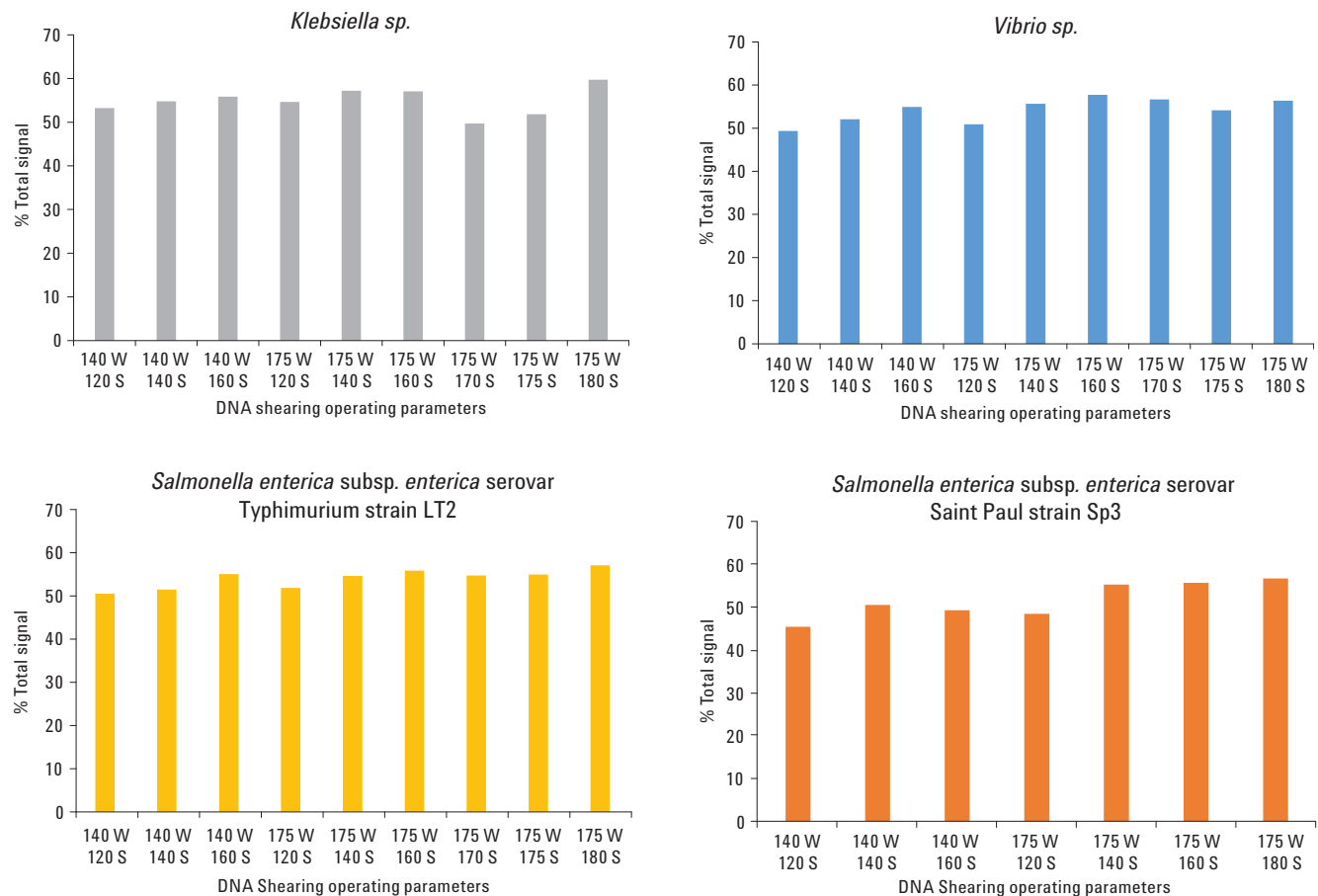


Figure 2. Optimization of the focused ultrasonication (Covaris) parameters for gDNA from four model bacteria. Each panel represents the results for each bacterium. Two parameters [peak incident power (W) and treatment time (S)] were modulated to optimize the proportion of gDNA fragments in the 150-350 bp size range. Agilent 2200 TapeStation and Agilent D1000 HS DNA ScreenTape Assay were used to acquire the data.

As peak incident power and treatment time increased, the proportions of DNA fragments in the 150–350 bp range increased (Figure 2). Figure 3 shows examples of 2200 TapeStation electropherograms of gDNA from *Salmonella enterica* subsp. *enterica* serovar Saint Paul strain Sp3 fragmented using different settings. As peak incident power and time increased, DNA fragments size distribution changed for

