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HighPrep PCR PB

Cleanup and Size Selection for Long Read Sequencing

Catalog Nos. PB-60001, PB-60005, PB-60050
Manual Revision 0
WI-72-125

- Magnetic bead-based chemistry
- No centrifugation or filtration
- Efficient cleanup and size selection

Protocol

Contents

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Product Description

HighPrep PCR PB is a paramagnetic bead-based reagent used in amplicon cleanup, DNA concentration, and purification. It can also be diluted with low salt buffer (Elution Buffer) to 35% (v/v) and then used for size selection of SMRTbell™ libraries.

Preparation

- **DO NOT FREEZE**
- Keep at room temperature for 30 min. prior to use
- Thoroughly shake the HighPrep PCR PB to resuspend the beads before use
- HighPrep PCR PB diluted with Elution Buffer to 35% (v/v) is stable for 2 months when stored at 2-8°C

Materials Supplied in the Kit

- HighPrep PCR PB

Kit Contents

HighPrep PCR PB Catalog No.	PB-60001 (Sample)	PB-60005	PB-60050	Storage
HighPrep PCR PB[1] volume	400 µL	5 mL	50 mL	2-8°C
Elution Buffer volume	1 mL	Must be purchased separately. See ordering and related product information on page 8.		15-25°C

Shipping and Storage

- [1] HighPrep PCR PB ships at room temperature. Store at 2-8°C.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). The SDS can be downloaded from the "Product Documents" tab when viewing this product at www.magbiogenomics.com.

Protocol: Amplicon Cleanup for SMRTbell™ Library Preparation

HighPrep PCR PB is based on paramagnetic bead technology designed for efficient purification of amplicons prior to SMRTbell™ library preparation. The purification of amplicon template consists of removal of salts, PCR buffers, excess primers, primer-dimers, and dNTPs. DNA fragments are selectively bound to the magnetic bead particles, and highly purified amplicons are eluted with Tris-HCl buffer (10 mM Tris-HCl, pH 8.5 – pH 9.0) which can be used in library preparation.

Equipment and Reagents to Be Supplied by the User

- ☐ 80% Ethanol (Prepared from non denatured Ethanol)
- ☐ 70% Ethanol (Prepared from non denatured Ethanol)
- ☐ Elution Buffer (10 mM Tris-HCl pH 8.5-9.0) or MagBio Genomics' Elution Buffer (see page 8)
- ☐ Magnetic separation device compatible with 96 or 384 well PCR plate (see page 8)
- ☐ Magnetic separation device compatible with 1.5 mL/1.7 mL/2 mL microcentrifuge tubes (see page 8)
- ☐ 96-well PCR plate or 384-well PCR plate
- ☐ 1.5 mL LoBind microcentrifuge tubes
- ☐ Wide-bore pipette tips

Things to do Before Starting

- ☐ Use the table below to determine the HighPrep PCR PB bead ratio which is required to select the desired insert size range
- ☐ Make sure the HighPrep PCR PB is equilibrated at room temperature before use

Insert Size Range (bp)	HighPrep PCR PB Bead Ratio/Volume Needed Per Sample
100-300	1.8X*
301-750	1.0X
751-3,000	0.6X
3,001-10,000	0.45X
15,000	

* The X represents the volume of the PCR sample to be purified. The required volume of HighPrep PCR PB is calculated as follows: for a 100-300 bp insert size, required volume of HighPrep PCR PB = 1.8 X (PCR reaction volume)

Protocol

1. Mix the **HighPrep PCR PB** and amplicon based on the bead ratio in the above table. Mix thoroughly 10-15 times using wide-bore pipette tips.

   Tip: Pipette a minimum of 15 µL of beads in order to get the best results. When calculating the amount of beads to use, if the volume is less than 15 µL then increase the sample volume to reach a bead volume above 15 µL.

2. Incubate the sample at room temperature for 15 min.

3. Briefly spin down the sample to collect the beads that may have splashed to the sides.

4. Put the tube/plate on a magnetic separation device to magnetize the beads. Wait for the solution to become clear.

5. With the sample tube/plate still on the magnetic separation device, remove and transfer the supernatant to another tube/plate. Avoid bead disturbance while pipetting out the supernatant (save the supernatant in case you may need this sample).

