

Fast, High-Resolution DNA Sizing with the Agilent Femto Pulse System



Accurately size high molecular weight DNA smears up to 165 kb in under 1.5 hours for generating large-insert SMRTbell libraries

The Agilent Femto Pulse system is a fast, high-resolution automated capillary electrophoresis (CE) platform that utilizes pulsed-field electrophoresis to separate high molecular weight DNA fragments. This platform allows important DNA quality checkpoints to be completed in less than 1.5 hours with minimal sample input for *de novo* large genome sequencing projects and other PacBio® applications leveraging multi-kilobase read lengths. The instrument can be used in place of gel-based pulsed-field electrophoresis (PFGE) systems to fully support generation of large-insert SMRTbell® libraries with accurate sizing to 165 kb. Alternative DNA sizing instruments cannot accurately resolve large DNA fragments in this range.

- Complete critical DNA quality checkpoints in under 1.5 hours
- Accurately size DNA fragments up to 165 kb
- Conserve sample with femtogram-level DNA input as low as 50 fg/μL for fragments and 5 pg/μL for genomic DNA smears
- 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 Femto Pulse system leverages capillary electrophoresis across the widest separation range to resolve high molecular weight genomic DNA up to 165 kb in under 1.5 hours. 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 multi-megabase contiguity. The Femto Pulse system offers a simplified workflow to generate large-insert size-selected SMRTbell libraries for sequencing in reduced time, and conserves sample by using femtogram range of input DNA.

The Femto Pulse system reduces QC time for a simplified SMRTbell library workflow

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

The Femto Pulse system can be used in place of PFGE to quickly assess the initial integrity of genomic DNA, evaluate shears, determine appropriate size-selection thresholds, and conduct final QC before preparing libraries for SMRT Sequencing (Figure 1).

	PFGE	Femto Pulse
Size Resolution	up to 10 Mb	up to 165 kb
Run Time	up to 20 hr	<1.5 hr
DNA Input	>50 ng	500 pg
Gel Staining	Yes	No
Accuracy	High	High
Quantification	Not automated	Automated

Table 1. Performance comparison between PFGE and Agilent Femto Pulse system.

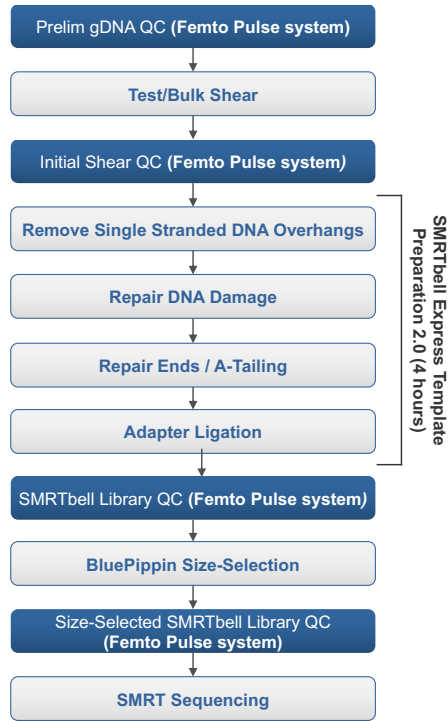


Figure 1. Large-insert library construction workflow with SMRTbell Express Template Prep Kit 2.0. Sizing with the Agilent Femto Pulse system replaces all PFGE to complete critical QC checkpoints in less than 1.5 hours (blue boxes).

Rapid run-times with minimal sample loss during genomics DNA and library QC

The Femto Pulse system quickly sizes DNA smears with high accuracy in under 1.5 hours using only 2 μ L of sample at 5 to 500 μ g/ μ L concentration (Table 1). Shown here are examples where the Femto Pulse system is used to assess the degree of genomic degradation and define optimum size-selection thresholds to prevent inadvertent sample loss on the Sage Science BluePippin system (Figure 2). Accurate DNA sizing with the Femto Pulse system informs appropriate follow-up steps for sample handling without having to wait for an overnight PFGE run to complete. As a final QC step, distribution of size-selected SMRTbell libraries are accurately quantified with the Femto Pulse system to prepare samples for sequencing. The accompanying Agilent ProSize data analysis software provides powerful analysis of data for automated quantification of fragment smears, with additional ease-of-use features to overlay sample profiles for visualization.

References

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

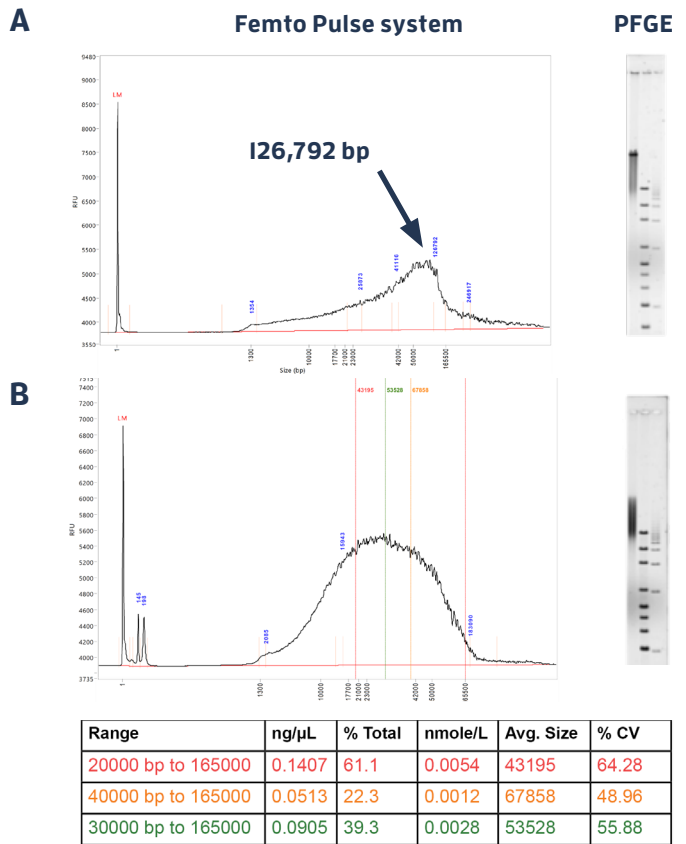


Figure 2. Electropherograms from the Agilent Femto Pulse system with corresponding PFGE results for several DNA QC checkpoints. (A) Initial genomic DNA QC with average fragment size >100 kb. (B) Large-insert SMRTbell library with no size-selection. Smear analysis allows selection of a particular size range to determine estimated percent and average insert size of the smear. In this example, 61.1 % of the smear is within 20 to 165 kb range, with average length of 43,195 bp as shown in the Smear Analysis table.

Product Recommendations and Specifications

FP-I002 Genomic DNA 165 kb kit

- Sizing Range: 1.3 to 165 kb
- Optimum input concentration for maximum sizing accuracy:
 - DNA fragments (0.3 to 30 μ g/ μ L);
 - DNA smear (5 to 500 μ g/ μ L)

Customers need to run updated data processing software (ProSize 3.0), available from from Agilent website or electrophoresis@agilent.com

Femto Pulse system