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Purification of pooled PCR amplicon libraries using SPRI beads

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Materials

MATERIALS

Agencourt AMPure XP **Beckman Coulter** **Catalog #**A63880

Mag-Bind RxnPure Plus **Catalog #**M1386-00

Troubleshooting

Prepare and bind DNA

1 Vortex AMPure XP or RXN pureplus SPRI beads to resuspend. Make your pooled library up to 100 µl if necessary.

2 Add 60 µl resuspended SPRI beads to 100 µl of pooled PCR product. Mix well by pipetting up and down at least 10 times. Vortex AMPure XP beads to resuspend.

3 Incubate for 5 minutes at room temperature.
00:05:00

4 Quickly spin the tube and place it on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (Caution: do not discard beads).
00:05:00

Wash DNA

5 Add 200 µl of 80% freshly prepared ethanol to the tube while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
00:00:30

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00:00:30

8 Air the dry beads for 10 minutes while the tube is on the magnetic stand with the lid open.
00:10:00

Elute DNA

9 Elute the DNA target from the beads by adding 30 µl of 10 mM Tris-HCl, pH 8.0 or 0.1X TE.

Note: Be sure not to transfer any beads. Trace amounts of bead carry over may affect the optimal performance of the polymerase used in the subsequent PCR step.

10 Mix well by pipetting up and down, or on a vortex mixer.

11 Quickly spin the tube and place it on the magnetic stand.

12 After the solution is clear (about 5 minutes), transfer 27ul a new PCR tube for amplification.

00:05:00