Procedure & Checklist – Sample Purification Using SMRTbell® Clean Up Columns v2 for **MagBead Loading**

Before You Begin

This document describes a procedure for purifying polymerase-bound complexes, using SMRTbell Clean Up Columns v2, for MagBead loading in the Sequel System. This is recommended for libraries or amplicons ≥ 2 kb.
For < 2 kb SMRTbell templates, use Loading Clean-Up Beads. Note that the purification step is performed after polymerase binding to remove excess unbound polymerase and polymerase bound to small DNA inserts and adapter dimers. Typical complex recovery, post purification, is 70%-90%.

Materials Needed

Item	Vendor	Part Number
SMRTbell® Clean Up Columns v2 Kit-Mag* which includes: • SMRTbell Clean Up Columns v2 • MagBead Wash Buffer Kit v2	PacBio	101-303-600 101-175-400 101-046-500
DNA LoBind 1.5 mL mircocentrifuge tubes	Eppendorf	002-43-1021

*Note that you can also use the GE HealthCare Life Science MicroSpin S-400 HR (PN: 27-5140-01) for this procedure.

Required Equipment

Item	Vendor	Part Number
Microcentrifuge	Any MLS	
VWR MiniVortex	VWR	14005-824

*Centrifuge must have 700 X g capability. Note that a swinging bucket is not necessary. Be sure to adjust the speed, based on the rotor size, to meet the spin speed of 700 X g.

Storage

SMRTbell Clean Up columns should be stored at 4˚C.

Column Preparation

1. Equilibrate MagBead Wash Buffer v2 and columns at room temperature prior to use.
2. Resuspend the resin in the column by vortexing and inverting to completely resuspend the column matrix.
3. Loosen the cap one-quarter turn and twist off the bottom cap from the column.
4. Place the column in the supplied collection tube.
5. Centrifuge the column at 700 X g for 2 minutes.
6. Discard the collected buffer and place the column back in the collection tube. Proceed directly to the Buffer Exchange section.

Buffer Exchange

The buffer exchange in this protocol uses 600 µL of MagBead Wash Buffer v2 from the in the SMRTbell Clean Up Columns v2 Kit-Mag.

1. Remove the cap and add 300 µL of MagBead Wash Buffer v2 to the column matrix.
2. Cap the top end of the column.
3. Resuspend the resin in the column by vortexing and inverting to completely resuspend the column matrix.
4. Loosen the cap one-quarter turn and centrifuge at 700 X g for 2 minutes.
5. Discard the collected buffer and place the column back in the same collection tube.
6. Remove the cap and add 300 µL of MagBead Wash Buffer v2 to the column matrix.
7. Cap the top end of the column.
8. Resuspend the resin in the column by vortexing and inverting to completely resuspend the column matrix.
9. Loosen the cap one-quarter turn and centrifuge at 700 X g for 2 minutes.
10. Discard the collected buffer. The column matrix should appear semi-dry at this step.
11. Place the column in a clean 1.5 mL LoBind microcentrifuge tube and proceed directly to the Purification section (Using SMRTbell Clean Up Columns v2). Note that if the columns are not used immediately, the matrix may dry out.

Purification Using SMRTbell Clean Up Columns v2

1. Dilute the polymerase bound complex to at least 50 µL (up to 100 µL) with with MagBead Wash Buffer v2. Do not exceed 100 µL. Maximum capacity of the column is 100 µL. Save 1 µL of diluted bound complex for the Qubit® Fluorometer.

2. Carefully and slowly apply the sample to the top-center of the matrix, being careful not to disturb the matrix bed. Do not allow any sample to flow along the inner wall of the column. Any sample deposited on the tube wall will pass through the collection tube without filtration.

3. Centrifuge at 700 X g for 2 minutes.

4. Remove the column and LoBind microcentrifuge tube from the centrifuge. The purified sample is at the bottom of the microcentrifuge tube.

5. Place the sample in ice as soon as the sample is collected.

6. Check DNA concentration before and after column purification, using a Qubit Fluorometer. Measure the volume and calculate yield. As noted above, typical recovery is 70%-90%.

7. The purified sample is now ready for MagBead loading following the instructions in the Binding Calculator or Sample Set Up SMRT Link v5.0.

PN 101-118-700-02