Shearing DNA with Confidence: A Robust Method for Generating 3 kb DNA

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Introduction

Implementation of Next Generation Sequencing (NGS) technologies enabled an evolution in the field of medical genetics. However, the short read lengths of currently used sequencing approaches can limit the identification of structural variants, sequencing repetitive regions, and distinguishing highly homologous genomic regions. These limitations may impede the accuracy of optimizing or developing targeted treatments for people with genetic disorders who have undergone standard NGS, like whole exome or even genome sequencing. Over the past decade, long-read DNA sequencing technologies have emerged as a powerful player in genomics, offering significant improvements in characterization of genetic variation and regions that are difficult to assess with prevailing NGS approaches. For long-read sequencing (also referred to as third generation sequencing), the input DNA should be fragmented into shorter sections prior to sequencing with the fragment length depending on the requirements of the sequencing platform. However, and despite all the benefits offered by long read sequencing, it can be challenging to develop a robust, reliable, and reproducible and easy to implement assay, regardless of the user expertise.

Getting to 3 kb DNA

Along with long-read sequencing, 3 to 5 kilo base-pairs (kb) has been used for research and in precision medicine. Until recently, while DNA sequencing technologies produced both short (up to 300 bases at 99% accuracy)[1,2] and long reads (10 to 100 kb at 75 to 90% accuracy),[3,4] those workflows were not amenable to automation, hence, failed to address throughput requirements. Highly accurate short reads are used for several applications; however, error-prone long reads are more appropriate for de novo genome assembly, haplotype phasing, structural variant identification, full-length mRNA sequencing, and mRNA isoform discovery. To increase the utility of noisy long-read sequencing, 3 to 10 kb DNA was reported to be optimal.[5] Similarly, *Thorstenson et. al*, reported an automated hydrodynamic process for controlled, unbiased DNA shearing with 3 to 5 kb DNA.[6]

Challenges

While 3 to 5 kb DNA offers several advantages to the accuracy of long-read sequencing applications, it can be challenging to generate high-throughput and automation-friendly DNA shearing in this range. Adaptive Focused Acoustics® (AFA®) technology has long been used for DNA shearing and is rated by many as the Gold Standard for DNA shearing, primarily for NGS applications. In this study, we report development of a workflow for robust, reliable, and reproducible generation of 3 kb DNA enabled by AFA technology. The method reported in here is a high throughput protocol compatible with most automated liquid handlers.

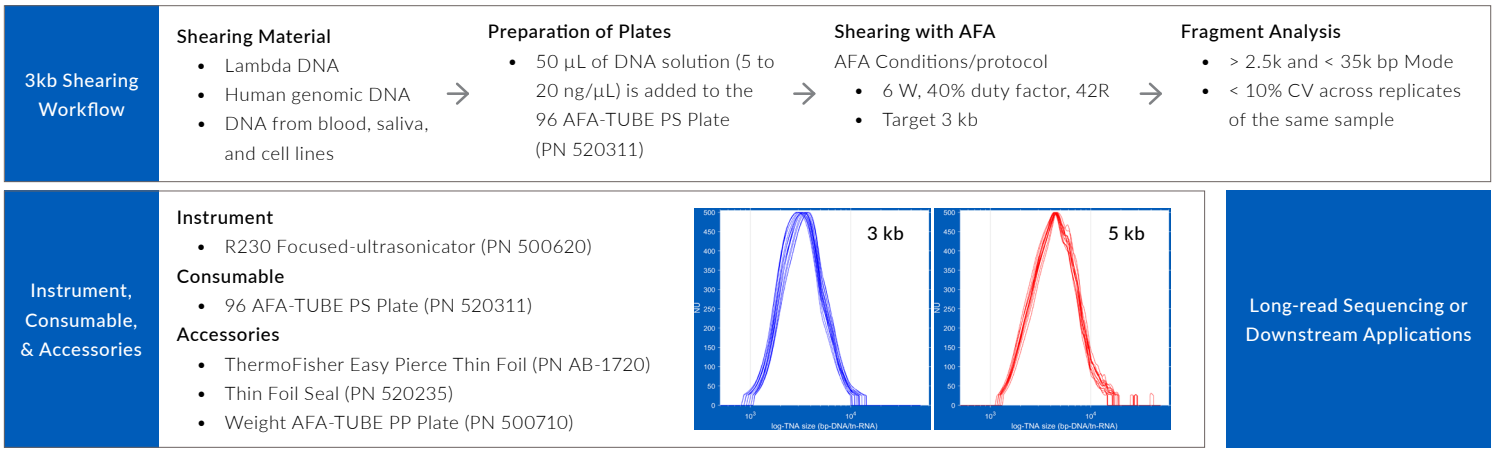

Figure 1. Overview of 3 kb DNA shearing workflow on the R230 Focused-ultrasonicator. A generalized workflow depicting the sequential steps involved in shearing process (top) and the required consumables and accessories (bottom) are designed for batch processing and are engineered for use with AFA Technology.

Materials and Methods

Preparation of Extracted Samples

Lambda DNA (NEB, N3011L), Human Genomic DNA (Promega, G3041) were diluted in 10 mM Tris-HCl 1 mM EDTA to concentrations between 5 ng/µL and 20 ng/µL. - DNA from saliva and blood samples stored in 10 mM Tris-HCl were diluted using 10 mM Tris-HCl 1 mM EDTA to 10 ng/µL.

