Procedure & Checklist – Using AMPure® PB Beads for Size-Selection

This procedure provides instructions for using AMPure PB beads for size-selection of SMRTbell libraries. AMPure PB beads are diluted with PacBio Elution Buffer to 35% (volume by volume) and then used for size-selection.

Important: To efficiently remove SMRTbell templates <3 kb or <5 kb, be sure to use this procedure **after** the first AMPure PB bead purification step (post-adapter ligation in the SMRTbell library construction workflow).

Required Materials

Item	Vendor	Part Number
AMPure PB Beads	Pacific Biosciences	100-265-900
Elution Buffer	Pacific Biosciences	101-633-500
15 mL High-Clarity Polypropylene Conical Tube	Corning	352096
Wide Orifice Tips (RT-LTS-A-200 µL-/F/L/W-960/10)	Rainin	30389241
1X dsDNA HS Assay kit and Qubit 2.0, 3.0 or 4.0 fluorometer	Thermo Fisher	Q33230

Dilute AMPure PB Beads with Elution Buffer

The final AMPure PB bead concentration is critical to the success of this procedure. Therefore, accurate pipetting is of utmost importance to achieve a final 35% (v/v) AMPure PB bead solution in Elution Buffer.

Reagent	Volume	✓	Notes
Elution Buffer	6.5 mL		
AMPure PB Beads (stock reagent)	3.5 mL		
Total Volume	10.0 mL		

1. Bring the AMPure PB bead stock to room temperature.
2. Vortex the stock solution for 30 seconds to mix well.
3. Using a P1000 pipette, transfer 6.5 mL of Elution Buffer into a 15 mL conical tube.
4. Add 3.5 mL of the stock AMPure PB beads to the Elution Buffer. When pipetting the viscous AMPure PB bead solution, pipette slowly to ensure that the volume aspirated is as precise as possible. Large residual AMPure PB beads adhering outside of the tip should be removed prior to adding to the Elution Buffer.
5. Vortex the diluted AMPure PB beads solution for 30 seconds to mix well before use. This solution may be stored at 4°C for 2 months for future use.

Size-selection using Diluted AMPure PB Beads

For effective size-selection using AMPure PB beads, accurate pipetting is necessary. Additionally, the DNA concentration of the SMRTbell library to be size selected must be **0.5–10 ng/µL**. Use of higher concentrations (>15-100 ng/µL) decreases the efficiency of reduction of short insert SMRTbell templates. Adjust the sample concentration so that the DNA concentration is within this range. The volume of diluted AMPure PB beads to use for size-selection depends on the desired molecular weight cutoff.
Please note that presence of ethanol in the sample impacts yield - therefore it is important to remove residual ethanol in the library prior to performing the procedure below.

Figure 1 shows the performance of diluted (35% v/v) AMPure PB beads for size-selection of a DNA sample with a concentration of 8 ng/µL at varying ratios of diluted AMPure PB bead volume:sample volume.

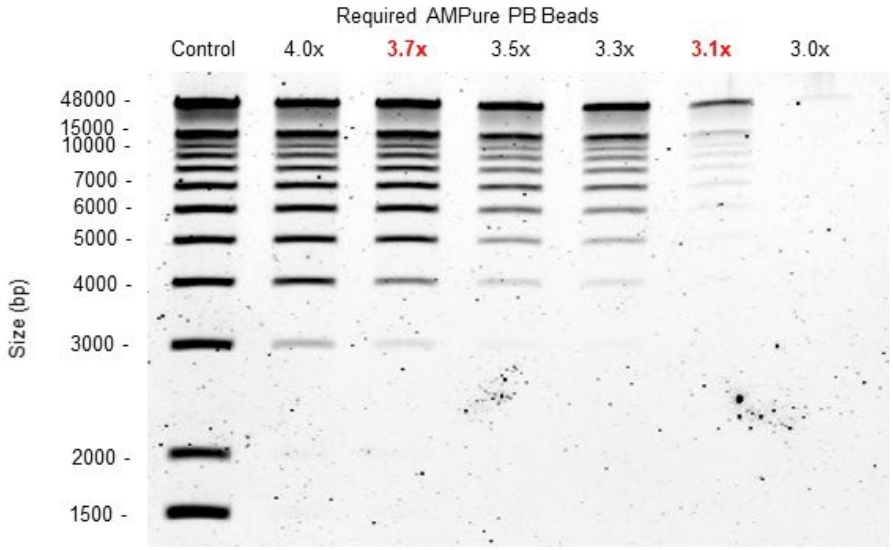

Figure 1: Performance of diluted AMPure PB beads for size-selection at varying ratios of diluted AMPure PB bead volume:sample volume. PacBio recommends using a 3.7X ratio of diluted beads:sample to remove <3 kb SMRTbell templates and a 3.1X ratio to remove <5 kb SMRTbell templates.

STEP	✓	Purify SMRTbell Templates	Notes
1		Before starting with the procedure, sample concentration must be between **0.5 – 10 ng/µL**. The most optimal concentration is 10 ng/µl. Dilute the sample if necessary.	
2		Measure the total sample volume accurately with a pipettor.	
3		To remove <3 kb SMRTbell templates, use 3.7X of diluted AMPure PB beads to the sample. To remove <5 kb SMRTbell templates, use 3.1X of diluted AMPure PB beads. **Important:** – It is critical to mix precise sample volumes and diluted AMPure PB beads to achieve successful size-selection. – When using 3.1X diluted AMPure PB beads, it is important to consider the total available library for size-selection. The 3.1X is an aggressive size-selection method which may impact recovery.	
4		Mix the bead/DNA solution thoroughly by pipette mixing 15 times with wide-bore pipette tips. For larger volumes, use a bigger tip. It is important to mix well.	
5		Quickly spin down the tube (for 1 second) to collect the beads.	
6		Incubate the mix on bench top for 15 minutes at room temperature.	
7		Spin down the tube (for 1 second) to collect beads.	
8		Place the tube in a magnetic bead rack to collect the beads to the side of the tube.	
9		Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the beads.	
10		Wash beads with freshly prepared 80% ethanol. Note that 80% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 80% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 80% ethanol against the side of the tube opposite the beads. – Do not disturb the beads. – After 30 seconds, pipette and discard the 80% ethanol.	
11		Repeat step 10.	
12		Remove residual 80% ethanol. – Remove tube from magnetic bead rack and spin. Both the beads and any residual 80% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. Beads will be on the side of the tube. – Pipette off any remaining 80% ethanol.	
13		Check for any remaining droplets in the tube. If droplets are present, repeat step 11.	

14		Add **10 µL** Elution Buffer volume to the beads. When adding 10 µL EB, dispense the volume directly to the beads. Do not let the beads dry. Pipette mix 15 times with wide-bore pipette tips. – Place at 37°C for 15 minutes to elute the DNA from the beads. – Spin the tube down, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the beads, transfer supernatant to a new 1.5 mL Lo-Bind tube. – Discard the beads.	
15		Verify total mass and concentration using a Qubit quantitation platform. – Using 1 µL of the purified sample, make a 1:10 dilution in EB. – Use 1 µL of this 1:10 dilution to measure the DNA concentration using a Qubit fluorometer and the dsDNA HS Assay kit according to the manufacturer's recommendations. – Use the remaining 9 µL of 1:10 diluted sample for DNA sizing QC.	
16		Purified SMRTbell libraries may be stored at -20ºC.	

Revision History (Description)	Version	Date
Initial release.	01	September 2019
Added note that measuring precise sample volumes and diluted AMPure PB beads is critical.	02	January 2020