Clean-up using AMPure XP beads

Bioline

Abstract

This is the suggested protocol for clean-up using AMPure XP beads. It is recommended as part of the JetSeq™ DNA Library Preparation Kit protocol for post ligation, post adaptor extenstion (PCR1), and post indexing clean-ups.

Citation: Bioline Clean-up using AMPure XP beads. **protocols.io**
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Protocol

Step 1.

Mix the reagent well so that the reagent appears homogeneous and consistent in color.

NOTES
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Use only room temperature AMPure XP beads.

Step 2.

Add 117 µL of homogenous AMPure XP beads to each adaptor ligated DNA sample (in either 1.5 mL LoBind tubes or 0.2 mL LoBind tubes). Mix well by pipetting up and down at least 10 times.

Step 3.

Incubate at room temperature for 5 min.

DURATION
00:05:00

Step 4.

Put the tube in the magnetic stand and wait for the solution to clear (which should take approximately 3–5 min).

Step 5.

Keep the tube in the magnetic stand. Do not touch the beads while you carefully discard the cleared

solution from the tubes.

Step 6.

Continue to keep the tube in the magnetic stand/rack whilst adding 500 µL (1.5 mL LoBind tubes) or 200 µL (0.2mL LobBind tubes) of 70% ethanol to each tube. (wash 1/2)

Step 7.

Let the tube sit for 1 min to allow any disturbed beads to settle, and remove the ethanol. (wash 1/2)

DURATION
00:01:00

Step 8.

Continue to keep the tube in the magnetic stand/rack whilst adding 500 µL (1.5 mL LoBind tubes) or 200 µL (0.2mL LobBind tubes) of 70% ethanol to each tube. (wash 2/2)

Step 9.

Let the tube sit for 1 min to allow any disturbed beads to settle, and remove the ethanol. (wash 2/2)

DURATION
00:01:00

Step 10.

Seal the tube or plate and centrifuge briefly (260 x g for 30 sec).

DURATION
00:00:30

Step 11.

Return the tube to the magnetic stand/rack and wait 1 min.

DURATION
00:01:00

Step 12.

Remove any remaining ethanol using a P20 pipette and tip, being careful not to touch the bead pellet.

Step 13.

Dry the samples on a 37 °C heat block for 3–5 min or until the residual ethanol completely evaporates. **IMPORTANT: Do not over-dry as this will decrease yield.**

DURATION
00:03:00

NOTES

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Bead pellet is dry when the appearance of the surface changes from shiny to matt.

Step 14.

Add 32 µL nuclease-free water directly to the bead pellet, mix well by pipetting up and down at least 10 times.

Step 15.

Incubate for 3 min at room temperature.

DURATION
00:03:00

Step 16.

Centrifuge briefly to consolidate the sample and place on a magnetic stand/rack for 2–3 min or until the solution is clear.

DURATION
00:02:00

Step 17.

Remove 30 µL of the supernatant and transfer to a fresh LoBind tube. The beads can be discarded at this time.