Product Information

5R-PLEX Beads

For PCR Purification

MBD6009

Product Description

The 5R-PLEX beads are suitable for purification and size selection of PCR products, including for NGS (16S and 18S) and 5R-PLEX library preparation (see 5R-PLEX kit MBD6000).

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store this product at 2-8 °C.

Protocol

Preparation

1. Prepare fresh 80% molecular grade ethanol. Add 1 mL molecular grade water to 4 mL ethanol.
2. Equilibrate all reagents for at least 30 minutes at room temperature.

   Consumables and reagents:

   - 5R-PLEX beads
   - Elution Buffer (EB) from the 5R-PLEX Kit (MBD6005) or Trizma® hydrochloride solution pH 8.5, DNase and Microbial DNA free, 10 mM (SBR00051)
   - Ethanol for molecular biology (1.08543 or equivalent)
   - Water for molecular biology (W4502 or equivalent)
   - Disposable RNase/DNase-free multichannel reagent reservoirs, PCR strips/tubes
   - Magnetic stand

Bead Purification

Complete all steps in the "Preparation" section before starting Bead Purification.

Important: The beads must be equilibrated to room temperature 30 minutes before use.

1. The beads must be homogenous. Vortex the beads thoroughly for 30 seconds to make sure that the beads are evenly dispersed.
2. Add 42.5 µL beads (0.85x) to 50 µL of pre-purified, pooled library (for 5R-PLEX KIT), or other PCR product.

   Note: If size selection is needed, choose from the following ratios:

   - 0.7x= 35 µL
   - 0.8x= 40 µL
   - 0.9x= 45 µL
   - 1.0x= 50 µL
   - 1.5x= 75 µL beads to 50 µL product.

   Ideally, the optimal ratio can be determined experimentally for each application since it will depend on the exact conditions of the experiment. As a guide, use the application data in **Figure 1**.
3. Pipette up and down 10 times, seal the tubes and incubate at room temperature for 5 minutes.
4. Place the tubes on a magnetic stand for 2-5 minutes or until the supernatant has cleared.
5. Remove and discard the supernatant.
6. With the tubes on the magnetic stand, wash the beads with freshly prepared 80% ethanol as follows:
   a. Add 200 µL of freshly prepared 80% ethanol.
   b. Incubate the tubes on the magnetic stand for 30 seconds.
   c. Carefully remove and discard the supernatant.

7. With the tubes on the magnetic stand, perform a second ethanol wash as described in step 6.

   IMPORTANT: Completely remove all traces of the ethanol wash after the second wash. To do this, briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol first with a 200 µL pipette, and then use a 10 µL pipette to remove any residual ethanol.

8. With the tubes still on the magnetic stand, allow the beads to air-dry for 3 minutes (time can change according to humidity and temperature in the lab).

   NOTE: When completely dry, the beads should have a "cracked" appearance. Do not over-dry the beads.

9. Remove the tubes from the magnetic stand. Add 20 µL of EB to each tube.
10. Gently pipette mix up and down 10 times until beads are fully resuspended.
11. Incubate at room temperature for 2 minutes.
12. Place the tubes on the magnetic stand for 2 minutes or until the supernatant has cleared.
13. Carefully transfer 18 µL of the supernatant (purified DNA) into a new tube. To avoid bead carryover, up to 2 µL of eluate can be left behind.

    NOTE: For an FFPE DNA library, it is highly recommended to repeat bead purification to completely remove all primer dimers. Excess of adapter dimer wastes sequencing reads and lowers the classification rate.

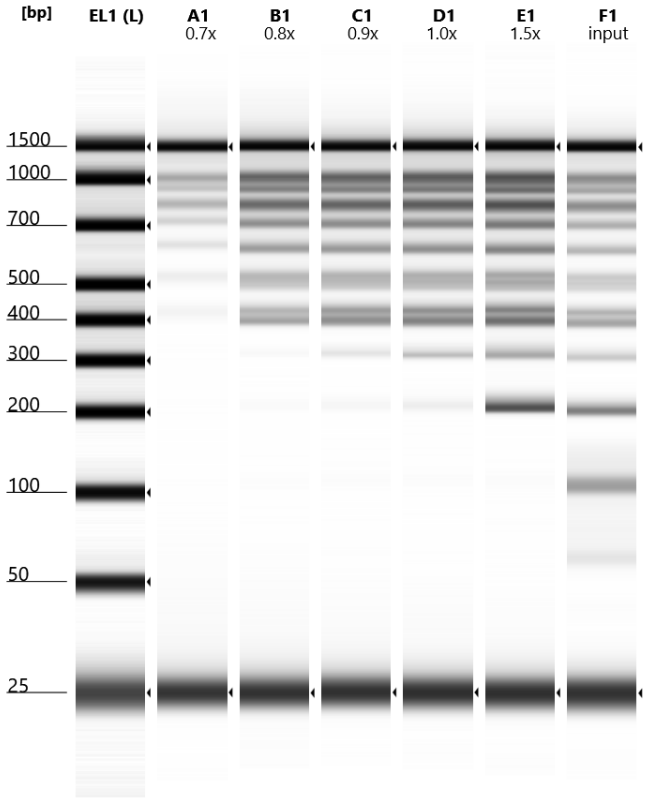
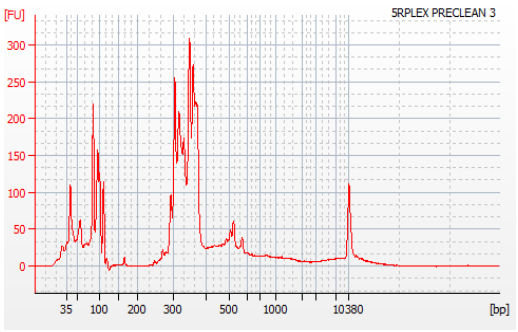


Figure 1: Automated electrophoresis image of a DNA ladder purified by the 5R-PLEX beads in different ratios. 5R-PLEX Beads in ratios between 0.7x1.5 were added to 50 µL diluted DNA ladder, purified and visualized by automated electrophoresis. EL1 is an electronic ladder. The bands at 25 and 1500 bp are the lower and upper markers (artifacts). A1-E1 are purified samples, F1 is the ladder without purification.

Application data: 5R-Plex Library of FFPE samples

5R-Plex library before cleanup



5R-Plex library after cleanup

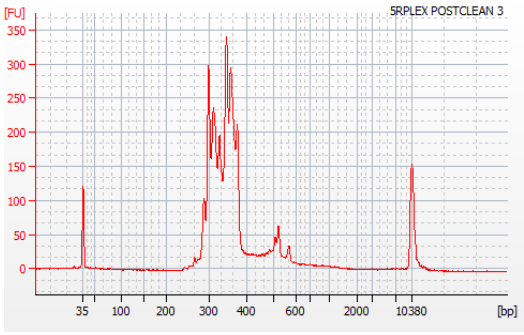


Figure 2: FFPE samples were processed according to the 5R-PLEX Kit protocol using a 0.85x 5R-PLEX beads-to-PCR products ratio. The results before and after bead cleanup were visualized on an Agilent Bioanalyzer. The figures show that before cleanup, the reaction mix contained a significant amount of primer dimers due to low DNA input and the high number of PCR cycles. The 5R-PLEX beads successfully removed these unwanted DNA fragments while retaining a high percent of the desired amplicons (between 200-400 bp). Removing primer/adaptor dimers is critical for the 5R-PLEX sequencing to get the maximum amount of amplicon reads.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

MBD6009pis Rev 01/23