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Ampure bead clean up for high molecular weight DNA

In 1 collection

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

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Guidelines

For sample, do not vortex or mix by pipetting throughout protocol. This is to avoid shearing DNA.

Materials

MATERIALS

Agencourt AMPure XP beads

Troubleshooting

Before start

Turn on a heat block to 55 °C

Add beads

1 Make sure beads are completely resuspended before use by vortexing vigorously.

1.1 Add ratio of resuspended beads specified in your main protocol.

Note

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

1.2 Flick tube gently to mix beads and sample.

Incubate

2 Incubate at room temperature for 5 minutes.

Room temperature 00:05:00

Note

Flick gently periodically throughout the incubation to prevent beads from settling to the bottom of the tube.

Separation

3 Place tubes on a magnetic rack.

- see below for example.

Equipment

Magnetic Stand	NAME
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 2 min for the beads and buffer to seaparate. Beads will stick to magnetic side of the tube and the solution should be clear.

3.2 Remove and discard the clear solution. DNA will remain in the tube bound to the beads.

Wash

4 Add 500ul 80% EtOH to each sample.

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

Note

Volume of 80% EtOH can be adjusted. Just so long as the amount added is enough to cover beads.

4.1 Incubate at room temperature for 30 seconds.

Room temperature 00:00:30

4.2 Remove 80% EtOH, being careful not to disturb the beads.

5 Repeat the step 4.

6 Spin down breifly and place back on the magnetic stand. Remove residual EtOH.

Dry Beads and Resuspend DNA

7 Leaving the caps open, remove tubes from magnet, and dry until surface of the beads has a matte finish.

Note

Caution: If the surface of beads appear cracked, they are overdrying! Resuspend *immediately*. Over-dried beads will not resuspend well.

8 Resuspend DNA by adding a minimun of 15ul water or low EDTA TE and gently mix by flicking. The exact resuspension volume should be specified in your main protocol.

8.1 Incubate for 2 minutes at 55 C.

55 °C 00:02:00

Collect

9 Place tubes back on magnetic rack

9.1 Wait 2 min for the beads to seaparate. Beads will stick to magnetic side of the tube and the solution should be clear.

9.2 Pipette out your specified volume of eluate and keep as your sample.

Note

Do not carry over any beads! They are a significant inhibitor of various downstream aplications. Carefully check to 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 ul.