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DNA Purification Magbeads (for NGS Size Selection)

Product Number: MB17C

Shipping and Storage

Store at 2-8℃ and transport at room temperature

Components

Component	MB17C-5ml	MB17C-50ml
NGS bead	5mL	50mL

Description

This kit provides a simple, rapid, and efficient method for nucleic acid purification. This product can be used for selective or non selective DNA recovery during second-generation sequencing library construction, as well as purification and recovery of PCR products. After mixing NGS beads with the sample in a certain proportion, magnetic beads selectively adsorb nucleic acids. After two steps of rinsing, the DNA obtained by elution has high purity, with an A260/A280 ratio between 1.7-1.9 and A260/A230

The ratio is usually above 2.0.The DNA purified by this kit is suitable for experiments such as PCR, Real Time PCR, sequencing, and southern blotting.

Table 1. DNA Recovery Efficiency

Sample type	Typical yield	Sample type	Typical yield
5000 bp segment	Up to 90%	1000 bp segment	Up to 90%
500 bp segment	Up to 80%	200 bp segment	Up to 70%

Self provided instruments and reagents

1. Magnetic frame
2. 80% ethanol.
3. Eluent: Buffer EB (10 mM Tris HCl, pH 8.0)
4. Deionized water (pH between 7.0-8.0)

Preparation and important precautions before the experiment

1. Freezing, centrifugation, and ultrasound can cause irreversible damage to the magnetic beads in NGS beads.
2. After long-term storage, the magnetic beads in NGS beads will aggregate into clusters, reducing the surface area of the beads and lowering the sample recovery rate. Before use, it is necessary to thoroughly mix the magnetic beads with vortex oscillation.
3. Before use, it is recommended to mix the NGS bead vortex and divide it into 1.5mL centrifuge tubes, with 1mL NGS bead per tube.
4. This kit is not suitable for purifying and recovering DNA fragments smaller than 100bp. If you want to recover DNA fragments smaller than 100bp, it is recommended to increase the amount of NGS bead to 4 times the sample volume.
5. When selectively recovering DNA, NGS beads are sensitive to the ion concentration in the DNA solution. The ion concentrations in the DNA solutions and PCR amplification products obtained from different manufacturers' second-generation sequencing library kits after adapter connection are different, so the amount of reagents used for DNA selective recovery using NGS bead varies.

Protocol

1. DNA purification steps

1.1. Vortex the NGS bead for 20 seconds to thoroughly mix it into a homogeneous solution.

1.2. Add purified DNA solution to a 1.5mL centrifuge tube.

1.3. Add 1 or 2 times the sample volume of NGS bead to the centrifuge tube in the previous step, vortex and shake for 5 seconds, and then let it stand at room temperature for 5 minutes.

1.4. Place the centrifuge tube from the previous step on the magnetic rack until the magnetic beads are completely adsorbed (approximately 5 minutes).

1.5. Keep the centrifuge tube fixed on the magnetic frame, completely discard the solution, and avoid contact with magnetic beads during the process.

1.6. Continue to fix the centrifuge tube on the magnetic frame and add 250 μ L of freshly prepared 80% ethanol to the centrifuge tube.

1.7. Keep the centrifuge tube fixed on the magnetic frame and completely discard ethanol after the suspended magnetic beads are completely adsorbed.

1.8. Repeat steps 6-7 twice

1.9. Keep the centrifuge tube fixed on the magnetic frame and let it stand for 5-10 minutes to completely evaporate the ethanol.

1.10. Remove the centrifuge tube from the magnetic rack, add 20-100μL of Buffer EB (self provided) or deionized water, vortex and shake to completely resuspend the magnetic beads in Buffer EB, and let it stand at room temperature for 5 minutes.

1.11. Place the centrifuge tube on the magnetic rack until the magnetic beads are completely adsorbed (approximately 5 minutes).

1.12. Transfer the eluent to a new 1.5mL centrifuge tube. At this point, the magnetic beads can be discarded.

2. DNA fragment sorting steps

Table 2. Reference conditions for DNA fragment sorting

DNA fragment size		200~300bp	300~400bp	400~500bp	500~600bp	600~750bp
Magnetic bead dosage	First choice	0.8×	0.7×	0.6×	0.5×	0.45×
	Second choice	0.2×	0.2×	0.2×	0.15×	0.15×

Taking the case of library fragment sizes ranging from 400 to 500bp as an example for fragment sorting, the specific steps are as follows:

2.1. Take out the NGS bead 30 minutes in advance and place it at room temperature. Shake and mix thoroughly before use;

2.2. Transfer the PCR reaction solution to a new 1.5mL centrifuge tube and fill the reaction system to 100μL.

2.3. Suck 60μL NGS bead into the PCR product, vortex and mix thoroughly, and let it stand at room temperature for 5 minutes.

