Electrophoretic transfer of RNA to nylon membrane (Semi-dry blotting)

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Abstract

Protocol for transfer of RNA to nylon membrane for subsequent Northern Blot analysis.

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Protocol

RNA separation

Step 1.

Separate RNA in a gel.

PROTOCOL

. Denaturing RNA Urea-PAGE

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Gel preparation

Step 1.1.

Before preparing the gel, clean all components with 70 % ethanol and RNase away.

Recipe for one small Hoefer gel (10 mL):

PAA percentage	5 %	6 %	8 %	10 %	12 %	15 %
40 % PAA (19:1)	1.25 mL	1.5 mL	2 mL	2.5 mL	3 mL	3.74 mL
Urea	5 g	5 g	5 g	5 g	5 g	5 g
10x TBE	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
10 % APS	80 µL	80 µL	80 µL	80 µL	80 µL	80 µL
TEMED	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL
ddH_2O	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

Gel preparation

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Step 1.2.

Dissolve 5 g urea in PAA, 10x TBE and H_2O. This can be done at RT or at 40˚C. Agitate/shake occasionally.

SAFETY INFORMATION

Wear vinyl gloves when handling PAA (neurotoxic!)

Gel preparation

Step 1.3.

Let the liquid cool down before pouring gel.

Assemble gel caster. Take a 1 mL aliquot of gel mixture, add 20 µL APS and 1 µL TEMED and quickly pour to prevent gel from running out.

Gel preparation

Step 1.4.

Add 80 µL APS and 12 µL TEMED to the rest of the gel and quickly pour. Insert comb immediately and clasp tight to prevent leakage. Let polymerize for an hour or over night.

Gels can be stored for a week by wrapping with wet paper towels and Saran wrap.

RNA preparation

Step 1.5.

For Northern Blot Analysis, at least 2-3 µg of RNA should be loaded per well.

Adjust concentration and volume of all RNA samples by adding RNase-free ddH2O. Add desired amount of 2x or 5x RNA loading dye.

Denature RNA at 95˚C for 5 min. Snap cool on ice.

DURATION

00:05:00

SAFETY INFORMATION

RNA loading dye contains formamide. Wear goggles/lab coat/ gloves!

RNA loading

Step 1.6.

Add running buffer (1x TBE). Remove comb. Before loading samples, wash all wells and each well individually just before loading with 1xTBE and a syringe or a pipette tip. Carefully pipet samples into wells.

Step 1.7.

Close the lid and plug electrodes into power supply.

Separate RNA at 20 mA/gel, 1 hour.

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Visualization

Step 1.8.

Pour out buffer, then disassemble gel.

Incubate gel in 0.5x TBE + GelRed for 10 minutes, then visualize.

DURATION
00:10:00

Materials required

Step 2.

Prepare the following materials

- 8 layers of 3MM whatman papers (1x gel size)
- Hybond N+ Nylon membrane (1x gel size)

Soak in 0.5 x TBE buffer.

Gel should also be incubated in 0.5 x TBE prior to electrophoretic transfer.

Assembly of blotting sandwich

Step 3.

Transfer is set up in a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad).

Prior to assembly, humidify both electrode plates with 0.5 x TBE buffer.

From the bottom up, stack the blotting sandwich as follows:

- 4 layers of 3 MM whatman papers
- Nylon membrane
- PAA gel → Remove all bubbles with a glas bar
- 4 layers of 3 MM whatman papers

Electrophoretic transfer

Step 4.

Close lid and plug in electrodes.

Run at constant current of 2 mA/cm^2 gel for 45 minutes.

DURATION
00:45:00

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