Procedure & Checklist – AMPure® PB Purification of Polymerase Bound SMRTbell® Complexes for Diffusion Loading

Before You Begin

This procedure is to be used with the SMRTbell Express Sample Setup worksheet. It describes a procedure for purifying polymerase-bound SMRTbell complexes using AMPure PB beads for Diffusion loading. This purification protocol is currently recommended for large insert libraries. Typical complex recovery yield, post purification, is >50%.

Required Materials and Equipment

Item	Vendor	Part Number
MagBead Binding Buffer Kit v2	PacBio	101-046-400
AMPure PB Beads	PacBio	100-265-900
DNA LoBind 1.5 mL microcentrifuge tubes	Eppendorf	002-43-1021
Wide Orifice Tips	Rainin	17014294 (Tips LTS W-O 200UL Fltr RT-L200WFLR)
Magnetic Rack	Invitrogen	Customer's Choice

PN 101-348-800 Version 02 (December2017)

Purification Using AMPure® PB Beads

1. Equilibrate the AMPure PB beads and MagBead Binding Buffer v2 to room temperature.
2. Enter your sample information in the SMRTbell Express Sample Setup worksheet.
3. Per the recommendations in the SMRTbell Express Sample Setup worksheet, dilute the complex with MagBead Binding Buffer v2.
4. Measure the diluted complex concentration using a Qubit® ds DNA High Sensitivity kit.
5. Add 0.6X of AMPure PB beads and gently pipette-mix. Pipette-mixing with wide orifice pipette tips is recommended for libraries > 15 kb.
6. To bind the polymerase-bound complexes to AMPure PB beads, incubate the mixture on the benchtop for 5 minutes. **Note**: Longer incubation times have not been tested and may have a negative impact on polymerase-template complex stability due to high salt concentrations.
7. Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear. Discard the supernatant. **DO NOT** wash the collected beads with ethanol.
8. Immediately resuspend the beads with the MagBead Binding Buffer v2. The required elution volume is determined by the SMRTbell Express Sample Setup worksheet. Pipette-mixing with wide orifice pipette tips is recommended for libraries >15 kb.
9. Elute the polymerase-bound complexes by incubating the sample on the benchtop for at least 15 minutes at room temperature.
10. Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.
11. Transfer eluate to a new LoBind microcentrifuge tube. Place the tube (containing the now-purified polymerase-bound complexes) on ice. Discard beads.
12. Measure the recovered DNA concentration using a Qubit dsDNA High Sensitivity kit. Typical recovery is >50%.

Revision History (Description)	Version	Date
Updated to include references to SMRTbell Express Sample Setup worksheet instead of specifying volumes.	02	December 2017