TECHNICAL BULLETIN

Protocol for DNA Purification From a Gel Slice or PCR Amplification Product

Description

The following protocol is for DNA purification from an agarose gel slice or PCR amplification product using the Gel and PCR Clean-up Kit (Catalog #79030). For complete instructions, refer to the Technical Manual (Document #10000005433).

Directions

1. Prepare a gel slice or PCR amplification product as indicated below.

Gel Slice

a) Following electrophoresis, excise DNA fragment from gel and place gel slice in a pre-weighed 1.7 mL microcentrifuge tube.

b) Add 10 µL Membrane Binding Solution per 10 mg of gel slice. For DNA fragments > 5 kb, mix gently by inversion; for DNA fragments < 5 kb, vortex to mix. Incubate at 50 - 65°C for 10 minutes or until the gel slice is completely dissolved. During incubation, mix the tube every few minutes to increase the rate of dissolution.

c) Proceed to step 2 for DNA purification.

PCR Amplification Product

a) Add an equal volume of Membrane Binding Solution to the PCR amplification product.

b) Proceed to step 2 for DNA purification.

2. Insert minicolumn into Collection Tube.
3. Transfer dissolved gel mixture or prepared PCR product to the minicolumn assembly. Incubate at room temperature for 1 minute.
4. Centrifuge at 16,000 x *g* for 1 minute. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Reinsert minicolumn in the Collection Tube.
5. Add 700 µL Membrane Wash Buffer (with ethanol added). Centrifuge at 16,000 x *g* for 1 minute. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Reinsert minicolumn in the Collection Tube.
6. Add 500 µL Membrane Wash Buffer. Centrifuge at 16,000 x *g* for 5 minutes. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Reinsert minicolumn in the Collection Tube.
7. Centrifuge at 16,000 x *g* for 1 minute to dry membrane.
8. Carefully transfer minicolumn to a clean DNase-free 1.7 mL microcentrifuge tube.
9. Add 50 µL nuclease-free water to the minicolumn. Incubate at room temperature for 1 - 2 minutes.
10. Centrifuge at 16,000 x *g* for 1 minute. Discard minicolumn and store eluted DNA at 2 - 8°C or -20°C.

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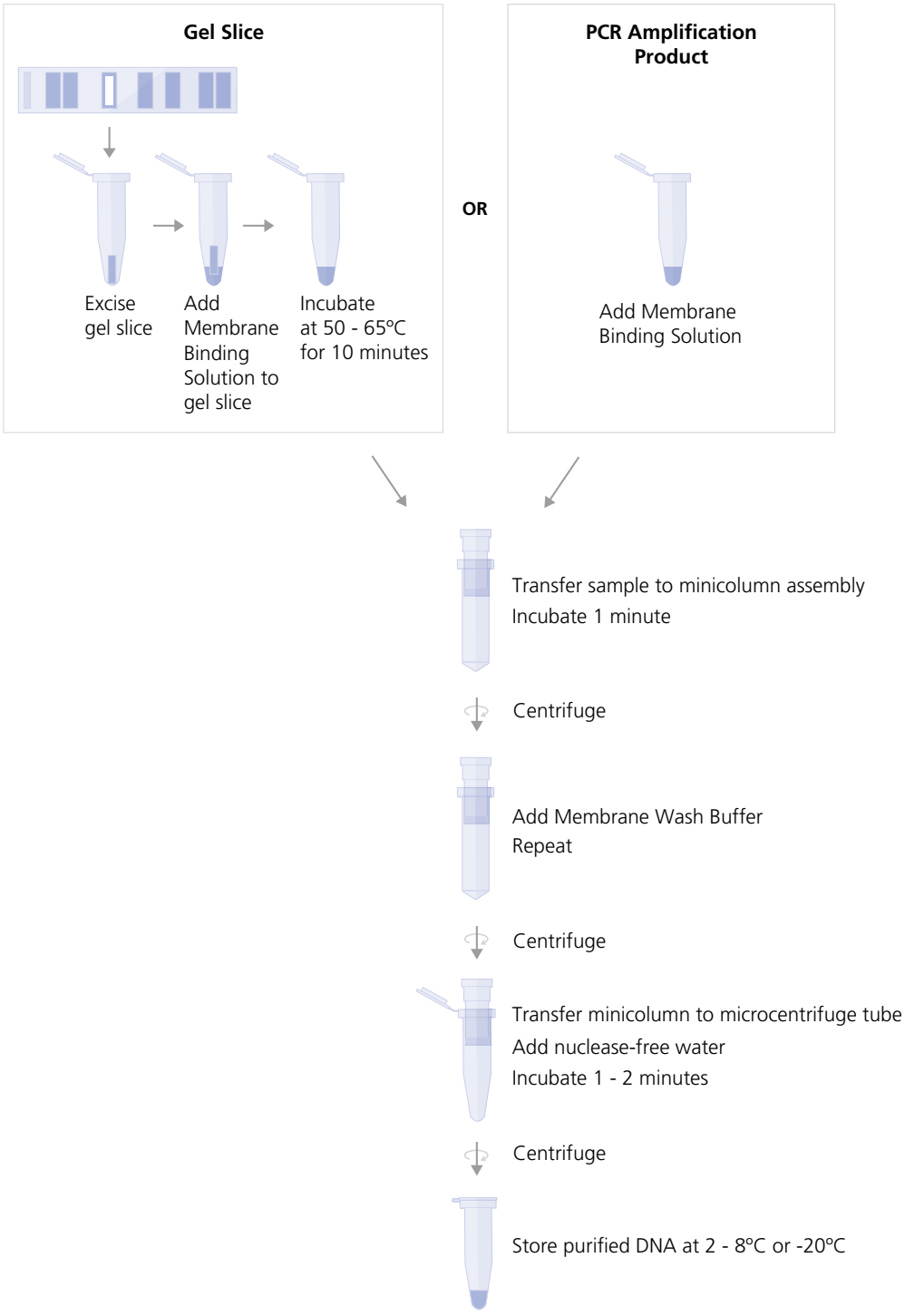

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