6. With the sample tube/plate on the magnetic separation device, add 200 µL of 80% Ethanol to each tube/well, and incubate for 30 seconds at room temperature.

   ⚠ *Make sure to use freshly prepared 80% Ethanol to achieve the best results.*

7. With the tube/plate still on the magnetic separation device, remove and discard the supernatant by pipetting.

8. Repeat steps 6 and 7 for a total of two 80% Ethanol washes.

9. Ensure that there is no remaining 80% Ethanol in the sample tube/well. If droplets are present, do the following:

   i. Spin the sample tube or plate down to bring the droplets down
   ii. Place the sample on the magnetic separation device
   iii. Use a fine pipette tip to remove the droplets without touching the beads

10. Air dry the beads for 30 to 60 seconds. Remove the sample plate/tube from the magnetic separation device and add 40 µL of elution buffer, 10 mM Tris-HCl pH 8.5-9.0, or MagBio Genomics' Elution Buffer (see page 8).

11. Vortex for 1-2 min. at 2,000 rpm. Spin down the sample tube/plate and place it on the magnetic separation device.

12. Wait for the solution to be clear and pipette out the supernatant to a new 1.5 mL Lo-Bind tube.

13. Discard the beads.

14. Store the DNA at -20°C or proceed to the next step of library preparation.

DNA cleanup during library preparation: Follow the PacBio protocol and replace AMPure PB with HighPrep PCR PB. Use the bead ratios suggested in the PacBio library preparation protocol.

Protocol: DNA Purification

Improvement of DNA Purity (A260/A230)
The quality of DNA can be a reflection on sequencing results. High quality and purity of DNA is of utmost importance in long read sequencing. HighPrep PCR PB can be used to cleanup genomic DNA of low sample purity (low A260/A230). Low A260/A230 of DNA can be attributed to carryover contaminants like salts, carbohydrates etc. The A260/A230 value should be between 2.0 and 2.2 to obtain higher quality libraries. Use HighPrep PCR PB at a ratio of 0.45X for DNA purification to improve purity.

Purification of Samples Treated with RNase A
If RNA is present in a DNA sample, RNase A treatment is needed prior to library preparation. After RNase A treatment, use HighPrep PCR PB at a 0.45X ratio for DNA purification.

Concentration of a Dilute DNA Sample Using HighPrep PCR PB
If the sheared DNA that is going to be used for SMRTbell™ library preparation is dilute, less than the required concentration, then sheared genomic DNA can be concentrated using HighPrep PCR PB. Use the 0.45X ratio of HighPrep PCR PB and adjust the elution buffer volume accordingly to obtain the desired concentration.

Equipment and Reagents to Be Supplied by the User

- ☐ 80% Ethanol (Prepared from non denatured Ethanol)
- ☐ 70% Ethanol (Prepared from non denatured Ethanol)
- ☐ Elution Buffer (10 mM Tris-HCl pH 8.5-9.0) or MagBio Genomics' Elution Buffer (see page 8)
- ☐ Magnetic separation device compatible with 96 or 384 well PCR plate (see page 8)
- ☐ Magnetic separation device compatible with 1.5 mL/1.7 mL/2 mL microcentrifuge tubes (see page 8)
- ☐ 96-well PCR plate or 384-well PCR plate
- ☐ 1.5 mL LoBind microcentrifuge tubes
- ☐ Wide-bore pipette tips
- ☐ Optional: RNase A (if RNA is present in DNA samples)

Things to do Before Starting

- ☐ Make sure the HighPrep PCR PB is equilibrated at room temperature before use

Protocol

1. Mix the **HighPrep PCR PB** and DNA solution based on a bead ratio of 0.45X. Mix thoroughly 10-15 times using wide-bore pipette tips.

