Purification of pooled PCR amplicon libraries using SPRI beads

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Abstract

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Materials

Agencourt AMPure XP A63880 by Beckman Coulter

Mag-Bind RxnPure Plus M1386-00 by Contributed by users

Protocol

Prepare and bind DNA

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

Prepare and bind DNA

Step 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.

Prepare and bind DNA

Step 3.
Incubate for 5 minutes at room temperature.

DURATION
00:05:00

Prepare and bind DNA

Step 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).

DURATION
00:05:00

Wash DNA

Step 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.

DURATION
00:00:30

Wash DNA

Step 6.
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.

DURATION
00:00:30

Wash DNA

Step 7.
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.

DURATION
00:00:30

Wash DNA

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

DURATION
00:10:00

Elute DNA

Step 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.

Elute DNA

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

Elute DNA

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

Elute DNA

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

DURATION
00:05:00