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Bead clean-up (single tube)

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ABSTRACT

Protocol for purification of DNA using SPRI beads

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID:
60811

Prepare reagents

5m

1 Prepare the following reagents/materials: 5m

Agencourt AMPure XP **Beckman Coulter Catalog #A63880** or Homemade SPRI beads
Fresh 70% Ethanol
Nuclease-free water or elution buffer
DNA sample/PCR product
Clean 1.5 mL tubes
Magnetic tube rack

Note

- Make sure beads are thoroughly mixed/vortexed to ensure they are well resuspended, the solution should be a homogenous brown color.
- Beads and sample must be brought to Room temperature before mixing.

Note

- Ethanol should be freshly prepared. Due to miscibility of EtOH and water, prepare by adding volumetric parts, not by bringing to volume.

Add beads to sample

7m

2 In a 1.5 mL tube, add resuspended beads to sample/PCR product in ratio specified in main protocol. 1m

Note

The ratio is dependent on the length of DNA you want to recover.

2.1 Flick tube gently to mix beads and sample. 1m

Note

Pipette mixing may fragment the sample. Do not vortex to mix.

2.2 Allow mixture to incubate for 00:05:00 at Room temperature 5m

Note

Place tube on slow rotation mixer or flick gently periodically throughout the incubation to prevent beads from settling to the bottom of the tube.

Separation

3m 30s

3 Place tube on a magnetic rack. 30s

Equipment

Magnetic Stand

Magnetic Stand	TYPE
Thermo Scientific	BRAND
MR02	SKU
https://www.thermofisher.com/order/catalog/product/MR02	LINK
Any magnetic rack that fits your tubes will suffice.	SPECIFICATIONS

3.1 Wait ~1-3 minutes for the beads and buffer to separate. Beads will stick to magnetic side of the tube and the solution should be clear. 2m

3.2 Using a pipettor, remove and discard the clear supernatant solution. DNA will remain in the tube bound to the beads. 1m

Ethanol Washing

2m 30s

4 Add 500 µL 70% EtOH to tube 30s

- Do not disturb the beads! Pipette into the opposite side of the tube.

Note

Volume of 70% EtOH can be adjusted. Just so long as the amount added is enough to cover beads.

4.1 Incubate at Room temperature for 00:00:30 30s

4.2 Using a pipettor, remove and discard the EtOH. Be careful not to disturb or aspirate the beads. 30s

5 Repeat the Step 4 EtOH wash. Try to remove as much EtOH as possible without disturbing the beads. 1m

Note

A quick spin-down may help to remove residual EtOH.

Air dry beads 1m

6 Leaving tube cap open, allow beads to dry for a maximum of 90 seconds. 1m

Note

-Do not over-dry beads. Over-dried beads do not resuspend well and can lead to a loss of DNA.
-If the surface of beads appear cracked, they are over-drying. Resuspend immediately.

Resuspend 1m

7 Remove tube from magnetic rack and resuspend DNA/beads by adding a minimum of 15 µL nuclease-free water or desired buffer. 30s

Note

The exact resuspension volume should be specified in your main protocol. Use a smaller volume to increase concentration of DNA

7.1 Flick tube gently to resuspend beads in elution. 30s

Incubate 5m

8 Incubate at Room temperature for 00:02:00 - 00:05:00 5m

Recovery 2m

9 Place tube back on magnetic rack. Allow beads and elution to separate for 00:01:00 . 1m

9.1 Slowly aspirate out clear supernatant, now containing DNA, and transfer to final clean container. 1m

Note

Do not carry over any beads. They are a significant inhibitor of various downstream applications. Carefully check your pipette tip for bead carryover. If there are beads, replace the sample into the tube and wait another 2 min. You may need to decrease the volume removed from the tube by 1-2 µL.