2. Incubate the sample at room temperature for 15 min.

3. Briefly spin down the sample to collect the beads that may have splashed to the sides.

4. Put the tube/plate on a magnetic separation device to magnetize the beads. Wait for the solution to become clear.

5. With the sample tube/plate still on the magnetic separation device, remove and transfer the supernatant to another tube/plate. Avoid bead disturbance while pipetting out the supernatant (save the supernatant in case you may need this sample).

6. With the sample tube/plate on the magnetic separation device, add 200 µL of 70% Ethanol to each well/tube, and incubate for 30 seconds at room temperature.

   ⚠ *Make sure to use freshly prepared 70% Ethanol to achieve the best results.*

7. With the tube/plate still on the magnetic separation device, remove and discard the supernatant by pipetting.

8. Repeat steps 6 and 7 for a total of two 70% Ethanol washes..

9. Ensure that there is no remaining 70% Ethanol in the sample tube/well. If droplets are present, do the following:

   I. Spin the sample tube or plate down to bring the droplets down
   II. Place the sample on the magnetic separation device
   III. Use a fine pipette tip to remove the droplets without touching the beads

10. Air dry the beads for 30 to 60 seconds. Remove the sample tube/plate from the magnetic separation device and add 40 µL of elution buffer, 10 mM Tris-HCl pH 8.5-9.0, or MagBio Genomics' Elution Buffer (see page 8).

11. Vortex for 1-2 min. at 2,000 rpm. Spin down the sample tube/plate and place it on the magnetic separation device.

12. Wait for the solution to be clear and pipette out the supernatant to a new 1.5 mL Lo-Bind tube.

13. Discard the beads.

14. Store the DNA at -20°C or proceed to the next step of library preparation.

Note: This protocol is for standard DNA purification. It is advisable to follow the bead ratio mentioned in the library preparation protocol that is being used at the time of the experiment.

Protocol: DNA Size Selection

Size selection is most effective when the DNA sample or SMRTbell™ libraries to be size selected are at a lower concentration of 0.5-10 ng/µL. It is important to adjust sample concentration before size selection to have efficient size selection. Using highly concentrated DNA outside of the range given will result in the beads binding to small fragments that are undesirable in long read sequencing. HighPrep PCR PB is diluted first to a concentration of 35% (v/v) using Elution Buffer.

Dilution of HighPrep PCR PB with Elution Buffer to 35% (v/v)

The final HighPrep PCR PB bead concentration is vital to the success of this procedure. Therefore, accurate pipetting is necessary in order to achieve a final 35% (v/v) HighPrep PCR PB bead solution.

Before performing size selection, determine the number of reactions needed and the volume of beads and Elution Buffer needed to dilute HighPrep PCR PB to 35% (v/v).

Reagent	Volume (mL)
HighPrep PCR PB	3.5
Elution Buffer	6.5
Total Volume	10

Ratio of HighPrep PCR PB 35% (v/v) Used in DNA Size Selection

SMRTbell™ Template Length Removal	HighPrep PCR PB 35% (v/v) Volume Needed
Remove < 3kb	3.7X
Remove < 4kb	3.3X
Remove < 5kb	3.1X

* The X represents the volume of the sample to be selected. The required volume of HighPrep PCR PB is calculated as follows: for removal of < 3kb, the required volume of HighPrep PCR PB = 1.8 X (sample volume)

Equipment and Reagents to Be Supplied by the User

- ☐ 80% Ethanol (Prepared from non denatured Ethanol)
- ☐ 70% Ethanol (Prepared from non denatured Ethanol)
- ☐ Elution Buffer (10 mM Tris-HCl pH 8.5-9.0) or MagBio Genomics' Elution Buffer (see page 8)
- ☐ Magnetic separation device compatible with 1.5 mL/1.7 mL/2 mL microcentrifuge tubes (see page 8)
- ☐ 1.5 mL LoBind microcentrifuge tubes
- ☐ Wide-bore pipette tips
- ☐ 1X dsDNA HS Assay kit
- ☐ Qubit 2.0, 3.0, or 4.0 fluorometer

Things to do Before Starting

- ☐ Dilute HighPrep PCR PB with Elution Buffer to 35% (v/v)
- ☐ Make sure the HighPrep PCR PB is equilibrated at room temperature before use

Protocol

1. Mix the **HighPrep PCR PB** and DNA solution based on the ratios shown in the table on page 6 (Ratio of HighPrep PCR PB 35% (v/v) used in DNA size selection). Mix thoroughly 10-15 times using wide-bore pipette tips.