Preparation of Plates

Fifty (50) µL of the diluted DNA solutions were added to each well of the 96 AFA-TUBE PS Plate (PN 520311) by piercing the seal with a 200 µL pipette tip, without ejecting the beads from the well. An adhesive foil seal was then used to re-seal the pierced wells. The plates containing samples were then centrifuged at 1600 x g for 60 seconds.

DNA Shearing with AFA Technology

The 96 AFA-TUBE PS Plate was then placed on the R230 Focused-ultrasonicator (PN 500620) and the Weight AFA-TUBE PP Plate (PN 500710) was placed on top of the consumable. The samples were sheared using the "*R230 96 AFA-TUBE PS Plate 3 kb Shearing*" method.

Fragment Size Determination

Control Samples were normalized to 2.5 ng/µL and measured for fragment distribution using the Fragment Analyzer High Sensitivity Large Fragment Kit (Agilent, DNF-493 (obsolete)), or Fragment Analyzer High Sensitivity Large Fragment 50 kb Kit (Agilent, DNF-464). Extracted samples were measured for fragment distribution at their sheared concentrations on the Fragment Analyzer. Smear Analysis was used in Prosize 4.0 (Agilent) to determine the Mean Fragment Size and % of DNA between 15 and 15,000 bp.

Results and Discussion

In this study, we optimized the AFA conditions and validated consumables to produce DNA size distributions with the mean fragment size of 3 kb. Purified DNA in 10 mM Tris-HCl with 1 mM EDTA buffer was used as a sample in a 96-well plate format with R230 Focused-ultrasonicator. DNA samples were sourced from multiple sources (purified control, cell-line, blood DNA) with a starting fragment size ranging between 10 and 40 kb. 3 kb fragment size was achieved using control samples (L-DNA, DNA from blood, saliva, and buccal swab) along with two different purification schemes (Chemagen and standard bead clean up) and with samples that were either newly extracted or few months old. DNA fragments within 10% deviation from the target size were generated with CV less than 15%. For the number of experiments that were conducted, 100% of 3 kb DNA were recovered from every experiment with CV less than 10%. The turnaround time was 140 minutes/plate, or less than 90 seconds/sample.

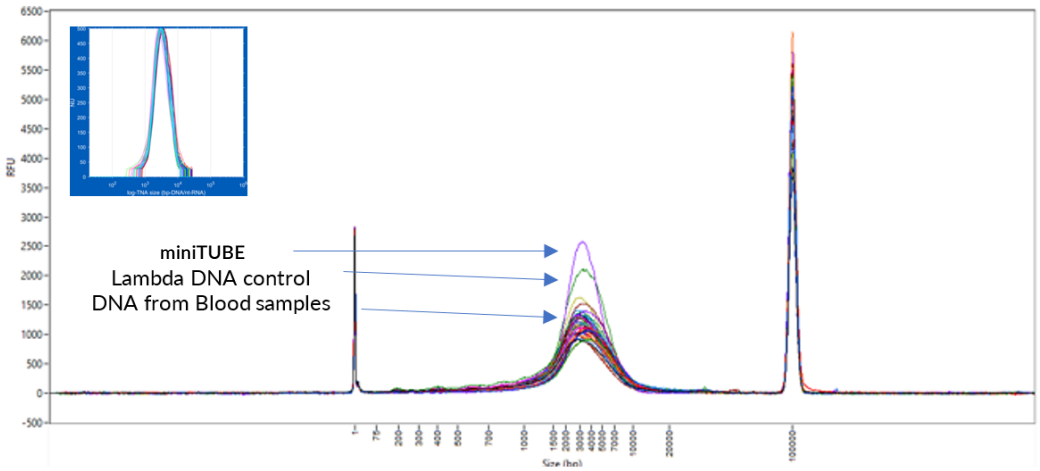

Figure 2. DNA shearing profile showcasing highly reproducible yield of 3 kb DNA obtained from different sample sources. Inset picture: 100% recovery of 3 kb DNA generation with remarkable reproducibility (%CV < 10).

Samples	Column	bp (Mode)	SD	%CV	% Target	Average (bp)
Saliva	c01	3333	351.5	10.5	111.1	N/A
Blood	c02	3158	262.5	8.3	105.3	N/A
Blood	c03	2940	215.5	7.3	98.0	N/A
Blood	c04	2890	220.4	7.6	96.3	N/A
Blood	c05	2946	151.0	5.1	98.2	3053.3

Table 1. List of 3 kb DNA generated from multiple samples along with their %CV and % value of target that was recovered.

Conclusion

Sheared DNA (3 to 5 kb) has been deemed as optimal by several research laboratories for genomic studies, primarily for human genetics research, including genome assembly, disease association and to the detection of larger sequence changes. Such consistent sizes will expand the breadth of applications on Illumina, Oxford Nanopore and PacBio platforms by enabling the most comprehensive view of the genome, including SNPs, indels and structural variants. However, generating 3 kb DNA from a variety of sources can be challenging. In this study, we report a robust, reliable, reproducible, and high-throughput assay in the ANSI/SBS format 96 AFA-TUBE PS Plate enabled by AFA technology. This method of generating 3 kb DNA also ensures faster turn-around-time compared to most other published and/or available methods, which can enable every single genomics laboratory regardless of their throughput requirements and user expertise.

Acknowledgements

The contributions and support of Zhenxi Zhang, PhD, (Laboratory Corporation of America Holdings) are gratefully acknowledged. He provided clinical specimens (e.g. blood, saliva) as well as valuable feedback on the development and optimization of the AFA Methods, contributed to the results shown in ***Figure 1*** and ***Table 1***, and helped improve the Focused-ultrasonicator controller software.

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