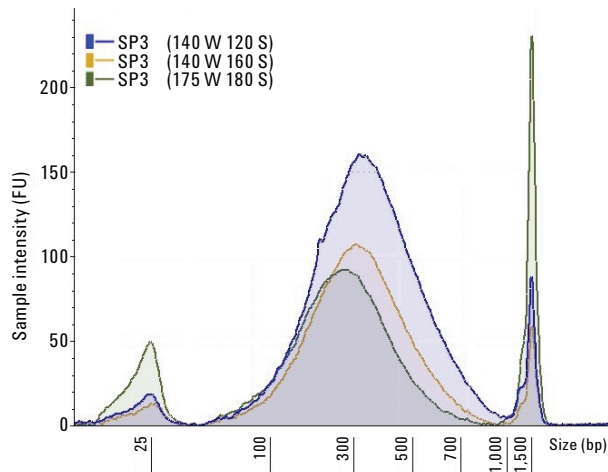


Figure 3. Overlaid TapeStation electropherogram examples of gDNA from *Salmonella enterica* subsp. *enterica* serovar Saint Paul strain Sp3 fragmented using different parameters of peak incident power and time. Peaks at 25 and 1,500 bp are internal standards. Broad peak from 50–1,000 represents the fragmented gDNA. An Agilent High Sensitivity D1000 ScreenTape assay was used to acquire the data.

each organism (data not shown). Based on these data, optimal parameters for shearing/fragmentation were selected to be peak incident power of 175 W and treatment time of 180 seconds. However, the settings selected gave consistent size distribution of DNA fragments for all four model bacteria with a maximum of approximately 300 bp (Figure 4).

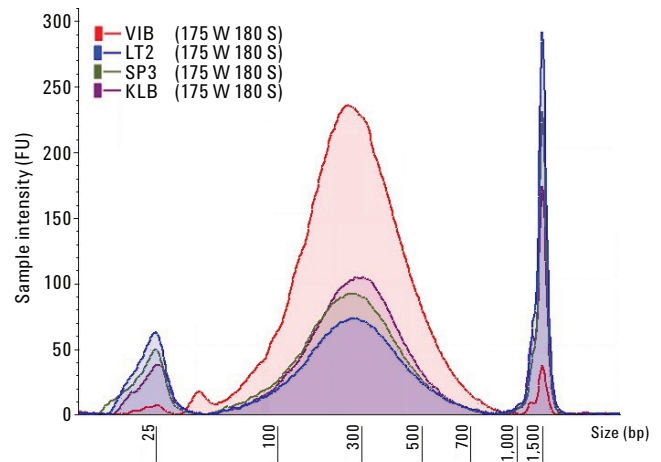


Figure 4. Overlaid TapeStation electropherogram examples of gDNA from four model bacteria fragmented in optimal conditions (175 W as peak incident power and during 180 seconds). Peaks at 25 and 1,500 bp are internal standards. Broad peak from 50–1,000 represents the fragmented gDNA. An Agilent High Sensitivity D1000 ScreenTape assay was used to acquire the data.

Using the Genomic DNA ScreenTape assay, we determined the efficiency of the focused ultrasonication in shearing gDNA. In Figure 5, extracted gDNA of *Klebsiella* sp. is completely fragmented using previously determined settings. The capability of the Genomic DNA ScreenTape assay in allowing the analysis of DNA over a broad size range (100 bp to 60 kp) is a notable advantage of the 2200 TapeStation in optimizing fragmentation parameters.

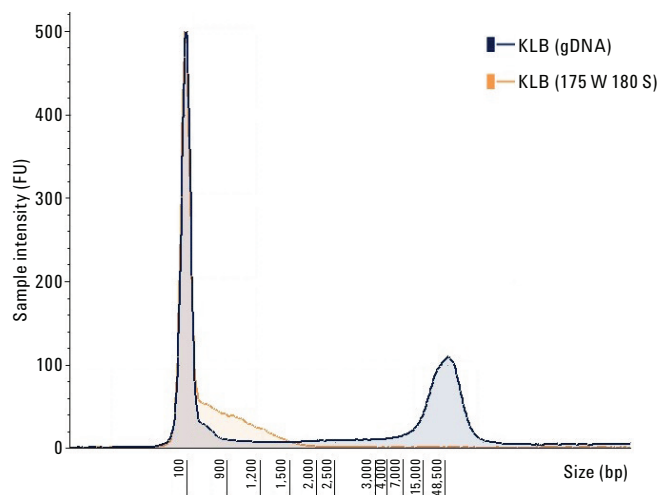


Figure 5. Overlaid Agilent 2200 TapeStation electropherograms of gDNA from *Klebsiella* sp. (KLB) before and after fragmentation. The 100 bp peak is an internal standard. An Agilent Genomic DNA ScreenTape assay was used to acquire the data.

## Conclusion

Shearing bacterial gDNA prior to library construction is a critical step in NGS workflow. Settings of Covaris E220 instrument used to shear gDNA were optimized to maximize the quantity of DNA fragments in the target size range (150–350 bp). Quantification and sizing of DNA fragments were assessed using an Agilent 2200 TapeStation and Agilent High Sensitivity D1000 ScreenTape assay. An Agilent Genomic DNA ScreenTape assay was also used to confirm the efficiency of the process in shearing all gDNA. The Agilent 2200 TapeStation system is suitable to determine size distribution after fragmentation of gDNA in a 96-well plate format. This is a format suitable for a high-throughput workflow and perfectly compatible with Covaris technology that could work in 96-well plate format.

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