

Before You Begin

To perform this procedure, you must have the PacBio®:

- MagBead Kit
- DNA Internal Control Complex

This procedure can be used to prepare MagBead-bound complexes for loading onto the instrument. The MagBead Kit can be used to sequence any library greater than 1 kb.

The SMRTbell™ complex must be first diluted at a concentration specified by the Binding Calculator before it can be bound to MagBeads. If a DNA Internal Control is required, it must also be diluted to an appropriate concentration as specified below.

| STEP | ✓ | Prepare Sample and DNA Internal Control | Notes |
|------|---|--|-------|
| 1 | | If required, dilute the DNA Internal Control (a 2-step dilution) using the MagBead Binding Buffer and the dilution set-up generated by the Binding Calculator. | |
| 2 | | Dilute the SMRTbell Complex using the MagBead Binding Buffer and the dilution set-up generated by the Binding Calculator. | |
| 3 | | Save both dilutions on ice until ready to use. | |

Wash MagBeads thoroughly prior to binding with the diluted complex. The MagBeads are washed in this order: MagBead Wash Buffer first, followed by Magbead Binding Buffer.

| STEP | ✓ | Prepare MagBeads for Binding | Notes |
|------|---|--|-------|
| 1 | | Resuspend the beads in the stock bottle by mixing vigorously (30 seconds on a VWR® vortex mixer at high speed). Before taking an aliquot of MagBeads from the stock bottle, mix contents thoroughly by vortexing the bottle. Follow the Binding Calculator output to add the appropriate volume of MagBeads to an empty LoBind microcentrifuge tube. Note that you can spin down contents of the tube with a quick spin. The MagBeads 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 rack until the MagBeads collect to the side of the tube (approximately 30 seconds). The supernatant should be clear before proceeding to the next step. | |
| 3 | | With the tube still on the rack, slowly aspirate cleared supernatant and discard. Avoid disturbing the MagBeads. | |

| STEP | ✓ | Prepare MagBeads for Binding | Notes |
|------|---|---|-------|
| 4 | | Remove the tube from the magnetic rack, and add MagBead Wash Buffer. <ul style="list-style-type: none"> – Resuspend the MagBeads by flicking the tube gently until the MagBeads are in solution. – Quickly spin down the tube. | |
| 5 | | Place the tube in a magnetic rack until the MagBeads collect to the side of the tube (approximately 30 seconds). The supernatant should be clear before proceeding to the next step. | |
| 6 | | Slowly aspirate the cleared supernatant and discard. Avoid disturbing the MagBeads. | |
| 7 | | Remove the tube from the magnetic rack, and add MagBead Binding Buffer. <ul style="list-style-type: none"> – Resuspend the MagBeads by flicking the tube gently until the MagBeads are in solution. – Quickly spin down the tube. | |
| 8 | | Place the tube in a magnetic rack until the MagBeads collect to the side of the tube (approximately 30 seconds). | |
| 9 | | Remove the cleared supernatant. Avoid disturbing the MagBeads. | |

Once washed and prepared, the MagBeads are ready for binding with the diluted complex.

| STEP | ✓ | Bind SMRTbell™ Templates to MagBeads | Notes |
|------|---|---|-------|
| 1 | | Ensure that the supernatant is removed. | |
| 2 | | Add the diluted complex mix to the MagBeads and mix gently by flicking the tube until the MagBeads are in solution. Avoid splashing to the side of the tube. | |
| 3 | | Place the tube in a rotator and incubate at 4°C for 20 minutes (up to 2 hours). | |

The SMRTbell-bound MagBeads must be washed thoroughly prior to loading on the instrument. The wash step requires washing MagBeads with the supplied Buffers in this order: MagBead Binding Buffer, MagBead Wash Buffer and MagBead Binding Buffer.

If required, the DNA Internal Control can be added here.

| STEP | ✓ | Wash MagBead-SMRTbell Complex | Notes |
|------|---|---|-------|
| 1 | | Remove the tube from the rotator and quick spin the tube. | |
| 2 | | Place the tube in magnetic rack until the MagBeads collect to the side of the tube (approximately 30 seconds). | |
| 3 | | Optional (but recommended if this is the first time this preparation is being attempted): you can set aside 5 µL of the supernatant to measure concentration with a Qubit® system. Note that the concentration should be close to zero. | |
| 4 | | Slowly aspirate cleared supernatant and discard. Avoid disturbing the MagBeads. | |

| STEP | ✓ | Wash MagBead-SMRTbell Complex | Notes |
|------|---|--|-------|
| 5 | | Remove the tube from the magnetic rack, and add MagBead Binding Buffer. <ul style="list-style-type: none"> – Resuspend the MagBeads by flicking the tube gently until the MagBeads are in solution. – Quickly spin down the tube. | |
| 6 | | Place the tube in a magnetic rack until the MagBeads collect to the side of the tube (approximately 30 seconds). The supernatant should be clear before proceeding to the next step. | |
| 7 | | Slowly aspirate the cleared supernatant and discard. Avoid disturbing the MagBeads. | |
| 8 | | Remove the tube from the magnetic rack, and add MagBead Wash Buffer. <ul style="list-style-type: none"> – Resuspend the MagBeads by flicking the tube gently until MagBeads are in solution. – Quickly spin down the tube. | |
| 9 | | Place the tube in a magnetic rack until the MagBeads collect to the side of the tube (which typically takes 30 seconds). The supernatant should be clear before proceeding to the next step. | |
| 10 | | Slowly aspirate the cleared supernatant and discard. Avoid disturbing the MagBeads. | |
| 11 | | Remove the tube from the magnetic rack, and add the final MagBead Binding Buffer for loading on the instrument using either the MagBead Standard Protocol or MagBead OneCellPerWell Protocol. Final volumes are specified by the Binding Calculator. <ul style="list-style-type: none"> – Resuspend the MagBeads by flicking the tube gently until the MagBeads are in solution. – Quickly spin down the tube. | |
| 12 | | If required, add the DNA Internal Control previously diluted at the start of the procedure. Mix gently by flicking the tube. Quickly spin down the tube, if needed. | |
| 13 | | For MagBead Standard Protocol, transfer the entire volume in a well of a 96-well plate. For MagBead OneCellPerWell Protocol, transfer 45 µL to one or several wells of a 96-well plate. | |
| 14 | | The sample-bound MagBeads with DNA Internal Control are now ready to load onto the instrument. | |

List of Reactions Per MagBead Kit

Note that the total number of reactions depend upon your run set up or configuration of SMRT® Cells/sample wells. The number of reactions listed is based on the Magbead Standard Protocol selection in the Binding Calculator

| Run Set Up | Total Number of SMRT Cells Supported per Kit |
|---------------------|--|
| 1 SMRT Cell/Sample | 32 |
| 4 SMRT Cells/Sample | 52 |
| 8 SMRT Cells/Sample | 56 |

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