Procedure & Checklist – AMPure® PB Bead Purification of Polymerase Bound SMRTbell® Complexes

Before You Begin

This document describes a procedure for removing excess polymerase and primer in the polymerase-bound SMRTbell complexes using AMPure PB beads. This procedure is also described in SMRT® Link Sample Setup (v5.1). It is recommended for all insert sizes.

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

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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 SMRT Link Sample Setup.
3. Per the recommendations in SMRT Link Sample Setup, dilute the complex with MagBead Binding Buffer v2.
4. Measure the concentration of the diluted complex using a Qubit® ds DNA High Sensitivity kit. Enter concentration and volume of diluted bound complex in SMRT Link Sample Setup.
5. Add 1.2X of AMPure PB beads and gently pipette-mix. Pipette-mixing with wide orifice pipette tips is recommended for libraries > 15 kb. For small insert libraries, flicking is sufficient but ensure that the sample/beads are mixed well.
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. **DO NOT** let the beads dry. The required elution volume is determined by SMRT Link Sample Setup. Pipette-mixing with wide orifice pipette tips is recommended for libraries >15 kb. For small insert libraries, flicking is sufficient but ensure that the sample/beads are mixed well.
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. Enter the volume and concentrations in SMRT Link Sample Setup.

Revision History (Description)	Version	Date
Updated to include references to SMRTbell Express Sample Setup worksheet instead of specifying volumes.	02	December 2017
Procedure is updated for consistency with the AMPure PB bead purification method outlined in SMRT Link v5.1 Sample Setup. The procedure now supports all insert sizes.	03	February 2018
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