



Fast, High-Resolution DNA Sizing with the Fragment Analyzer™ System

Accurately size DNA up to 50 kb in 1 hour for large-insert SMRTbell™ libraries

The Fragment Analyzer™ instrument is a fast, high-resolution benchtop capillary electrophoresis (CE) platform that utilizes proprietary markers to accurately size fragments ranging from 10 bp up to 50 kb. This platform allows important DNA quality checkpoints to be completed in 1 hour for *de novo* large-genome sequencing projects and other PacBio® applications leveraging multi-kilobase read lengths. The instrument can be used in place of time-consuming QC steps involving Pulsed Field Gel Electrophoresis (PFGE), saving time by avoiding multiple overnight gel runs when preparing large-insert SMRTbell libraries. Alternative DNA-sizing instruments cannot accurately resolve large DNA fragments in this range.

- Reduce important DNA quality checkpoints down to 1 hour
- Accurately size DNA fragments up to 50 kb with proprietary markers
- Conserve sample for sequencing with minimal 2 ng input requirement
- Improve overall workflow efficiency for large-insert SMRTbell library preparation

Introduction

Accurate DNA quality measurements of large fragments are needed to optimize project outcomes and maximize sample recovery with long-read SMRT® Sequencing. The Fragment Analyzer instrument leverages capillary electrophoresis across the widest separation range to resolve genomic DNA up to 50 kb in 1 hour. This is especially useful for several PacBio applications requiring information contained within multi-kilobase reads to characterize complex structural variations, phase SNPs, infer haplotypes and span highly repetitive regions. Spanning long, complex repeat structures is necessary to obtain high-quality reference genomes with megabase contiguity. With 1 hour runs, the Fragment Analyzer System (FA) offers improved workflow management to achieve large-insert size-selected SMRTbell libraries ready for sequencing in reduced time.

The Fragment Analyzer System improves SMRTbell library workflow at key quality checkpoints

Multiple critical quality control checkpoints are recommended when preparing large-insert SMRTbell libraries, and the FA offers a time-saving alternative to overnight PFGE runs while still preserving the ability to accurately size large fragments (Table 1).

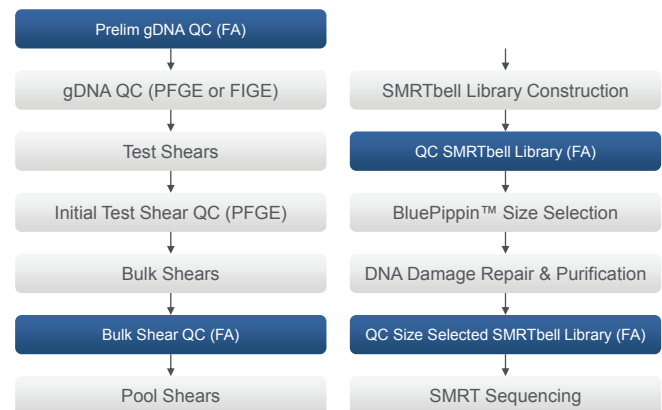


Figure 1 - PacBio large-insert library construction workflow overview. FA sizing replaces PFGE to complete critical QC checkpoints in 1 hour (blue boxes).

The FA can be used to quickly assess the integrity of genomic DNA, confirm bulk shears, determine appropriate size-selection thresholds, and conduct final QC before preparing libraries for SMRT Sequencing. While pulsed-field gel electrophoresis (PFGE) is still recommended to characterize high-molecular-weight DNA for shearing, the FA can be used in place of PFGE for improved expediency by eliminating overnight gel runs once samples have been sheared to the desired range (Figure 1).

	PFGE	Fragment Analyzer
Size Resolution	up to 10 Mb	up to 50 kb
DNA Input	3 µg	2 ng
Time	16 Hr	1 Hr
Gel Staining	Yes	No
Accuracy	High	High
Quantification	No	Yes

Table 1 - Performance comparison of PFGE and FA.

Rapid run-times with minimal sample loss during DNA fragment QC

With a one-hour run time, the FA system offers an attractive solution to quickly size DNA smears using only 2 ng of sample, especially to inform key decision steps for size-selection of SMRTbell libraries (Table 1). Shown here are two examples where the FA is used to assess the degree of degradation of genomic DNA samples and define optimum size-selection thresholds to prevent inadvertent sample loss on the Sage Science BluePippin™ system (Figure 2). Accurate DNA sizing with the FA informs the appropriate follow-up steps for sample handling without having to wait for an overnight PFGE run to complete. As a final QC, size-selected SMRTbell libraries can also be quantitatively sized with the FA instrument to confirm distribution profiles and obtain precise sizing information important for preparing samples for sequencing. The accompanying PROsize™ software also provides additional ease-of-use features to overlay sample profiles for visualization.

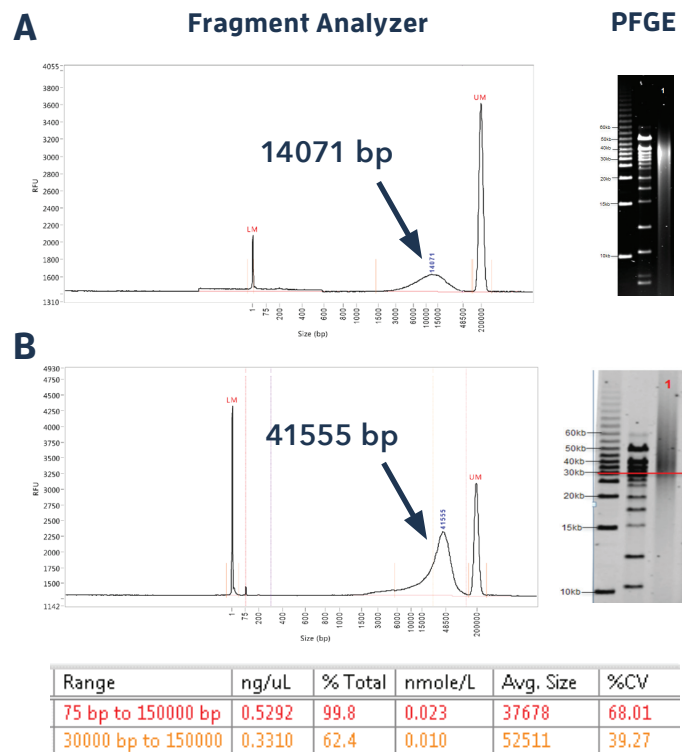


Figure 2 - FA plots with corresponding PFGE results at two DNA QC checkpoints to determine appropriate follow-up steps. (A) Initial sample screen detection for sample degradation with fragments <50 kb. (B) SMRTbell library QC prior to size-selection to determine appropriate thresholds. QC run shows 62.4% of templates fall in the range of 30 to 150 kb and are 52.51 kb in length on average.

Product Recommendations and Specifications

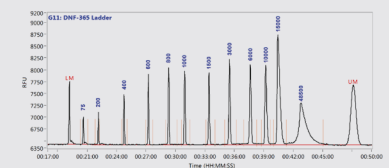
DNF-464 High Sensitivity Large Fragment 50 kb Analysis Kit

- Sizing Range: 75 bp - 50,000 bp
- Optimum input conc. for maximum sizing accuracy: 1 ng/µl

Compatible with all versions and capillary array format options of the FA. Customers need to run updated Fragment Analyzer software (Prosize 2.0.0.57), available from AATI website or support@aati-us.com.



Fragment Analyzer™ Automated CE System



References

1. Fragment Analyzer product page
2. Preparing >30 kb SMRTbell Libraries Using the Megaruptor Shearing and BluePippin Size-Selection System