Procedure & Checklist - Purification of Polymerase-Bound Complexes Using SMRTbell™ Clean Up Columns for Sequel™ MagBead Loading

Before You Begin

Performing purification using SMRTbell Clean Up Columns has shown to improve Sequel data quality. The purification step is performed after polymerase binding to remove excess unbound polymerases and polymerase molecules which are bound to small DNA inserts (e.g., 400 bp).

This protocol requires 80 µL of polymerase-bound complex. Typical complex recovery, post purification, is 50%-80%. If desired, increase the on-plate concentration in the Binding Calculator by 20-50% to take into account the anticipated loss.

Materials Needed

• SMRTbell™ Column Conditioning Buffer Kit	PN 100-912-000
• SMRTbell™ Clean Up Columns (5 each)	PN 100-912-100
• BD Falcon Round Bottom tubes (14 mL) or	352059 (Becton Dickinson)
• 15 mL Falcon™ Conical Tube	Any vendor
• Tweezers (may be required)	

Required Equipment

We recommend the following rotors to allow the sample to pass through the column matrix uniformly. Fixed-angle rotors force the sample to slide down the inner side of the column instead of passing through the gel matrix, resulting in reduced or inconsistent purification.

- Swinging bucket rotor or
- Any horizontal rotor
- Centrifuge tube adapter that holds 1.5 - 2.0 mL tubes or 14 mL round bottom or 15 mL Falcon Conical tubes.

Buffer Exchange

The Buffer exchange in this protocol uses 2.7 mL of SMRTbell Column Conditioning Buffer from PacBio.

1. Invert the Clean Up Columns several times to completely resuspend the column matrix.
2. Remove the top cap first and then the bottom cap from the column (note that the bottom cap is removed by twisting and pulling). Save the top cap. Place the bottom tip of the column gently into a 2 mL microcentrifuge tube provided.
3. Discard any buffer that immediately collects in the 2 mL microcentrifuge tube. Place the column into the same microcentrifuge tube.
4. Centrifuge the column in a swinging bucket rotor at 700 x g for 3 minutes. If a centrifuge tube adapter for 2.0 mL tubes is not available, use a tube adapter that can hold 14 mL or 15 mL Falcon tubes.
    a. For convenience, place the column and collection tube in a 14 mL or 15 mL Falcon tube for centrifugation.
    b. Use tweezers to insert and remove the column from the Falcon tube.
5. Discard the collected buffer from the 2 mL tube. Place the column back in the microcentrifuge tube.
6. Add 1 mL of SMRTbell Column Conditioning Buffer to the column matrix. Cap both ends of the column and resuspend the matrix completely by inverting the column several times. The bottom caps are provided with the kit.
7. Remove caps and place into a 2 mL tube and centrifuge at 700 x g for 3 minutes.
8. Discard the collected buffer in the 2 mL collection tube and place the column back in the same 2 mL tube. Note: Do not invert the column or resuspend the matrix at this stage.
9. Add 1 mL of SMRTbell Column Conditioning Buffer gently to the column matrix.
10. Centrifuge at 700 x g for 3 minutes.
11. Discard the collected buffer in the 2 mL collection tube and place the column back in the same 2 mL tube.
12. Add 700 µL SMRTbell Column Conditioning Buffer.
13. If the column will not be used immediately, it can be stored at 4°C for future use. For the procedure on how to store the columns, see Storing Conditioned Columns in the next section.
14. If column will be used immediately, spin for 700 x g for 2 minutes, and empty and replace the collection tube. Note: Do not invert the column or resuspend the matrix at this step.
15. Spin once more at 700 x g for 2 minutes and discard collected buffer. Column matrix should appear semi-dry at this step.
16. Place spin column in a clean 2 mL tube and proceed directly to Purification using Spin Columns.

Storing Conditioned Columns

1. If the column will be saved for later use, close the column with the bottom cap and replace the top cap tighly.
2. Invert the column several times to resuspend the column matrix.
3. Store the column at 4°C in an upright position. Do not freeze. Note that the columns can be stored for up to 24 hours (before use) with good results. Longer storage times, post buffer exchange, have not been tested.
4. When ready to use, equilibrate the column at room temperature. Centrifuge at 700 x g for 2 min, and empty and replace the collection tube.
5. Spin once more at 700 x g for 2 minutes and discard collected buffer. After centrifugation, the column matrix will appear semi-dry. This step purges the conditioning buffer from the column and reestablishes the matrix bed.
6. Remove the Clean Up Columns and collection tube from the centrifuge rotor, and discard the collection tube and conditioning buffer.
7. Once the column bed is established, place the Clean Up Column in a clean 2 mL microcentrifuge tube contained in the kit.
8. Go to the next section for sample clean up.

Purification using Clean Up Columns

1. Carefully and slowly apply the sample to the center of the gel bed's flat surface. Do not allow any sample to flow along the inner wall of the column.
   a. Recommended volume is 80 µL.
   b. Any sample deposited on the tube wall will not undergo filtration. Note: For particularly small sample volumes (e.g., <30 µL, a conventional, tapered 1.5 mL microcentrifuge tube can be substituted for the 2 mL collection tube. This will allow the sample to be confined to a narrower area for easier handling.
2. Centrifuge at 700 x g for 5 minutes.
3. Remove the Clean Up Columns and collection tube from the centrifuge. The purified sample is at the bottom of the collection tube.
4. Transfer purified sample to a LoBind tube and place in ice as soon as the sample is collected.
5. Optional: To determine recovery, check DNA concentration before and after column purification, using a Qubit® Fluorometer. As noted above, typical recovery is 50%-80%.
6. The purified sample is now ready for MagBead binding following the binding calculator protocol.

   Note: Purified samples have been stored at 4°C, for up to 48 hours, with good results.

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