2. Incubate the sample at room temperature for 15 min.

3. Briefly spin down the sample to collect the beads that may have splashed to the sides.

4. Put the tube on a magnetic separation device to magnetize the beads. Wait for the solution to become clear.

5. With the sample tube still on the magnetic separation device, remove and transfer the supernatant to another tube. Avoid bead disturbance while pipetting out the supernatant (save the supernatant in case you may need this sample).

6. With the sample tube on the magnetic separation device, add 200 µL of 80% Ethanol to each tube and incubate for 30 seconds at room temperature.

   ⚠ *Make sure to use freshly prepared 80% Ethanol to achieve the best results.*

7. With the tube still on the magnetic separation device, remove and discard the supernatant by pipetting.

8. Repeat steps 6 and 7 for a total of two 80% Ethanol washes.

9. Ensure that there is no remaining 80% Ethanol in the sample tube. If droplets are present, do the following:

   I. Spin the sample tube down to bring the droplets down
   II. Place the sample on the magnetic separation device
   III. Use a fine pipette tip to remove the droplets without touching the beads

10. Remove the sample tube from the magnetic separation device. Add 10 µL of elution buffer, 10 mM Tris-HCl pH 8.5-9.0, or MagBio Genomics' Elution Buffer (see page 8).

11. Perform warm elution by heating the sample tube at 37°C for 15 min. to elute the DNA from the beads.

12. Briefly spin down the sample and place the sample tube on the magnetic separation device. Wait for the solution to be clear and pipette out the supernatant to a new 1.5 mL Lo-Bind tube.

13. Discard the beads.

14. Measure SMRTbell™ library concentration using 1 µL of the 10 µL eluted DNA. Use the Qubit fluorometer and the dsDNA HS Assay Kit according to the manufacturer's instructions.

15. Store the remaining 9 µL SMRTbell™ library at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: US/Canada, +1 301-302-0144. Europe, +49 7250 33 13 403
Email: US/Canada, support@magbiogenomics.com. Europe, info.europe@magbiogenomics.com

Symptoms	Possible Causes	Comments
DNA recovery is too low	Highly fragmented sample	A significant portion of the sample should have fragments larger than 10 kb, otherwise DNA cleanup/purification will remove the small fragments leading to low recovery of larger fragments. The quality of DNA input determines the DNA recovery.
	Sample heterogeneity	If the input sample is heterogeneous and contains fractions of DNA that are not fully solubilized, recovery will be affected. Verify homogeneity by pipetting to ensure that no viscous jellies are present in the sample.
	Mishandling of beads during sample purification	The magnetic beads are often invisible. If the bead pellet is disturbed during the wash steps, it's possible to accidentally aspirate it into the pipette tip. Make sure that proper care is taken with tube orientation during pipetting steps such that pipetting is always performed on the opposite side of the tube from the beads.
A260/A230 not good	Contaminants present in the sample	Perform a few more rounds of 0.45x purification. Sometimes more than one round is needed to achieve the desired sample purity.

Ordering

HighPrep PCR PB

Catalog No.	Product
PB-60005	HighPrep PCR PB (5 mL)
PB-60050	HighPrep PCR PB (50 mL)

Related Products

Elution Buffer

Catalog No.	Product
EB-20	Elution Buffer (20 mL)
EB-250	Elution Buffer (250 mL)

High Molecular Weight DNA Isolation Kit

Catalog No.	Product	Description	Preps
HPHMW-D96	HighPrep HMW DNA Kit (96 Preps)	High molecular weight DNA extraction size range of 50-300+ kb from whole blood, saliva, buccal cells, cultured cells, tissues, and bacteria	96
HPHMW-D96X4	HighPrep HMW DNA Kit (384 Preps)		384

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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