Procedure & Checklist - Testing the MagBead Station Using 2 kb Lambda Library Complex

Before You Begin

This procedure provides instructions for diluting the 2 kb Lambda Library Complex, binding to MagBeads and sequencing in the PacBio® system. There are no performance specifications to meet other than to test the functionality of the MagBead Station. Low sequencing yield (<5000 reads) indicates that the MagBead Station is not spinning. If so, contact Pacific Biosciences by filing a case through the Portal.

The volume of sample prepared here is enough for 2 SMRT® Cells. We recommend running 1 to 2 SMRT Cells to verify that the Magbead Station is functioning.

Handle the 2 kb Lambda Library Complex with care and store at -20ºC at all times. Return to -20ºC immediately following use (it can then be stored for up to several months). Note that you should remove the Lambda Library Complex from -20ºC only when ready to take an aliquot (always keep the tube on ice).

- Lambda Library Complex (2 kb) PN 100-530-000
- MagBead Kit PN 100-133-600
- DNA Sequencing Kit PN 001-554-002
- SMRT Cells (8Pac) v3 PN 100-171-800
- Mixing Plate (384-Well Plate) PN 001-349-132
- Sample Plate Septa mat (96-Well) PN 001-378-690
- 96-Well PCR Plate PN 001-474-228
- 0.5 mL VWR®/Eppendorf® DNA LoBind tubes (used for all reactions) PN 001-474-555

Dilute the 2 kb Lambda Library Complex

The 2 kb Lambda Library Complex (6 nM) must be diluted to 15 pM (final concentration). Thaw and vortex the MagBead Wash Buffer and MagBead Binding Buffer, then follow the table below to dilute the Lambda Library Complex. A two-step dilution, is necessary.

First Dilution (to 500 pM)	Step
18.3 µL	Add the MagBead Binding Buffer to an empty LoBind microcentrifuge tube (label as #1).
1.7 µL	Add the 2 kb Lambda Library Complex to the LoBind tube prepared above (#1) and mix well by tapping the tube and then doing a quick spin.
20 µL	Total Volume

Second Dilution (to 15 pM)	Step
49.5 µL	Add the MagBead Binding Buffer to an empty LoBind microcentrifuge tube (label as #2).
1.5 µL	Add the mix from tube #1 to tube #2 and mix well by tapping the tube and then doing a quick spin.
51.0 µL	Total Volume

Bind the Lambda Complex to MagBeads

STEP	✓	Prepare MagBeads	Notes
1		Mix beads in the stock bottle by mixing vigorously (30 seconds on vortexer at high speed). Transfer 74.0 µL of MagBeads to an empty LoBind microcentrifuge tube. Note that you can spin down contents of the tube with a quick spin. The beads will pellet at the bottom but are collected on the side of the tube when placed in the magnetic rack.	
2		Place the tube in a magnetic bead rack until the beads collect to the side of the tube (approximately 30 seconds).	
3		Slowly remove cleared supernatant and discard. Avoid disturbing the bead pellet.	
4		Remove the tube from the magnetic bead rack, and add 74.0 µL of the MagBead Wash Buffer. – When adding the MagBead Wash Buffer, be sure to dispense it on the pellet to wash the beads. – Mix by tapping the tube gently so that the beads are in solution.	
5		Place the tube in a magnetic bead rack until the beads collect to the side of the tube (which typically takes 30 seconds).	
6		Slowly remove cleared supernatant and discard. Avoid disturbing the bead pellet.	
7		Remove the tube from the magnetic bead rack and add 74.0 µL MagBead Binding Buffer. – When adding the MagBead Binding Buffer, be sure to dispense the buffer on the pellet to wash the beads. – Mix by tapping the tube gently so that the beads are in solution.	
8		Slowly remove cleared supernatant and discard. Avoid disturbing the bead pellet.	
9		Add 19 µL of the diluted 2 kb Lambda Library Complex (from tube #2 of Second on page 1) and mix gently by tapping the tube so that the beads are in solution.	
10		Place the tube in a rotator and incubate at 4ºC for 20 minutes (up to 2 hours).	

Sequence

STEP	✓	Prepare for Sequencing	Notes
1		Once the beads and complex are bound, do a quick spin to collect the beads at the bottom of the tube.	
2		Place the tube in magnetic bead rack until the beads collect to the side of the tube (approximately 30 seconds).	
3		Slowly remove cleared supernatant and discard. Avoid disturbing the bead pellet.	
4		Remove the tube from the magnetic bead rack, and add 19 µL of the MagBead Binding Buffer. – When adding the MagBead Binding Buffer, be sure to dispense it on the pellet to wash the beads. – Mix by tapping the tube gently so that the beads are in solution. Note that you can spin down contents of the tube with a quick spin. The beads will pellet at the bottom but are collected on the side of the tube when placed in the magnetic rack.	
5		Place the tube in a magnetic bead rack until the beads collect to the side of the tube (approximately 30 seconds).	
6		Slowly pipette off cleared supernatant and discard. Avoid disturbing the bead pellet.	
7		Remove the tube from the magnetic bead rack and add 19 µL of the MagBead Wash Buffer. – When adding the MagBead Wash Buffer, be sure to dispense the buffer on the pellet to wash the beads. – Mix by tapping the tube gently so that the beads are in solution.	
8		Place the tube in a magnetic bead rack until the beads collect to the side of the tube (which typically takes 30 seconds).	
9		Slowly remove cleared supernatant and discard. Avoid disturbing the bead pellet.	
10		Remove the tube from the magnetic bead rack, and add 95 µL of the MagBead Binding Buffer. This is enough for two SMRT Cells. – When adding the MagBead Binding Buffer, be sure to dispense it on the pellet to wash the beads. – Mix by tapping the tube gently so that the beads are in solution.	
11		Place the tube on ice until ready to use. For 1 SMRT Cell, transfer a 45 µL aliquot into the 96-well plate. For 2 SMRT Cells, transfer two 45 µL aliquots into two wells of a 96-well plate. Prior to transferring the beads to a 96-well plate, ensure the beads are well suspended. Sequencing complex-bound beads that are more than 1 day old is not recommended.	

For more information, refer to the *RS Remote Online Help* system or *Pacific Biosciences Software Getting Started Guide*. Follow the touchscreen UI to start your run.

Run Parameters for 1 SMRT® Cell

RS Remote Option	Selection
Protocol	MagBead OneCellPerWell v1
DNA Control Complex	None
Movie Time	1 x 30 for PacBio RS II
Insert	2000
# of SMRT Cells	1 to 2