

Product note

FAST, ACCURATE DNA SIZING WITH THE AGILENT FEMTO PULSE SYSTEM FOR HIFI WGS

Improve your HiFi WGS sequencing workflow with the Femto Pulse system

Getting the most out of your long-read whole genome sequencing (WGS) workflow requires accurately characterizing the size distribution of your genomic DNA and SMRTbell® libraries. The **Agilent Femto Pulse system** is the recommended quality control (QC) technology for PacBio long-read sequencing using the new **SMRTbell prep kit 3.0** workflow.¹ There are 3 recommended QC checkpoints: genomic DNA, post-shearing, and final SMRTbell library (figure 1).



Key benefits of the Agilent Femto Pulse system

- High-resolution DNA sizing through 165 kb in under 1.5 hours
- Ultra-sensitive analysis using only picogram amounts of DNA
- Reduced project cost with accurate genomic DNA and SMRTbell library QC

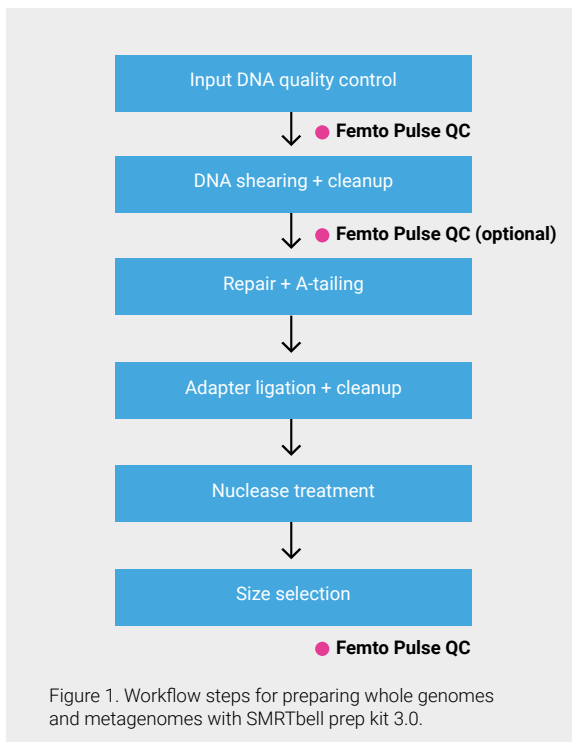


Figure 1. Workflow steps for preparing whole genomes and metagenomes with SMRTbell prep kit 3.0.

QC checkpoint: genomic DNA

Qualify genomic DNA samples by accurately measuring the degree of degradation (figure 2). For human, plant, and animal genomes, we recommend that 50% of the mass is greater than 30 kb and at least 90% is greater than 10 kb. For small genomes with fewer long repeats like microbial isolates or metagenomic samples, we recommend that 90% of the mass be greater than or equal to 7 kb.

	Human, plant, and animal	Microbial	Metagenomes
Genomic DNA QC	50% \geq 30 kb 90% \geq 10 kb	90% \geq 7 kb	90% \geq 7 kb

Table 1. Recommended QC thresholds for genomic DNA size distributions when using SMRTbell prep kit 3.0.

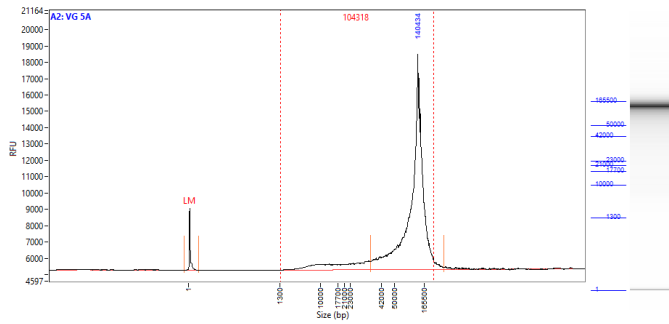


Figure 2. Genomic DNA quality check with Femto Pulse system for a human whole blood sample extracted using the Nanobind CBB kit.

QC checkpoint: DNA shearing

Verify that the sheared DNA has the appropriate size distribution for optimal HiFi sequencing. The Femto Pulse system is the best technology to accurately resolve fragments in the WGS range. Proceeding with DNA outside the recommended ranges (table 2) can result in lower yields either because the reads are too short and lead to lower genome coverage, or too long and thus do not receive sufficient sequencing passes for the minimum quality consensus.

	Human, plant, and animal	Microbial	Metagenomes
Target modal fragment lengths	15–18 kb	7–12 kb	7–12 kb

Table 2. Sheared DNA should resemble a bell-shaped distribution with a peak within the size ranges shown in the table below for the different types of genomes.

QC checkpoint: SMRTbell library

Perform a smear analysis on the Femto Pulse system to estimate the average molecular weight of your final SMRTbell library. Accurately estimating the molecular weight of your sample is necessary for preparing libraries for sequencing. Inaccurate sizing can result in inaccurate molarity, which affects SMRT® Cell loading and sequencing yields.

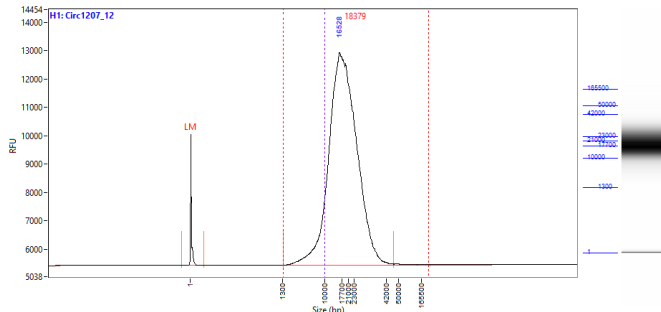


Figure 3. Electropherogram of the final SMRTbell library of the human whole blood sample shown in figure 2. A smear analysis between 1,300 and 165,000 bp demonstrates that the average mass of the library is close to 18 kb.



Agilent Femto Pulse system

Size resolution	Up to 165 kb
Run time	<1.5 hours
Samples per run	11
DNA input	500 pg
Recommended kit	FP-1002, Genomic DNA 165 kb kit

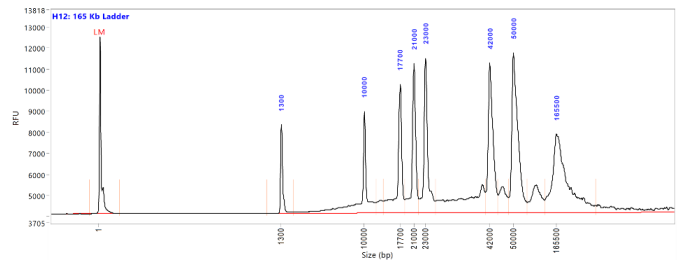


Figure 4. The FP 165 kb Ladder shows the ability of the Femto Pulse system to separate large DNA fragments up to 165 kb in 1.5 hours.

KEY REFERENCES

1. Procedure & checklist – Preparing whole genome and metagenome libraries using SMRTbell® prep kit 3.0 PacBio documentation.

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