Titration of AmPure XP Beads for Removal of Fragments <400bp

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Abstract

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Guidelines

Note: AmPure XP beads are normally used at 80:100 ratio* (beads:DNA) to remove fragments less than 100bp and retain all DNA above 100bp. If you lower that ratio. then DNA will not bind to the beads as efficiently (due to decreased amount of PEG present) and you will lose some of the DNA. To recover all DNA, make sure the starting DNA is not too concentrated and hold onto the residual sample after beads first adhere to magnet; in this way you can re-bind any unbound DNA to fresh beads.

*see step 17 annotation for more info

Agencourt® AmPure® XP beads: Beckman Coulter #A63880
100bp DNA ladder: New England Biolabs (NEB) #N3231S (comes with 6xGLB)
6x Blue Orange Loading Dye: Promega #G190A (optional)
Magnetic Particle Concentrator (MPC): Invitrogen Dyna-Mag2 #123-21D

Materials

- Agencourt AmPure XP beads A63880 by Contributed by users
- 100 bp DNA Ladder - 100 gel lanes N3231S by Contributed by users
- Blue/Orange Loading Dye, 6X G190A by Contributed by users
- Magnetic Particle Concentrator (MPC): Dyna-Mag2 12321D by Contributed by users

Protocol

AmPure XP Bead Titration

Step 1.
Label 11x1.5 ml tubes 40-45-50-55 etc. to 90 (= ratio of beads to DNA)

AmPure XP Bead Titration

Step 2.
Mix 48µl NEB 100bp ladder with 1152µl Molecular Biology Water.

AmPure XP Bead Titration

Step 3.

Pipet 100µl of mixture into each of the 11 tubes.

AmPure XP Bead Titration

Step 4.

Vortex bottle of AmPure XP beads and dispense 800µl into fresh tube.

AmPure XP Bead Titration

Step 5.

Using same pipettor used for step #3, pipet volume of beads into each tube (i.e., 40µl into tube labled 40, 45µl into tube labed 45, etc.)

AmPure XP Bead Titration

Step 6.

Close lids, vortex tubes and incubate 5 minutes at room temperature.

DURATION

00:05:00

AmPure XP Bead Titration

Step 7.

Place tubes in MPC rack; wait 5 minutes for beads to adhere to magnet side of tubes.

DURATION

00:05:00

AmPure XP Bead Titration

Step 8.

Pipet off supers and discard them.

AmPure XP Bead Titration

Step 9.

Wash the beads 2x with 500µl each 70% ethanol with 30 second incubation.

DURATION

00:00:30

AmPure XP Bead Titration

Step 10.

Remove all supers from tubes.

AmPure XP Bead Titration

Step 11.

Place tubes (with caps open) into 37°C heat block to dry beads (evaporate residual ethanol).

AmPure XP Bead Titration

Step 12.

Add 10µl Qiagen EB to each tube, close lids.

AmPure XP Bead Titration

Step 13.

Vortex to resuspend beads.

AmPure XP Bead Titration

Step 14.

Place tubes back into MPC rack and let beads adhere to magnet sides of tubes.

AmPure XP Bead Titration

Step 15.

Pipet off supers (containing eluted DNA) into fresh tubes.

AmPure XP Bead Titration

Step 16.

Run samples in 2% agarose gel (1x TAE) using 5µl DNA and 1µl 6x gel loading buffer.

AmPure XP Bead Titration

Step 17.

Photograph results and determine what ratio of beads to DNA to use to exclude lower range of DNA.

NOTES

VERVE Team 31 Jul 2015

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