Technical note

HIFI WGS PERFORMANCE WITH AMPURE PB BEAD SIZE SELECTION

AMPure® PB bead size selection is a simple, quick, and scalable approach to deplete molecules less than 5 kb in a SMRTbell® library. For high-quality DNA, the sequencing performance and application metrics for assembly and variant calling are comparable to gel cassette size-selection methods.

Key benefits of AMPure PB bead size selection

- Depletion of molecules below 5 kb
- High DNA recovery
- Lower DNA input requirements
- Fast workflow time
- Scalable
- No additional capital expenses
- Automation-friendly

Best practices for HiFi WGS with AMPure PB bead size selection

- Use protocol: **Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0**
- Use high-quality DNA samples
- QC input genomic DNA and final SMRTbell library on the Agilent Femto Pulse system
- Shear DNA to a modal size between 15 to 18 kb using the Diagenode Megarupter 3 system
- Use SMRTbell prep kit 3.0 (SPK3)
- Sequence using the latest software release

Results

Stat	HG002 (6 libraries, SMRT® Cells = 17)		
	HiFi Yield (Gb)	Mean HiFi read length (kb)	Median Read Quality
Min	28.5	12.7	Q32
Mean	35.8	13.8	Q34
Max	39.8	15.1	Q36
CV	8.7%	6.2%	2.7%

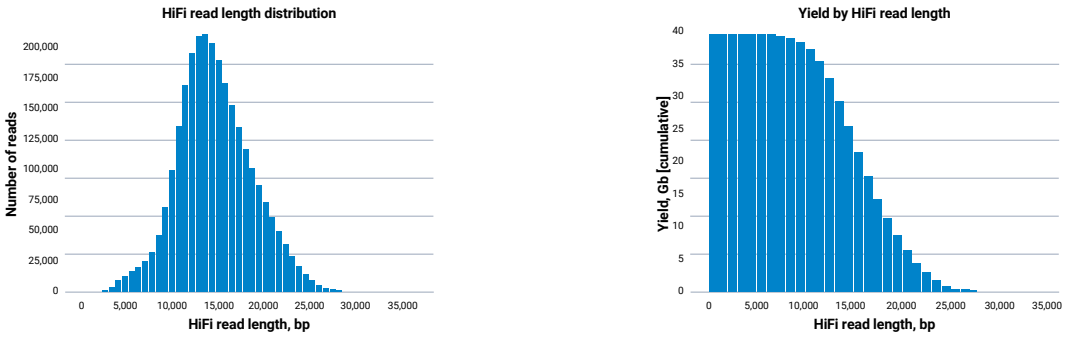
Table 1. Six SMRTbell libraries were prepared from HG002 human DNA following the protocol **Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0**, which utilizes size selection with AMPure PB beads. A total of 3 µg of genomic DNA was used per library and each library was sequenced across 3 SMRT® Cells except for one library that used only 2 SMRT Cells. Each library used an on-plate loading concentration of 85 pM.

Stat	Human whole blood 8-plex (SMRT Cells = 12)		
	HiFi Yield (Gb)	Mean HiFi read length (kb)	Median Read Quality
Min	24.3	12.4	Q33
Mean	30.0	13.6	Q35
Max	32.8	14.6	Q37
CV	8.2%	4.5%	3.3%

Table 2. A multiplex pool of 8 SMRTbell libraries prepared from human whole blood was sequenced across 12 SMRT Cells. The same workflow as described for HG002 was followed. Each multiplexed pool used an on-plate loading concentration of
85 pM.

PacBio

A. HG002 SMRTbell library



B. Human whole blood (multiplex of 8 samples) SMRTbell libraries

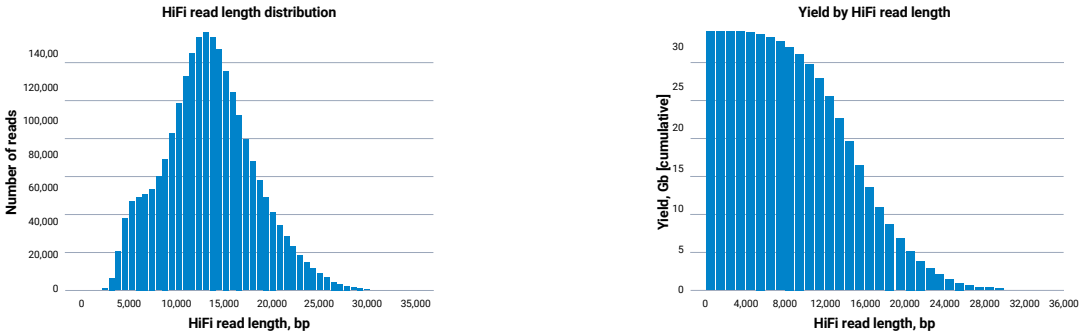


Figure 1. HiFi read length distribution and cumulative yield plots for samples prepared with SMRTbell prep kit 3.0 using AMPure PB bead size selection. Top row (A) shows the result for one of the six HG002 libraries while the bottom row (B) is the result for the multiplexed library of 8 multiplexed human whole blood samples. These read length distributions and cumulative yield plots are representative of the data generated from the other SMRT Cells.

	Metric	Library prep kit	
		SPK3	TPK2
Reads	Size selection method	AMPure PB beads	Gel cassette
	DNA input	3 µg	5 µg
	Coverage	32.2×	30.0×
	Average read length	12,824 bp	16,844 bp
Variant calling	SNV F1	99.9%	99.9%
	Indel F1	99.5%	99.3%
	SV F1	95.7%	95.0 %
Assembly	Primary contig N50	89.5 Mb	92.3 Mb
	Haplotig N50	503.6 kb	306.4 kb

Table 3. Sequencing and application performance for HG002 is comparable between the SMRTbell prep kit 3.0 workflow with bead-based size selection (SPK3) and the Express SMRTbell template prep kit 2.0 using a gel-based size selection method (TPK2), both sequenced with Sequel® II chemistry 2.0 and binding kit 2.2. Bead size selection leads to shorter average read lengths but has little impact on sequencing yields. Variant calling was performed with DeepVariant v1.2 (SNV, indel) and pbsv v2.8.1 (SV) and evaluated against Genome in a Bottle small variant benchmark v4.2 and SV benchmark v0.6. Assembly was performed with hifiasm v0.15.

Conclusions

Bead size selection has many advantages over gel size selection: high recovery, scalability, low cost, and no capital equipment requirement. While bead size selection does not provide a stringent size cutoff, sequencing yields and application performance are comparable to libraries selected with gel casettes, even with a lower average read length. For most cases, the benefits in improved workflow outweigh the small tradeoffs in read length. For high quality samples, PacBio recommends bead size selection.

102-326-525 REV01 30JUN2022