Magnetic particle based DNA purification

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Abstract

This method describes magnetic particle based DNA purification.

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Guidelines

Materials:

80% Ethanol, made fresh

'beads' - 1.5 mg/mL Sera-mag magnetic particles or Agencourt AMPure XP beads, Beckman Coulter A63880/1/2

DNA sample needing purification and/or concentrating

10 mM Tris-HCl (pH 8.0 - 8.5)

Equipment:

Stand or plate magnet compatible with sample size such as:

DynaMag-2 from Thermo-Fischer, 12321D

http://www.eandkscientific.com/Magnet-Plates/

Before start

Make 80% Ethanol. Do not use 80% Ethanol that is more than 3 days old.

Protocol

Step 1.

Bring beads to room temperature for 30 minutes prior to use.

Step 2.

Mix beads by inverting container and swirling until no aggegated beads remain stuck to the container, or in suspension.

Step 3.

Add beads to DNA sample. Mix gently by pipetting up and down 10 times, by inverting tubes, or by slight vortexing. If there are more than a few samples done in serial, mix beads between every 3 - 5 samples. If droplets are stuck to sides of container, centrifuge quickly and gently.

NOTES
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Bead to sample ratios:

Optimum bead to sample ratios should be determined individually for each sample type, bead prep, and process.

In general, 0.5x retains 500 bp and above, 1.0x retains 250 bp and above, and 2.0x retains 100 bp and above.

Step 4.

Incubate at room temperature for 10 - 30 minutes.

Step 5.

Transfer to magnet. Incubate until full separation has occurred. Separation time will vary based on sample volume, magnet strength, and bead type. Typical separation time is 10 minutes or less.

Step 6.

Remove and discard supernatant. On magnet, wash two times with 80% ethanol. Allow a 30 second bead/ethanol incubation for each wash. Use a volume that will completely cover the pellet, typically 1 mL for the 1.5 mL Eppendorf tubes and 200 µL for PCR plates and small strip tubes.

Step 7.

Off magnet, allow pellet to air dry for approximately 5 minutes. Do not over-dry. Pellet should not become so dry that it cracks. If pellet is big enough to see easily, a difference in sheen can be observed when the beads become less wet. Immediately after the difference in sheen happens is the right time to resuspend.

Step 8.

Resuspend pellet with 10 mM Tris-HCl by pipetting up and down until beads are free from the container sides and not aggregated. If droplets are stuck to sides of container, centrifuge quickly and gently.

Step 9.

Incubate for 5-10 minutes, off magnet and at room temperature.

Step 10.

Transfer bead suspension back to magnet. Incubate until full separation has occurred. Separation time will vary based on sample volume, magnet strength, and bead type. Typical separation time is 10 minutes or less.

Step 11.

Elute sample from beads to a clean container.