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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 μ l if necessary.
- 2 Add 60 μ l resuspended SPRI beads to 100 μ l 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 μ l 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
- 7 Add 200 μ l 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
- 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 μ l 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 27 μ l to a new PCR tube for amplification.

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