PACBIO®

Fast, High-Resolution DNA Sizing with the Agilent 5200, 5300, and 5400 Fragment Analyzer Systems

Agilent | Trusted Answers

Accurately size DNA up to 50 kb in One hour for large-insert SMRTbell® libraries

The Agilent 5200, 5300, and 5400 Fragment Analyzer systems are fast, high-resolution benchtop capillary electrophoresis (CE) platforms that utilize proprietary markers to accurately size fragments ranging from 10 to 50 kb. This platform allows important DNA quality checkpoints to be completed in one hour for *de novo* whole 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 one 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 Single Molecule, Real-Time (SMRT®) Sequencing. The Fragment Analyzer systems leverage capillary electrophoresis across the widest separation range to resolve genomic DNA up to 50 kb in one 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 one hour runs, the Fragment Analyzer system offers improved workflow management to achieve large-insert size-selected SMRTbell libraries ready for sequencing in reduced time.

The Fragment Analyzer systems improve SMRTbell library workflow at key quality checkpoints

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

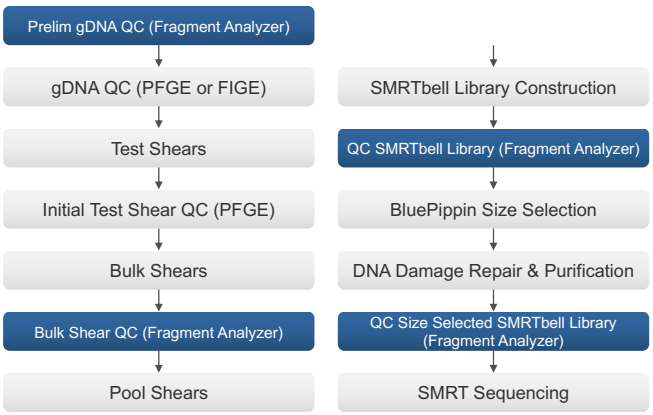

Figure 1. PacBio large-insert library construction workflow overview. *Agilent Fragment Analyzer system sizing replaces PFGE to complete critical QC checkpoints in one hour (blue boxes).*

The Fragment Analyzer systems 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 PFGE is still recommended for characterizing high-molecular-weight DNA for shearing, the Fragment Analyzer systems 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).

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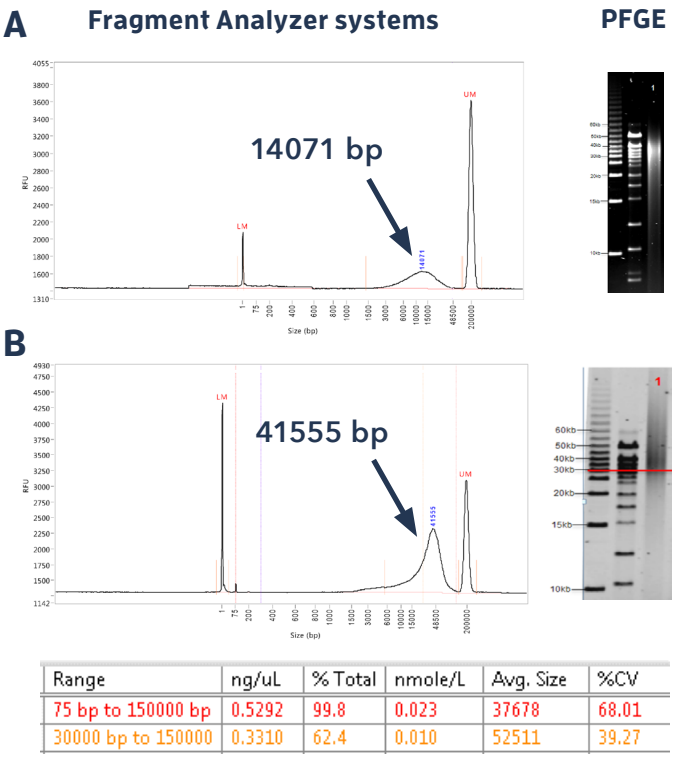
	PFGE	Fragment Analyzers
Size Resolution	Up to 10 Mb	Up to 50 kb
DNA Input	50 ng	2 ng
Time	16 hr	1 hr
Gel Staining	Yes	No
Accuracy	High	High
Quantification	No	Yes

Table 1. *Performance comparison of PFGE and Fragment Analyzer systems.*

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

With a one-hour run time, the Fragment Analyzer systems offer 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 Fragment Analyzer systems are 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 Fragment Analyzer systems 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 Fragment Analyzer systems to confirm distribution profiles and obtain precise sizing information important for preparing samples for sequencing. The accompanying ProSize data analysis software also provides additional ease-of-use features to overlay sample profiles for visualization.



Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
75 bp to 150000 bp	0.5292	99.8	0.023	37678	68.01
30000 bp to 150000	0.3310	62.4	0.010	52511	39.27

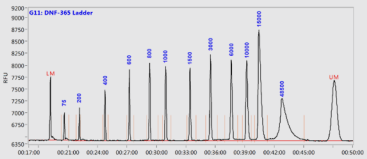
Figure 2. Agilent Fragment Analyzer system plots with corresponding PFGE results at two DNA QC checkpoints to determine appropriate follow-up steps. *(A) Initial sample screen detection for sample degradation with fragments <50 kb. (B) SMRTbell library QC prior to size-selection to determine appropriate 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 HS Large Fragment 50 kb kit

- Sizing range: 75 to 50,000 bp
- Optimum input concentration for maximum sizing accuracy: 1 ng/µL

Compatible with all versions and capillary array format options of the Fragment Analyzer systems. Customers should run updated Fragment Analyzer control software and ProSize versions, available from the Agilent website or electrophoresis@agilent.com.



Fragment Analyzer Systems

References

1. Fragment Analyzer product page
2. Procedure & Checklist - Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0

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