

# 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 27 µl to a new PCR tube for amplification.

 DURATION

00:05:00