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Magnetic bead cleaning of PCR products with Cytiva Sera-Mag Select

Forked from Magnetic bead cleaning of PCR products

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

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Disclaimer

Abstract

Magnetic bead cleaning of PCR products, adapted for Cytiva Sera-Mag Select beads. Forked from an older protocol using AMPure XP beads with minimal changes.

Guidelines

Work at post-PCR lab.
You can use PCR tubes/strips or PCR-plates but for easiest work with magnetic beads flat-bottom 96-well microplates are recommended.

Troubleshooting

1 Calculate the volume needed for the beads. For normal length primers a bead:sample ratio of 0.8:1 is likely OK. For long primers you can use a ratio of down to 0.65:1 (probably even lower). If you can still see primer dimers on a gel after cleanup, try a lower bead:sample ratio.

0.8 * sample volume =

2 Shake the magnetic bead buffer bottle to fully resuspend magnetic particles.

3 Prepare 85% ethanol. You need 400 µl per sample, but prepare some extra.

Remember that when mixing ethanol and water, the total volume decreases (e.g. 15 ml water + 85 ml 100% ethanol < 100ml 85% ethanol). For the same reason, measure ethanol and water separately before mixing

4 Add Sample.

5 Add magnetic beads. Pipette mix 10 times and Incubate at room temperature for 5 minutes.

00:05:00

6 Place the reaction plate onto a Magnet Plate for 2 minutes to separate beads from solution. (Keep on the magnet plate until step 11).

00:02:00

7 Aspirate the supernatant from the reaction plate and discard.

8 Dispense 200 µL of 85% ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. You can discard the ethanol by inverting the plate keeping the magnetic plate on the bottom of it.

200 µL

00:00:30

9 Repeat the washing step as above: dispense 200 µL of 85% ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard. You can discard the ethanol by inverting the plate keeping the magnetic plate on the bottom of it.

200 µL

00:00:30

10 Remove the remaining ethanol by pipetting.

11 Take the plate off the magnet

12 Dry off the remaining ethanol. To speed up the process, you can place the plate on a warm (30°C) heat block.
Drying takes at least 30 minutes. Check that drying was complete before continuing.
00:30:00

13 Add 40 µL of elution buffer (PCR water or 10mM Tris), pipette mix 10 times. Make sure the buffer reaches up to the beads!
40 µL

14 Incubate at room temperature for 2 minutes.
00:02:00

15 Place the reaction plate onto a Magnet Plate for 1 minute to separate beads from solution.
00:01:00

16 Transfer purified product to a new PCR plate or strips. Ensure that no beads are carried over when transferring, as it may inhibit later steps.