2.4. Briefly centrifuge, place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution becomes clear (approximately 5 minutes), carefully aspirate the supernatant, and avoid contact with magnetic beads that have already bound to the target DNA during this period.

Note:Do not discard the supernatant.

2.5. Add 20μL of well mixed NGS bead to the supernatant, vortex for 5 seconds, and let it stand at room temperature for 5 minutes.

2.6. Perform a brief centrifugation and place the centrifuge tube on a magnetic rack to separate the magnetic beads from the supernatant solution until the solution becomes clear (approximately 5 minutes). Carefully aspirate the supernatant and avoid contact with magnetic beads that have already bound to the target DNA.

Note:Do not discard magnetic beads.

2.7. Keep the centrifuge tube fixed on the magnetic frame, add 250μL of freshly prepared 80% ethanol, let it stand at room temperature for 30 seconds, and discard the supernatant.

Note:Please use the newly configured ethanol, otherwise it may affect the experimental results.

2.8. Repeat step 7 once, and try to absorb the liquid at the bottom of the tube as much as possible for the last time. If there is a small amount of residue on the tube wall, the centrifuge tube can be centrifuged instantly. After separation on the magnetic frame, use a small range pipette to absorb the liquid at the bottom of the tube.

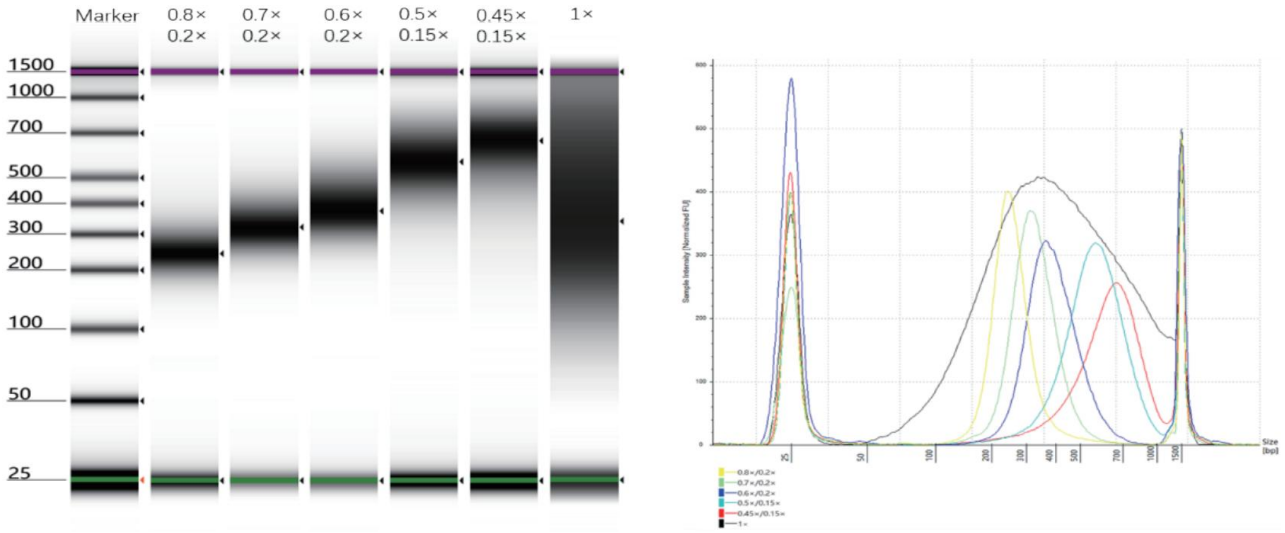
Note:Do not suck magnetic beads to avoid affecting production.

2.9. Keep the centrifuge tube fixed on the magnetic frame, open the non stick tube cover, and dry at room temperature for 3-5 minutes until the magnetic beads have no reflection or cracking.

Note:Do not heat dry and do not excessively dry the magnetic beads, otherwise it will affect the yield.

2.10. Remove the centrifuge tube from the magnetic rack, add 20-100μL of deionized water, vortex and oscillate to completely resuspend the magnetic beads in the deionized water, and let it stand at room temperature for 5 minutes.

2.11. Briefly centrifuge, place the centrifuge tube on a magnetic rack until the solution is clear (approximately 5 minutes), and transfer the clear solution to a new PCR tube.



According to the conditions in Table 2, NGS bead was used to sort the PCR library enrichment products, resulting in libraries of different fragment sizes. The above figure shows the results of Agilent 4200 TapeStation system analysis of libraries of different sizes.